

The British Society for Dental Research (A Division of the IADR) 43rd Annual Meeting April 10-13, 1995 University of Manchester England

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1 T.R. AMESS* (Department of Physiology, University of Bristol, U.K.):

The Effects of Topical Applications of Lignocaine to Dentine in the Cat.

The present experiments investigate the diffusion of lignocaine into cat dentine. They were carried out on lower canine teeth of young adult cats, anaesthetised throughout with sodium pentobarbitone (42 mg/kg I.P. followed by 4 mg/kg I.V. as required). Dentine was exposed by removing 1 mm from the canine's tip with a diamond disc under Ringer's solution and the cut surface etched with acid. A Perspex cap was sealed over the tip of the tooth to provide a reservoir for the solutions to be tested. The effects of lignocaine (10% w/v in Ringer's) applied at atmospheric and 32.5 cm H₂O were determined on the antidromic action potential recorded from the tooth, with a pair of Ag/AgCl electrodes, during electrical stimulation of fibres in the inferior alveolar nerve (I.A.N.) and on the discharge evoked by the application of pressure stimuli. The size of the antidromic single unit and compound action potentials began to decrease 3 mins after application of the lignocaine to the dentine at atmospheric pressure. These effects reversed when the lignocaine was replaced with Ringer's. Raising the pressure of the lignocaine to 32.5 cm H₂O increased the rate at which the size of the action potentials reduced. Hydrostatic pressures of ± 200 mm Hg were applied to the dentine for alternate 3 s periods. The nerve activity evoked was recorded as before and also from functional single pulpal fibres in filaments dissected from the I.A.N. No pulpal nerve response could be elicited during pressure stimulation after the application of lignocaine for 3 min. When the lignocaine solution was replaced with Ringer's limited recovery began after 10-15 min of total blockade. Total recovery of the dentine to control levels was not achieved due to deactivation of the pulpal nerves by the repeated pressure stimuli. It can be concluded that 10% lignocaine applied to dentine for 3 mins will block the response of the intradental nerves to ± 200 mmHg hydrostatic pressure stimulation.

2 D ANDREW* (Department of Physiology, University of Bristol, U.K.):

Dentinal fluid flow and sensory transduction in intradental nerves of the cat.

Displacement of the contents of the dentinal tubules has been implicated in the receptor transduction mechanism in teeth. Experiments were carried out to determine the relationship between fluid flow through dentine and the discharge of intradental nerves in the cat. Animals were anaesthetised with sodium pentobarbitone and dentine exposed by removing 1 mm from the tip of a canine tooth with a diamond disc. The smear layer was removed by etching with acid. In one series of experiments the rate of fluid flow through dentine was recorded simultaneously with single fibre and multi-unit activity evoked in pulpal nerves whilst stimulating the exposed dentine with hydrostatic pressure within the range ± 500 mm Hg. In a second series of experiments neural activity was recorded in the same way during the application of a range of different stimuli. With these stimuli, fluid flow could not be recorded at the same time for technical reasons. Following completion of the electrophysiological recordings dentinal fluid flow was recorded *in vitro* during stimulation of the exposed dentine. In the first series of experiments, of 45 single units isolated, (33 A-fibres cv: 10.2 - 55.1 m/s, 12 C-fibres) 17 (cv: 10.6 - 55.1 m/s) responded to pressure stimuli. C fibres were insensitive to flow through dentine evoked by pressure stimulation. All units were more sensitive to outward than inward flow. In the second series of experiments 55 units were isolated, 9 units (cv: 5.9 - 24.8 m/s) responded to either cooling or mechanical stimulation of dentine only and of 10 other units (cv: 25.6-54.8 m/s) which responded to thermal, hydrostatic pressure, mechanical and drying stimuli all responded in accordance with the hydrodynamic hypothesis. Neural responses of these units to cooling were smaller than those expected from the observed flow; this may be due to flow through unopened as well as opened tubules. It is concluded that these units responded to a common transduction mechanism which involved flow through tubules.

3 V BOOTH* (Guy's Hospital, UMDS, London University): Monoclonal antibodies specific to *Porphyromonas gingivalis* and their use in passive immunisation.

The aim of the current study was to characterise the MAb 61BG (Gmür *et al* 1988; Oral Microbiol. Immunol. 3:181-186) and assess its use in passive immunisation against *P. gingivalis*. The 3 major bands recognised by MAb 61BG in Western blots had M_r consistent with those reported for *P. gingivalis* haemagglutinins. Binding of MAb 61BG in a radioimmunoassay was adsorbed by a protease preparation and the MAB could inhibit haemagglutination of erythrocytes by that preparation. 14 patients with adult periodontitis and indigenous *P. gingivalis* were treated by root planing and a course of metronidazole. Patients were randomly assigned, in a double blind study either to a group who were subgingivally immunised with a solution of MAB 61BG or to a control group who were sham immunised with saline. The applications were repeated on 4 occasions 3-4 days apart. Plaque samples were collected before treatment, at baseline, and at 2 and 6 weeks, 3, 6 and 9 months after immunisation. The level of *P. gingivalis* in the samples was assessed by immunofluorescence. Clinical measurements of controlled force probing, plaque and bleeding were recorded before treatment, 6 weeks and 6 months after immunisation. At 6 and 9 months after immunisation there were significantly lower levels of *P. gingivalis* in deep pockets in the immunised patients (Mann Whitney U $p < 0.01$). However, 2-way analysis of variance showed no differences in the clinical parameters of the 2 groups after immunisation. Topical application of MAB 61BG, which recognised a haemagglutinating antigen of *P. gingivalis* prevented recolonisation of deep pockets by the microorganism 6 and 9 months after immunisation.

4 D DYMOCK (Department of Oral and Dental Science, University of Bristol, Bristol, UK): Molecular analysis of microflora in dento-alveolar abscess.

Dento-alveolar abscesses are mixed infections predominated by gram-negative anaerobes and streptococci. Approximately half of the oral flora is uncultivable and it is therefore likely that uncultivable bacteria are involved in dento-alveolar infections. The aim of this study was to use molecular analysis to determine the flora associated with dento-alveolar abscesses. Pus was obtained by aspiration from abscesses in 3 patients. Part of the sample was processed for bacterial culture. Universal primers were used to amplify 16S rRNA genes, both from the cultivated bacteria and directly from the pus sample, by the polymerase chain reaction (PCR). The PCR products were cloned and then digested with restriction endonucleases to generate 'fingerprint' patterns. By comparison of patterns obtained from amplified genes from the cultivated bacteria and directly from the sample, 5 clones of putative uncultivable organisms were identified. These genes were fully sequenced and compared to databases of 16S rRNA sequences. Two of the clones were identified as *Porphyromonas endodontalis* and *Prevotella oris* respectively. The remaining clones did not correspond to any known organisms; one was related to *Zoogloea ramigera*, a water-borne organism, the second to the genus *Prevotella* and the third to *Peptostreptococcus*.

In conclusion, molecular analysis of the flora associated with dento-alveolar abscesses has been successfully performed and three putative uncultivable bacteria have been identified. The results obtained using this technique have profound implications for all areas of microbial ecology.

5 D O MORRIS (Orthodontic Department, Child Dental Health, The Royal London Hospital, Whitechapel, London, UK): The effects of functional appliances on the soft tissue facial form.

The purpose of this prospective study was to quantitatively assess, cephalometrically and by the use of a 3-D facial laser scanning technique, the treatment changes on the facial soft tissue profile and form produced by three different functional appliances. Fifty-eight patients were randomly allocated to the treatment groups (Twin Block, Bionator or Base appliance) with a further 20 'temporarily untreated' subjects used as controls. All exhibited Class II Division 1 type malocclusions. Each patient had a lateral cephalogram radiograph and a facial laser scan taken at the start of the study and again after 9 months. The radiographs were digitized and 32 hard and soft tissue measurements were calculated and analysed. Groups were compared using two-way analysis of variance (ANOVA) with probability values of 0.05 or less being taken to be statistically significant. A Scheffé's test determined differences among the groups. An 'average face' scan was produced for each of the groups. Each 'averaged' appliance group was compared with the 'average' control group face. The Twin Block group achieved superior changes in their facial soft tissues compared with the other two appliances. Significant advancement and lengthening of the lower lip was seen combined with some forward movement of the chin point and increase in the soft tissue lower and total face height. Each of the functional appliances produced further changes in the soft tissue profile and form than would otherwise have been expected. The laser scanning technique proved to be a simple, non-invasive method of measuring three-dimensionally and provided information comparable to the cephalometric data. The long term significance and ultimate stability of these changes must however be considered with caution.

6 P A MOSSEY*, G M VINTNER and R ARNGRIMSSON (Department of Dental Health*, University of Dundee, UK): Transforming growth factor alpha and orofacial clefting in the West of Scotland.

A sample of 76 parents who volunteered to undergo DNA analysis was derived from a completely ascertained sample of 286 cleft probands in the West of Scotland. Polymerase chain reaction (PCR) amplification and restriction enzyme digestion with three restriction enzymes (Qian J *et al*, *Am J Hum Genet*, 53: 168-175, 1993) was carried out. Allele frequency at the transforming growth factor alpha (TGFA) locus in this parental group was compared to that of a British control population (Holder S E *et al*, *J Med Genet*, 29: 390-392, 1992). The frequency (f) of the TGFA/TaqI 1.7 kb allele (C2) in the parents of cleft lip and palate children (f = 0.13) was statistically significantly higher when compared to the control group (f = 0.04, $p = 0.024$). The isolated cleft palate (CP) parental group showed a similar statistically significant increase in the C2 allele frequency (f = 0.15, $p = 0.013$). The TGFA/BamHI 10 kb allele (A1) frequency (f = 0.25) was also found to be significantly increased relative to the control (f = 0.13) in the parents of children with CP ($p = 0.05$). Conversely, the A1 allele frequency was slightly but not significantly reduced in the CL(P) parents (f = 0.08, $p = 0.26$). There was a highly significant difference in the A1 allele frequency between the CP and CL(P) parental groups (f = 0.25 and 0.08 respectively, $p = 0.0075$).

In conclusion, there is an association between the TGFA gene locus and orofacial clefting in the West of Scotland with differences in allele frequency between the parents of children with CL(P) and isolated CP.

7 F NOHL*, D SETCHELL (Department of Conservation, Eastman Dental Institute, UK): Discrete field stress analysis of intact teeth in-vivo using electrical strain gauges.

An intact maxillary central incisor (3 subjects) and an intact mandibular first molar (1 subject) were chosen as test teeth. A rosette strain gauge was attached to the buccal surface of each test tooth. Whilst occluding on the test tooth through a load cell, subjects attempted to attain steady target loads indicated by labels on a computer screen. All incisor recordings were repeated with a fresh gauge. For each tooth occlusal load and surface strain were recorded simultaneously. Maximum and minimum principal strains, shear strains and their orientations were calculated for each gauge placement at each load. Results showed that the magnitude of strains was consistent with the results of a previous benchtop study (Meredith N, PhD Thesis, University of London 1992). Repeat recordings with the separate gauges were consistent. There was a linear relationship between load and strain. Tensile and compressive surface strains were found. The orientations of strains remained constant with increasing load, in some cases differing from those suggested by the geometry of the teeth and site of load application. The loads attained by the subjects were accurate and steady enough for the purposes of this experiment.

It was concluded that it was possible to measure surface strains on teeth in-vivo. It was also concluded that there was a linear relationship between load and strain, and that strain orientations were not readily predictable from observation of tooth shape and loading site.

8 J DAVEY* (Leeds Dental Institute, University of Leeds, Leeds, UK): Induction of white-spot hypoplasias by hypobaric conditions - a role for albumin?

Chronic exposure to conditions of high altitude (hypobaric hypoxia) is known to result in developmental defects of enamel in the form of white-spot hypoplasias (Angmar-Mansson B and Whitford G, *Caries Res* 24: 420, 1990). In a recent paper, Robinson *et al* (*J Dent Res* 71: 1270-1274, 1992) showed that albumin inhibited crystal growth in vitro and suggested that failure to remove albumin from enamel prior to secondary crystal growth may result in such defects. The aim of this investigation was to determine the albumin content of rat enamel formed under conditions of hypobaric hypoxia. Rats were kept at simulated high altitude for 4 weeks. The animals were killed by anaesthetic overdose, the incisors removed and the entire developing enamel dissected as a series of contiguous particles. Protein was extracted using 0.1M phosphate buffer and by demineralisation. Albumin content was determined by ELISA using polyclonal antibodies to rat serum albumin. Phosphorus content was measured in the same samples (Chen *et al*, *Analyt Chem* 28: 1756-1758, 1956). Qualitative assessment of the protein was effected using SDS PAGE coupled with Western Blotting. The results showed a massive increase in the amount of intact albumin and breakdown products during the transition / early maturation stage of enamel development compared with controls. These persisted into the maturation stage where albumin is usually absent. In conclusion, the mechanisms operating to degrade and remove albumin from enamel may have been unable to cope with the massive influx occurring under hypobaric conditions. The persistence of albumin into the maturation stage may inhibit crystal growth, resulting in the eruption of enamel with white spot hypoplasias.

9

M DUNCALF¹, G M HUMPHRIS², E A FIELD¹ (Departments of Dental Clinical Sciences¹ and Clinical Psychology², University of Liverpool): The development of a questionnaire to assess patient knowledge of oral cancer

The aim of this study was to validate a recently designed questionnaire constructed to assess an individual's knowledge about oral cancer. This work follows the design of a patient information leaflet concerning oral cancer. It is intended to use the leaflet in primary care and clinic settings. The scale was constructed to assess knowledge of: (i) the risks associated with oral cancer, (ii) the screening procedure, (iii) signs of oral cancer and (iv) general features associated with the disease and consisted of 36 questions with a true/false answering format. Some additional questions concerning health behaviour, age and gender were also included. The following predictions were made to test the validity of this scale: (a) trainee dentists would be more knowledgeable than the general public, (b) subjects reading the leaflet would show an increase in knowledge. A multi-group experimental design was employed to remove the practice effect of repeated questionnaire administration. Subjects (n=136, mean age=28 years [sd=11], 65% female) were second year dental students (n=82) and members of the public (n=54). Analysis of variance and paired t statistics were computed to test the above predictions. The results confirmed predictions. Those subjects with access to the leaflet showed raised knowledge levels in comparison with subjects not in receipt of the leaflet (F(130,2)=61.50, p<0.001). Trainee students were more knowledgeable than members of the public (F(130,1)=4.75, p<0.05). Both students and members of the public benefited to a similar degree from reading the leaflet. It is concluded that the measures obtained from the questionnaire behaved as expected.

10

M FOXWORTHY (Department of Oral Medicine and Pathology and Division of Biochemistry and Molecular Biology, UMDS Guy's Hospital, UK):

Structure-function relationship of antibacterial sequences in human lactoferrin

The bactericidal properties of lactoferrin have been attributed to a peptic fragment named lactoferricin. The aims of this study were to determine the site and structure of the lactoferricin peptide in intact human lactoferrin and to identify the antibacterial domain within its sequence. Computer modelling of the 3-D structure of human lactoferrin revealed lactoferricin to be in the N-terminal lobe and that a 15 residue loop was exposed on the surface. A synthetic peptide corresponding to this loop was synthesised. Antibacterial activity was determined by incubating concentrations of peptide, lactoferrin and pepsin-digested lactoferrin up to 500µM with 10⁶ colony forming units of *E. coli* serotype O-111 (NCTC 8007) in phosphate buffered saline at 37°C. Samples were removed at varying times and viability assessed by serial dilution and drop counting and compared to controls using the Mann-Whitney U test. Only the peptide had significant antibacterial activity. The effect was time-dependent and significant at 30 minutes at concentrations above 100µM. Assay of β-galactosidase released from a lactose permease-deficient β-galactosidase-constructive *E. coli* mutant revealed that killing was not associated with bacterial permeabilisation.

It is concluded that a loop domain within human lactoferricin resides on the surface of lactoferrin. A homologous synthetic peptide exerts antibacterial effects which are much more potent than the native protein or peptic digest.

Supported by the Newland Peckley Fund, UMDS & The Wellcome Trust (grant VS/94/UMD008).

11

C. LAWSON (School of Biological Sciences, University of Manchester, UK): Molecular genetic studies of blepharophimosis sequence.

The aim of the investigation was to delineate the critical region encompassing the blepharophimosis sequence (BPES) locus on human chromosome 3. BPES is an autosomal dominant disorder the features of which are small palpebral fissures (blepharophimosis), drooping eyelids (ptosis) and a skin fold arising from the lower eyelid (epicanthus inversus). DNA samples from two individuals, one with an interstitial deletion (case 1) and one with a balanced translocation (case 2) involving chromosome 3, were analysed. Allele loss studies using microsatellite markers in case 2 suggested that the D3S1292-D3S1298 interval was the most likely region to contain the deletion [46,XX,del(q23q25.1)]. Subsequently, the derived chromosomes resulting from the translocation in case 2 were segregated into somatic cell hybrids. Polymerase chain reaction analysis of these hybrids showed that the DNA marker D3S1549 was retained in the derived chromosome 3, whereas the marker D3S1580 was retained in the derived chromosome 4. In neither case was the marker present in the reciprocal hybrid. These markers lie 6 centimorgans apart (Gyapey G. *Nat Genet* 7: 246-339, 1994).

It is concluded that both of these markers are contained within the deletion and define the critical region as being approximately 8 megabases of DNA. This study lays the foundation for the isolation of candidate genes for BPES.

12

W. McLEAN*, B.J. MOXHAM, R.C. HALL (Anatomy Unit and Basic Dental Sciences UWCC and UWCM, Cardiff). Localisation of Decorin and Versican within rat palatal shelves during normal palatogenesis.

Palatal shelf elevation is thought to involve an "intrinsic shelf-elevation force", generated by hydration of the mesenchymal extracellular matrix within the shelves. In the present study we have begun to analyse the proteoglycan population in Wistar rats, at different stages of palatogenesis, by the immunolocalisation of decorin, biglycan and versican. Six rat fetuses (age E15 and E16) were fixed (95% ethanol, 24h), wax embedded, serial sectioned and immunolabelled with polyclonal antibodies. Immunoreactivity was visualised with a fluorescein-conjugated secondary antibody. The findings indicate that decorin and versican (but not biglycan) are present in the palatal shelves, both before and after elevation. Decorin was only localised in the anterior region (i.e. the presumptive hard palate). Prior to elevation, decorin was localised to the basement membrane, with mesenchymal extension in the anterior palatal shelf. Post elevation, localisation of decorin along the basement membrane was not so marked. Instead, decorin appeared to be present within the central region of the anterior palatal shelf. *The presence of decorin anteriorly and its absence posteriorly may be related to the fact that, for the rat, it is only the anterior portion of the palatal shelves that elevated. This is consistent with its association with collagen fibrillogenesis and binding of growth factors. The widespread pattern of distribution of versican may relate to its role in binding hyaluronan, which has been implicated in shelf elevation.*

13

S. MacMILLAN (Department of Adult Dental Care, University of Glasgow, U.K.): Factors associated with shear bond strength of composite resin to human enamel.

The effect of enamel surface preparation before etching has been studied and the relationship of etchant pH and surface topography as related to shear bond strength of composite resin to enamel. Groups of 10 enamel specimens were treated with four etching regimes: 5 groups received mechanical surface preparation before a bonding resin and a hybrid composite resin was applied. After 7 days, the specimens were thermocycled and the magnitude of the composite enamel bond tested under shear load. Variations in the etchant pH at the enamel surface were measured over a 2 minute period and correlated with bond strength. Etched enamel surfaces were studied under SEM and the topography correlated with the shear bond strength. Results showed that surface preparations did not significantly improve the bond strength and if nitric acid conditioner were allowed to desiccate on the enamel surface the resultant bond strength was significantly reduced compared to all other etchant materials. No correlation was observed between the etchant pH and the bond strength but the surface topography of the etched enamel specimens on SEM correlated moderately well. *It is concluded that enamel preparation and etchant pH have no effect on the mean shear bond strength but that the surface topography of the etched enamel correlates moderately well.*

14

J McWILLIAM (Department of Oral Sciences, University of Glasgow, UK): A laminated assessment of fluoride in white spot lesions after either *in situ* or *in vitro* fluoride exposure.

The aim of this study was to measure the fluoride concentration in artificial white spot lesions at depth stages of 20µm, and further to assess if the F concentration would change after exposure to F either intra- or extra orally over a period of 8 days. Eight blocks of bovine enamel (1mm²) were allocated to each of 4 protocols: sound enamel (SE), control lesion (CL), *in vitro* remin (IVR) or *in situ* remin (ISR). Artificial lesions were created by exposing the blocks to a standard demin solution for 5 days. Microradiographs were taken of a sample of each lesion to determine accurately the lesion depth using image analysis. The IVR involved daily exposure for 8 days to an 1100ppm NaF dentifrice slurry (5:1). The ISR involved the mounting of the blocks in an *in situ* appliance and were exposed twice daily to the NaF dentifrice for 8 days. A lesion sampling technique was developed based on that of Weatherell *et al.* (1985), by mounting the blocks on to the vertical probe of a Mitutoyo micrometer. Thin serial samples of mineralised tissue were then abraded from the natural enamel surface inwards at 20µm stages (±1µm). Image analysis indicated that the lesions were never deeper than 230µm. The enamel powder was weighed and dissolved in 0.5M perchloric acid and the fluoride concentration calculated using an Ion Selective electrode. The mean total F concentrations (ppm) for each of SE, CL, IVR and ISR were: 952.5 (±30.0), 665.7 (±16.7), 897.7 (±17.5) and 830.6 (±14.4) respectively. The individual fluoride concentrations at the various depths throughout the lesions was mimicked closely by the mineral content distribution for both control and remineralised samples. At a depth of 200µm, the fluoride concentration became similar for all lesions. *This study has shown that it may be possible to correlate the fluoride profile with the mineral content of porous enamel.*

15

L.K.S.TAN¹, G.R.OGDEN¹ & S.F.DOWELL² (Dept of Otolaryngology, Ninewells Hosp & Med School, Dept of Dent Surgery & Pathology, University of Dundee). Antigen retrieval of p53 in lesions of the head and neck.

The tumour suppressor gene p53 is frequently over-expressed in neoplasia, such overexpression is often associated with gene mutation. p53 over-expression can be detected in paraffin sections of tumours using immunohistochemistry. Microwave antigen retrieval of sections prior to immunohistochemistry can increase antigenicity and therefore the rate of detection of p53 over-expression. Paraffin sections of 58 benign and 45 malignant lesions of the oral cavity and larynx were immunostained for p53 using D01 at a 1:50 dilution, with and without antigen retrieval. The antigen retrieval technique was microwave boiling (750W) in 10mM citrate buffer for 10 minutes. Without antigen retrieval p53 over-expression was observed in 1/58 (1.7%) benign lesions and 19/45 (42%) malignant lesions. After antigen retrieval 56/58 (97%) benign lesions and 37/45 (82%) malignant lesions showed positive staining. In benign conditions staining was mainly confined to basal and parabasal cells. Stromal cells remained negative in all specimens. We postulate that proliferating cells may have an increased level of wild-type p53 which, although not normally detected in routine immunocytochemistry, is seen after antigen retrieval. *Conclusion: Antigen retrieval increases the sensitivity of p53 immunohistochemical detection, but such staining should be interpreted with extreme caution.*

16

DW THOMAS*, P STEPHENS, S LIM¹ and JP SHEPHERD (Departments of Oral Surgery, Medicine & Pathology and ¹Haematology, UWCM, Heath Park, Cardiff, UK): T-Cell Receptor (TCR) Vβ gene usage in intraoral squamous cell carcinoma.

In the evolution of an anti-tumour immune response, clones of antigen-reactive CTL evolve, which have been shown to exhibit specific anti-tumour cytotoxicity *in vitro* (Yasumura *S et al*, *Int J Cancer* 57: 297-305, 1994) and represent potential targets for inductive immunotherapy. This study used reverse transcription polymerase chain reaction (RT-PCR) to analyse the TCR Vβ repertoire in tumours, regional metastases and peripheral blood of patients with HNSCC and normal control patients. Biopsy specimens were divided into two, one portion snap-frozen and the remainder routinely processed. Lymphocytes were harvested from peripheral blood by density centrifugation. Total RNA was isolated from specimens using the RNazol method. cDNA was synthesised using Mol.V reverse transcriptase and random hexamer primers. PCR was performed for fragments of cDNAs encoding twenty-five 5'Vβ primers together with the corresponding C-region 3' primer specific for 24 TCR Vβ families. Negative controls included the omission of the cDNA; positive controls included amplification with β-actin and Ca-specific primers. Amplified products were identified by agarose gel electrophoresis and visualised utilising ethidium bromide staining.

It was concluded that restricted TCR usage is not a feature of normal oral mucosa but in the primary tumours restricted usage may occur in primary HNSCC and is a feature of tumour-bearing lymph nodes.

- 17** P.A. ROBINSON*, H. KAZEMI, J. LEEK, W. HUME & A. MARKHAM. (Leeds Dental Institute, *Mol Med Unit, Univ. of Leeds, UK): Chromosomal localisation of terminal differentiation genes differentially expressed between keratocyst and normal oral epithelia. Multiple odontogenic keratocysts are often associated with the autosomally inherited nevoid basal cell carcinoma syndrome (NBCCS). We compared the transcription of genes between odontogenic keratocysts and normal oral palatal mucosa using differential hybridisation. A number of genes were found to be differentially expressed including two small proline-rich protein genes (SPRC and SPRK, cornified envelope proteins) that are associated with the formation of cornified envelopes. SPRC and SPRK genes demonstrate strong homology to SPRR1 and SPRR3, respectively (Gibbs et al., [1993] Genomics 16, 630-637). SPRK transcription appeared to be down regulated whereas SPRC gene transcription was upregulated. The chromosomal localisation of these genes were determined using Human-rodent somatic cell hybrid panels were screened by PCR. The Leeds Molecular Medicine Yeast Unit artificial chromosome (YAC) library was screened to isolate YACs containing the SPRC. One out of three YACs that contained the SPRC gene (400kb) also contained the involucrin gene. FISH on metaphase spreads of lymphoblastoid cells was performed using biotinylated YACs. The chromosomal localisation of the SPRC/involucrin gene was 1q21. It is now known that this region of chromosome 1 is also the location from which many other terminal differentiation genes are transcribed. Loss of regions of chromosome 1q are often lost in HNSCC as well as carcinomas of the breast, bladder and uterus. We are constructing a yeast YAC contig of this cluster to identify novel terminal differentiation genes and to investigate the frequency with which this region is lost or translocated in tumours of NBCCS patients and in HNSCC.
- We have demonstrated that differential hybridisation can be employed to identify differences in gene transcription between odontogenic keratocysts and normal palatal mucosa. A YAC contig of the terminal differentiation gene cluster at 1q21 is now being generated to identify and map other genes transcribed from this region in normal and diseased tissue.*

- 18** NJ LENCH*, AS HIGH, WJ HUME and PA ROBINSON (Leeds Dental Institute, Leeds, UK) Deletion of chromosome 9q DNA markers in odontogenic keratocysts.

The nevoid basal cell carcinoma syndrome (NBCCS) or Gorlin syndrome is a complex pleiotropic autosomal dominant disorder, associated with a broad spectrum of developmental abnormalities, and a predisposition to a number of different neoplasms, in particular basal cell carcinomas (BCCs). The NBCCS locus has been mapped to chromosome 9q22.3-q31 using genetic linkage studies. The consensus DNA marker order in the region is: cen-D9S196-D9S287-D9S180-D9S176-D9S109-D9S127-tel. Loss of heterozygosity studies of both hereditary and sporadic BCCs have demonstrated deletion of large regions of chromosome 9q although to date, deletion end points within 9q22.3 have not been observed. In order to define the critical region containing the NBCCS locus, we have chosen to examine DNA from odontogenic keratocyst tissue for loss of heterozygosity. Odontogenic keratocysts occur at a very early age and may be one of the first clinical manifestations of homozygous inactivation of the NBCCS gene. We have analysed DNA, microdissected from tumour and normal paraffin-embedded tissue sections, for loss of heterozygosity by PCR of the microsatellite markers D9S196, D9S287, D9S180, D9S173, D9S176 and D9S127. Preliminary results indicate that there is loss of heterozygosity for some or all of these markers in odontogenic keratocyst tissue from various patients.

Combination of data from all patients tested define a minimum region of deletion defined by the markers D9S287 centromeric and D9S176 telomeric, a distance of approximately 350kb.

- 19** C.L. WU*, L. ROZ*, S. HOLLAND, P. SLOAN, A.P. READ, N.S. THAKKER. (University of Manchester, *Eastman Dental Institute, London): Two discrete areas of loss of heterozygosity on chromosome 9q in oral squamous cell carcinomas.

The genes for the basal cell nevus syndrome (BCNS), multiple self-healing epitheliomas (BSS1) and Fanconi's anaemia type C map to chromosome 9q. Furthermore, loss of heterozygosity (LOH) studies indicate presence of a tumour suppressor gene involved in bladder carcinoma in this region. We have examined for LOH on chromosome 9q in 36 oral squamous cell carcinomas (SCCs) using 13 highly informative microsatellite polymorphisms. Overall, ~40% of the samples showed LOH and at least two non-contiguous regions were identified. The more proximal of these regions maps to 9q22.3-31 and is centred around D9S180; ~30% of informative samples show LOH at this locus. The distal region encompasses 9q31-34.3 with ~35% of informative samples showing LOH at D9S177 and/or D9S164.

These data indicate involvement of two tumour suppressor genes on chromosome 9q in oral carcinogenesis. The two regions of deletion identified closely overlap the BCNS/BSS1 locus (9q22.3) and the area of deletion in bladder carcinoma (9q31-34.1) respectively.

- 20** C.L. WU, L. ROZ*, S. HOLLAND, P. SLOAN, A.P. READ, N.S. THAKKER*. (University of Manchester, *Eastman Dental Institute, London): Two discrete areas of loss of heterozygosity on chromosome 8p in oral squamous cell carcinomas.

Loss of heterozygosity (LOH) studies in various tumours including colorectal, lung and prostatic carcinomas indicate the presence of two tumour suppressor genes on the short arm of chromosome 8. We have constructed a detailed deletion map for chromosome 8p in oral squamous cell carcinoma (SCCs) using 14 highly informative microsatellite polymorphisms. Thirty-six SCCs were examined for LOH. Overall, ~55% showed LOH at one or more loci on chromosome 8p. Interstitial deletions were identified in a majority (~95%) of tumours showing LOH. These specified two non-contiguous regions of deletions; the proximal region is defined by the markers D8S133 and D8S298 at 8p21. Approximately 50% of the samples informative at either of these 2 loci showed LOH. The distal region is between D8S265 and D8S261 at 8p22. Every case that had the distal region of deletion showed LOH (where informative) at D8S254 which maps between D8S265 and D8S261.

These data indicate the presence of two tumour suppressor genes on chromosome 8p that are involved in oral carcinogenesis. These genes map to 8p21 and 8p22. The regions of deletion identified show a close overlap with those observed in carcinomas of other tissues.

- 21** A. DIRANIA*, E.D. THAKKER*, N.S. THAKKER*, P. SLOAN*, S.E. HOLLAND*, J.V. SOAMES*. (Universities of Manchester* and Newcastle*): A preliminary study of nm23H1 expression in primary oral squamous cell carcinoma.

nm23 is a putative metastasis suppressor gene whose expression has been inversely correlated with metastatic potential in experimental systems and human breast carcinoma (Royds JA, et al., J. Pathol 173: 211-212, 1994). The aims of this study were to determine whether nm23H1 was expressed in oral squamous cell carcinoma (SCC) and to compare its expression in a series of metastatic and non-metastatic tumours. Paraffin sections from 50 oral primary SCCs, 25 of which showed regional metastases, were reacted with a monoclonal antibody to nucleoside diphosphate kinase A² (which has sequence homology with nm23H1) using an immunoperoxidase technique. The stained sections were assessed consensually by three observers. nm23H1 expression was demonstrated in 22 of the non-metastatic and 20 of the metastases proven SCCs. Variations in staining pattern and intensity were seen within and between tumours. There was evidence of both nuclear and cytoplasmic positivity. Further studies of the lymph node metastasis are in progress.

nm23H1 is expressed in oral SCCs. However this study has not demonstrated any correlation between expression in the primary tumour and its metastatic potential.

*Novocasts Laboratories Ltd.

- 22** J.P. MCCABE* and R.M. BASKER* (Universities of Newcastle-upon-Tyne and Leeds, UK): Characterisation of viscoelastic properties of long-term soft lining materials.

The purpose of this 3-centre trial was to evaluate methods which could be used for standard specification testing of long-term soft denture liners. Four materials¹ were subjected to tests including depth of penetration (at 5s and 30s) and elastic recovery at both room temperature and 37°C. The softness of materials as determined by penetration at 5s was A 0.65mm, B 0.49mm, C 0.97mm and D 0.56mm. Material C was significantly softer than the other materials (p<0.05 ANOVA). There was good agreement between test centres. The penetration ratio (30s value/5s value) for each material was A 1.49, B 0.99, C 1.23 and D 1.08. There was significant inverse correlation between this ratio and elastic recovery (p<0.05) indicating that the viscoelastic properties of materials could be characterised by a single penetration test. For some materials (eg A) penetration values were highly dependent on test temperature, whilst for others (eg B) penetration was independent of temperature. This indicates the importance of standardising temperature in these tests. A simple penetrometer test performed at 37°C can be used for the meaningful characterisation of long-term soft lining materials.

¹ A-Coe Super Soft, B-Molloplast B, C-Palasisiv, D-Movus.

- 23** M.G.J. WATERS* and R.G. JAGGER (Cardiff Dental School, UK): Properties of a new experimental silicone soft lining material.

Previous work has shown that an experimental room-temperature vulcanising silicone soft lining material had favourable properties. (Waters M.G.J. and Jagger R.G. J Dent Res 73: 807). However the material was deemed unacceptable because of its high water absorption. A modification of this material has since been developed incorporating a new surface treated silica filler. In this study the new modified material was compared with Molloplast-B and the original material. The hardness, wettability, bond to denture base, resistance to tear, viscoelastic properties and water sorption properties were investigated. All specimens were prepared using a conventional dough moulding technique. The experimental material was cured for 24 hours at room-temperature and Molloplast-B was cured according to manufacturers instructions. The new formulation had low water absorption and solubility. It was slightly softer than the original formulation and slightly firmer than Molloplast-B, and its wettability characteristics were similar to both. The tear resistance and bond to the denture base of the new material however were greatly superior to both the original formulation and Molloplast-B. The new materials resilience and response to load was also improved over the original formulation, and was closer to that of Molloplast-B. The new experimental silicone soft lining material has shown favourable properties in comparison to Molloplast-B and the original formulation. Clinical studies are now needed to further assess its potential as a new material.

¹ Regneri GmbH and CO., Germany.

- 24** J.T. MCGILL*, N.J. JEPSON, J.F. MCCABE. (University of Newcastle upon Tyne): Influence of dietary stimulating solvents on viscoelasticity of temporary soft lining materials.

Clinical changes in the viscoelasticity of temporary soft lining materials are characterised by a more rapid and increased reduction than observed in-vitro. A solvent effect acting clinically is a possible explanation. In this investigation four temporary soft lining materials (Coe Soft, Coe Comfort, GC Soft Liner and Viscogel) were immersed at 37°C ± 1°C in 8% alcohol, 50% alcohol, Heptane and Corn oil, solutions chosen to simulate dietary solvents. Viscoelastic changes over time were compared to a controlled immersion in distilled water. Three 2 mm thick specimens of each material for each immersion were prepared on cast acrylic sheet bases. Three sites on each sample were subject to a 28.7g load applied for 5s and then removed using a penetrometer modified to continuously record creep strain and strain during recovery. Testing was performed at 2 hours, 24 hours, 2,4,7,14,21 and 28 days after sample preparation. Results indicated that all immersion solutions caused a significant reduction in compliance values of each material over time (p<0.01). However only heptane and corn oil immersants produced the rapid and increased reduction in compliance which is evident clinically. Changes in the elastic recovery were less pronounced.

The results suggest that a clinical solvent effect is taking place with a dietary source being a possibility. Heptane and corn oil may form the basis of a more clinically relevant immersion regime used to evaluate temporary soft lining materials.

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A SHORTALL*, E HARRINGTON (The School of Dentistry, The University of Birmingham, U.K.). Influence of light intensity on polymerisation of three light cured composites.

Light activated composites rely on adequate intensity of the light source to cause polymerisation through the bulk of material. Material factors such as filler size and loading, resin composition and shade are influential in determining the effective depth of cure. This study compared upper and lower surface microhardness of 2mm thick and 4mm diameter samples of three light cured composites 1-3 cured with twelve light curing units. Composite samples were irradiated for 40s at zero distance from the light guide tips. Light intensity was measured with a commercially available dental radiometer. Mean light intensity for the light activation units tested ranged from 101 to 725 (mW/cm²) at full optic diameter. Upper surface microhardness was little affected by changes in light intensity. Lower surface microhardness was related to light intensity and the correlation between lower surface microhardness and light intensity was most pronounced with the microfilled composite³.

Material composition is a very important variable at low light intensity in determining adequate polymerisation in bulk.

- | | |
|--------------------------|------------------------|
| 1. Herculite XR, Kerr | 2. Z100, 3M Dental |
| 3. Silux Plus, 3M Dental | 4. Cure-Rite, Efos Inc |

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D.J.WITTER¹, P.F.ALLEN², A.F.KAYSER¹, N.H.F.WILSON² (Oral Function, University of Nijmegen, The Netherlands; ²Restorative Dentistry, University of Manchester, UK). Dentists' attitudes to the shortened dental arch concept.

This study investigates the attitudes and experiences of a group of dentists in Nijmegen towards the shortened dental arch concept (SDA). A questionnaire was sent to 65 dentists employed in the University Dental Hospital of Nijmegen. Forty one responses were received - a 65% response. The results indicate that 98% (n=40) of the respondents believe that SDA has a place in contemporary clinical practice, with 83% (n=34) indicating current criteria to be satisfactory. This belief does not appear to be influenced by either time spent or special interests in clinical practice. Thirty eight (93%) of the respondents reported having prescribed SDA in the last 5 years. Thirty (75%) of the respondents reported patients being considered for SDA having voiced concerns regarding treatment outcome. However, 38 (90%) of the respondents indicated that SDA provides a satisfactory outcome in terms of oral function, comfort and dental appearance in c.90% of cases. Thirty one respondents (76%) reported having to provide dentures following SDA-27 (66%) indicating that this was necessary in <10% of cases treated.

It is concluded that the results lend further support to the view that the shortened dental arch concept has a place in contemporary clinical practice and may be found to provide a satisfactory outcome in the majority of cases.

27

D J JOHNSTON*, J G MCGIMPSEY AND R B LONGMORE (School of Clinical Dentistry, The Queen's University of Belfast, UK); Cross Infection: Comparison of attitudes of dentists, dental students and patients in N Ireland.

This study investigated the attitudes of dentists to cross-infection control and compared them with those of dental students. Patient's awareness and reactions to these measures were also sought. Questionnaires covering aspects of cross-infection including gloves, masks and eye protection were completed by dentists, dental students and patients. The study confirmed that dentists, dental students and patients had a high awareness of the necessity for cross infection control. The positive attitude of dentists to the wearing of gloves at all times (72%) was better than that shown by Glenwright and Shovelton (1987) but not as high as the results achieved by Burke et al, 1994², which gave a positive response of 83%. The percentage of dentists who thought gloves should be worn for all patients decreased significantly with age: whereas 82% of dentists under 31 years thought gloves should be worn for all patients this had fallen to 43% of dentists over 51 years of age. 79% of patients thought that dentists should wear a new pair of gloves for each patient, but only 25% of dentists thought likewise - most dentists indicated that they would decide when to change or wash their gloves. Dental students were strongly in favour of wearing gloves for all patients (87%) and all three groups felt the wearing of gloves afforded protection to both patient and dentist. The majority of participants were in favour of masks being worn by the dentist during treatment although 13% of patients felt intimidated by the wearing of a mask and protective glasses. Most patients (71%) and dental students (65%) favoured the wearing of eye protection at the dentist's discretion but 65% of dentists thought that eye protection should be worn at all times.

The study confirmed that patients have a high level of expectation and acceptance of measures to control cross infection which may not be fully recognised by dentists and dental students.

28

TLP MATTS* and L MILLARD (Department of Periodontology, and Educational Adviser, UHDS (Guy's), London, UK); Personality profiles and staff-student interaction.

This study examined the possible effect of personality differences on staff-student relations in a large dental school. A complete fourth year of dental students (n=87), and those teachers whom they met regularly (n=80), were asked to participate. Subjects completed a form of the Myers-Briggs personality questionnaire simplified for use in education, and were asked to assess their relationship with persons in the other group. All students and 75% of staff returned usable data. There was close similarity between staff and student personality profiles, and perception of working relationships by both groups was largely independent of personality factors and temperament. There were minor differences in staff perception of their relationship with extrovert and introvert students. Students showed minor differences in their perception of staff relationships with respect to two personality factors.

These findings indicate a substantial similarity between personality profiles for staff and students, and suggest a mature and stable relationship between people in the two groups.

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A A QUAYLE*, C UCER and H CARTER (Department Dental Medicine and Surgery, University of Manchester UK); Effects of autogenous bone graft on bone regeneration in osteopromotion. A laboratory study.

The aim of this study was to study the effects of autogenous bone graft on osteoneogenesis beneath barrier membranes composed of polydioxanone¹. Rabbits were anaesthetised with halothane (2%), nitrous oxide (20%) and oxygen. Non-self-healing cranial defects were created in rabbits. Non-permeable sheets of PDS were applied mono-cortically and bi-cortically to cover the defects, which were filled with autogenous bone chips harvested from the skull. Control defects were filled with blood clot only. Animals were sacrificed at intervals up to 24 weeks by an overdose of pentobarbitone, administered intraperitoneally, and the excised specimens were studied histologically. This experiment demonstrated that bone regeneration occurs with equal facility at bone control and experimental sites. At the latter, however, even after 24 weeks, non-vital bone fragments representing the free grafts were evident, incorporated in the newly formed vital bone trabeculae.

This study suggests that the use of autogenous bone chips may delay completion of osteoneogenesis in the membrane technique.

¹PDS (R)-Ethicon

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C UCER*, H CARTER and A A QUAYLE (Department of Dental Medicine and Surgery, University of Manchester UK); Bone Regeneration using a non-permeable resorbable barrier membrane: A laboratory study.

The aim of this study was to investigate the efficacy of a non-permeable resorbable membrane (Polydioxanone)¹ as a barrier membrane in osteopromotion. Rabbits were anaesthetised with halothane (2%), nitrous oxide (20%) and oxygen. Non-self-healing cranial defects were created bilaterally. Non-permeable sheets of PDS were applied mono-cortically and bi-cortically to cover the defects, which were filled with blood clot. Animals were sacrificed at intervals up to 24 weeks by an overdose of pentobarbitone, and the excised specimens were studied histologically. This experiment demonstrated that bone regeneration occurs beneath such membranes. The rate of bone formation is rapid and develops a mature trabecular pattern with bicortical laminae. The PDS membranes were completely resorbed by hydrolysis and phagocytosis by macrophages within the period under investigation.

This study suggests that polydioxanone (PDS (R)) may be a suitable material for use as a barrier membrane in osteopromotion in dental implantology.

¹PDS (R)-Ethicon

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S TABIBI*, H CARTER and A A QUAYLE (Department of Dental Medicine and Surgery, University of Manchester UK); Bone regeneration using a macroporous resorbable barrier membrane: A laboratory study.

The aim of this study was to investigate the efficacy of a macroporous resorbable membrane (Polyglactin 910)¹ as a barrier membrane in osteo-promotion. Bilateral non-self healing cranial defects were created in rabbits, which had been anaesthetised with halothane (2%), nitrous oxide (20%) and oxygen. Porous polyglactin 910 membrane was applied monocortically and bicortically to cover the defects, which were filled with blood clot or autogenous bone chips. Animals were sacrificed at intervals up to 24 weeks by an overdose of pentobarbitone administered intraperitoneally. Excised specimens were examined histologically. This experiment demonstrated that bone regeneration occurs beneath such membranes, but that it develops primarily as individual islands of bone and does not have the mature trabecular pattern observed when Gortex(R) or PDS(R) membranes are employed.

It is concluded that Polyglactine 910 may be unsuitable for use as a barrier membrane in osteopromotion in dental implantology.

¹Vicryl(R)-Ethicon

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LA SALATA, GT CRAIG, IM BROOK* (Universities of Sao Paulo, Brazil, and Sheffield, UK); *In vivo* evaluation of a new membrane (Gengiflex®) for Guided Bone Regeneration (GBR).

GBR became established as a successful technique in the late 80s. Membranes of varying types have been proposed depending on the clinical situation, with Gore-Tex¹ (GT), an expanded polytetrafluoroethylene membrane being extensively used to date. Gengiflex² (GF), a new alkali-cellulose membrane produced by biotechnological process has been shown to have similar biological properties to GT *in vitro* (Salata, LA, et al, J Dent Res, 72:729,1993). We compared the biological performance of GT and GF using the *in vivo* non-healing bone defect model proposed by Dahlin, C, et al (Plast Reconstr Surg, 81:672-676,1988).

Under anaesthetic (halothane³ 2% in oxygen 25% and nitrous oxide 75%), holes 3mm in diameter were drilled through both mandibular ramus of twenty Sprague-Dawley rats. On one side either GF or GT were applied both lingually and buccally. The contralateral site was used as a control, and left to heal unaided. Five animals from each group were killed at 4 and 10 weeks following surgery. Specimens were fixed in formal saline, wax embedded and stepped sections at 50 µm intervals were used for histologic analysis. The area occupied by new bone within the defect was quantified using computerized histomorphometric analysis⁴. The inflammatory reaction to both membranes was also assessed using a semi-quantitative method. GT membrane was associated with significantly less inflammation, both GT and GF membranes promoted the same amount of bone formation at both time periods. A greater amount of bone formation was present in bone defects protected by either GT or GF membranes, when compared to the control sites. GT membrane is better tolerated by the tissues than GF. New bone formation was essentially the same for both membranes.

- | | |
|--|--------------------------------|
| 1. Gore-Tex®, Gore and Associates, USA | 3. Halothane®, May & Baker, UK |
| 2. Gengiflex®, BioFill, Brazil | 4. Optimas® 4.1, Biosoft, USA |

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C WITTWER*, C W I DOUGLAS, P V HATTON and I M BROOK (Biomaterials Group, School of Clinical Dentistry, University of Sheffield, UK): Release of Clindamycin and Cephalixin from Ionomeric and Acrylic Cements.

Acrylic bone cements containing antimicrobial agents have been successfully used in joint replacement surgery. Novel ionomeric cements have several potential advantages over acrylics. The aim of this study was to compare the release of two antibiotics from ionomeric¹ and acrylic² cements. Discs 12 mm x 2.5 mm containing 1% to 10% w/w of cephalixin or clindamycin were prepared from both cements. The discs were placed into phosphate buffered saline (PBS) at 37°C eluted and sampled between 1 hour and 9 months. Released drug was measured using uv spectrophotometry and MIC determinations were performed using *Staphylococcus aureus* (Oxford strain), *Streptococcus sanguis* NCTC 7863, *Escherichia coli* NCTC 10418 and *Bacteroides fragilis*. Cephalixin was readily eluted from ionomeric discs, but at low drug loading it was poorly released from acrylic. Clindamycin was released well from both matrices, however, the release being much better from acrylic. Within the first 10 days therapeutic concentrations of clindamycin were released from all discs. For cephalixin a drug load of 5% was necessary in acrylic and 3% in ionomeric cements to achieve therapeutic concentrations. For both matrices the activity of cephalixin was found to be slightly diminished after 10 days of elution and was almost lost after 1 month. In contrast clindamycin retained almost full activity for 3 months. *Ionomeric cements are suitable matrices for delivery of cephalixin and clindamycin. The discs under study were active against the strains tested for at least 10 days.*

1. V-O CEM, Experimental Ionomeric cement, Ionos GmbH and Co KG, Germany
2. Simplex Rapid Clear®, Howmedica Ltd, UK

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K K JOHAL*, G T CRAIG, A J DEVLIN, R G HILL*, E de BARRA*, P V HATTON, G HENN, and I M BROOK (Universities of Sheffield, UK & *Limerick, Ireland): Ionomeric cements: in vivo response to set rods with increasing sodium content.

Ionomer cements (ICs) have potential as bone cements and substitutes as they exhibit rapid, non-exothermic setting, high compressive strength, adherence to bone and metal and release biologically active ions. This investigation aimed to evaluate a defined series of glasses for use as ionomeric bone cements. These glasses were based on the general formula $1.58\text{SiO}_2 \cdot 0.5\text{P}_2\text{O}_5 \cdot \text{Al}_2\text{O}_3 \cdot (1.0-Z)\text{CaO} \cdot 0.75\text{CaF}_2 \cdot \text{ZnO}$, where Z ranges from 0 to 0.2 and is the mole fraction. Under anhydrous conditions (May & Baker, UK); in oxygen 25% and nitrous oxide 75% set cement rods (2mm x 1mm in diameter) were implanted in the midshaft of weaned Wistar rat femora via a 1mm diameter burr hole. After four weeks, femora were harvested, fixed in 10% formal saline, decalcified in 10% formic acid and wax embedded. Serial sections (7µm) were cut using a microtome, and stained using haematoxylin and eosin. Tissue responses were studied using inverted light microscopy linked to an image analysis system (Optimas 4.1, Biosoft, USA). New bone was observed apposed to the surface of the implanted ICs. In some cases, foci of foreign body giant cells were observed at the surgical site. Increased levels of new bone formation were observed as the sodium content of the glass increased. The control material (LG2) which contained no sodium was associated with the least new bone formation, while the material with the greatest sodium content (LG63a) was associated with the greatest level of new bone formation. *These model IC formulations were osteoconductive. Increased levels of new bone formation were observed with increasing sodium content. This correlates with the increased fluoride ion release that accompanies increasing sodium content.*

35

PA BRUNTON*, S RICHMOND and M WILSON (Restorative Dentistry and Orthodontics, University of Manchester, UK): Quality assessment of preparations for resin-bonded restorations.

It is considered that inter- and intra-operator inconsistencies contribute to wide variations in preparations for resin-bonded inlays and porcelain laminate veneers (PLVs). In this study random samples of dies for resin-bonded inlays and PLVs were assessed by two groups of clinicians in respect of six characteristics: depth (veneers only), taper (inlays only), finish, design, contour, space for restoration and overall quality. The 40 dies in each of the two samples were assessed twice by the members of the groups working independently and according to a prescribed schedule under standardised conditions. The assessments were compared collectively and separately to investigate the agreement within and between the groups regarding the various characteristics of the preparations. Kappa values of between 0.09 and 0.8 were obtained in respect of the PLV preparations and of -0.14 to 0.84 for the inlay preparations. Data obtained using a reflexmograph was compared with the subjective assessments of the clinicians. The level of agreement between and within the groups was generally poor (Kappa values <0.4). *It is concluded that further work may confirm the need to develop techniques to improve consistency among clinicians in the preparation of teeth to receive resin-bonded inlays and PLVs.*

36

JF ALLSOPP*, JB MATTHEWS, PM MARQUIS and JW FRAME (School of Dentistry, University of Birmingham): Research in general dental practice.

To utilise the wealth of potentially useful data generated by general dental practitioners (GDPs), the Postgraduate Board appointed a 'Research Facilitator' to co-ordinate/initiate research in general practice. The aim of this report is to show the potential of GDP-based research by reporting the results of a pilot study on current use of amalgam following recent adverse media coverage.

GDPs interested in participating in practice-based research projects (n=31) were asked to complete a simple questionnaire covering various aspects of the choice and use of amalgam alloy. Analysis of the responses revealed that perceived quality of an amalgam alloy is significantly more important (p<0.05, Wilcoxon) in its choice than either cost or availability. The majority (83.3%) had used only one brand of amalgam alloy in the previous year, with 85.7% using an amalgamator as opposed to a encapsulated delivery system. Group members placed 22.7±10.1 amalgams/week with the age of replaced amalgams being 9.7±1.7 years. Although the majority (64.3%) did not perceive any 'at risk' groups associated with the use of amalgam, most reported increasing use of alternative restorative materials which was limited by patient charge (92.9%). The mean rate of patient inquiries into the safety of amalgam was 5.5/week. Although social class I only accounted for 21.4% of such inquiries they accounted for 45.5% of the alternative restorations placed.

This study demonstrates the financial limitations on the placement of alternative restorative materials and indicates the potential of a general dental practitioner-based research group.

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R J McCORMICK*, N MEREDITH and M SHERRIFF* (University of Bristol and UMDS, London, UK): The Effect of Lossy Compression on the Diagnostic Value of Digital Radiographic Images - A Pilot Study.

A pilot study has been designed to investigate the effects of image compression on the diagnostic value of digital radiographic images subjected to lossy compression algorithms. Forty conventional bitewing radiographs were used, half of the sample displaying a carious lesion within enamel and the other half considered normal. These films were converted to a digital format using a viewing box and a 3 chip Sony CCD camera (DXC 930P), producing a composite video signal. Analogue to digital conversion was performed by a Video Vue* card, and the images saved as 8 bit Targa* files, resolution 640 x 480 pixels. These files were then duplicated and saved in JPEG* file format at 2 different levels of compression. These 120 images were displayed, in random order, on an IBM compatible personal computer, at full resolution. A clinician was asked to diagnose caries from these images using a confidence scale. The results were compared to the baseline diagnosis and the diagnostic accuracy analysed using relative operating characteristic (ROC) methodology. This work suggests that storing digital images using lossy compression will cause image degradation that is likely to effect the diagnostic quality of such images.

The results from this pilot study suggest that digital diagnostic images should not be subjected to lossy compression.

* Video Associates Laboratories; * Truevision Incorporated; * Joint Photographic Experts Group.

38

A J MORRIS (School of Dentistry, University of Birmingham, UK): Intra and inter examiner variation in use of examination lamp.

This study investigated variation in the illumination of the mouth for epidemiological surveys when using the Dury Versatile Medical Light¹. The amount of light falling on the mouths of the subjects of 38 examiners was measured using a light meter² held at a defined point on each subject. The values recorded during the study ranged from 320 Lux to over 20,000 Lux. The mean light intensity for individual examiners was 2143 (SE ±319) Lux. The distance between lamp and mouths of subjects varied between examiners (range 30-92 cm), the mean distance being 60 (SE ±3) cm. The distance also varied in the same examiners with different subjects. Individual subjects were not consistent for light intensity or distance from lamp when with different examiners. There was poor correlation between distance between lamp and mouth and mouth illumination ($r_s = -0.22$, $p = 0.10$).

There is no evidence from this study that use of a standard type of lamp results in uniformity of illumination of the mouths of subjects in dental epidemiological surveys. The distance between lamp and subject was not the only factor accounting for this variation.

¹Dary Lighting Ltd.

²TES 1330, RS Components Ltd

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A McMICHAEL (Dental Public Health, University of Birmingham, UK & North West HA): Factors affecting general practitioners' provision of NHS orthodontics.

This study distinguished practices. It aimed to distinguish practices which provided NHS orthodontics in Hereford & Worcester (UK). Dental Practice Board data showed that 56% of dentists undertook no orthodontics, while only 6% saw thirty or more cases per annum. A questionnaire was sent to all 250 dentists in the country: the response rate was 89%. Most felt their orthodontic experience and training was deficient and 36% had no orthodontic training beyond undergraduate level. They wanted more clinical attachments and reported that they tended to direct their referrals to specialist practices, rather than hospital departments. There was little support for the concept of hospital consultant 'outreach' clinics. A methodology was developed to categorise dental practices into 'providers' and 'non-providers'. Orthodontic providers tended to be older and to work in larger practices. Distance from specialist centres did not appear to influence practices' orthodontic capacity. Specialist providers identified remuneration as a particular issue of concern.

There is a need to address the training and remuneration of general and specialist orthodontic practitioners. The sector profile of specialist orthodontic practice needs to be recognised and counteracted.

40

A HALLAS* (Dental Public Health, University of Birmingham, UK): Parents' perceptions of school screening and their children's dental state.

A questionnaire was sent to the parents of all 5 and 8 year old children resident in Bassetlaw. The aims of the study were to ascertain the value placed, by parents, on the recommendations of the dental school inspection and to determine the level of knowledge held about the dental needs of their child. Parents were asked to examine their child's teeth to determine the number decayed or filled. The children also received a dental examination using criteria as described by Palmer et al (Community Dental Health 1 55-61). 80% of parents believed that dental screening was important but only 50% reported that their child attended a dentist as a result. Parents overestimated the number of fillings and underestimated the number of carious teeth. The clinical examinations demonstrated that of 40% of 5 year olds and 50% of 8 year olds had untreated decay.

It concluded that parents underestimated the dental needs of their children. Furthermore although most parents perceive school screening as useful, only half report that it acts as a trigger for positive action.

41

D WHITE, R J ANDERSON, G BRADNOCK* (Dental Public Health, University of Birmingham, U.K.): Children's dental health under the capitation scheme.

A study was undertaken of 7870 eight-year-old children resident in the city of Birmingham, using the standard BASCO epidemiological diagnostic procedures. Positive consent was obtained from every parent or guardian, including permission to link the findings with the Dental Practice Board's records as to whether each child was registered under the NHS capitation scheme for the provision of primary dental care to children. The dental state of those who were registered in the capitation scheme, (54.5%), was compared with those who were not, (45.5%). Overall, there were only very small differences between the caries state of registered and non-registered children. Combined results for the primary and permanent dentitions show that registered children had, on average, 0.68(SE±0.02) decayed teeth, 0.51(SE±0.02) missing teeth and 0.65(SE±0.02) filled teeth compared with the non-registered children who had, on average, 0.94(SE±0.03) decayed teeth, 0.52(SE±0.02) missing teeth and 0.65(SE±0.02) filled teeth. However 32% of those children registered in the scheme still had active decay which was not restricted to the primary dentition.

Overall, children registered under the capitation scheme had slightly better dental health than those who were not registered. However, a third of all the children in capitation had active decay which was not restricted to their primary teeth.

We gratefully acknowledge support from Department of Health grant no. 128/0051 for this research.

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D WHITE, R J ANDERSON* (Dental Public Health, University of Birmingham, U.K.): Children's dental health under capitation by ethnic and residential classification.

A study of 7870 eight year old children in Birmingham investigated the differences between children who were registered and who were not registered under the NHS capitation scheme, in terms of their residential neighbourhood classification and ethnic background. Combined results for the primary and permanent dentitions show that the registered children in the most deprived neighbourhoods had, on average, significantly less decayed teeth, 0.89(SE±0.03) than their non-registered colleagues, 1.08(SE±0.03). Registered Asian children in the most deprived neighbourhoods had the greatest increase in the average number of filled teeth, 1.02(SE±0.08) when compared with their non-registered counterparts, 0.77(SE±0.04). Unfortunately this group had the lowest proportion of children, 26%, who were registered in the capitation scheme compared with 54% of the total sample.

The group of children who would benefit most from being registered under the capitation scheme had the lowest proportion who were actually registered.

We gratefully acknowledge support from Department of Health grant no. 128/0051 for this research.

43

J G STEELE* (Dental School, Newcastle upon Tyne, UK): Clinical dental factors affecting satisfaction and function in the elderly.

Amongst the increasing population of dentate elderly subjects, clinical perfection and complete disease control are often neither feasible nor affordable. This study aimed to identify the main clinical variables which can influence function and satisfaction.

Clinical and questionnaire data from a random selection of 1211 dentate over 60 year old subjects in three parts of England were analysed. A model of oral health in the elderly was constructed with subject dissatisfaction as the major outcome. Potential independent risk factors were entered into a series of stepwise multiple logistic regression analyses.

Overall dissatisfaction was primarily influenced by dissatisfaction with aesthetics and function, but symptoms, speech difficulties and partial dentures were significant risk factors ($p < 0.05$) for being dissatisfied. Unrestored anterior spaces were the major risk for aesthetic dissatisfaction (Odds Ratio 3.6), but other significant variables included age, gender and geography ($p < 0.05$). Dissatisfaction with function was related to difficulty eating which was, in turn, correlated with number of teeth, number of decayed and periodontally involved teeth, symptoms, gender and geography. The proportion of the sample wearing partial dentures showed a sharp reduction where more than 17 teeth or 3 posterior contacts were present.

In elderly people overall oral satisfaction is most likely to be achieved with natural teeth where there are more than 17 teeth, 3 posterior contacts, no unfilled anterior spaces, and where disease and symptoms are under control. These findings suggest shortened dental arch philosophy is likely to be an appropriate strategy for the elderly.

This research was supported by the Department of Health, grant no. 121/2392

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D. W. WILLIAMS (Department of Oral Surgery, Medicine and Pathology, University of Wales College of Medicine, Cardiff, UK). Identification of *Candida* species using the polymerase chain reaction and restriction fragment length polymorphism analysis.

Traditional methods for the identification of *Candida* species can be time consuming and may be unreliable due to variable expression of phenotypic characteristics. Genotypic analysis may provide a more consistent method of identification. The aim of this study was to develop a rapid technique to identify *Candida* species based on size and sequence differences in the variable spacer regions within the ribosomal RNA operon. A total of 84 strains of *Candida*, species by the API-20C system, were studied. PCR amplification using primers targeting conserved regions of the 18S and 25S rRNA genes revealed inter-species size variation. Unique product sizes were obtained for all strains of *C. guilliermondii* (n=3), *C. glabrata* (n=13) and *C. pseudotropicalis* (n=5). The remaining test strains, consisting of *C. albicans* (n=41), *C. tropicalis* (n=6), *C. stellatoidea* (n=1), *C. parapsilosis* (n=5) and *C. krusei* (n=10), produced PCR products of similar electrophoretic mobility and therefore could not be differentiated on the basis of product size alone. However, analysis of restriction fragment length polymorphisms (RFLPs), obtained following digestion of the PCR products with the restriction enzymes *Bfa* I, *Dde* I and *Hae* III, permitted differentiation of all these strains. The results obtained by this technique were reproducible and could be acquired within a working day.

It is concluded that RFLP analysis of the candidal ribosomal RNA operon provides a rapid and reliable technique for the identification of *Candida* species.

DWW is in receipt of an MRC studentship.

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SL CHEESEMAN, AJ WRIGHTMAN and WG WADE* (Dental School, Bristol, UK and Pure and Applied Biology, UWCC, Cardiff, UK): Phylogeny of oral asaccharolytic *Eubacterium* species.

Oral asaccharolytic *Eubacterium* species are slow-growing, non-sporing, anaerobic gram-positive bacilli rarely found in oral health but which make up a significant proportion of the flora of subgingival plaque in advanced periodontitis. The aim of this study was to determine the phylogeny of this group of organisms. *E. brachy* ATCC 33089, *E. nodatum* ATCC 33099, *E. sapientum* ATCC 49989, *E. timidum* ATCC 33093 and strains W1471, SC87K and SC3D were studied. 16S ribosomal RNA genes were amplified by the polymerase chain reaction and cloned. The genes were sequenced by means of the automated ALF sequencer. Sequences were aligned with a panel of bacteria representative of all groups in the bacterial domain and with a subset of low G+C% gram positives. The PHYLIP suite of computer programmes was used to perform the phylogenetic analysis. All strains except 3D formed a unique, and deep-branching, line of descent most closely related to the genus *Peptostreptococcus*. Similarity values between strains were low, suggesting that a number of genera may be represented. 3D clustered with a group of intestinal *Eubacterium* species.

In conclusion, the oral asaccharolytic *Eubacterium* species appear to have evolved as a distinct line of descent: the lack of similarity between strains taken with the known heterogeneity of cell wall composition and metabolic end-products suggests that all strains studied are representatives of distinct genera.

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S HIOM*, W G WADE AND A J WRIGHTMAN (Dental School, Bristol, UK, and PABIO, UWCC, Cardiff, UK.): A polyphasic approach to the taxonomy of oral asaccharolytic *Eubacterium* species.

The aim of this study was to clarify the taxonomy of oral asaccharolytic *Eubacterium* species which is currently unsatisfactory. The sole use of an individual analysis method may be misleading and therefore a polyphasic approach was used where information obtained from phenotypic and genotypic analyses were combined. 40 *Eubacterium* strains, including representatives of known species, were examined by means of API Rapid ID 32A kits, gas chromatography of metabolic end-products and protein profiling. In addition, 16S rRNA genes were amplified by the polymerase chain reaction and then digested with restriction endonucleases *Cfo* I, *Hae* III, *Hph* I and *Bst* II to determine fragment length polymorphisms (PCR-RFLP). The combined results demonstrated nine separate groups: five contained the type strains of *E. nodatum*, *E. timidum*, *E. brachy*, *E. lentum* and *E. sapientum*; three were un-named but previously characterised groups (*E. Cluster 1*, *E. Cluster 2* and *E. Newf*); and one was a new group. PCR-RFLP patterns were characteristic for each group.

In conclusion, the polyphasic approach gives confidence to the classification of the oral asaccharolytic *Eubacterium* species and has demonstrated that with this group of organisms, PCR-RFLP analysis discriminates at the "species" level.

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D. BEIGHTON*, KA HOMER, A BOUVET¹ & AR STOREY. (Oral Microbiology, KCSMD & ¹Service de Microbiologie, 1, Place du Parvis Notre-Dame, 75181, Paris, France): Further characterisation of nutritionally variant streptococci.

The nutritionally variant streptococci (NVS), members of the normal oral flora and associated with infective endocarditis, were first isolated as fastidious streptococci forming satellite colonies around other microorganisms. The NVS consists of two species, *Streptococcus adjacens* and *Streptococcus defectivus*, which may be differentiated from each other, and from other viridans streptococci, using routine tests or commercial streptococcal identification kits. Previous phenotypic studies have been restricted to an examination of strains using a small set of tests to determine their ability to ferment carbohydrates and for the presence of pre-formed enzyme activities. To extend these studies we have examined *S. defectivus* (n=10) and *S. adjacens* (n=19) strains for the presence of an extensive range of pre-formed enzyme activities, especially peptidase activities, using 7-amido-4-methylcoumarin (AMC) and for glycosidase activities using 4-methylumbelliferyl (MU) linked substrates. *S. adjacens* strains produced a very wide range of proteolytic activities hydrolysing phe-, pro-, leu-, met-, orn-, thr-, cyst-, tyr-, ser-, his- and hyp-AMC while *S. defectivus* strains hydrolysed only phe-, pro- and leu-AMC. *S. defectivus* produced neuraminidase and α -fucosidase and *S. adjacens* produced α -fucosidase and N-acetylhexosaminidase activities. These data provide additional phenotypic characteristics for distinguishing between these two species and suggest that they have different nutritional requirements.

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ASSINDER, S. J., EYNSTONE, L. (MRC Dental Group, Dental School, Lower Maeslin Street, Bristol). End-product inhibition of acid production in *Streptococcus mutans* R9.

We have shown that the glycolytic end-products lactic acid and acetic acid inhibit acid production of oral streptococci non-competitively and competitively respectively (S J Assinder and G H Dibdin, Caries Res. 27: 240, 1993; Caries Res. 28: 223, 1994). The mechanisms and reversibility of such inhibition in *S. mutans* R9 were further investigated here by measuring the effects of varying concentrations of undissociated lactic or acetic acids, at a fixed pH, on the initial rates of acid production in the pH stat. Lactic acid (at pH 5.5, 2.34% of total acid undissociated) was shown to cause mixed inhibition with inhibition constants of K_{ii} (uncompetitive) 4.45 mmol/L and K_{ic} (competitive) 2.77 mmol/L. Inhibition was partially reversible, depending on the concentration of inhibitor. The greater the inhibitor concentration, the less activity was retrievable (e.g. 51% was retrieved after removal of 2.75 mmol/L lactic acid). Acetic acid (at pH 6.0, 5.4% of total acid undissociated) also caused mixed inhibition with K_{ii} 4.68 mmol/L and K_{ic} 7.89 mmol/L. Inhibition was again partially reversible, with 42% being retrieved after removal of 8 mmol/L acetic acid.

In conclusion both lactic and acetic acids caused mixed, partially reversible inhibition. The irreversibility of inhibition is probably due to cytoplasmic acidification and the sensitivity of glycolytic enzymes to reduced pH.

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G.J.ROBERTS,¹ * P.B.LONGHURST,¹ and P.B.HEWITT,² (¹ UMDS Guys Dental School, ² Guy's and St Thomas Hospital Trust, London) : Bacteremia following Local Anaesthetic Injections.

Objectives : The purpose of this study was to investigate the relationship between local anaesthetic injections and bacteraemia.

Patients and Methods : Ninety-three children undergoing outpatient general anaesthesia for extractions had a 21 gauge cannula placed in a vein in the antecubital fossa. Prior to any dental treatment each child was given one of three local anaesthetic injections selected at random and blood drawn thirty seconds later. The blood was injected into aerobic and anaerobic broth cultures (Bactec) and lysis filtration vials (Isolator). Isolates were speciated using standard methods.

Results : The proportion of positive cultures from the Bactec were 15.6% for Infiltration Analgesia, 46.9% for the Modified Intra-ligament Analgesia and 96.6% for Intra-ligament Analgesia. Statistically significant differences were found using Chi Square, $p < 0.001$, 2 degrees of freedom. For the counts of colony forming units per ml (cfu/ml) the mean value was 252 (sd 645) for the intra-ligament technique, with a cfu/ml of zero for the modified intra-ligament and infiltration techniques.

Conclusions : Local anaesthetic techniques commonly used in paediatric dentistry cause a bacteraemia. Antibiotic prophylaxis should be used on children at risk of septicaemia from a dental bacteraemia and to children at risk of contracting bacterial endocarditis when administering local anaesthesia.

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RM MIRCHANDANI (Dept of Conservative Dentistry, UMDS, Guy's Hospital, London Bridge SE1 9RT, U.K.): A confocal evaluation of the granular layer of Tomes and its significance in root porosity.

Conventional light microscopy is limited by lack of resolution for studying the granular layer of Tomes (GLOT). By producing high resolution optical tomograms in reflected and fluorescent modes using a confocal light microscope, this investigation aimed at analysing the structure and relationships of GLOT. 75 human teeth sectioned longitudinally, transversely and *en face* were studied in reflective mode. Structural morphology and interconnectedness of GLOT with adjacent structures e.g. cementocytes, interglobular spaces and looped dentinal tubule configurations were observed. Extended through focus imaging determined any lateral connections around the root periphery within GLOT in longitudinal and transverse sections. Through focusing and serial grinding of *en face* samples determined any pulpal connections between GLOT and the dentinal tubules. Pulpal connections in fluorescent mode were studied by introducing rhodamine B dye into the pulp chamber of 10 human molar teeth and observing dye movement. Results from *en face* imaging revealed a 'sponge-like' structure formed by the incomplete fusion of calcospherites and not one caused by the random looping of the terminal ends of dentinal tubules (Ten Cate, 1972). Reflected and fluorescent light imaging indicated preferential connection laterally rather than pulpally, which was also verified by demineralisation experiments. Early mineral loss in root caries may be more easily directed laterally and therefore afford a protective function towards the pulp. It is concluded that GLOT represents incompletely fused calcospherites and forms a better interconnected system within itself (lateral connection) than with dentinal tubules (pulpal connection); this could be of significance in root caries.

51

M PRING (Department of Oral and Dental Sciences, University of Bristol, UK): Control of transcription factor expression by transforming growth factor β 1 in rat oral keratinocytes.

The signal transduction pathways that elicit the diverse biological effects of TGF- β have not been determined. Previous studies in epithelial cells have linked the inhibition of cell proliferation to *c-myc* and the elaboration of extracellular matrices to *jun-B*. This study examined the effect of TGF- β 1 on the transcription factors *jun-B* and *c-myc* in rat oral keratinocytes with variable degrees of cellular differentiation and a range of inhibitory responses to this ligand. *Jun-B* expression was upregulated in response to exogenous TGF- β in all cell lines ($n=7$). The downregulation of *c-myc* expression by TGF- β 1 predominantly reflected growth inhibition as determined by tritiated thymidine incorporation assays; in 2 cell lines *c-myc* expression was not downregulated despite growth inhibition by TGF- β 1. The regulation of both *jun-B* and *c-myc* by TGF- β 1 did not reflect the degree of cellular differentiation.

The results of this study indicate that TGF- β 1 can inhibit cell cycle progression by mechanisms other than c-myc downregulation. By contrast, TGF- β 1 consistently upregulates jun-B expression, an effect that appears independent of cell cycle control.

This work was supported by a grant from the Nuffield Foundation (NUF-URB94)

52

J RICHARDSON* (Dundee Dental Hospital and School, University of Dundee, UK): Random and systematic variation among dental treatment decisions *in vitro*.

Several studies have examined the extent of variation among treatment decisions made by dentists. Many factors may contribute to disagreement, but in general two sources of variation are involved; systematic (e.g. differences in opinion about treatment needed) and random (e.g. diagnostic or other errors). The aim of this study was to assess the relative contribution of the two types of variation in order to determine what strategies could be adopted to reduce disagreement. Twenty five general dental practitioners planned treatment for 160 extracted pre-molar and molar teeth mounted in phantom heads on two separate occasions under standardised conditions. The hypothesis was that inter-dentist disagreement on treatment planning would stem from both systematic and random variation but intra-dentist agreement would mainly stem from random sources. Combining all tooth surface-specific treatments together (extractions, restorations and some items of preventive care) the mean intra-dentist agreement assessed by kappa was 0.58 (Range 0.31-0.84), the mean inter-dentist agreement was 0.46 (Range 0.15-0.73). *The findings suggest that random variation may be a more frequently encountered problem than systematic variation. Containment of random variation may be helped by encouraging dentists to be more confident about being circumspect in planning interventional treatment.*

53

S ROBB (Department of Dental Health, University of Dundee, Scotland, UK): Towards a predictor of dental compliance in childhood.

Many models of health behaviour have been developed to explain compliance with preventive advice and use of health services. However, little is known of the role of personality and dental attitudinal variables in predicting compliance. The aim of this study was to investigate whether psychological profiles as determined by the psychometric variables of dental anxiety, dental indifference, extraversion and neuroticism could serve as reliable predictors of the dental compliance achieved by children in relation to oral health preventive procedures. 78 primary school children (49 girls, 29 boys) aged 10-11 years completed standard questionnaires measuring the psychometric variables, eg Eysenck Junior Personality Questionnaire (Eysenck SBG and Eysenck HJ, Hodder and Stoughton, 1975). A week following baseline examinations and comprehensive oral hygiene instruction, the level of dental compliance was represented by the reduction in plaque scores attained. After controlling for initial plaque levels, partial correlations revealed a significant association between extraversion and dental compliance ($p = 0.04$).

In conclusion, this study is the first to suggest that child extraversion may be useful as a predictor of the level of dental compliance of that child, with the more extroverted being likely to comply to a greater extent with dental instructions and advice.

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KH TAYLOR. (Clinical Dental Sciences, The University of Liverpool, L69 3BX UK). Evidence of novel sites of tumour suppressor genes in squamous cell carcinoma of the head and neck.

Specific sites of frequent allelic loss have been recognised in many types of malignancies associated with loss of function of nearby tumour suppressor genes (TSG). Cytogenetic and molecular evidence has indicated the presence of putative TSG's on chromosomes 1 and 9. The aim of this study was to assess loss of heterozygosity (LOH) on these two chromosomes in squamous cell carcinomas of the head and neck (SCCHN) using a set of informative polymorphic microsatellite markers (12 on chromosome 1 and 13 on chromosome 9). Tumour and normal DNA specimens were isolated from 50 SCCHN and the polymerase chain reaction (PCR) followed by gel electrophoresis were used to determine regions of loss. LOH on chromosome 1 was found in 40% (20/50) of informative cases. The most frequent loss was found on the p arm at D1S159 (16%) and D1S167 (18%), giving a cumulative LOH of 24% (14/46) and LOH of markers telomeric and centromeric were found to be 0%. This indicates a specific region of interest at 1p31.2-p21.1, which has previously been shown to contain cytogenetic breakpoints. Chromosome 9 has been investigated with 13 markers in 35 SCCHN specimens, 10 markers on 9p and 3 on 9q. The highest losses were found at D9S157 (37%) (10/27), D9S161 36% (11/31) and D9S162 23% (6/26). A minimal area of loss was found on 9p which includes the markers (D9S156-D9S157-D9S162-D9S171-D9S161) at 9p21-p22 with a LOH of 60% (21/35). This area corresponds to the IFNA region and also contains the putative tumour suppressor gene p16. *Two new regions containing putative tumour suppressor genes have been identified by LOH analysis on chromosomes one and nine in SCCHN.*

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K TINDLE* (The Dental School, The University of Wales College of Medicine, Cardiff, UK): Fibroblast attachment to titanium discs coated with glycosaminoglycans.

The formation of a stable connective tissue-implant interface may be an important factor in the successful osseointegration of dental implants. Proteoglycans and their component glycosaminoglycans (GAG) have been implicated in adhesion events. This study investigates the influence of GAGs, typical of periodontium and bone, on the attachment of fibroblasts to titanium discs.

Commercially pure titanium discs were pre-coated with the GAGs chondroitin-4-sulphate (C4S), dermatan sulphate (DS) and heparan sulphate (HS). They were then incubated in a suspension of human gingival fibroblasts, passage 5 of a cell line produced for this study, at a concentration of 10^5 /ml in DMEM. The number of cells adhering to the surface of the discs was estimated at 2, 4, 6 and 12 hours, after ethidium bromide/acridine orange staining. The number of fibroblasts adherent to the culture well, uncoated control discs and discs coated with DS and HS showed only a small increase in adherent cells between 2 hours (DS 59.7 cells/mm² SD±6.68) and 12 hours (DS 84.4 cells/mm² SD±7.38) while the number of cells adhering to C4S coated discs increased significantly ($p < 0.01$) over the same time period (55.5±5.5 - 121.2±7.36 cells/mm²). Uncoated discs incubated with the GAGs in the culture medium showed no significant difference from control discs.

This study indicates that fibroblasts adhere more readily to titanium pre-coated with chondroitin-4-sulphate, the primary GAG component of alveolar and basal bone. The absence of altered adherence to discs coated with dermatan sulphate or heparan sulphate may be of interest since these GAGs are associated with non-mineralised periodontal tissue.

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P H R WILSON (Dept. of Oral Sciences, University of Glasgow Dental School, Glasgow, UK): A novel purification strategy for the basic proline-rich protein Ps1.

Basic proline-rich protein (BPRP) Ps1, is fascinating in that it interacts with certain oral bacteria and binds specifically to buccal epithelial cells. Although incompletely characterised, its migration on SDS-PAGE is well documented and its pI is ≥ 8.4 . The aim of this project was to isolate Ps1 from parotid saliva by means of a novel three-stage electroelution/ion-exchange process. The proteins were visualised throughout by 10% addition of dansylated parotid salivary proteins in order to make them UV-fluorescent.

The proteins were first separated by semi-preparative SDS-PAGE (Beesley J A *et al*, *Electrophoresis* 12: 1032-1041, 1991) and the Ps1 band excised under UV transillumination. The excised band was subjected to a novel PAG-electroelution procedure and the protein exhaustively dialysed to remove residual SDS. Finally, DEAE-cellulose ion-exchange chromatography was employed to remove minor contaminants and dansylated marker protein. The product was analysed by SDS-PAGE, spectrophotometry and the ninhydrin reaction.

Purified Ps1 had a A_{max} at 206 nm and was high in proline. It corresponded to Ps1 on SDS-PAGE but there was some conversion to a faster migrating species. Whilst proteolysis might be involved, deglycosylation or conversion to an apo-form could also have occurred.

This novel purification strategy provides an alternative method for the purification of salivary BPRPs. It offers the prospect of modification for other salivary proteins.

Supported by the Scottish Office Home and Health Department.

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D. J. REID & A. D. BEYNON* (Department of Oral Biology, University of Newcastle upon Tyne, U.K.): Stria slope and spacing in human and great ape molar teeth.

In human teeth striae of Retzius show variation in slope and spacing in different parts of the crown, with greater angles and closer spacing towards the cervix. (Shellis R. P., Archs. Oral Biol. 29: 697-705, 1984). There are no reports on these parameters in hominoid teeth. This preliminary study on molar teeth in modern humans and great apes was designed to establish whether there are consistent differences in stria morphology and mechanisms of crown formation in these taxa. Stria width (SW) was measured along the prism length at 100µm from the external surface; angle of stria (SA) was measured tangential to the enamel dentine junction at occlusal (O), lateral (L), and cervical (C) 1/3 of the crown in axial ground sections of molars in Gorilla (n=12; M₁ x4, M₂ x2, M₃ x2, M₄ x3, M₅ x1), Pongo (n=6; M₁ x2, M₂ x2, M₃ x1, M₄ x1), Pan (n=7; M₁ x3, M₂ x1, M₃ x3), and human (n=10; M₁ x5, M₂ x5). The SA increased from O through L to C levels in all taxa. In human M1 SA values were very similar in upper and lower molars (M₁M₂; O,L,C: 13±1.6, 13±1.4; 28±1.2, 26±1.3; 33±1.4, 32±2.3), ratio (1:0.2:1:2.5). Ape specimens showed smaller O values (7±2.8-9±3.5) and larger and more variable L (24±6.5-33±9.8) and C (31±9.5-44±6.7) values, with larger ratio range (1:3.2-3.8:4.4-9). SW showed progressive reduction from O through L to C levels in all taxa, with gorilla and orangutan showing greater values at all levels compared to humans and chimpanzees. These results suggest that there may be differences in stria slope and width between modern humans and great apes which suggests systematic differences in manner of crown formation between these taxa.

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H PERRY* and A J SMITH (School of Dentistry, University of Birmingham, Birmingham, UK): Comparison of the mineral content of primary and tertiary human dentines.

Modulation in odontoblast secretory activity during tertiary dentinogenesis can give rise to morphological changes in the tissue. Differences in the composition of the organic matrix of tertiary as compared to primary dentine have been previously reported and in the present study, we have investigated whether this has an influence on the mineralisation of the tissue. A micro-punching technique was used to prepare discs to sample human primary (n=20) and tertiary dentine (n=20) from beneath carious lesions. After measurement of volume, mineral was extracted from specimens with 0.2M-acetate buffer, pH 3.8 at room temperature for 48 hours. Calcium and phosphorus levels in these extracts were determined spectrophotometrically and fluoride by use of an ion-selective electrode. Results were analysed by the students t-test. Whilst the levels of calcium and phosphorus were lower in the tertiary than primary dentines, these differences were not statistically significant. The levels of fluoride in the tertiary dentines were more than twice those observed in the primary dentines and this difference was highly statistically significant. Individual variation between teeth for all analyses was greater for the tertiary than primary dentines.

We conclude that the degree of mineralisation of tertiary dentines is unlikely to influence the morphological appearance of the tissue, although its raised fluoride content indicates differences as compared to primary dentine.

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A J SMITH*, C GARDE, N CASSIDY, J V RUCH* and H LESOT† (Dentistry, Birmingham, UK and †Institut de Biologie Médicale, Strasbourg, France): Solubilisation of dentine extracellular matrix by calcium hydroxide.

Calcium hydroxide is used widely for induction of reparative dentinogenesis in exposed dental pulps. Whilst the molecular basis for its action is not well understood, the growth factor, Transforming growth factor-β (TGF-β) and isolated dentine extracellular matrix (ECM) components have been shown to be able to induce odontoblast differentiation. The aim of this study was to investigate solubilisation of these components from dentine by calcium hydroxide. Pools of powdered dentine prepared from rabbit and human teeth were extracted at 4°C with 0.01M- and 0.02M-solutions of calcium hydroxide, pH 11.7 and also 10% EDTA, pH 7.2 as a control. Non-collagenous proteins and glycosaminoglycans in the solubilised material were analysed by dye-binding assays and TGF-β isoforms by enzyme-linked immunosorbent assay. The calcium hydroxide solutions were able to solubilise these components although the yields were not as great as with EDTA. Non-collagenous proteins of the tissue were more soluble in calcium hydroxide than the glycosaminoglycans. TGF-β₁ was solubilised by all of the extractants.

It is concluded that calcium hydroxide solutions of low concentration can solubilise ECM components and TGF-β from dentine *in vitro* and this may be the basis of action of calcium hydroxide in the induction of pulpal repair *in vivo*.

Supported under the CEC-SCIENCE programme (ERBSC1*CT91-0680).

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K G TUCKER*, M ADAMS, L SHAW and A J SMITH (School of Dentistry, Birmingham, UK): Acid dissolution of the human permanent dentition.

In developing an *in vitro* model of dietary erosion in the human dentition, possible variation in relative solubility of the hard tissues of different teeth is important. We present here an examination of the susceptibility of different surfaces of human teeth of differing morphologies to acid dissolution. The buccal/labial and lingual/palatal surfaces of both human mandibular and maxillary incisors, canines, premolars and molars were examined (n=12 in each case). Citric acid (1% w/v) was pumped over circular, exposed areas on the teeth (5mm in diameter) for up to 20min at 37°C. Calcium dissolution was assayed by atomic absorption spectrometry, and the results compared by analysis of variance to investigate inter-tooth variability and also, any possible variation in the rate/amount of calcium dissolution between different faces of the mandibular and maxillary teeth of differing morphologies. The results illustrated that although the process of calcium dissolution appeared linear in all cases, there were some differences in the rate of dissolution. Dissolution of calcium from the palatal surface of the different maxillary teeth tested appeared fairly constant, however, there was more variation when considering their buccal/labial surface. Also, there was considerable variation in the various mandibular teeth investigated, when examining both surfaces. It is concluded that differences do exist in the susceptibility of human teeth from the permanent dentition to acid dissolution. This might be due to a number of factors which are important in an investigation on this subject.

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A. McDONALD*, G.J. PEARSON, N. CLAFFEY, W. BLAU, (Eastman Dental Institute, UK and Trinity College Dublin, Ireland): A Qualitative Evaluation of the Effect of Nd:YAG Pulse duration on Dentine Interaction.

The aim of this study was to evaluate the effect of laser pulse duration on laser dentine interaction. Sound third molars were collected and stored in saline. Each crown was sectioned transversely midway between the cusp tips and the DEJ. The lower section was mounted on a fixed optical stage prior to exposure to one of four Nd:YAG lasers. Each laser operated at a different pulse duration; 7ms., 150µs, 30ns., and 35ps. The pulse repetition rate was 10Hz for µs, ps lasers, 10.5 for the ns, ns lasers. The total energy delivered to each dentine target site was maintained for each pulse duration. All lasers were used in a non contact form. The 150µs laser was delivered via a silica fibre, all other lasers were focused by means of a lens. After irradiation target sites were sectioned longitudinally dehydrated, sputter coated and viewed under an SEM. The results showed that pulse durations of 7ms and 150µs produced craters with rounded carbonized edges. The crater walls appeared fused, carbonized, with some evidence of crazing. This carbonization did not extend sub-surface. Pulse durations of 30ns and 35ps produced "punched out" craters with no carbonization or fusion internally. Variations occurred in crater size at all pulse durations.

It was concluded that laser dentine interaction is dependent on pulse duration. With shorter pulse durations a reduction in tissue damage occurred. Variation in crater depth occurred at all pulse durations.

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P DREXLER, P ANDERSON* and J C ELLIOTT (Dept of Child Dental Health, The London Hospital Medical College, London E1 2AD, UK.): Influence of CO₂ partial pressure on enamel dissolution.

The aim of this *in vitro* study was to investigate the influence of CO₂ partial pressure on the rate of enamel dissolution. Scanning microradiography (SMR) was used to monitor continuously the rate of mineral loss in twelve 200 µm thick sections with their cut surfaces exposed to a demineralising solution (0.1 moles L⁻¹ acetic acid buffered to pH = 5, flow rate = 0.4 mL min⁻¹). CO₂ at a partial pressure of 0.035 bar was bubbled through the solutions flowing past half of the sections whilst the others acted as control. The results showed that there was no difference in the rate of demineralisation between the two groups of sections.

In conclusion, the presence of 0.035 bar partial pressure of CO₂ does not affect the rate of enamel demineralisation at pH=5.

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A O'DONNELL*, M HUKANNEN†, J POLAK†, FJ HUGHES. (Dept. Periodontology, London Hosp. Med. College, and †Dept. Histochim., Royal Postgrad Med. School). Cytokine-induced synthesis of prostaglandins in osteoblasts: the role of nitric oxide.

Recent evidence suggests that nitric oxide (NO) production is an important mediator of cytokine-induced effects on bone metabolism. Prostaglandins (PG) are also induced by pro-inflammatory cytokines, and are potent mediators of bone resorption. The aim of the study described here was to investigate the relationship between NO and prostaglandin synthesis by osteoblasts in response to exposure with interleukin-1 (IL-1), tumour necrosis factor-α (TNF-α), and interferon-γ (IFN-γ). Primary rat osteoblast cultures were obtained by sequential collagenase digestion of rat calvariae, and grown to confluence. In order to determine if NO stimulated PG synthesis, cells were stimulated with cytokines for 48 hours in the presence or absence of the specific NO inhibitor L-nitro-arginine methyl ester (L-NAME) at 1mM concentration. Conditioned medium was then assayed for PGE₂ synthesis by enzyme immunoassay. Conversely, in order to determine the effects of PGs on NO synthesis, cells were stimulated with cytokines in the presence or absence of indomethacin (10⁻⁶M), and cells were directly stimulated with exogenous PGE₂. Medium was then analysed for NO production by measuring nitrite levels using the Griess Reaction. Interleukin-1 and IFN-γ both induced marked synthesis of PGE₂ (eg controls < 0.5 pg/ml; 1000u/ml IL-1 90 ± 16 pg/ml), although this was not seen with TNF-α. Addition of L-NAME did not significantly affect PGE₂ synthesis in IL-1 treated cultures. However L-NAME completely inhibited IFN-γ induced PGE₂ synthesis. Addition of exogenous PGE₂ to cultures did not affect NO synthesis as determined by nitrite measurements. The results demonstrate that NO can induce PGE₂ synthesis in osteoblasts in response to stimulation with IFN-γ, and suggest one important mechanism of action of NO in bone metabolism. However PGE₂ synthesis may also be induced during inflammation by mechanisms independent of NO.

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A. TSIRLIS¹ and S. PATEROMICHELAKIS *² (¹University of Thessalonike, Greece; ²KCSMD, London, UK): The toxicity of i.a. and i.v. local anaesthetic injections in the rat.

The accidental intravascular injection of local anaesthetics and vasoconstrictors may cause serious adverse systemic reactions. This study, conducted on thirty two anaesthetised rats (urethane, 1.4 mg/kg), examined the consequences of the following two types of 0.5 ml injections in the external jugular vein and the external carotid artery: (a) plain lignocaine 2 mg (LGN, n=8 for each route) and (b) lignocaine 2 mg with adrenaline 2 µg (LGNADR, n=8). Mean arterial blood pressure (BP) and heart rate (HR) were measured via a pressure transducer connected to the cannulated right common carotid artery. Post-injection values (maximum divergence from pre-injection baselines) were subjected to the paired t-test. Experiments were terminated with a local anaesthetic overdose. Lignocaine i.a. did not cause significant changes in either BP or HR (p > 0.05) but LGNADR via the same route raised BP by 13% (p < 0.05) without affecting HR values (p > 0.05). Significant reduction of BP followed i.v. LGN injections (-17%, p < 0.01); HR also fell (p < 0.05). The mixture of LGNADR, given via the i.v. route, raised BP by 36% (p < 0.01), i.e. significantly higher than when it was administered into the ext carotid artery (p < 0.01), and slowed the heartbeat by 15% (p < 0.05).

These results suggest that lignocaine / adrenaline i.v. injections are considerably more toxic than similar i.a. injections or intravascular injections of plain lignocaine.

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J P ROOD* and M L SHARMA (University of Manchester UK): The influence of lignocaine and adrenaline on peripheral blood flow: an animal study.

To measure the effects of lignocaine and of adrenaline on skin blood flow in the rat, 28 rats were anaesthetized using fentanyl and midazolam and positioned under a collimated NaI (TI) scintillation probe coupled to a multichannel analyser and set in a multiscalar mode with a dwell time of 30 seconds. A radioactive tracer, ^{99m}Tc -DTPA, was mixed with (i) saline (control), (ii) adrenaline, (iii) lignocaine or (iv) a combination of lignocaine and adrenaline. The volume injected subcutaneously was 100 μ l. Immediately following the injection, data acquisition was carried out over 30 minutes and activity clearance curves obtained. Elimination rate constants (ERC) and half-clearance time (T_{1/2}) in minutes were obtained from the decay corrected data and a statistical analysis was carried out using unpaired T-test. The saline only group (i) (controls) gave $ERC = 0.066 \pm 0.0016$ $\times 10.5 \pm 2.51$. The test solutions produced the following results (p values when compared with controls): (ii) adrenaline, there was a biphasic clearance with an early slow elimination phase $ERC = 0.007 \pm 0.003$ ($p < 0.05$) $T_{1/2} = 100.39 \pm 46.10$ ($p < 0.05$) (iii) lignocaine; $ERC = 0.007 \pm 0.002$ ($p < 0.05$) $T_{1/2} = 93.45 \pm 25.38$ ($p < 0.05$) and (iv) lignocaine + adrenaline $ERC = 0.010 \pm 0.003$ ($p < 0.05$) $T_{1/2} = 72.31 \pm 19.42$ ($p < 0.05$).

Radioactivity dispersed slowly when technique was injected with adrenaline, showing that the blood flow was reduced in the presence of the vasoconstrictor. Similarly, the slow loss of radioactivity in the presence of lignocaine showed that this drug also produced vasoconstriction in this model.

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R J MIDDLEHURST* and J P ROOD (Unit of Oral and Maxillofacial Surgery, University Dental Hospital of Manchester): Electrocardiographic responses to lignocaine 2% with adrenaline 1:50,000 and vasopressin 0.25 IU/ml.

This investigation was part of a large and pragmatic study, examining the effects of local anaesthesia upon haemodynamic and electrocardiographic performance, in subjects with heart disease. One hundred and twenty five patients, 75 cardiac and 50 controls, were scheduled for dental/oral surgery and in 50 patients, 25 cardiac and 25 controls, local anaesthesia was supplemented by intravenous midazolam. Dynamic ECG tapes (Holter Tracker Recorder) were analysed (Raynolds Medical Pathfinder 3 Model P31 ECG Analyser) and comparative criteria: counted arrhythmic events, prevalence for arrhythmic events, "benign and malignant" arrhythmias and graded ventricular arrhythmias were used to derive a further measure, the "severity index". This was examined statistically by non-parametric methods. Results showed that the use of midazolam sedation significantly improved the electrocardiographic performance for all subject groups ($P = 0.01$).

Conclusion: The prescription of midazolam sedation is recommended for the minimisation of electrocardiographic risk in patients with heart disease.

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P COULTHARD* and J P ROOD (Department of Dental Medicine and Surgery, University of Manchester UK): Analgesic efficacy and safety of tramadol for patients undergoing oral surgery.

This study evaluates the efficacy and safety of the centrally acting analgesic tramadol administered intravenously for patients undergoing day-case oral surgery under intravenous sedation and local anaesthesia. A double-blind, placebo controlled, randomised study of twenty patients undergoing oral surgery to remove one impacted lower third molar tooth involving bone removal and tooth division was undertaken. Half the group received 100 mg tramadol intravenously and half an intravenous placebo. This was administered immediately prior to the induction of sedation with intravenous midazolam which was then titrated according to patient response up to a maximum of 10 mg. A short acting local anaesthetic block was administered using 4.4 ml of 4% prilcaine. The mean VAS pain scores and postoperative time to escape analgesia data demonstrate that tramadol significantly reduced postoperative pain. The mean dose of midazolam titrated for sedation was the same for those receiving tramadol as those receiving placebo (0.11 mg/kg). There were no adverse incidents and there was no clinical or statistically significant difference between the arterial oxygen saturation and blood pressure measures of the two groups. One patient receiving tramadol reported postoperative nausea.

The data from this pilot study indicate that tramadol significantly reduces mild to moderate postoperative pain, without sedative effect and safety.

*Brunenthal GmbH

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A RENEHAN*, M MCGURK and E N GLEAVE (University of Manchester, Dept Maxillofac Surg, Guy's Hosp, Christie Hosp, Manchester): Comparisons of survival and recurrence rates between carcinomas of the parotid gland and minor salivary glands.

Carcinomas of the salivary glands are uncommon. Some confusion exists in the literature regarding the relative prognoses of minor salivary carcinomas compared to parotid carcinomas. A series of 244 salivary carcinomas treated at the Salivary Gland Clinic, Christie Hospital, between 1952 and 1992 is currently under review. Survival and local control analysis was performed on a selected group; stage I to III, previously untreated cases who received definitive surgical treatment with or without adjuvant radiotherapy. There were 78 parotid cases and 15 cases arising from minor salivary glands. All the latter cases arose within the oral cavity. The median follow-up time was 14.5 years (range 1-32). Mucoepidermoid carcinoma was the commonest histopathological type in the parotid group, adenoid cystic carcinoma was the commonest in the minor salivary carcinoma group. Five and 10 year determinant survival rates for parotid carcinomas were 91% and 75%, while in the minor salivary group, the rates were 88% and 78% respectively. Local-regional failure rates were almost identical in both groups, 20.5% for parotid and 20% for minor glands. The median time to recurrence tended to be longer for minor salivary carcinomas but this was not significant.

It is concluded that survival and loco-regional failure rates are similar for parotid and oral cavity minor salivary gland carcinomas.

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K FAN*, C HOPPER, P SPEIGHT, S BOWN (National Medical Laser Centre & Maxillofacial Surgery, UCL Medical School, Oral Pathology, Eastman Dental Institute): Photodynamic therapy for oral cancer and precancer using aminolaevulinic acid.

In the UK there are 2400 new cases of oral cancer diagnosed each year. Despite advances in management, five year survival rates remain poor. The potential for malignant transformation in oral dysplasia is well recognised, however, no satisfactory therapy exists for these lesions. In photodynamic therapy (PDT) tumour damage results following activation of a previously administered photosensitising agent by an appropriate wavelength of laser light. This therapy has been suggested as an alternative modality in the management of premalignant and malignant lesions within the oral cavity, since it offers minimal morbidity combined with site specific therapy. The aim of this study is to examine the efficacy of PDT using 5-aminolaevulinic acid (ALA), the precursor of the photosensitiser protoporphyrin IX, for the management of premalignant and malignant oral epithelial lesions. Seven oral squamous cell carcinomas and 13 dysplastic epithelial lesions were treated. All patients were given 60mg/kg ALA orally in 3 fractions over 2 hours. This was followed by irradiation at 628nm up to 200J/cm² at powers up to 200mW/cm². Biopsies were taken prior to irradiation, to allow fluorescence detection of accumulated photosensitiser, and 2-4 days post treatment to assess depth of necrosis.

Of the seven squamous cell carcinomas complete response was established in the two cases of microinvasive tumours with no evidence of disease after eleven and twelve months. In the other five more advanced cases the PDT failed to eradicate the disease, and had no more than a superficial effect. In all 13 premalignant lesions there was full thickness epithelial necrosis and elimination of dysplastic epithelium at the treatment sites (follow up 2-12 months).

We therefore conclude that PDT using ALA may have a useful role in the management of oral epithelial dysplasia and microinvasive carcinomas.

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C HOPPER*, K FAN, P SPEIGHT, W GRANT, S BOWN (National Medical Laser Centre & Maxillofacial Surgery, University College London, Oral Pathology, Eastman Dental Institute): Photodynamic therapy in the management of field cancerization.

Photodynamic therapy (PDT) involves a non thermal photochemical reaction between a photosensitizer and light, resulting in tissue necrosis. Healing of normal mucosa without scarring takes place as a result of the preservation of collagen and elastin which act as a framework for regeneration. Field cancerization is a condition in which patients develop multiple synchronous and metachronous tumours and carries a poor prognosis (9 - 22% five year survival). Treatment is to a large extent unsatisfactory, with patients undergoing multiple surgical procedures with increasingly mutilating surgery as the disease progresses. PDT has been proposed as a means of managing this condition, but no long term follow up data is available to establish whether it is an effective treatment in terms of survival, or for that matter if patients are spared invasive procedures. Materials and methods: 11 patients with field cancerization were treated using PDT and followed for a period of 22 - 38 months (mean 30 months). All have been rebiopsied to evaluate treatment response and 2 patients have required further treatment for their disease.

Results: 6 patients are alive and well and completely free of disease either on clinical examination or by biopsy. 2 patients are clinically well but with some residual dysplasia and 2 have obvious tumour. Of these, one has refused treatment and has a T2 cheek lesion which is very slow growing and one has had surgery for an extensive area of tumour (T3) both of these recurring at a previously treated site. One patient has died of a squamous cell carcinoma of the tongue outside of the treated area.

Conclusion: PDT would appear to offer a clear alternative to surgery, but the grave prognosis for this condition is confirmed. Until such time as the oral mucosa can be stabilized in field cancerization, PDT offers the best treatment currently available.

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ST J CREAM* A POWIS (Eastman Dental Institute and University College London Hospitals Departments of Oral & Maxillofacial Surgery): Peripheral Total Parenteral Nutrition (TPN) in Maxillofacial Surgery.

Following major maxillofacial surgery, the oral intake of food may be impossible and alternative routes are required. Questionnaires were sent to 150 UK maxillofacial units enquiring as to the preferred method of non-oral nutritional support for up to a 4 week period. There were 60 replies (40%), of whom 40 (67%) advocated the use of nasogastric feeding of which 13 (32.5%) would subsequently convert to percutaneous endoscopic gastrostomy (PEG) feeding. There were 18 (30%) who would use PEG from the beginning of the post operative period. The 2 remaining units used central venous nutritional support. No units recommended the use of peripheral TPN, which involves the insertion of a paediatric fine bore silicone catheter into a suitable arm vein under aseptic conditions. Up to 2,500 calories per day of specially prepared feed¹ is delivered, with the majority of the kilocalories in lipid form, which provides pleiotropic properties delaying resiting of line for up to 23 days. (Kohlhart S R, *BJ Surgery* 81: 66-70, 1994). Nasogastric tubes and PEGs are not without complications, eg. aspiration and peritonitis respectively, therefore peripheral TPN is a method that deserves serious consideration in the specialty.

It is concluded that peripheral TPN is a method of nutritional support that is under-utilised in maxillofacial surgery and in view of the complications of other feeding methods, it should be seriously considered as an alternative.

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J LASZLO*, V SIVARAJASINGAM*, M GREEN*, G OGDEN* (Dept of Dental Surgery & Paediatric and Mathematics, University of Dundee UK): Knowledge and uptake of Hepatitis B vaccination in Dundee medical and dental students.

The 1996 C.V.C.P. guidelines indicate that all prospective Medical and Dental students should be immunised against Hepatitis B prior to admission in 1996 (Kingman BMJ 1994 308:876). This follows in the wake of similar U.K. Dept of Health (1994-1995) guidelines covering all personnel conducting invasive procedures in the NHS. The University of Dundee advises all new Dental and Medical students to be vaccinated against Hepatitis B. An investigation was conducted in the summer of 1994 among the 224 Dental and 649 Medical students in Dundee to monitor their uptake of vaccination and background knowledge of Hepatitis B and H.I.V. The Deans of Dentistry and Medicine approved the distribution of a confidential structured questionnaire to all undergraduate students. The reply rate (53% of medicals, 69% of dentals) was sufficiently high to apply normal distribution theory in our analysis. Nearly all students (99%) knew about the vaccination yet 34% of Dental students and 19% of the Medical students had not completed the vaccination regimen. Of those who did complete the full course of vaccination 39% of Dental students and 52% of the Medical students failed to take an antibody titre test post vaccination. Of those who were titre tested, 8% of Dental students and 11% of Medical students failed to seroconvert. Differences between students' ethnic grouping and completion of the immunisation schedule were noted. We conclude that medical and dental schools need to alter their advice to current students, to ensure they obtain immunisation against Hepatitis B.

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R MCKINLAY* and C PINE (Lothian Health Board and Department of Dental Health, University of Dundee, Scotland, UK): Young people's knowledge of infant dental health in deprived areas of Edinburgh.

Water fluoridation is unlikely to be available to the Scottish population this century. Therefore, behavioural aspects of caries prevention by carers of infants becomes crucial in reaching Scottish targets for child dental health by the year 2000 (*A National Policy Statement*, March 1991). Scottish Office Home and Health Department, Edinburgh, HMSO). A cross sectional study in deprived areas of Edinburgh, with high levels of child caries experience, assessed comparative levels of knowledge of the infant oral situation in young people aged 14-15 years; with present day mothers of infants. The teenager group ($n=651$) had fundamental deficiencies in knowledge of infant dental development. Awareness of major events in the transitional chronology of the dentitions was low. Longevity, value and function of deciduous teeth were poorly understood. Conversely levels of understanding for oral self care were high, toothbrushing and dental visiting being considered as social norms confirmed by reported behaviour patterns. The mothers' group ($n=57$) was more knowledgeable but serious gaps in understanding were also found. The longevity of the primary dentition was widely underestimated. Mothers wished for more information preferring post natal venues for oral health education, such as baby clinics; whilst talks and television presentations were the preferred channels.

In conclusion, a need for oral health education in both teenagers and mothers was demonstrated. A novel model for approaching teenage oral health promotion was formulated supporting the need for critical evaluation of classical school health education packages.

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B G SALOLE¹ and V I BINNIE² (¹ Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow; ² Department of Adult Dental Care, University of Glasgow, UK): Oral health awareness of community pharmacists in Scotland.

With the aim of assessing the potential of community pharmacists as collaborators in an interdisciplinary approach to oral health promotion in Scotland, a survey was undertaken to ascertain their oral health awareness. A self-administered questionnaire was mailed to a random sample of a third of the community pharmacies in the country (excluding Boots the Chemists), stratified across the mainland Health Boards. There were 250 usable returns (76.7% response rate).

Analysis of the Likert-type scores showed that community pharmacists had good knowledge of dental health issues; overall, their awareness of the factors affecting caries and periodontal disease was at least as good as that of general medical practitioners in Scotland (Birnie V I and Kay E J, *J Dent Res* 70: 695, 1991). Over 80% of respondents were consulted at least occasionally about common oral health problems and felt at least adequately competent to advise their customers about them; a similar proportion of pharmacists routinely offered sugar-free paediatric medicines, believed that oral healthcare was part of their role, and were interested in becoming more involved in oral health promotion.

It was concluded that the great majority of community pharmacists in Scotland are well aware of the factors affecting oral health. In view of their high-profile and accessibility to the public, better advantage should be taken, at both local and national levels, of their preparedness to become more involved in oral healthcare.

75

H FRENKEL* and A HARRISON (Restorative Dentistry, Dental School, Bristol, UK): Oral care attitudes and practices of staff caring for dependent clients resident in nursing homes.

Previous studies in countries in the developed world have shown that the levels of oral and denture hygiene among dependent institutionalised individuals are lower than in persons of comparable age dwelling in the community. This study aims to assess the factors affecting the way in which care staff carry out clients' oral care. A qualitative data collection method was chosen in order to explore more deeply the carers' attitudes, feelings and motivation towards clients' personal oral care. Semi-structured interviews were carried out with 20 care assistants and 14 qualified nurses from a random sample of nursing homes in the Bristol area. The main aspects of oral care which were investigated were nursing home policy, teaching methods, delivery, problems solving and educational needs. The data was analysed by coding and discourse analysis.

Results showed that none of the nursing homes had access to professional dental health education, oral care was a low priority and there was inadequate understanding, teaching and supervision of care assistants' delivery of personal oral care. Carers felt that they lacked training and expertise in oral care, and some experienced psycho-social barriers connected with contamination and crossing physical boundaries. A perceived need for professional dental help in oral health promotion and staff training was expressed by 73% of trained nurses and 55% of care assistants.

It is planned to develop and evaluate an oral health promotion programme which reflects the special needs of both nursing home care staff and their clients.

76

P M MCGOLDRICK* and C PINE, Tayside Area Clinical Psychology and Department of Dental Health, University of Dundee, Scotland, UK: Dental Student's Self-Assessed Cognitions, Emotions, and Actions relevant to Unhealthy Dietary Behaviours.

The link between sugar use and dental caries has been well documented in epidemiological studies. Furthermore, its prevention requires a knowledge of behaviour modification skills that not only incorporates health education but considers the alteration of damaging health behaviours (Levine RS, *The Scientific Basis of Dental Health Education*, HRA, 1989). Self-management training offers a way of teaching students the skills to influence behaviour change by modifying their own maladaptive dietary behaviours. Therefore, the aim of this preliminary study is to measure dental students' ability to report self-assessed cognitive, emotional and behavioural factors significant in the antecedence of their own sugar-based snacking behaviours. The results presented are gathered from the three-day diet diaries of 46 preclinical dental students. Interim results reveal that the predominant antecedents to snacking behaviours measured were competently identified by the students. One hundred and twenty-three sucrose-containing snacks were consumed by the group over three days. Negative emotions were present in 60% of snacking with stress-related cognitions in 59%. Behavioural settings were 63% social-related and 30% study-related, with the remaining 7% occurring in various other settings.

It is concluded that preclinical dental students can identify antecedents to snacking behaviours on several levels and this experience is relevant to the behaviour modification techniques that they will use in clinical practice. Training in the application of these skills to their own maladaptive behaviour as well as their patients' behaviour will provide a strong educational tool.

77

G M HUMPHRIES*, S KANEY*, D BROOMFIELD*, J D LILLEY* (Depts of Clinical Psychology¹ and Postgraduate Dental Education², University of Liverpool): Preferences of Junior Hospital Dentists and Doctors for Stress Management.

The assessment of stressors in medical and dental personnel has attracted increased attention. This study however focuses on dental and medical junior hospital staff preferences for personal support and services to assist them in coping with work-related stress. A menu of four services were rated by dental and medical JHS ($n=250$) in a cross-sectional questionnaire survey on Merseyside. The four services included: independent counselling, stress management workshops, computer aided self-assessment and a telephone help-line. The questionnaire also included a number of multi-item self-reported health assessment and job-related attitudinal scales. A response rate of 72% was achieved. Results showed that there were no differences between dentists and medicals in their preferences. The most favoured service was counselling although those staff who were classified in a state of burnout (using Maslach and Johnson's criteria) were less likely ($p<0.005$) to indicate a preference for this service despite controlling for grade, sex, discipline, work pressures and job satisfaction.

We conclude that the introduction of a support service requires a training component to increase awareness and acceptability of that service. However a preventive approach is strongly indicated particularly for those in posts susceptible to burnout.

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J D LILLEY*¹ G M HUMPHRIES*, S KANEY* and D BROOMFIELD* (Depts. of Postgraduate Dental Education¹ & Clinical Psychology², Univ. of Liverpool): Burnout among Junior staff of 3 dental hospital specialties.

The degree of emotional exhaustion, depersonalisation and sense of accomplishment (i.e. burnout) was assessed by the Human Services Scale, and job satisfaction by the Occupational Stress Inventory in a questionnaire survey of Junior Hospital Staff from three dental specialties, namely: restorative ($n=21$), oral surgery ($n=10$) and orthodontics ($n=11$). A number of other standardised measures of general health and work-related psychological constructs were also collected. The response rate for these dental staff was 84%. Depersonalisation varied significantly across specialties ($F(2,38) = 4.25, p<0.02$). Orthodontists exhibited less distancing from the emotional demands of their patients than their colleagues from the other two specialties. Four of the six job satisfaction sub-scales varied significantly by specialty ($p<0.02$) indicating that restorative staff were less satisfied with their work in comparison with the orthodontists and oral surgeons. The relationship of the three burnout variables to job satisfaction in this sample was strongly negative (all Pearson's r 's $> -0.4, p<0.01$). We conclude that the nature of the training of some specialties in dental hospitals may contribute to differences in burnout and be a factor in reducing job satisfaction.

79

JR MAIN*, R FREEMAN and FJT BURKE (Restorative Dentistry, University of Manchester, UK and Queen's University of Belfast, UK): Factors associated with premature retirement from dentistry due to illness.

The aim of this study was to evaluate reasons for premature retirement due to illness from the practice of dentistry. Details of the reasons for premature retirement due to illness were obtained from one society of 10,000 members (Dentists' Provident Society, London, UK), with individual members not being identified. Other UK-based organisations which were contacted were unable or unwilling to provide the necessary information. Data were obtained on 393 premature retirements from 1981 to 1993. Reasons for premature retirement were identified from this data and grouped where possible according to the following basic categories - cardiovascular, respiratory, digestive, tumours, musculoskeletal, neuroses, accidents, and disease of skin, eyes, ears and nervous system. These illnesses were subdivided and analyzed with respect to dentist's age and year of retirement. Results indicate that of the categories identified, musculoskeletal disease accounted for 30% ($n=116$), cardiovascular disease 21% ($n=83$), neuroses 17% ($n=65$) and tumours 8% ($n=30$). The most common specific condition given for early retirement in this study was depression and anxiety, while 82.6% of the dentists taking premature retirement due to illness were over 50 years of age.

It is concluded that the most frequent causes of premature retirement of the dentists surveyed are musculoskeletal problems, cardiovascular disease and neuroses, with the over 50 age group being particularly affected.

80

S YEGANEH*, C MORRIS-CLAPP, L ZOU, V JOVANOVSKEI and E LYNCH (Department of Conservative Dentistry, LHM, UK): Three-Dimensional quantification of plaque on tooth surfaces.

Clinical dentistry involves the continuous subjective analysis of the forms of oral structures: the surface morphology of teeth; their relationship to each other; and to the gingivae and the distribution of plaque and calculus. The aim of this study was to assess a method to quantify plaque thickness on tooth surfaces and the associated gingivae. For this preliminary investigation, exposed root surfaces of at least 1.5mm were selected in patients and impressions were taken in order to produce study models. Individual jigs specific to the exposed root surfaces were made using peripheral-seal material to stabilise rectangular brass tubes of 12mmx12mm. A replica of the site was then taken using light body addition polysiloxane (Extrude-Kerr). A second replica of the site was then obtained following manual plaque removal. The replicas were scanned to collect the co-ordinates of the surface points at a density of 20µm spacing using the Renishaw OP2 laser probe. The plaque thickness and volume were measured by superimposing the data from the two replicas. Plaque thickness ranged from 0.010-0.131mm. Reproducibility of the system measuring a 25mm diameter precision sphere was 24.9996 \pm 0.0024 (mean \pm SD).

This preliminary study illustrates a method of quantitative measurement of plaque thickness distribution and volume on root surfaces and its relationship to adjacent gingivae.

81

REEM DABABNEH-KHOURI & ROGER G SMITH* (Restorative Dentistry, Dental School, Bristol, UK) The development of a new method of plaque scoring

Many plaque indices for recording supragingival plaque have been reported. Detailed scoring methods, whilst enhancing index sensitivity, may be neither easy nor quick to perform. A new method of plaque scoring (NMPS) has been devised to be simple but sensitive. For scoring purposes a horizontal boundary is imagined on the smooth tooth surface between the gingival third (A) and coronal two-thirds, the latter being subdivided vertically into thirds (B,C,D). B includes the mesial, and D the distal, embrasure areas; C is middle third. Depending on the extent of plaque coverage, whole number scores 0-3 are assigned to A,B,D and 0-1 for C. Total score possible is 10. (1) Simulated plaque on 8 study casts was scored twice by 10 examiners using the criteria of NMPS and Turesky *et al* [J Periodontol 41:41-43, 1970]. (2) The time taken for the examiners to score 4 selected surfaces on 1 cast using NMPS and 3 other indices was also measured. NMPS in (1) was found to have less variability within and between examiners than Turesky, and in (2) took the least time to score. In conclusion NMPS appears to combine the benefit of sensitivity with simplicity: clinical testing is warranted.

82

D.L.GUTTERIDGE (Division of Restorative Dentistry, Dental Institute, Leeds): Rapidly progressive and chronic adult periodontal disease: a survey of referred cases.

Rapidly progressive periodontal disease (RPP) was first recognised as a distinct clinical condition in 1983. It affects young adults leading to advanced, generalized bone loss and presents a challenge in terms of initial management and subsequent maintenance. Clinicians are increasingly aware of the need for efficient and effective delivery of care and the management of these cases can place considerable demand on resources. This study aimed to survey the case mix of patients attending a consultant periodontal referral clinic and assess their treatment needs. Hospital notes of 172 patients, attending between July and October 1993, were assessed to record age, sex, smoker/non-smoker status, source of referral, periodontal status at initial examination including CPITN and the number of teeth with pocketing greater than 5mm. Radiographs were examined to determine how many quadrants had greater than 30% bone loss and the final diagnosis was noted. There were 61 (35%) male and 111 (65%) female patients in the survey, mostly referred from general dental practitioners (83%). 31% were smokers and 49% non-smokers, the status of 15% being unrecorded. Some 27% had bone loss greater than 30% in all 4 quadrants, 8.6% in 3 quadrants, 12.4% in 2 quadrants and 16% in 1 quadrant. Comparison of RPP and chronic adult periodontal (CPD) patient groups revealed mean ages of 29 and 41 respectively. Smokers and non-smokers were evenly distributed in the RPP group but non-smokers predominated in the CPD group (38/25). The diagnosis most commonly made was of chronic adult periodontitis (61%). Gingivitis constituted 8% of the total and RPP or suspected RPP 8%. Localised gingival recession was a smaller feature (3%). In conclusion, 8% of general practitioner referrals within the period of the study were patients affected by RPP. In total, 27% of patients presented with bone loss of more than 30% affecting 4 quadrants. This allows effective treatment planning and use of resources.

83

M P S BONAKDAR*, P M BARBER* and H N NEWMAN (E M Unit* & Periodontology Dept, Eastman Dental Institute, UK): Quantitative vascular changes in pocket soft tissues in Chronic Inflammatory Periodontal Disease.

Expansion and remodelling of the blood vessels (BV) in the pocket soft tissues may be significant in the pathogenesis of Chronic Inflammatory Periodontal Disease (CIPD) (Zollner H, J Oral Pathol Med 20: 433-7, 1991). The aim of the present study was to quantify BV within the pocket soft tissues from CIPD lesions.

For this study twelve interdental biopsies were obtained, six from CIPD patients and six from healthy volunteers. All specimens were sliced, fixed in 3% glutaraldehyde, post-fixed in 1% buffered osmium tetroxide, dehydrated, and embedded in araldite. Thick sections (1 µm) were cut and differentially stained (Humphrey CD, Stain Technol 43: 83-87, 1974).

The numerical density of BV for the sub-epithelial, superficial and deep connective tissue layers was then obtained by counting the number of BV per unit area. There was a significant increase in the numerical density of BV ($P < 0.01$) in the CIPD specimens (204.16 ± 46.14), as compared to healthy specimens (146.66 ± 21.84), with more BV in sub-epithelial layer than others.

In conclusion this study indicates that there is an increase in the numerical density of BV in CIPD lesions.

84

P BALDWIN*, K S LAST and N PENDER (Department of Clinical Dental Sciences, School of Dentistry, The University of Liverpool, UK): The effect of extraction during orthodontic treatment on gingival crevicular fluid flow.

Gingival crevicular fluid (GCF) flow is increased by active tooth movement (Samuels RHA, Pender N, Last KS, J Clin Periodontol 20: 371-377, 1993) and reduced by fixed retention (Pender N, Samuels RHA, Last KS, Eur J Orthod in press). The effect of adjacent tooth extraction on GCF flow in the early phase of orthodontic treatment is unknown. This study compared GCF volumes collected around teeth next to an extraction site (XT) with non-extraction (NX) sites. GCF was collected for 15-min from the disto-buccal aspect of one maxillary canine in 35 patients undergoing fixed appliance therapy (FA). Three samples were taken and Gingival Index (GI) scored before and during the first 10 weeks of FA. GCF increased ($P < 0.001$) over this period, GI did not ($P = 0.2$). Mean \pm sem GCF in the XT group before FA placement and three weeks post-extraction at $0.9 \pm 0.3 \mu\text{l}$ did not differ from ($P = 0.97$) the NX group, $0.9 \pm 0.5 \mu\text{l}$ before FA. Four weeks later, XT GCF ($2.6 \pm 0.4 \mu\text{l}$) did not differ ($P = 0.98$) from NX GCF ($2.8 \pm 0.9 \mu\text{l}$). Ten weeks after FA, XT GCF ($2.8 \pm 0.4 \mu\text{l}$) did not differ ($P = 0.35$) from NX GCF ($3.7 \pm 1.1 \mu\text{l}$).

The increase in GCF found in the early stages of orthodontic treatment is not influenced by either an increased Gingival Index or the proximity of a recent extraction site.

85

N AHMED*, TLP WATTS and RF WILSON (Department of Periodontology, UMDS (Guy's), London, UK): Validity of attachment level measurements with Florida probe.

There is a need for information on the validity of probing depth and attachment level measurements made with automated probes. In this study, 34 teeth in 9 patients were measured with the Florida probe from points marked with a bur prior to extraction. After extraction, connective tissue attachment levels were measured from the same points with a dissecting microscope. Mean measurements of attachment level were smaller with probing ($5.13 \pm 2.08\text{mm}$) and laboratory ($5.18 \pm 2.26\text{mm}$) assessments. The correlation between these measurements was high ($r = 0.72$; $p < 0.001$) and a t-test of the paired measurements showed no difference ($t = 0.44$; $p = 0.66$). The Florida probe thus showed high validity for this group of measurements of advanced periodontitis. However, there was substantial lack of agreement between individual probing measurements and the validity criterion of laboratory attachment level measurements.

It was concluded that the Florida probe was suitable for studies in which measurements were averaged, but not for longitudinal study of individual sites.

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PG ROBINSON*, S CHALLACOMBE, A SHEEHAN, JM ZAKRZEWSKA (Dept. Epidemiology and Public Health, UCL, and Dept. Oral Pathology and Medicine, UMDS): Periodontal attachment levels associated with HIV infection.

Aim and Method: To assess the prevalence, extent and severity of attachment loss associated with HIV infection, periodontal examinations were conducted on convenience samples of homosexual men attending a genito-urinary medicine clinic with the examiner blinded to their HIV status.

Results: 792 men were examined (312 HIV+ve, 260 HIV-ve and 220 untested) in a 15 month period. Men with HIV were slightly older than those without (35.5y. Vs 31.2y. $p < 0.001$, MWWT) and were of higher socio-economic status ($p < 0.001$, Chi sq.) but had similar numbers of teeth, plaque scores and tobacco use.

Forward stepwise multiple and logistic regression revealed strong associations between HIV infection and summary measures of attachment loss and pocketing (all $p < 0.005$). Among men with HIV, CD4+ve T lymphocyte counts were associated with summary measures of attachment loss (all $p < 0.001$) but not with pocketing.

Conclusions: This cross-sectional study suggests the prevalence, extent and severity of attachment loss are associated with progression of HIV disease whereas pocketing is associated with HIV infection but not with disease progression. Longitudinal studies are required to confirm these findings.

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P.M. FRESHAW*, P.J. KELLY*, P.A. HEASMAN* (Department of Restorative Dentistry, *Department of Medical Statistics, University of Newcastle upon Tyne, UK): Calibration methodology for the Periotron 6000®.

The Periotron 6000® is used to quantify volumes of gingival crevicular fluid (GCF) sampled on periopaper strips. Previously, for calibration purposes, a linear relationship has been demonstrated between Periotron units and fluid volume (Bickel *et al* J Periodontol Res 19: 313-316, 1984). The aim of the present study was to investigate further this relationship to provide a reliable and accurate method of calibration. Known volumes of liquid (human serum or 0.9% w/v saline) were dispensed onto periopapers using a Hamilton 7000 series microtitre syringe, and then transferred to the Periotron within 2-3 seconds. A total of 75 different volumes of serum, ranging from 0 to 1.60 µl, and 60 different volumes of saline, ranging from 0 to 1.50 µl, were used. Each volume was measured in (at least) triplicate, to provide over 300 individual data points for serum and 200 data points for saline. Regression analysis revealed that a quadratic curve produced a very good fit ($r^2 = 0.99$ for serum and 0.94 for saline). The non-linearity of the data was confirmed by examining the significance of the pure error term via regression analysis ($p < 0.0001$). Using a linear regression rather than the quadratic regression produced differences of up to 35% when serum was used. The regression equation can be used to determine 95% prediction intervals (PI) for estimating volume given a certain periotron value.

GCF volumes sampled using periopapers can be most accurately determined when calibration is undertaken with serum, and using a quadratic regression equation.

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AWG WALLS*, F OTIBENO, JF McCABE (Dental School, Newcastle upon Tyne, UK): Bonding of light activated glass ionomer cements to dentine.

Light-activated glass ionomer cements have been shown to exhibit resin-mediated hygroscopic expansion after setting which could disrupt the bond formed between the cement and dentine. The purpose of this investigation was to determine the influence of water storage on the bond strength of these cements to dentine. Three cements were used Chemfill Superion(C) a conventional acid/base material, and two light activated materials Fuji II LC (F) and Photac-Bond (P). Dentine from caries free human third molar teeth were used as the bonding substrate. The bond strength of 21 specimens of glass ionomer cement was measured in shear using an Instron Universal Testing Machine at a cross-head speed of 1mm per minute. Bond strengths were determined after 15 minutes, 24 hours and 14 days storage in dry conditions and after 14 days storage in wet conditions. The bond strength of P was low, with limited bonding to superficial dentine alone [$2.7 (1.3)$ MPa @ 15 minutes, $4.9 (2.53)$ MPa @ 24 hours]. This material bonded very poorly to the deeper layers of dentine. Both C and F formed bonds to all dentine levels. The mean bond strength for these materials increased with time ($p < 0.001$ ANOVA) on dry storage. The maximum bond strengths achieved were after 14 days of dry storage [C $10.39 (2.89)$ MPa, F $16.79 (6.75)$ MPa]. The mean bond strengths for F were consistently higher than those for C. Storage of both materials in water for 14 days resulted in a reduction in bond strength when compared to the 14 day dry specimens. This reduction was significant ($p < 0.05$, student t-test) for material F but not for C. There was a trend in all materials for the bond strength to superficial dentine to be greater than those to deep dentine.

The bond strength of a light activated glass ionomer cement was significantly reduced by storage in water for 14 days.

1 Dentistry UK, 2 GC Japan, 3 ESPE Germany

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N MEREDITH* (Department of Oral and Dental Science, University of Bristol, U.K.) and A WENNEBERG (University of Gothenburg, Sweden) Measurement of the surface roughness of etched enamel using laser scanning confocal microscopy.

A freshly extracted caries-free lower first permanent molar was embedded in a cold-curing resin (Simplex Rapid¹) and sections were prepared bucco-lingually in the sagittal plane using a water-cooled diamond saw. Two sections from the mid portion of the tooth were then ground with progressively finer silicone carbide paper up to 1,000 grit. The specimens were then mounted on the stage of the confocal scanning microscope (Topscan 3D²). XY scans of the enamel surface were then made over areas of 250 µm². The surface parameters were recorded on a PC and a 1 x 99 x 33 binomial filter was applied to eliminate noise. Measurements were repeated on the same specimens following the application of 36% phosphoric acid gel to the enamel surface for periods of 30, 60, 120, 180 and 240 s. The gel was applied to the surface which was then washed and dried thoroughly. Reference points enabled repeatable relocation on the stage of the microscope. The results indicated that there was a significant increase in the mean surface roughness (Ra) of the enamel surface with an increase in etching time. The Ra value of the unetched enamel surface was 0.136 µm and this rose to a maximum of 1.227 µm after an etching period of 180 s. The increase in roughness appeared to level off after an etching period of 60 s (0.826 µm).

It is concluded that the application of 36% phosphoric acid gel to a prepared enamel surface results in an increase in surface roughness which can be related to the etching period.

¹ Hwemica; ² Heidelberg GmbH.

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EH DAVIES*, GJ PEARSON, HM ANSTICE & C MORONFOLU* (Eastman Dental Institute, University of London, UK. * University of Greenwich): Studies on release/absorption from resin-modified glass-ionomers and related materials.

The fluoride release and water absorption of four materials, two resin-modified glass-ionomer cements (VM, F) and two other resin-modified materials (VG, D) were investigated, in distilled water or artificial saliva, at 37°C. To investigate the fluoride release, discs were made from each of the materials according to the manufacturers' instructions. The discs were then matured for 1h at 37°C. To monitor fluoride release the discs were suspended in a known volume of test solution. Aliquots of solution were removed at specific times and the concentration of fluoride determined. For the measurement of absorption, discs were prepared in a similar manner. After the maturation period, the specimens were weighed accurately and then placed upright in the appropriate storage solution. At specific times the specimens were removed from the solutions, blotted and then weighed, before being replaced in the solution. In both experiments the specimens were monitored for at least 6 months. In distilled water F showed the greatest sustained fluoride release, followed by VM. The release from VG and D was minimal. F also showed the greatest absorption (6% by mass), VM also had a large absorption closely followed by VG. D had the smallest increase in mass. In artificial saliva the release rates were far lower, but the relative order of release was maintained for the materials. However it should be noted that all the materials gradually degraded when stored in artificial saliva, stabilising after about 6 months storage. Resin-modified GIC have a good fluoride release rate, but have a high water absorption which develops during the first 24h. VM Vitremer (3M), F Fuji II LC (GC), VG Variglass & D Dyract (Dentsply)

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A J DEVLIN*, K K JOHAL*, R G HILL*, E de BARRA*, S GRIFFIN*, G HENN*, G T CRAIG, P V HATTON and I M BROOK (Universities of Limerick, Ireland & Sheffield, UK.): Fluoride ion release from glass-ionomer cements.

The mechanisms of fluoride ion release from glass ionomer cements (GICs) remain obscure. This investigation aimed to improve information on the relationship between glass composition and fluoride ion release to provide an understanding and enable predictable control of fluoride release from GICs. Two series of glasses were produced based on the general formulae $1.5SiO_2 \cdot 0.5P_2O_5 \cdot Al_2O_3 \cdot (1.8-Z)CaO \cdot 0.75CaF_2 \cdot ZnO$ and $(P/SiO_2)(Q/Al_2O_3) \cdot 3P_2O_5 \cdot (S-X)CaO \cdot (X)CaF_2$ where P, Q, X and Z range from 0 to 5 and are mole fractions. Ions were eluted under sink conditions from set cement discs (20mm x 2 mm) into sterile high purity water at 37°C, samples being collected between 3 and 84 days. Total and free fluoride ion concentrations were determined using a calibrated ion selective electrode with or without TISAB III buffer. All other ions were determined by atomic absorption spectroscopy. The results showed that virtually all the fluoride was released as free fluoride ions and was not chelated to metal ions. Fluoride release correlated with the sodium concentration of the glass, which appeared to support a counter ion mechanism. However, totalling the number of positive and negative ions released and calculating the cumulative ion balance indicated that there was a surplus of negative ions over positive ions leaving the cement at all times. Thus, the dominant fluoride release mechanism appeared to be based on an ion exchange process. The negative ion balance increased as the sodium content of the glass increased, indicating that sodium ions facilitated the rate of ion exchange, possibly by disrupting the cement matrix and thereby increasing ion mobility. In contrast to a previous study (Meyers S D and Smith A J, *Int. Endod. J.*, 17: 16-24, 1984), there was a strong correlation between the fluoride content of the glass and the release of fluoride from the set cements.

Fluoride ion release from GICs can be enhanced by increasing the fluorine and sodium content of the constituent glasses. This ion release process appears to be predominantly via an ion exchange mechanism.

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E DE BARRA*, S GRIFFIN, G HENN, R HILL (University of Limerick, IRELAND), J DEVLIN*, K JOHAL*, P HATTON* and I BROOK*: The Mechanism of Fluoride Release From Glass(Ionomer) Polyalkenoate Cements.

Fluoride release has been postulated to occur by a counter ion mechanism (Wilson A D et al Biomaterials 6: 431-33, 1985) involving the simultaneous release of an alkali metal cation. To test this hypothesis a range of model glasses were produced in which (i) the fluorine content and (ii) the alkali metal content of the glass was varied. Cements were made from these glasses and both the free fluoride ion and total fluoride released measured under sink conditions. At the same time the silicon, aluminium, calcium, sodium and phosphate ions released were measured. Aluminium release from the set cements was negligible and there was no significant difference between the free fluoride and the total fluoride released. This suggests that little or no fluorine is released in the form of aluminium complexes. The cements with no alkali metal content still released fluoride indicating that a soluble counter ion is not required. At all times the cements demonstrated a negative ion balance where there were more negative ions leaving the cement than positive ions.

The results demonstrate that the principal release mechanism for all the cements was not the counter ion mechanism, but an ion exchange mechanism where a fluoride ion in the cement is exchanged for a hydroxyl group from water in the elution medium.

+ School of Clinical Dentistry, University of Sheffield, England.

This study was funded under the Brite-EuRam Scheme Project No. BE6062 Contract BRE2-349.

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W S McLAUGHLIN*, J G MEECHAN, P A HEASMAN & J F McCABE (The Dental School, The University of Newcastle upon Tyne, U.K.) The release of flurbiprofen from dental materials.

Flurbiprofen is a potent anti-inflammatory and analgesic compound. Its incorporation and subsequent release from dental materials may have a topical benefit in certain dental procedures. The aim of this study was to measure its release from 2 commercially available dental cements; a calcium hydroxide lining material (Life) and a glass ionomer cement (Chemfil II Express²). 10 x 2 mm diameter discs of each set material were prepared in triplicate, incorporating either 0.02g, 0.01g, 0.001g or 0.0001g of flurbiprofen. Discs with no drug served as negative controls. Each disc was suspended in 15ml of distilled water. Positive controls comprised the same weights of flurbiprofen dissolved directly in vials containing 15ml of distilled water. 200µl samples in triplicate were removed from each solution at 1, 7 and 28 days. No obvious disintegration of the discs occurred with time. The samples were assayed for drug concentration using high performance liquid chromatography. Flurbiprofen was released at increasing levels from each material sample at every time interval. The maximum amounts of flurbiprofen being detected at one month were 46.5% of that dissolved in distilled water, and 15% and 7.5% of the quantity incorporated in Life and Chemfil II Express respectively.

It is concluded that flurbiprofen is released at an increasing concentration with time from both a glass ionomer cement and a calcium hydroxide lining material.

¹ Kerr U.K.; ² Dentsply U.K.

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M A CURTIS* (MRC Molecular Pathogenesis Group, Department of Oral Microbiology, LHM, London E1 2AA, UK): Microbial Proteases and Periodontal Disease.

The production of extracellular proteolytic activity is a widely-used strategy by a range of human parasites, including bacteria, protozoa and helminths, to facilitate survival in the colonised host. The advantage to the organism may arise from the increased availability of readily transportable substrates through the degradation of host macromolecules, the disablement of the local immune and non-specific host defences and the exposure of previously shielded substrata as potential sites for colonisation.

Much interest is now focussed upon the role of proteases derived from bacteria in subgingival plaque on the tissue destruction in the periodontal diseases. The potential destructive mechanisms can be divided into three categories. First, direct proteolysis of the structural proteins of the underlying connective tissue. Second, deregulation of the inflammatory response and normal homeostatic mechanisms operating within the tissues thereby leading to host derived damage. Third, an undermining of the local host defences to an extent which renders the site susceptible to damage by other agents from the subgingival plaque. Supportive *in vitro* evidence for each of these mechanisms is now accumulating in the form of physiologically relevant reaction rates for host macromolecule destruction/inactivation by purified microbial proteases.

However in the context of the disease process several issues remain unresolved. These include the level of microbial protease gene expression *in vivo* and its correlation with destructive disease, the role of host and microbially derived inhibitors of these activities and the ability of the specific immune response to neutralise their effects. The current research efforts aimed towards the structural and genetic characterisation of subgingival plaque microbial proteases should provide the means to address these questions.

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S. TODRYK*, C. G. KELLY, H. PARSONS AND T. LEHNER (Div. of Immunology, UMDS at Guy's Hospital, London SE1 9RT: Targeting immune responses to functional determinants of streptococcal antigen I/II).

Colonisation of the oral cavity with *Streptococcus mutans* can be prevented by antibodies against streptococcal antigen I/II (SA I/II), a cell surface adhesin. An adhesion binding site has been mapped to residues 816-1213 (fragment 3) of SA I/II. The aims of this study were to map the adhesion epitopes of SA I/II and to investigate the use of recombinant polypeptides to induce immune responses to these functional determinants. To identify adhesion epitopes a panel of synthetic peptides spanning residues 803-1174 was analysed for *in vitro* inhibition of adherence of *S. mutans*. A recombinant polypeptide comprising T cell and adhesion epitopes (TA) was expressed in *E. coli* and groups of mice of different MHC haplotypes were immunised either with fragment 3 or with the TA construct. Two 20-residue peptides consistently inhibited adherence of *S. mutans in vitro*. Immunisation with fragment 3 induced antibody responses both to fragment 3 and SA I/II, however responses to the adhesion epitopes were weak. In contrast, immunisation with the TA polypeptide induced strong antibody responses to the adhesion epitopes. The antibodies also recognised intact SA I/II and whole cells of *S. mutans*.

These results demonstrate that antibody responses may be induced to functional determinants of SA I/II by immunisation with a recombinant polypeptide construct comprising T cell and adhesion epitopes.

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J.K.-C. MA*, V. ROBERTS* and T. LEHNER (Dept. Immunology, UMDS Guy's Hospital, London UK; *The Scripps Research Inst., CA, USA): Sequence and structure prediction of Guy's 13, a monoclonal antibody that prevents *Strep. mutans* adherence....

Oral colonisation by *Streptococcus mutans* is mediated through a cell surface antigen SA I/II, and can be prevented by topical application of a specific monoclonal antibody (Guy's 13) which prevents adherence. The aims of this investigation were to clone, sequence and construct a structural model for this protective antibody, which would help to determine the nature of the protective mechanism at a molecular level. The genes encoding the light and heavy chains of Guy's 13 were amplified using RT-PCR from mRNA extracted from the hybridoma cell line and cloned into the Bluescript plasmid for sequence determination. Primary amino acid sequence prediction demonstrated a number of unusual features relating to the variable region of this antibody that are predicted to influence antigen binding. The structural model of Guy's 13 was built using a database containing antibodies for which the crystallographic structure has previously been solved. The key findings were that a) the 3rd hypervariable loop of the heavy chain (H3) is extremely short, which is unusual, as H3 normally has extensive and important contacts with the antigen; b) the majority of the residues which contact the antigen and determine the binding affinity are tyrosine residues, except for a key arginine residue on the light chain that is predicted to extend directly into the antigen binding pocket; c) the antigen binding site is predicted to be flat, rather than to form a groove or pocket.

Based on these findings, it may be possible to introduce mutations into the antibody variable region of Guy's 13, in order to modify the interaction with antigen and improve the clinical efficacy of this antibody.

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J TAYLOR*, DR GEATCH, DA ROSS and PA HEASMAN (Departments of Oral Biology and Restorative Dentistry, The Dental School, University of Newcastle upon Tyne, UK): Expression of T-cell receptor β (TCRB) genes in periodontal disease.

T-cell receptor (TCR) genes encode T-cell surface proteins which are involved in antigen recognition during the immune response to pathogens. We have investigated the structure of expressed TCR genes in periodontal disease using reverse-transcriptase PCR (RT-PCR). Restriction of the TCR repertoire at the site of inflammation would imply a role for selected subsets of T-cells in the pathology of the disease. Total RNA was extracted from gingival biopsies of patients undergoing surgical treatment for chronic adult periodontal disease. cDNA was prepared by reverse transcription using an oligo-dT primer and the presence of amplifiable cDNA confirmed using a PCR for a constitutively expressed gene ($\beta 2$ -microglobulin). Expressed TCR β chain (TCRB) genes were detected using PCR for an internal sequence in the TCRB constant (C) region genes. TCRB gene rearrangements were detected in the same samples using a PCR which employed a constant region primer and a panel of primers specific for TCRB variable region gene families. cDNA obtained from the T-cell line MOLT-4 acted as a positive control. Using this approach we have isolated expressed TCRB gene rearrangements which employ genes from TCRBV2, V6 and V8 families. The pattern of expression was different in each of the 3 biopsies examined thus far.

It is concluded that RT-PCR for TCRB is a useful approach in the investigation of T-cell repertoires in periodontal disease.

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S P SWEET*, S J CHALLACOMBE, P ROBINSON, M M COOGAN and C RACHANIS (Oral AIDS Unit, Dept Oral Medicine and Path, Guy's Hospital, UK): Oral yeasts and candidiasis in HIV infected men in London and Johannesburg

HIV infection is strongly associated with an increased prevalence of oral candidiasis. However, the aetiology and the prevalence of oral candidiasis in different HIV infected populations is unclear. The aims of this study were to determine the prevalence of oral candidiasis, oral yeast carriage rates and the species of infecting yeasts, in two population groups. HIV-antibody positive and negative subjects were studied in the two groups, which consisted of white homosexual and black heterosexual men attending STD clinics in London, UK and Johannesburg, SA, respectively. Whole saliva was collected and yeasts were isolated on Sabouraud's agar and speciated using the API 20C AUX system. The mean CD4 counts of the HIV infected patients in the UK group was 321 compared with 290 in the SA group. Oral candidiasis was clinically diagnosed in 35% of HIV infected UK men and 6% of HIV infected SA men ($P < 0.01$). Negligible levels were diagnosed in HIV negative males. Yeast isolates from the UK group consisted predominantly of *Candida albicans* (97%). In contrast, the SA isolates (HIV+ and HIV-) consisted of 40% *Saccharomyces cerevisiae*, 3% *C. albicans* and many isolates that could not be reliably identified ($P < 0.01$).

This study suggests that SA heterosexual men are less susceptible to HIV associated oral candidiasis than UK homosexual men. This may be associated with an increased prevalence of less pathogenic, non-C. albicans, oral yeasts in South African men, although factors related to race, sexual preference or geographic location remain to be studied.

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J HAMBURGER*, GM LAWRENCE* & R OAKES* (School of Dentistry, The University of Birmingham, West Midlands Cancer Registry, Queen Elizabeth Medical Centre, UK): Clinical features of lichenoid drug reactions.

The term "lichenoid reaction" (LR) is applied to those cases of lichen planus (LP) believed to have been provoked by drugs. Clinically and histologically, the differentiation between LP and LR is subjective. The aim of this study was to determine whether the clinical features of histologically diagnosed LR and LP differ.

Clinical data on 544 patients with oral lichen planus were analysed retrospectively with regard to medical history, nature and distribution of lesions. Statistical analysis was performed using Epi Info. 201 patients underwent biopsy, 36 of whom were diagnosed histologically as LR. 30 of these patients were taking drugs known to be associated with LR, 6 demonstrating a marked clinical improvement on drug withdrawal. There were no statistically significant differences in site distribution nor in the frequency of erosions between the LR and LP patient groups as diagnosed histologically. However, comparison of these clinical parameters in patients taking drugs and those receiving no medication revealed a higher prevalence of erosive lesions and labial involvement in the group on medication ($\chi^2 = 7.58$; $p < 0.01$ & $\chi^2 = 3.99$; $p < 0.05$ respectively).

The data suggest that although the clinical features of histologically diagnosed LR and LP show no statistically significant differences, the frequency of erosions and labial involvement is greater in those patients on medication.

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DI PETRUCCI*, DH FELIX, DG MACDONALD, D WRAY* and A FORSYTH* (Glasgow Dental Hospital and School, Glasgow Royal Infirmary): Associations of lichenoid reactions with exposure to environmental and dietary allergens.

A series of 46 patients (14 male, 32 female; mean age 50.8 years) with biopsy confirmed symptomatic lichenoid reactions involving the oral mucosa were investigated to identify contributing factors in the aetiology of this common mucosal disorder. Histopathological diagnostic criteria included keratosis and a chronic inflammatory cell infiltrate with obvious immunologically mediated damage to the epithelium. Where possible, drug therapy was altered to exclude possible contributing agents. Venous blood was removed for a full blood count together with assays of plasma ferritin, red cell folate, serum vitamin B₁₂ and glucose. All patients were subsequently patch tested to the standard European series.

Allergens were identified in 40 of 46 patients (87%). Common allergens identified fell into two broad groups: dental materials (particularly mercury and organic mercurials) and food additives and flavouring agents (most commonly benzoic acid and cinnamon). Thus reactions to mercury were identified in 26 patients and allergies to dietary additives in 14. Some patients had evidence of multiple reactions.

It is concluded that patch testing to determine the presence of reactions to dietary and/or environmental allergens may be a useful investigation in the management of patients presenting with symptomatic lichenoid eruptions involving the oral mucosa. This investigation requires further evaluation.

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B E MCCARTAN*, P-J LAMEY, D G MACDONALD AND R M MCKIE (Trinity College Dublin, Queen's University, Belfast and Glasgow University UK): Oral lichenoid reactions and basal cell cytoplasmic auto-antibodies.

This prospective study sought to ascertain the value of basal cell cytoplasmic (BCC) autoantibodies in differentiating oral lichen planus from oral lichenoid reactions. One hundred and sixty one patients were studied (42 male and 119 female mean ages 9 years and 55 years respectively). Mucosal biopsy was undertaken in 144 patients and in addition to clinical assessment of their oral condition, all had analysis of BCC autoantibodies using an indirect immunofluorescence assay. Drug therapy was also documented in detail. Clinically unilaterality of lesion(s) was a good discriminator of oral lichenoid reactions versus oral lichen planus ($p < 0.02$). Histologically oral lichenoid reactions can be difficult to diagnose and a range of histological changes were recognised. The presence of BCC circulating autoantibodies was significantly higher ($p < 0.01$) in the lichenoid reaction group.

The data suggests that BCC circulating autoantibodies are a useful adjunct when attempting to determine if an oral lichenoid reaction has been precipitated by drug therapy.

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CM HEALY*, AT CRUCHLEY and MH THORNHILL (London Hospital Medical College and University of Manchester, UK): Adhesion molecule expression in recurrent oral ulceration (ROU).

Recurrent oral ulcers are characterised histopathologically by an early mononuclear cell inflammatory infiltrate which becomes mixed with the influx of neutrophils as the lesions develop. The recruitment of inflammatory cells at sites of inflammation is mediated by adhesion molecules on endothelial cells (EC). Adhesion molecules on other cells may help maintain the infiltrate. This study was carried out to determine the expression of the adhesion molecules, vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin, in ROU lesions and to compare this with their expression in normal oral mucosa (NOM) and non-specific ulcers (NSU).

Biopsies of ROU were obtained from 14 patients (mean age: 31 \pm 13 years, 9 males, 5 females), NSU from 5 individuals (mean age: 35 \pm 8 years; 3 males, 2 females) and NOM from 11 individuals (mean age: 58 \pm 26 years; 7 males, 4 females). Serial cryostat sections were obtained and sequentially incubated with primary monoclonal mouse anti-VCAM-1, anti-ICAM-1 or anti-E-selectin antibody, biotinylated polyclonal rabbit anti-mouse antibody, alkaline phosphatase-conjugated streptavidin biotin complex and finally alkaline phosphatase substrate. The sections were examined by two observers, the distribution and intensity of antigen expression semi-quantitatively graded and the expression in the groups compared using the Mann-Whitney U test. Higher levels of VCAM-1 staining were seen in ROU lesions compared to in NSU ($p < 0.005$) or NOM ($p < 0.0005$). Similarly E-selectin staining was increased in ROU compared to in NSU ($p < 0.05$) or NOM ($p < 0.0005$). ICAM-1 was strongly expressed constitutively on EC in NOM, but was upregulated in ROU and NSU and keratinocyte (KC). ICAM-1 was a prominent feature in ROU.

Upregulation of VCAM-1 and E-selectin expression on EC and ICAM-1 expression on KC in ROU suggest that these molecules are important in the recruitment and maintenance of the predominantly lymphocytic infiltrate of early ROU lesions.

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BK BLOOR*, SV SEDDON, L SU, Y ALAM and PR MORGAN (Department of Oral Medicine & Pathology, UMDS (Guy's), London Bridge, UK) Keratin 4 and 13 protein and mRNA expression in oral dysplasia and malignancy.

An important factor in estimating the extent of differentiation in squamous cell carcinoma and premalignant lesions is the degree of keratinisation and, in mucosal carcinoma, the expression of mucosal keratins, K4 and K13. We set out to determine the level of regulation at which these keratins were expressed in normal, dysplastic and malignant oral epithelia using a combination of immunocytochemistry and *in situ* hybridisation techniques.

Fresh frozen sections of normal mucosa (n=9), dysplasias (n=23) and malignant lesions (n=20) were fixed in paraformaldehyde and processed for *in situ* hybridisation using specific radiolabelled riboprobes to K4 and K13. Adjacent sections were fixed in acetone for immunoperoxidase staining using monoclonal antibodies to each of these keratins. Levels of expression of mRNA and protein for both keratins in these groups were compared.

In normal epithelia and mild dysplasias, mRNA for K4 and K13 was present in the suprabasal cells with some expression in the basal layer, which was more extensive for K13 than K4. Protein expression paralleled that of mRNA. In moderate dysplasia, there was increased expression of K4 and K13 mRNAs and proteins in suprabasal cells but reduced expression in basal cells. In severe dysplasia expression of K4 and K13 mRNAs and proteins was either reduced or abolished. Expression of keratin mRNAs and proteins in carcinomas varied greatly, poorly differentiated SCCs expressing minimal K4 and K13 protein in one third of cases and moderately to well differentiated SCCs expressing high levels of keratin mRNAs and proteins, thus conserving near normal expression.

These results indicate that, in general, expression of K4 and K13 is regulated transcriptionally, although individual cases of dysplasia and carcinoma show evidence of post-transcriptional regulation.

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P SLOAN*, N S THAKKER, E D THAKER and H TURNER (Departments of Oral Pathology and Medical Genetics, University of Manchester, UK): Collagen type VI in infantile systemic hyalinosis.

Infantile systemic hyalinosis is a rare, fatal, autosomal recessive disorder associated with widespread deposition of hyaline material in connective tissues. Previous reports have implicated a defect in collagen type VI in its pathogenesis. The aim of the study was to characterise the hyaline material. Gingival tissues from an affected four year old (for clinical details see Devlin *et al*, *J. Oral Pathol. Med.* in press) were snap frozen and examined by immunofluorescence or processed for electron microscopy with immunogold labelling. Polyclonal antibodies raised against the $\alpha 1$ and $\alpha 2$ chains of collagen type VI were used. In the light microscope there was intense labelling of the gingival connective tissue with both antibodies, most marked in the subepithelial zone. In the transmission electron microscope the abnormal matrix appeared as ordered arrays of fine filaments with banding at 100nm intervals. Immunogold labelling for collagen type VI was related to the filaments and to cytoplasmic vacuoles in fibroblasts.

The findings suggest that infantile systemic hyalinosis may be due to accumulation of collagen type VI. The genetic defect may relate to molecules regulating its assembly or degradation.

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D P LANDES* and A J CLAYTON-SMITH (Leeds Dental Institute and Fosse Health Trust): The value of pre-general anaesthetic assessment for patients undergoing dental extractions.

This study was undertaken to investigate the value of seeing patients referred from general dental practice to the community dental service at a separate appointment prior to any administration of a general anaesthetic for dental extractions. A sample of record cards of patients seen at assessment appointments was drawn from a twelve month period, 593 patients record cards were used. 498 patients were referred for reason of dental decay, 21 for decay and orthodontic reasons, 73 purely for orthodontic reasons and of those 31 were for deciduous extractions only. 17% of cases required some further investigations before an anaesthetic could be given, 264 cases were undertaken as requested by their GDP, 208 cases had altered treatment plans, 49 cases had extractions performed with local anaesthesia and 41 cases were discharged without any treatment. Of those prescribed an anaesthetic, less than 5% failed to have the anaesthetic.

The pre-general anaesthetic assessment appointments reduced the need for general anaesthesia for 15% of all referrals and reduced the number of failures for patients prescribed an anaesthetic, from 11% to 5%.

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K. RIJAL*, K. MILSOM and M. A. LENNON (Department of Clinical Dental Sciences, School of Dentistry, The University of Liverpool, UK): Dental caries experience of 12-year-old children in Nepal in 1994.

This survey of 12-year-old school children followed the WHO pathfinder methodology and used examination criteria from the 4th Edition of the WHO Basic Oral Health Surveys (In press). Twelve schools were selected at random; four in the capital Kathmandu, and two in each of two large towns selected at random from the ten largest towns in Nepal; a further four schools were selected from four randomly selected villages, with at least one village located in each of the three major geographical regions. The schools were selected so as to include two private schools in Kathmandu and one private school in each of the two other large towns. At each school, thirty children (plus five substitutes) were selected at random. Drinking water samples were obtained from all appropriate sources for subsequent fluoride analysis. Three hundred and sixty children were examined. The DMFT for all children was 0.91. Generally, DMFT was slightly higher in Government schools than in private schools. (0.95 v 0.74), in girls than boys (1.01 v 0.83) and in villages than in towns (1.03 v 0.84). Fluoride levels were generally low except in the town Birnagar where it was 0.6 mg F/L.

It was concluded that DMFT in 12-year-old school children in Nepal in 1994 was very low.

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E. PAVI*, E. J. KAY*, K. W. STEPHEN*, A. HAYWOOD*, and D. EADIE* (Dept. of Adult Dent. Care, Univ. of Glasgow*, Acad. Group of Oral Med., Univ. of Manchester*, ARU, Univ. of Strathclyde*): Area deprivation and adult oral health in Glasgow.

This study investigates the effect of social environment on dental and periodontal health status and treatment needs, among adult populations.

Of a random sample of 852 16- to 65-year-olds residing in affluent and in deprived areas, 512 (60.1%) were examined clinically. The SPEED data collection system was used, supplemented by a CPTN measurement. The analysis examined the differences in dental and periodontal health, and in treatment costs (expressed in Resource Related Index units) (Hill F J, Thesis, Univ. of Manchester, 1974), between 'affluent' and 'deprived' respondents.

The dentallessness rate was 2.8% among the 'affluent' and 22.7% among the 'deprived' ($p < 0.001$). While no significant differences could be detected in the mean DMFT score, deprived populations had significantly higher ($p < 0.001$) numbers of decayed and missing teeth than their affluent counterparts. In contrast, the affluent had significantly higher ($p < 0.001$) numbers of filled teeth. Of the 'affluent', 6.8% were periodontally healthy, while of the 'deprived' only 1.8% exhibited no signs of periodontal disease ($p < 0.05$). However, no difference between the two groups could be detected for advanced periodontal disease. The mean cost of treatment required to render individuals dentally fit amounted to 37.4 RRI units for the 'affluent' and 55.0 RRI units for the 'deprived' ($p < 0.001$).

The results confirm that social deprivation relates to higher levels of dental and periodontal disease. Area deprivation analyses can identify population groups and areas of greater treatment needs. Resources for oral health promotion and disease prevention should be directed in these areas.

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H. E. FYFFE* and C. DEERY (Dental Health Services Research Unit, University of Dundee, UK): Developmental defects of enamel in regularly attending adolescents in Scotland.

The aim of this investigation was to assess the prevalence of developmental defects of enamel in a group of regularly attending adolescent dental patients in Scotland. The study sample consisted of 485 subjects (52% female, 48% male), with mean age 13.5 years. The SCOTS Index (Pitts NB, Stephen KW, J Dent Res 70: 683; 1991) was used to assess the prevalence and type of developmental enamel defects. The SCOTS assessment involves a clinical examination to determine the prevalence of defects and a subject component to determine patient perception. Overall 68 subjects (14%) claimed to have some 'marks on their front teeth which would not brush off'. There was no difference between males and females in the reported level of marks on teeth. Clinical examination showed 114 females (48%) and 112 males (50%) had defects as measured by the SCOTS Index. In 62% of subjects with defects these were symmetrical and in 74% defects affected at least one entire tooth surface. The majority of the defects were either 'demarcated' (47%) or 'diffuse' (46%). Of the 68 subjects reporting marks on their teeth 75% had SCOTS defects whilst 44% of the subjects claiming to have no marks had defects as measured by the SCOTS Index.

Developmental defects of enamel affected approximately half of this sample of regularly attending adolescents; yet few (14%) seemed aware of the defects.

Supported by the Scottish Office Home and Health Department

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P. J. HOLLOWAY*, M. ASHTON and H. V. WORTHINGTON (Dental Health Unit, University of Manchester, UK): Preventive technologies in general dental practice.

Previous work (Holloway P J and Clarkson J, Int Dent J 44: 317-322, 1994) investigated the views of general dental practitioners on the most cost-effective preventive technologies in general practice. This study sought to confirm these opinions by direct observation in a selection of established general dental practices. One of the authors (M A) observed working sessions at 22 group and four single-handed practices, recording on schematised data sheets the preventive procedures carried out on 94 children and 68 adults, 134 of whom were asymptomatic attenders. Ten sessions were spent with dentists, eight with hygienists, three with qualified dental health educators and five with a combination of these. Oral hygiene instruction was received by 32 children and 32 adults taking a modal time of 2-3 minutes (median=4.0). Dietary advice was given to 64 children and 15 adults, with a modal time of 2 minutes (median=2.5). Fifteen patients received fissure sealants, fluoride supplements were prescribed for 17 patients and five received applications of topical fluoride.

It is concluded that many dental practitioners utilise preventive technologies at a superficial level only.

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G. C. BAILEY*, S. CUNNINGTON*, P. BARBER and D. J. SETCHELL (Department of Conservative Dentistry, Eastman Dental Institute, London, UK): Ultrasonic condensation of gutta-percha: Quality of root canal filling.

The aim of this study was to compare the homogeneity of ultrasonically condensed (Enac OE-3 Ultra Endo Device) and cold laterally condensed gutta-percha root canal fillings. A single tooth model was designed to enable repeated obturation of the root canal and to facilitate retrieval of intact root fillings. Three different ultrasonic power settings and activation times were compared with cold lateral condensation. The sectioned root fillings were subjected to image analysis (Quantimet 500) to compare the percentage area of voids present in the apical, mid-root and coronal levels. Results showed that at the higher power settings and longer activation times tested significantly smaller voids ($P = 0.05$) were observed than for cold lateral condensation at all levels of the root canal.

Under the conditions of this study it was concluded that ultrasonic condensation can produce more homogeneous root canal fillings than cold lateral condensation.

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K. GULABIVALA*, B. LONG (Department of Conservative Dentistry, Eastman Dental Institute, UK): Apical microleakage of lateral condensation*, Alpha-Seal*, Thermafil* and JS Quick-Fill*.

The aim of the study was to compare apical microleakage when root canals were obturated using one of 4 techniques. A comparison was also made between obturation of canals with Alpha-Seal (Alpha phase only) when prepared with hand files (I) and using McSpadden's Nickel Titanium automated files (II). 130 canals in extracted human teeth were prepared using a Step-down technique and sodium hypochlorite irrigation and divided into 5 groups of 23 leaving 15 as positive and negative controls. The groups were balanced by matching canal curvature and diameter after preparation. The canals were obturated in a standard way after coating them with Roth's sealer*. Manufacturers instructions were followed when appropriate. The roots were immersed in Indian ink for 3 days and rendered transparent by clearing to evaluate apical leakage.

The number of specimens with no leakage were as follows: lateral condensation-12, Thermafil-11, Quickfill-5, Alpha Seal I-15, Alpha Seal II-16. There were statistically significant differences between Alpha Seal and Quick-Fill ($p < 0.01$) and lateral condensation and Quick-Fill ($p < 0.05$).

*Kerr UK Ltd, Peterborough, UK.

*Niti Co, Chattanooga, USA.

*Thermafil Products Ltd, Tulsa, Oklahoma, USA.

*Opident Ltd, West Yorkshire, UK.

*Roth's International Ltd, Chicago, USA

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P. J. LUMLEY*, AD WALMSLEY, PM, MARQUIS and L. BLUNT. (School of Dentistry, The University of Birmingham, UK): Cutting ability of sonic files.

The aim of this study was to investigate the cutting ability of files powered by the MM 1500¹ handpiece. Three widths of file (15, 25, 35) of the Heliosonic, Rispisonic and Shaper files were evaluated at three different loads of 25, 50 and 100 grams and three power settings (1/2 3/4, full opening of the air inlet ring). A 3rd full factorial analysis with two replications was performed making a total of 162 experimental units. The substrate used was 1 mm thick sections of bovine bone and the linear speed of the files was set at 2mm/sec. Instrumentation time was for 10 seconds.

Analysis of the data revealed all the variables to be significant (ANOVA, $n=54$, $p < 0.01$). Load was the most significant variable to affect cutting followed by file type, power and file width. There was no significant difference between the Rispisonic or Shaper files (Two sample t test, $n=54$, $p > 0.05$) or 3/4 or full power setting (Two sample t test, $n=54$, $p > 0.05$).

In conclusion load was the most significant variable to affect cutting and operators need to be aware of this when using the instrument clinically.

Micro Mega, Prodonta, Geneva, Switzerland.

113 R DEVALI, M WAPLINGTON*, P J LUMLEY and L BLUNT (Schools of Dentistry and Mechanical Engineering, The University of Birmingham, UK): Factors affecting the cutting ability of sonic retro tips.

Sonic retro tips can now be used to prepare class I cavities in the root ends of apicoect teeth. The aim of this study was to investigate the cutting ability of retro tips powered by a sonic handpiece. The following variables were evaluated: a) power setting with the air inlet valve half or fully open, b) loading of 25 or 50 g, c) length of tip at 2 or 3 mm, d) orientation of tip, perpendicular or parallel to the substrate surface, e) operator or non operator assisted movement f) tip size of ISO 35 or 55. The substrate used was 1 mm thick sections of bovine bone held in a load cell. Cutting was performed for a standard 10 second period and water irrigation was used in all cases. A 2nd full factorial analysis was performed with two replications making a total of 128 experimental units. The resultant depth of cut was measured using a stereomicroscope¹ at x16 magnification. Analysis of the data indicated that all variables had a significant effect on cutting (ANOVA $n=64$, $p<0.05$). The most significant factor was operator assisted movement, followed by power, length, tip orientation, width and load. An increase in loading resulted in tip constraint and a reduction of cutting ability. Little cutting was noted without operator assisted movement. In conclusion, sonically activated retro tips were found to cut satisfactorily providing operator assisted movement was used.

¹Micro Mega 1500, Prodontia, Geneva, Switzerland.

²Wild M3C, Wild Heerbrugg Ltd., 9435 Heerbrugg, Switzerland.

114 A D WALMSLEY*, P J LUMLEY, W T JOHNSON and R E WALTON (School of Dentistry, Univ. of Birmingham & College of Dentistry, Univ. of Iowa, USA): The breakage of ultrasonic root end preparation tips.

Modified ultrasonic tips can be used to prepare retrograde cavities following removal of the root apex during endodontic surgery. The tips may include single or double bends of 45° to 90° and may be of different thicknesses. Such designs places strain on the tips during oscillation. The purpose of this study was to determine whether the design of the ultrasonic retro tip (URT) would be susceptible to breakage under clinical use. Ten different URT designs were driven by an ultrasonic unit at full setting. These designs were grouped as follows: four-apical preparation; three-isthmus preparation; two-removal of obstructions and one-amalgam condensation. A tooth was instrumented, the apex removed and placed horizontally on one arm of a pan balance. The apex was instrumented by each URT for 15 minutes duration. Loading was placed on the opposite arm of the balance being increased by 100g every 5 mins. Two URTs and the amalgam condenser which had curvatures of 70° or greater fractured within the first five minute loading period. Breakage occurred 2 to 3 mm from the end and always at a bend. SEM of fractured surfaces indicated brittleness with little change in the original shape of the remaining section of the URT. Two tips bent; one isthmus preparation tip and one silver point removal tip. This occurred at 200g or above and after at least 7.5 mins usage. All other URTs were deemed as satisfactory after completion of the experiment. It was not possible to involve greater numbers due to the cost of the retro-tips. Fracture of URT can occur and is related to the degree of bending and thickness. Fracture of a tip may lead to clinical problems especially identification of the small fractured part in order to remove it from the surgical site.

115 JM WHITWORTH*, AK KHAN (Restorative Dentistry, University of Newcastle upon Tyne): Gallium Alloy GF, *in vitro* evaluation as a mercury-free alternative to amalgam in endodontic surgery.

The apical marginal seal of amalgam (A) and Gallium Alloy GF (G)¹ was evaluated *in vitro* in sheep incisors. Following ultrasonic debridement and orthograde obturation with gutta percha and sealer², root and cavities were prepared and filled with A ($n=50$) or G ($n=50$). Twenty five teeth from each group plus controls were subjected to immediate dye leakage assessment under vacuum conditions with methylene blue dye (2%, pH 7). The other 25 teeth in each group were incubated for 12 weeks in Ringer's solution before assessment. Linear dye penetration was measured following longitudinal splitting. Mean dye penetration was: A 5.17mm (2.78) at baseline; 2.33mm (2.87) after 12 weeks. G 2.21mm (1.98) at baseline; 1.41mm (1.02) after 12 weeks. Two way ANOVA showed that the seal of both materials improved following incubation ($P<0.001$). G was significantly better than A at baseline and after 12 weeks incubation ($P<0.001$). Subjectively, G was more difficult to manipulate than A. Gallium Alloy GF performed significantly better than amalgam in the current *in vitro* leakage study. This material is worthy of further investigation as a mercury-free alternative to amalgam in endodontic surgery. Sheep incisors provide a useful source of uniform mammalian teeth for use in certain endodontic dye leakage studies.

¹Sybraloy, Kerr. ²Tokuriki Honten, Japan. ³Tubliaseal, Kerr.

116 J A HOBKIRK (Eastman Dental Institute, London, UK): Dental implants: Do we need any more research?

Between 1986 and 1991 the annual number of papers on dental implants in Medline increased from 5 to 390; since then apparent activity has fluctuated but remained at similar levels. The original discoveries by Brånemark preceded this period, more recent work has yielded less significant findings and the high and predictable success of modern clinical techniques might suggest no need for further research. There are however gaps in our knowledge. The performance of implant stabilised prostheses has been well studied, the cost benefits of providing them have received less attention. Osseointegration remains the goal of dental and increasingly orthopaedic implantation, yet alternative types of host-implant interaction may be more beneficial, especially if they are associated with controlled cellular differentiation and tissue formation. A wide range of mechanisms for initiating and maintaining these processes has been proposed, which will depend on more sophisticated designs and materials. Implants are also increasingly providing a test bed for biomechanical studies involving bone deformation and transducer development. Further research in this field is justified on clinical grounds. The great potential of dental implants to provide models both for investigating basic cellular processes and also for applications in other areas such as orthopaedics must also be recognized. Continued activity will extend our knowledge; further discoveries are by nature unpredictable, they will increasingly fall to the comprehensively trained, gifted and well founded research worker.

117 LEUNG T*, HOBKIRK JA, THOROGOOD P (Prosthodontics Dept., Eastman Dental Institute and Developmental Biology Unit, Institute of Child Health, U. of London): A new organ culture model for studying bone-biomaterial interactions.

In vitro culturing of isolated bone cells on many biomaterials has been used to study bone responses to these substrata. It is not certain whether mineralized nodules formed under specific culture conditions exhibit all the characteristics of osteogenesis *in vivo*. *In vitro* organ culture models for bone tissue often show necrosis, reduced growth and mineralization due to the absence of vascularization. The objective of the present study was to develop a new organ culture model for studying bone-biomaterial interactions. Bone tissues were grafted onto the chorioallantoic membrane (CAM) of chick eggs incubated for between 8 and 10 days and then cultured for a maximum of 10 days on the CAM. Bone vitality and growth were maintained, thus providing a test bed for the study of bone-biomaterial interactions. Using microdissection techniques, commercially pure titanium and a glass ceramic material, Apoceram, were inserted into chick bone tissues prior to their grafting onto the CAM. Recovery rates of around 80% was achieved. Histologic findings suggest that the CAM graft model provides a rapid and inexpensive technique for assessing early bone reactions to implant materials.

118 D T. HUGHES WASSELL* and G. EMBERY (Dept. of Basic Dental Science, Cardiff Dental School, UK): Adsorption of bovine serum albumin to titanium powder.

It is generally accepted that one of the initial events in the interaction of implant materials with bone is the adsorption of plasma proteins to the surface. This study is important because it will provide an improved understanding of the bone-biomaterial interface. The adsorption of bovine serum albumin (BSA) to titanium powder (>99.5% purity) has been investigated using various solution conditions. Adsorption of BSA (0.01-1.0g L⁻¹) to titanium powder (0.4g) was carried out in 0.02M sodium acetate pH range 5.15-7.15 for 4hrs at 37°C. Solution protein concentration was measured using the Bio-rad assay. The influence of calcium ions on the adsorption process was investigated (calcium acetate 0.002M-0.2M). Desorption studies were carried out using 0.02M sodium acetate and 0.36M disodium hydrogen orthophosphate. An "overshoot" in the adsorbed amount of BSA was shown as a function of time and an inflection observed in the isotherm. These results are indicative of possible conformational changes in BSA. The maximum adsorption (Ad_{max}) at pH 6.8 was 1.13mg m⁻². The amount of BSA adsorbed increased 1) with decreasing pH, (at pH 5.15 Ad_{max} = 1.31mg m⁻²), 2) in the presence of calcium ions (at pH 6.8 for 0.002M Ad_{max} = 1.73mg m⁻²). BSA was found to be more readily desorbed in the presence of phosphate ions compared to buffer only, although some desorption occurred for the latter depending on protein concentration and pH. The results suggest that both electrostatic and hydration effects are important in the adsorption of BSA to titanium powder.

119 I Q BENINGTON*, P A BIAGIONI, P CROSSBY, P J LAMEY and S SHERIDAN (School of Clinical Dentistry, The Queen's University of Belfast, Northern Ireland): Electronic infrared thermography measurement of bone preparation for osseointegration.

Studies of the heat generated during implant site preparation have shown that a temperature of 47°C or greater may hinder osseointegration. Different techniques have been utilised in the quantification of temperature that develop in the preparation of bone. Electronic infrared thermography is in essence a non-invasive real-time scanning thermometer which allows the investigator to quantitate and visualise how heat generated is emitted from the bone surface. Electronic infrared thermography was previously used to assess the temperature changes overlying the drill channel as viewed from above. To try and refine this, thermographic measurements were recorded perpendicular to the drilling channel, over a sectioned surface 0.5 mm from the outer edge of the final channel wall. This allowed for quantification around the cutting edge of the bur during the drilling sequence. Bovine mandibular bone was used to provide cortical bone of similar quality to human. A conventional handpiece was used with a Nobelpharma¹ motor. One operator conducted all drilling procedures and manufacturer's specifications were followed but no irrigation was employed. An Agema thermovision 900 system² was used to measure the temperature generated whilst a round bur, 2 mm twist drill and a 3 mm pilot drill were in operation.

Results ($n=10$ drill sequences). The mean maximum temperature rises from baseline were: round bur 17.8° ± 8.9°C, 2 mm twist drill 70.0° ± 20.5°C and 3 mm pilot drill 82° ± 6.5°C

Conclusion: Large temperature rises were observed along the drill channel with the highest being recorded around the drill cutting edge.

¹ (Nobelpharma, Uxbridge, England)

² (Agema, Danderyd, Sweden)

120 D CHESHIRE*, N J MORDAN, P M BARBER, and M WILSON† (Prosth. Dept*, E M Unit, Microbiol. Dept†, Eastman Dental Institute, London, UK): Ultrastructural features of the material surrounding percutaneous Brånemark implants.

Percutaneous osseointegrated titanium implants (POTI) are increasingly being used to retain facial prostheses. Clinical observation of these patients indicates a variable skin response. The aim of this study was to characterise the material which forms the superficial exudate around POTI. Two clinically healthy sites and three clinically inflamed sites from 2 patients were examined. After careful removal, specimens were fixed in 3% glutaraldehyde in 0.1M cacodylate buffer and post-fixed in 1% buffered osmium tetroxide. They were then dehydrated and embedded in Araldite. 1µm sections were cut for orientation and ultrathin sections were cut, stained and viewed on a JEOL 100CX TEM. Paper points were taken from the same sites and cultured on blood agar plates both aerobically and anaerobically. Colonies on the plates were identified by standard methods. Specimens from both healthy and inflamed sites showed the same ultrastructural features. There was a general lack of structural organisation with keratinised epithelial cells, inflammatory cells and erythrocytes present in various stages of degeneration. Connective tissue and epithelium were observed in the exudate from 2 sites. Gram-positive organisms were present and a yeast-like structure was often found associated with the keratinised epithelial cells. Only *Staphylococcus epidermidis* was cultured from the paper points. These results indicate that there is a degree of inflammation associated with clinically healthy POTI. They also suggest that *S. epidermidis* and an as yet unidentified organism are present in the superficial exudate from POTI.

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RM PALMER, BJ SMITH, PJ PALMER*, PD FLOYD (UMDS Guy's Hospital London); ASTRA single tooth implants: results after 1 year.

A new design of single tooth implant (AstraTech, Molndal Sweden) featuring a microthreaded conical neck and Tio-blasted (tm) surface was evaluated clinically and radiographically after 1 year in function. 15 patients (age range 16 to 48) with missing maxillary anterior teeth (6 central incisors, 8 laterals, 1 bicuspid) had four 13mm and eleven 15mm implants placed under local anaesthesia and left for a period of 6 months before exposure and abutment connection (stage 2)/crown fabrication. All patients were seen at 2 to 3 monthly intervals for hygienist maintenance. Radiographs using Rinn holders and a long cone technique were taken at the crown insertion and 1 year later. The radiographic data is based on 13 cases as 2 patients were unavailable at 1 year.

All implants were successfully integrated at stage 2. The internal conical seal design of the abutment/implant interface facilitated connection and no subsequent soft tissue problems were observed. At crown insertion the mean bone level was 0.30 mm (95% C.I. -0.16, 0.75) apical to the top of the implant and at 1 year it was 0.40mm (95% C.I. 0.08, 0.71). T tests for paired samples did not show a significant difference (95% C.I. -0.29, 0.48). Three cases accounted for most changes in bone levels with apical loss of 0.75mm, 0.9mm and 1.35mm.

In conclusion, this study of single tooth Astra implants were highly successful and bone changes within the first year of function were comparable with other systems reporting high long term success rates.

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R.M. BROWNE*, W. McKENNA and R.L. SAMMONS [Units of Oral Pathology and Biomaterials, The School of Dentistry, University of Birmingham, U.K.]: In vitro cytotoxicity of some root-canal filling materials.

Although there have been several previous studies of the in vitro cytotoxicity of root canal filling materials, their effects upon osteoblasts have not been studied. The aim of this study was to investigate the cytotoxicity of Apexit¹, AH26², Endamethasone³ and Grossman's Sealer on osteoblasts in suspension cultures and on rat calvaria. Negative and positive control specimens were also studied. Standard sized samples were prepared and tested after 1, 7 and 14 days setting. Osteoblasts were obtained from three day old rat calvaria and cultured in Fitton Jackson's modification of Bigger's medium. Samples were placed in cell suspensions of approximately 2×10^5 cell/ml and examined after 24, 48 and 72 h. The proportion of spread (living) and rounded (dead) cells in four areas equidistant from the specimen centre was measured. Only Apexit permitted the growth of spread cells, being no different from the negative control. The duration of set did not affect the cytotoxicity. Samples of Apexit were also tested in calvarial cultures according to the method of Matsuda & Davies, 1987, over 14 days and cell growth assessed by scanning electron microscopy. Bone cells in contact with the material exhibited toxic changes even after only 3 days. These results show that all the materials except Apexit are cytotoxic to osteoblasts in suspension cultures. Apexit is also cytotoxic in calvarial cultures.

¹Ivoclar-Vivadent; ²DeTrey Dentsply; ³Septodont

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R V CURTIS*, D BROWN, A S JUSZCZYK, J D WALTER (Departments of Dental Materials Science & Prosthetic Dentistry, UMDS, London, UK): Titanium alloy dental implant superstructures with passive fit.

Current technology is not able to guarantee passive fit for dental implant superstructures produced using the lost wax investment casting technique despite meticulous attention to detail (Gold G. J. *Prosth Dent* 66: 377-384, 1991). A novel metal forming technique known as superplastic forming has been used as an alternative to the lost wax investment casting process to fabricate dental implant superstructures in titanium alloy using ceramic dies. The use of a ceramic die in itself is novel because the technique which is currently used in the aerospace industry in general uses metal dies. These ceramic dies are durable enough to withstand at least four consecutive pressings. The titanium alloy beam formed in this way is then cut from the metal sheet using conventional dental cutting tools and is polished on the under surface. The fit of these titanium alloy beams to Branemark implant abutments has been quantified and is found to be excellent. The process that has been developed enables strong, light-weight beams to be produced for fixed bridge cases.

Titanium alloy superstructures for fixed bridge cases have been produced using a metal forming process known as superplastic forming. The fit of these superstructures to Branemark implant abutments was quantified and found to be passive.

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O.A. ABU-HAMMAD*, A. HARRISON and A.C. WATKINSON (Restorative Dentistry, Dental School, Bristol, U.K.): A three dimensional finite element study of compressive stresses around cylindrical and vent cylindrical implants.

This study examined differences in compressive stress values generated around two different dental implant designs (cylindrical and vent cylindrical) using the three dimensional finite element analysis software program Display III¹. The implants' dimensions were those of the ITI² system. Each of the two implant designs was installed in an identical bone structure. To avoid the creation of distorted elements, the bone cores inside the implants were made cylindrical (i.e. hollow in the middle). Cortical bone properties ($E=13700$ Mpa, $\nu=0.3$) were allocated to the upper and lower layers of the bone geometry along with a 1 mm thick interface zone around the implant. The rest of the bone was allocated cancellous bone properties ($E=1370$ Mpa, $\nu=0.3$). The implant was allocated the properties of titanium ($E=103400$ Mpa, $\nu=0.35$). The outer nodes of the lower cortical plate were restrained and the implant was loaded by vertical and horizontal single forces of 100N magnitude. Third principal stresses were examined around both implant designs in the crestal bone region at varying distances from the implant surface.

The results showed that values of the third principal stresses around the neck region of the vent cylindrical implant were slightly higher than their values around the cylindrical implant. Nevertheless the stress picture at the cervical region of both implants was similar.

¹ Engineering Mechanics Research Corporation, P.O. Box 698, Troy, Michigan, USA.
² Institut Straumann A.G. Waldenburg, Switzerland.

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H.CHANA, R. IBBETSON*, G. PEARSON and A. EDER (Department of Conservative Dentistry, Eastman Dental Institute, UK): The Influence of Film Thickness on the Tensile Bond Strength of Two Resin Cements.

Eleven paired cast nickel-chrome cylinders were cemented with 2 different resin luting cements¹ at film thicknesses between 20 and 310 micrometres; these were controlled by means of a cementation jig. Specimens were stored in water at 37°C for 24 hrs prior to testing to failure in a universal load testing machine². The results in MPa (S.D.) for Panavia Ex and Panavia 21 Ex respectively at 20 µm were 48.8 (7.68) and 57.2 (7.39); at 50 µm, 47.3 (6.84) and 55.2 (11.13); at 100 µm, 38.2 (5.63) and 58.4 (10.32); at 150 µm, 22.5 (4.87) and 44.9 (7.32); at 210 µm, 14.2 (2.22) and 45.2 (6.45); at 250 µm, 11.7 (1.98) and 38.4 (7.84) and at 310 µm were 10.2 (1.74) and 23.7 (3.75). Tensile bond strengths were greater at lower film thicknesses whilst Panavia 21 Ex produced greater bond strengths than Panavia Ex at all film thicknesses tested. The Mann-Whitney Test showed the differences between the cements to be statistically significant at all film thicknesses ($P < 0.01$). These results may have implications when data from different in-vitro studies of tensile bond strength are compared.

It is concluded that an increase in film thickness resulted in a decrease in bond strength for both resin cements and that Panavia 21 Ex had a greater bond strength than Panavia Ex at all film thicknesses evaluated.

¹Panavia Ex and Panavia 21: Kuraray Co Ltd, Japan. ²Hounsfield Testing Equipment, Surrey.

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RJ COOK and TF WATSON* (Dept of Conservative Dentistry, UMDS, Guy's Hospital, London): Bur Blade Concentricity and High-Speed Tooth Cutting Interactions: a Confocal Microscopic Study.

Error in bur concentricity may arise from mal-alignment of the steel shaft and carbide head in a two piece construction bar. Cutting flutes rotate at multiple radii from the shaft axis, potentially producing vibrations and damage to the cut substrate. Techniques now allow the manufacture of one piece tungsten carbide burs with adequate strength to withstand lateral loading. A comparison of tungsten carbide dental cutting tools, revealed the true extent of concentricity errors. Variation in alignment of the cutting head and shaft in the two part constructions incurred between 20 - 50 µm of additional axial error. High-speed cutting interactions with dental enamel between carbide burs were studied using a video-rate confocal microscope. A cutting stage fitted to a Tandem Scanning Microscope, allowed real time dynamic image acquisition. Images were captured and retrieved using a low light level camera recording directly to S-VHS video tape. Video tape showing the interactions of high speed rotary cutting instruments (at 120,000 rpm) were taken under simulated normal wet cutting environments, and the consequent damage to the tooth tissue was observed as it occurred. Concentrically engineered bur types produced a superior quality cut surface at the entry, exit and advancing front aspects of a cavity as well as less sub-surface cracking. Imaging of the coolant water film local to recent cutting operations, showed regular spherical cutting debris of 6-18 µm diameter from the concentric tools, whereas the less well engineered burs produced ragged, irregular swarf, with 25-40 µm diameter debris, indicating far more aggressive cutting actions. This study has shown that there is reduced substrate damage with high concentricity carbide burs.

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PJ MULLAN* and WG WADE (Division of Oral Medicine, Pathology and Microbiology, Dental School, Bristol, UK): Antimicrobial and antidiatheric properties of agents with potential for use in plaque control.

The aim of this study was to screen potential active agents for plaque control for their antimicrobial and antidiatheric properties *in vitro*. The agents tested were alkyl polyglycosides (AP) 1200 and 2000, polyhexanide (P), polidocanol (PO), glycidil-I (G), hexamidine (H), sodium lauryl sulphate (SLS), triclosan (T) and chlorhexidine gluconate (CG). Antimicrobial activity against a panel of 20 bacteria representative of those found in oral health and disease was assessed by an agar diffusion method. The results were expressed as the Maximum Inhibitory Dilution (MID) of solutions at in-use concentration. The effect of the test agents on the adherence of *S. sanguis* NCTC 7863, *S. oralis* NCTC 7864, *S. gordonii* NCTC 7865 and fresh isolates of these species to hydroxyapatite was determined using radiolabelled bacteria and hydroxyapatite beads. T and SLS displayed the most antibacterial activity (MIDs 160-5120 and 80-1280 respectively). The remaining agents all displayed moderate activity. AP 1200 and 2000, PO, CG, G, H and SLS had no effect on the adherence of the streptococci. P had a variable effect; adherence ranged from 65-444% of the no-treatment control, depending on the species tested.

In conclusion, although in-vitro tests do not necessarily predict in-vivo efficacy, a number of compounds have been identified with potential for use as anti-plaque agents.

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J GREENMAN*, A ELWORTHY and M ADDY. (University of the West of England and Dental School, Bristol, UK): Effects of oral hygiene products on salivary bacteria.

The persistence of action, or substantivity, of antimicrobial agents in the mouth is a major variable influencing plaque inhibition and can be assessed by measuring the duration and magnitude of the suppression of salivary bacterial numbers. The aim of this study was to record the substantivity of oral hygiene products as a screening of efficacy. Nine formulations or products were chosen namely, 1 chlorhexidine rinse, 4 CPC rinses, 2 toothpastes with and without stannous fluoride, a C31G rinse (TheraSol), a minus CPC control rinse, with water as the placebo. The study was a blind 10 cell, randomised, crossover, single rinse design balanced for carryover and involving 20 volunteers. Mouthrinses were 15 ml volumes and toothpastes 3 gms in 10 ml slurries rinsed for 60 seconds. Salivary bacterial counts were obtained at baseline and at periods up to 7 hours following rinsing. All rinses except water and the minus CPC control produced significant falls in counts to 30 minutes. Compared to water, the chlorhexidine rinses were substantive to 7 hours and C31G to 60 minutes. The CPC rinses were more substantive than their control rinse to 3 hours. The stannous fluoride and control paste were similarly substantive to 5 hours with the stannous fluoride remaining substantive compared to water to 7 hours.

It is concluded that the variable substantivity of the different products will be reflected in different plaque inhibitory efficacy.

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L. HARTER*, N. CLAYDON, J. MORAN, W.G. WADE, M. ADDY (Dental Schools, Bristol and Cardiff, UK): Effects of 0.1% and 0.2% Delmopinol mouthwashes on plaque, gingivitis, calculus and staining.

Delmopinol has proved effective against plaque and gingivitis in short term studies. The aim of this study was to determine the adjunctive benefits and safety of Delmopinol rinses used alongside toothbrushing. The study was a 6 month home use placebo controlled, double blind, randomised, parallel design evaluating 0.1% and 0.2% Delmopinol rinses. A total of 450 dentate male and female subjects participated who had moderate levels of plaque and gingivitis. At baseline, 3 and 6 month subjects were scored for plaque, gingivitis, toothbrushing and calculus. A baseline prophylaxis was provided and subjects instructed to rinse twice daily for 60 seconds with 10ml volumes of the allocated rinses. Demographic features of three groups were similar and losses to trial small. Adverse reports included numbness of the tongue, tooth and tongue staining and merely mucosal soreness. There were no significant shifts in haematological or biochemical parameters measured at the beginning and end of the study. All groups showed marked improvement in oral hygiene and gingival health with differences in favour of the 0.2% rinse compared to placebo some of which reached significance for gingivitis and most for plaque. Staining was significantly increased in the Delmopinol groups but not calculus. It is concluded that 0.2% Delmopinol rinses has the potential to provide adjunctive benefits to oral hygiene and gingival health.

Study supported by Biosurface, Kabi Pharmacia, Sweden.

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A J ELWORTHY*, R EDGAR, J MORAN, M ADDY and W G WADE (Dental School, Bristol): A six-month home-use trial of 0.1% and 0.2% delmopinol mouthwashes. II Effects on the plaque microflora.

Delmopinol has been shown to be an effective antiplaque agent in short-term studies. In this six-month home-use study, the effects of 0.1% and 0.2% delmopinol mouthwashes on supragingival plaque flora were investigated. 147 subjects were studied, from whom plaque was collected at baseline, 12, 24 and 36 weeks. Samples were examined by microscopy and cultured for total counts and for specific bacterial groups including black-pigmented Prevotella and Porphyromonas species, *Fusobacterium nucleatum* and streptococci. There were no consistent effects on microscopic or total counts. However, there was a significant reduction in the proportion of dextran-producing streptococci in the active groups compared to the control group throughout treatment. There was no opportunistic colonisation by *Candida* species or Gram negative aerobic bacilli in the active groups nor was there any decrease in susceptibility to delmopinol.

Delmopinol does not appear to cause major shifts in bacterial populations with long-term use, although dextran-producing bacteria, which play a primary role in plaque formation, appear to be affected. This may have relevance to the mode of action of the agent.

This study was supported by Biosurface AB, Sweden.

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W CHANDRACHUD*, J BAGG*, D FELIX* (Departments of Oral Sciences and Oral Medicine, Glasgow Dental Hospital, UK): The effect of tetracycline mouthwash on the intra-oral candidal load and candidosis.

Tetracycline mouthwash may be used in the management of patients with recurrent aphthous stomatitis. The aim of the present study was to investigate the effect of this method of treatment on the intra-oral candidal load and subsequent candidal infection (Meads, Rowe and Haslam, *Archs Int Med* 1951; 87: 533-40). Sixty patients were enrolled. Each patient provided an oral rinse prior to treatment (Samaranayake *et al.*, *J Oral Pathol* 1986; 15: 386-388). A tetracycline mouthwash (250 mg qds for 1 month) was then prescribed and further oral rinses collected and processed at regular intervals. The rinses were cultured and quantified by standard methods for yeasts, coliforms, *Staphylococcus aureus* and oral streptococci and total bacterial counts were derived. Thirty-two patients completed the study. Fourteen had no *Candida* species isolated before or after treatment; nine non-carriers had a measurable candidal load following treatment and *Candida* species were isolated from nine patients both before and after treatment. Only one patient developed clinical oral candidosis.

It is concluded that candidal infection is not a common complication following usage of a tetracycline mouthwash in the treatment of recurrent aphthous stomatitis.

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S J BROOKES*, J KIRKHAM, R C SHORE, W A BONASS AND C ROBINSON (Division of Oral Biology, Leeds Dental Institute, University of Leeds, Leeds, UK): The mineral-binding properties of albumin during enamel development.

Both albumin and amelogenin are known to inhibit apatite crystal growth *in vitro* and are degraded during the transition stage of enamel development, presumably allowing crystal growth to occur. During secretion, however, the relationship between albumin, amelogenin and mineral is still unclear. This has been investigated using selective extraction procedures. Rat incisor enamel from each developmental stage was extracted in synthetic secretory stage enamel fluid (Aoba T & Moreno EC, *Calcif Tis Int* 41: 86-94, 1987). Insoluble residues were extracted with 0.1M phosphate buffer (pH 7.4). Extracted proteins were subjected to SDS PAGE and Western blotting employing polyclonal anti-rat albumin antibodies. Albumin was extracted by synthetic enamel fluid from the secretory and early transition stage enamel. However, albumin in late transition and maturation was only extracted with phosphate buffer, presumably by desorption from crystal surfaces. Prior extraction of secretory stage enamel with chaotropic agents rendered albumin insoluble (i.e. mineral bound) in synthetic enamel fluid. The data suggests that albumin is not bound to enamel crystals during secretion but becomes mineral bound during transition and maturation due to increased access to the crystal surfaces following *in vivo* matrix removal. Reports suggesting that albumin is mineral bound during secretion (Lineback H *et al.*, *Bone Min Res* 4: 235-241, 1989; Strawich E & Glimcher M J, *Eur J Biochem* 191: 47-56, 1990) may be misleading. These studies involved treatment of enamel with chaotropic agents which appear to induce artificial albumin binding due to removal of the amelogenins. Amelogenins may function to protect crystal surfaces from crystal growth inhibitors such as albumin during secretion.

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I L C CHAPPLE*, N TYLER, H D GLENWRIGHT AND J B MATTHEWS (School of Dentistry, The University of Birmingham, UK): Circular fluid alkaline phosphatase isoenzymes as markers of bone involvement in periodontitis.

Alkaline phosphatase (ALP) within GCF is a promising marker of periodontal disease activity (Page R, *J Periodontol* 63: 356-366, 1992), but the source of the enzyme is unknown. We have developed a highly sensitive chemiluminescent assay for GCF ALP (Chapple I L C *et al.*, *J Perio Res* 28: 266-273, 1993) and demonstrated local production of ALP within the gingival crevice and that GCF ALP levels will predict gingivitis before overt clinical signs of disease (Chapple I L C *et al.*, *Biomol & Chemilum* pp 281-285, 1993). The aim of this study was to investigate the major source of ALP found within GCF. The production of ALP by 30 putative periodontopathogens and commensals was investigated and their levanisole inhibition profiles compared with ALP in GCF and serum. Isoenzyme analysis of GCF ALP was performed by agarose gel electrophoresis (\pm neuraminidase) with liver, bone and PMNL ALP. Finally, since oestradiol is thought to modulate osteoblast activity (Liel Y, *et al.*, *Endocrinol* 30: 2579-2601), GCF and serum ALP and oestradiol levels were measured throughout the menstrual cycle in female subjects who did ($n=5$), and did not ($n=5$) take the oral contraceptive. Whereas bacterial ALP was generally not inhibited (mean <14% inhibition) by levanisole, GCF and other mammalian enzymes tested were inhibited by >79%. Electrophoretic profiles for GCF ALP produced bands consistent with the enzyme being derived from bone or polymorphonuclear leukocytes (PMNL's). Although significant decreases in serum ALP occurred with decreases in oestradiol-17 β ($p<0.003$), GCF ALP was unaffected ($p>0.3$). We conclude that GCF ALP is largely mammalian in origin, and that PMNL's or bone are the major source.

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D TENORIO* & F J HUGHES (Department of Periodontology, The London Hospital Medical College, UK): Cementoblasts express parathyroid hormone (PTH) receptors.

The cementoblast phenotype has not been fully defined but shares many of the features of the osteoblast phenotype. We have investigated the expression by cementoblasts of cell surface receptors for parathyroid hormone (PTH) by means of immunocytochemistry. For this (1) unlabelled PTH was bound to tissue sections and subsequently detected with anti-PTH monoclonal antibodies and biotinylated antibodies; (2) Digoxigenin (DIG)-labelled PTH was applied to the sections and the bound hormone detected with anti-DIG alkaline phosphatase conjugated antibodies. The mandibles of 25 to 30 day old Wistar rats were removed following cervical dislocation, fixed in 4% paraformaldehyde, decalcified in EDTA and processed for paraffin embedding. 4 μ m sections were rehydrated and subsequently incubated with human synthetic PTH (10ng/4 μ l - 0.1ng/4 μ l) or DIG-labelled PTH. The use of non-radioactive DIG-labelled PTH represents a novel approach for the immunodetection of PTH receptors *in situ*.

PTH binding sites were detected immunocytochemically in the cells of the alveolar bone and to a lesser degree those of cementum. Positive staining was particularly strong in the osteoblast lining bone surfaces and those located in the periodontal ligament surface of the alveolar bone. In cementum, positive reaction was detected in some cementoblasts of well developed roots. Cementoblasts associated with acellular cementum were negatively stained. In the developing root, newly differentiated cementoblasts were largely negatively stained. The binding of DIG-labelled PTH to sections of developing toothperms confirmed the results found with the immunolocalisation of unlabelled PTH binding sites. These results show that cementoblasts of functional erupted teeth express PTH receptors and may be responsive to PTH stimulation. The results further support the idea that cementoblasts and osteoblast share a similar phenotype.

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S MEGHJI*, SP NAIR*, M WILSON, Y SONG, HENDERSON B (Maxillofac Surg and Microbiology, Eastman Dental Institute UK): *Staphylococcus aureus* from patients with infected implants has potent osteolytic surface proteins.

We have shown that type strains of *Staphylococcus aureus* have easily solubilized surface proteins with potent osteolytic activity. The aim of this study was to determine if clinical isolates of this organism had similar proteins and to examine the patients antibody response. Pus was obtained from three patients with infected orthopaedic prostheses and one with infective arthritis. *S. aureus* was isolated and grown in bulk. Surface-associated material (SAM) was obtained by gentle extraction in saline, followed by dialysis against distilled water and lyophilization. Blood was obtained from each patient and sera prepared. Antibody levels to SAM in sera from the patients and healthy controls were determined by ELISA. Bone resorbing activity of the SAM and the neutralizing activity of the patients sera were tested by the murine calvarial bone resorption assay.

All the clinical isolates examined had SAM with bone resorbing activity although there was marked variation in the potency and efficacy. Both control sera and patients sera contained IgG antibodies to the SAM. When control or patients serum was included in the bone resorption assay two of the latter sera inhibited bone resorption while the controls were without effect.

Clinical isolates of *Staphylococcus aureus* contain SAM which has osteolytic activity. Patients with bone infections can produce a neutralizing antibody response. These findings may be of prognostic value.

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M. HARRIS*, S. MEGHJI, A. BOYDE*, N. SHAN*, M. ARORA* S. J. JONES*, AND B. HENDERSON (Maxillofac Surg, Eastman Dental Institute and *Anatomy and Develop. Biology, UCL, London): SAM from *S. aureus* activates isolated osteoclasts.

S. aureus is the commonest causative organism of acute osteomyelitis, septic arthritis and implant failure and is associated with localised bone breakdown. Surface associated material (SAM) from *Staphylococcus aureus* has been isolated and been shown to be a potent osteolytic agent in the murine calvarial bone resorption assay (Song *et al.*, *J. Dent Res* 72:689, 1993). It also stimulates the osteoclast formation in murine bone marrow cultures (Meghji *et al.*, *J. Dent Res* 72:729, 1993).

This study tested the possible direct stimulatory effect of SAM on osteoclasts *in vitro*. Osteoclasts were released mechanically from pre-hatch chick or from rat long bones. Rat osteoclasts were exposed to SAM and tested for stimulation of tartrate resistant acid phosphatase (TRAP) activity. Chick osteoclasts were cultured on sperm whale dentine slices for 18 hours with various dilutions of SAM. The slices were fixed and axial scan confocal mapping was used to measure the resorption pits. In both assays the SAM dose-dependently stimulated a significant increase in rat osteoclast TRAP activity and in the numbers, volumes and volume:area ratios of osteoclastic resorption pits.

S. aureus SAM may directly stimulate osteoclast activity.

137 S NAIR*, KIRBY A, MEGHJI S, *WILSON M, HENDERSON B. (Maxillofac Surg and *Microbiology Eastman Dental Institute, London): GroEL from *Actinobacillus actinomycetemcomitans* stimulates alveolar bone resorption.

We have established that surface-associated material (SAM) from *Actinobacillus actinomycetemcomitans* has potent osteolytic activity and that this activity is blocked by monoclonal antibodies P2 and P3 raised to the whole bacterium (Kirby AC, J Dent Res 73: 787, 1994).

We have now fractionated the SAM by various chromatographic techniques including antibody-affinity, anion exchange and ATP-Sepharose affinity chromatography and have isolated a 62kDa protein which contains the bone resorbing activity of the SAM. Amino acid N-terminal analysis has revealed that this protein has 95% homology with the molecular chaperone GroEL from *E. coli* and 65% homology to HSP65 of *Mycobacterium tuberculosis*. Purified GroEL from both *Actinobacillus actinomycetemcomitans* and *Escherichia coli* potently stimulated murine alveolar bone resorption. In contrast the mycobacterial GroEL (HSP65) from *M. tuberculosis* and *M. leprae* failed to promote bone resorption.

GroEL in *A. actinomycetemcomitans* is a surface-associated protein with potent osteolytic activity.

139 S T LOKE* and N PHUNT (Department of Orthodontics, Eastman Dental Institute and Hospital, London): Freeway space and EMG activity of masticatory muscles at different head positions.

The aim of this prospective quantitative electromyographic (EMG) study was to investigate the association between masticatory muscle activity and freeway space at altered head positions. Forty adult subjects (20 males and 20 females) with Class I skeletal relations and average facial proportions underwent simultaneous bipolar EMG and mandibular kinesiographic recordings using a Myotronics K6-1 diagnostic system. EMG activity was recorded from the anterior and posterior temporalis muscles, masseter and digastric at rest in the natural head position and at 10, 20 and 30 degrees head extension and flexion. Both the within visit and between visit reproducibility of the system was established. The results revealed that the freeway space was maintained at the different head positions. Both masseter and digastric activity increased at head extensions greater than 10 degrees from natural head position. Masseter muscle activity reduced during flexion, whilst digastric activity again showed a significant increase but only beyond 20 degrees. Both the anterior and posterior temporalis muscles showed no statistically significant change in EMG activity at the different head positions investigated.

It is concluded that the size of the freeway space is maintained at differing head positions and, of the masticatory muscles studied, masseter and digastric appear to be primarily responsible for the maintenance of freeway space.

*Myotronics Inc, Seattle, USA

141 W.P. BOCK* and M.S.B. ABDULLAH (School of Dentistry, University of Birmingham, UK): Effect of four variables on the shear bond strength of orthodontic brackets.

Orthodontic appliances must resist shearing forces up to 100N during mastication. This in vitro study measured the shear forces needed to dislodge etch retained orthodontic brackets from human premolars. Variables were adhesive type (autopolymerized* or light-cured*), cure time (30s or 60s), debond interval (15 min or 24 hrs) and bracket base design (mesh† or undercut‡). Following previous work (Abdullah MSB and Rock WP, Brit J Orthod, In Press) all specimens were etched for 30s. Bond strengths varied from 7.7 MPa to 25.3 MPa. Overall the two materials produced similar bond strengths. Light cured adhesive gave higher bond strengths after 24 hrs than 15 min, $p < 0.01$. Auto-polymerized adhesive gave lower bond strengths on undercut base brackets, $p < 0.01$.

The combination of variables that gave the weakest bonds was autopolymerized resin and undercut base brackets, tested after 15 min. The best combination was light cured resin cured for 60s and mesh base brackets, tested after 24 hr.

*Right On, TP Orthodontics. †Transbond, 3M Unitek. ‡DynaLock, 3M Unitek, *A Company, Orthologic.

143 F A KHAN and J PEDLAR* (Division of Dental Surgery, Leeds Dental Institute, UK): Generalised joint hypermobility as a factor in the clicking temporomandibular joint.

The aims of this study were to test the relationship between internal derangement of the temporomandibular joint (TMJ) (as measured with a standardised index) and generalised joint hypermobility in a British population.

26 patients (19 female) with clicking or locking of the TMJs and 28 age- and gender-matched controls were compared for generalised joint hypermobility using Beighton's scoring system (Beighton P, Solomon L, Soskolne CL, Ann Rheum Dis 32: 413-418, 1973). TMJ function was assessed with the Friction Craniomandibular index (Friction JR, Schiffman EL J Dent Res 65: 1359-1364, 1986).

There were significantly higher hypermobility scores in the TMJ patients (Mann Whitney U test, $p = 0.001$), but there was no apparent correlation between the severity of TMJ dysfunction and the hypermobility score within the patient group (Spearman rank correlation) and 10 of the 26 patients showed no sign of generalised joint hypermobility. Although numbers were small, dysfunction scores appeared significantly higher in female patients than males (Mann Whitney, $p < 0.05$).

This study supports the concept that generalised joint hypermobility is a factor in some but not all patients with TMJ internal derangement.

138 D R YOUNG*, N PRICE and J H NOAR (Department of Orthodontics, Eastman Dental Hospital and Institute, London and Mount Vernon Hospital, Middlesex): Plaque removal by chewing gum in fixed orthodontic patients

This pilot study investigated plaque removal effects of chewing sugar free gum in fixed orthodontic patients. 30 patients wearing fixed appliances¹ with rectangular stainless steel wires were chosen at random. Each patient was seen before a routine appointment and allocated to one of three equal size groups by random permuted blocks. Malocclusion and sex were not taken into account. Group 1 brushed with an Oral B toothbrush and MentoDent P-Sensitive toothpaste, Group 2 chewed one stick of Orbit(R)² chewing gum only and Group 3 chewed gum for ten minutes and brushed their teeth. All patients were disclosed at the start and their hygiene score recorded as the number of plaque free surfaces/number of surfaces measured x 100. Their individual cleaning regime was then carried out prior to re-disclosing and scoring. Single factor ANOVA statistics revealed no statistical differences between groups for age, length of treatment or starting hygiene scores. Brushing alone had a 38% improvement, chewing gum alone 26% and the brushing/chewing group 77% improvement in hygiene score. Paired two sample t-tests showed improvements to be highly significant for all groups. No damage to any appliances was found. Analysis was made to determine differences between the arches and individual tooth surfaces. It is concluded that chewing sugar free gum and toothbrushing together is significantly better at removing plaque deposits from fixed appliance patients than either toothbrushing or chewing gum alone.

¹ A-Company ² Wrigley's

140 G E READ-WARD*, S P JONES and E H DAVIES (Department of Orthodontics, Eastman Dental Institute and Hospital): Comparison of the static frictional resistance of self-ligating and stainless-steel ligated systems.

The static frictional resistance of 3 types of self-ligating bracket was compared with a conventionally ligated Ultratrim[®] bracket. A frictionless piston system which enabled bracket to archwire angulations of 0°, 5° and 10° was used. The effects of archwire size (0.020", 0.019" x 0.025", 0.021" x 0.025"), angulation (0°, 5°, 10°) and the presence of unstimulated human saliva, were investigated. Modified ligature locking pliers were used to standardise the ligation force for the Ultratrim[®] brackets. The study demonstrated that both increases in wire size and bracket/archwire angulation resulted in increased static frictional resistance, with the presence of saliva having a variable effect. In comparison of the individual bracket types:

Mobil-Lock Variable Slot[®] had the least friction for all wires for 0° angulation. With the introduction of angulation, values were comparable with the other brackets. Active[®] brackets had the second lowest frictional resistance, although high values were found with the 0.019" x 0.025" wires, possibly reflecting bracket/archwire binding. SPED[®] brackets demonstrated low forces with round wires, although with rectangular wires, or in the presence of angulation, friction was greatly increased. Ultratrim[®] brackets produced large individual variation in the friction, confirming the difficulty in standardising ligation force, although under certain conditions significantly larger frictional forces were observed with these brackets.

It was concluded that self-ligating brackets showed lower frictional resistance than stainless-steel ligated brackets, although there was great variation between the individual bracket types.

142 S ARICI and D R WILLMOT* (Dept of Clinical Dentistry, Sheffield, UK): Comparison of bond strengths of a new orthodontic bracket base with a conventional base.

Metal orthodontic bracket bases form a micro-mechanical interlock between the base and the adhesive. Many types of bracket base have been designed eg. foil mesh, milled, photoetched etc. Recently a manufacturer introduced a new base¹ which consists of macro retention structures and micro irradiated quartz particles. This study investigates the in vitro bond strength of the bracket in shear and tensile and compares it with a conventional foil mesh base². 40 brackets of each type were bonded to 80 extracted human premolars with various adhesives. Bond strength tests were carried out with a Lloyd Mark 5 testing machine with a crosshead speed of 2mm per min. In shear and tensile. Bracket base area was measured by computer image analysis and expressed in N/mm².

Beta brackets produced a mean shear bond strength of 11.8 N/mm² compared with 11.4 N/mm² for conventional brackets using the same adhesive³. Mean tensile bond strengths of 3.7 N/mm² were recorded for Beta brackets and 6.4 N/mm² conventional brackets ($p < 0.01$). The bond failure site was predominately at the bracket-adhesive interface, with the Beta group showing slightly more adhesive-enamel failures.

The new micro irradiated quartz particle orthodontic bracket base (Beta) in vitro produced similar shear bond strengths to conventional brackets, but lower tensile bond strengths. The results demonstrate bond strengths which are clinically acceptable.

¹ Beta bracket, Hudson Ltd. ² Dyna-Bond bracket, Unitek Ltd. ³ Eurobond, Hudson Ltd.

144 R J M GRAY*, S J DAVIES (Department of Dental Medicine & Surgery, University of Manchester, UK): Anterior repositioning splint usage in the management of internal derangement of the temporomandibular joint.

Temporomandibular disorders (TMDs) comprise a multitude of conditions which affect the articulator system. One of the most frequently reported complaints is the joint sound of clicking, indicative of an internal derangement involving the intra-articular disc (disc displacement with reduction). Over 50% of the population experiences this sign at some stage during life. The anterior repositioning splint (ARPS) is the splint of choice for treatment of a patient suffering from disc displacement with reduction. The pattern of usage of such an appliance has not been validated. The AIM OF THE STUDY was to determine whether or not such a splint was successful in the management of this condition and to investigate various patterns of usage. MATERIALS AND METHODS. 70 patients diagnosed as having disc displacement with reduction were entered on to the trial. The patients were randomly distributed into one of three groups. Each group wore the splint according to a previously determined prescription, either 24 hours a day, only during the daytime, or only at night. Reviews were regularly undertaken over a three month period. RESULTS. Of the 70 patients, 48 improved, 22 did not. The overall success rate was 68.6%. Group 1 patients wore the splint 24 hours a day; 22 of 25 (88%) improved. Group 2 patients wore the splint during the day; 13 of 25 (52%) improved. Group 3 patients wore the splint at night; 13 of 20 (65%) improved.

CONCLUSIONS. The results indicated that this splint is a successful conservative treatment for patients suffering from disc displacement with reduction. Significantly more patients improved in the group who wore the splint 24 hours a day when compared with the two other groups. The difference in improvement when comparing day with night wear was not statistically significant.

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S J DAVIES*, R J M GRAY (Department of Dental Medicine & Surgery, University of Manchester, UK): Investigation into the significance of the pattern of wear of a stabilisation splint in the treatment of patients with pain dysfunction syndrome.

Temporomandibular Disorders (TMD) are relatively common. Epidemiological data indicates that between 50 - 75% of the population exhibit some signs of a TMD, between 20 - 25% of the population have symptoms and 3 - 4% of the population seek treatment. Pain Dysfunction Syndrome (PDS) is a common TMD. Many different designs of occlusal appliances have been used in the treatment of this condition. The stabilisation splint (SS) is an established treatment for patients with PDS. The pattern of wear for this splint has not been satisfactorily validated. The aim of this study was twofold, first to test the hypothesis that the aforementioned splint was an appropriate treatment. Secondly, to examine whether or not there was any significance in the pattern of usage of this splint. 70 patients with pain dysfunction syndrome were examined. This population was divided into three treatment groups and wore the splint either 24 hours a day, just during the day or only at night. Patients were reviewed over a three month period. 84.3% of patients treated with splint improved.

CONCLUSION There was no statistically significant difference in improvement relative to the pattern of usage.

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A. JOHNSON, Department of Restorative Dentistry, University of Sheffield, UK. The effect of sprue design on the fit of three unit bridges.

The effect of three sprue designs on the marginal accuracy of three unit metal/ceramic bridges has been assessed. The three sprue designs were as follows: 1) Three straight sprues attached to the pattern and having a button of excess metal after casting, 2) As design 1 but with no button of excess metal linking the sprues after casting, 3) An indirect sprue design comprising two main sprues, a reservoir bar and three feeder sprues. Three metal/ceramic alloy categories were used (gold, palladium and Ni-Cr based alloys). Results showed that design 2 produced better marginal accuracy in all alloy categories, although this was only significant in the gold ($p=0.0078$ Student *t*-Test) and palladium ($p=0.018$ Student *t*-Test) alloy categories. It was seen that there was a significant increase in the accuracy of fit of the gold based alloy over the Ni-Cr based alloy ($p=0.013$ Student *t*-Test), and although the gold based alloy was showed more accuracy than the palladium based alloy no other comparison of combinations proved significant.

These findings lead to the conclusions that, sprue design 2 will improve the marginal fit of three unit metal/ceramic bridges, and that using a gold based metal/ceramic alloy will give better fit than the other two alloy categories used.

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J VERRAN¹, S KOSSE² and J F McCORD* (¹Biological Sciences, Manchester Metropolitan University and ²Prosthodontics, University Dental Hospital of Manchester, U.K.). A microbiological assessment of risk areas in dental technology laboratories.

The aim of this study was to investigate the microbiological status of certain risk areas in a dental technology laboratory.

Samples of duplicating agar, pumice slurry and of water in a water bath were placed in nutrient media which were used to select for staphylococci, yeast and total faecal flora and non-faecal flora bacteria. All plates were incubated at 30°C for 48 hours. No growth occurred on plates incubated with agar. Although plates incubated with freshly made slurry remained free from microbiological growth, it was found that after 3 days use, there was considerable contamination (10^6 cells) of pumice slurry.

It was concluded that laboratory managers should be constantly aware of potential cross-contamination hazards in dental laboratories.

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J SELLARS* & FJ HUGHES. (Dept. Periodontology, London Hospital Medical College). Effects of nifedipine and cyclosporin on fibroblast proliferation *in vitro*.

Nifedipine (Nf) and cyclosporin (Cs) are both known to cause gingival overgrowth in many patients receiving this medication, although the effects of their combined administration is less clear. The aims of this study were to investigate the effects of Nf and Cs on proliferation of gingival fibroblasts *in vitro*.

Eight different human oral fibroblast lines were isolated by explant cultures of tissue removed during periodontal surgery or mucosal biopsy. Cells were stimulated with Nf or Cs for 24 hours both in the presence or absence of foetal calf serum and proliferation was estimated by measuring DNA synthesis by assay of uptake of the thymidine analogue bromodeoxyuridine (BrdU). Uptake was quantified using a commercially available ELISA kit (Boehringer Mannheim) and results quantified by spectrophotometer. The cell lines showed marked variations in basal proliferation rate in unstimulated cultures. The lines tested did not show any response to either drug in the absence of serum. However in the presence of serum 2 of the lines showed marked increases in proliferation in response to both Nf and Cs. EG. Control (mean optical density \pm SD) 0.685 ± 0.122 ; 10⁻⁶M Cs 1.512 ± 0.425 ; 10⁻⁶M Nf 1.878 ± 0.500 . Combinations of Nf and Cs did not show additive effects. There was inhibition of proliferation in one of the lines tested, and 5 of the lines showed no significant response to either drug. The results demonstrate wide variations in responses to Nf and Cs between different cell lines, which supports the proposed presence of "responding" and "non-responding" sub-populations of fibroblasts to drug stimulation. The absence of effects in serum free conditions suggest that these drugs do not directly stimulate proliferation but act by modulation of other factors present in serum. **We conclude that the variations in responses seen may partly explain differences in susceptibility to Nf and Cs-induced gingival overgrowth.**

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I SHAHEEN* & FJ HUGHES. (Dept. of Periodontology, London Hospital Medical College). Effects of BMP-2 on osteocalcin expression in periodontal ligament cells.

Periodontal ligament (PDL) cells are thought to contain osteoblast and cementoblast progenitors in addition to fibroblastic cells. In view of this, bone morphogenetic proteins (BMP) may be able to promote PDL cell differentiation both *in vitro* and during periodontal regeneration *in vivo*. The aim of the study here was to investigate the effects of recombinant human BMP-2 on the expression of osteocalcin, a protein found specifically in bone and cementum matrix, in PDL cell cultures. Furthermore we have also tested the effects of dexamethasone, which is known to promote osteoblastic differentiation *in vitro*.

Cells were stimulated with dexamethasone and BMP-2 from 48 hours prior to confluence and sampled at time points up to 4 days after confluence. The percentage of cells expressing osteocalcin was determined by immunocytochemistry. The total osteocalcin synthesised was determined by radioimmunoassay of culture medium. The number of cells expressing osteocalcin increased after confluence in both test and controls cultures. (EG at confluence, control cultures $4.2 \pm 0.5\%$, 4 days after confluence $45 \pm 1\%$). In addition both BMP and dexamethasone increased cells expressing osteocalcin (EG at confluence, control cultures $4.2 \pm 0.5\%$, 100ng/ml BMP-2 $15 \pm 1.3\%$; 10⁻⁷M dex $12 \pm 2\%$). In addition the total osteocalcin in culture medium was stimulated by BMP-2 as determined by radioimmunoassay (EG, 4 days after confluence, controls 14.52 ± 1.82 ng/ml; 100ng/ml BMP-2 24.84 ± 3.4 ng/ml).

The expression of osteocalcin in PDL cultures is consistent with the presence of osteoblast and cementoblast progenitors. Furthermore BMP-2 and dexamethasone stimulation increased osteocalcin expression. **We conclude that PDL cells are responsive to BMP-2 stimulation and suggest a possible role for this factor in periodontal regeneration.** rhBMP-2 was a generous gift of Dr J Worley, Genetics Institute, Cambridge MA USA.

150

M. SOORY* and S. AHMAD (Periodontal Dept., King's College School of Medicine & Dentistry, London, UK): Metabolism of 14C-4-androstenedione by gingival tissue in response to bacterial culture supernatants.

The anabolic effects of the potent androgen 5 α -dihydrotestosterone (DHT) on matrix tissue are well documented. DHT synthesis is elevated during inflammation and may have some bearing on repair processes. It was therefore relevant to study the effect of culture supernatants derived from P.intermedius (Pi), P.gingivalis (Pg) and A.actinomycetemcomitans (Aa) on 14C-4-androstenedione metabolism by human gingival tissue. Duplicate incubations were performed with human gingival tissue (wet weight established) together with 14C-4-androstenedione and optimal stimulatory concentrations of Pi, Pg and Aa culture supernatants in Eagle's MEM+10% FCS. Incubations were performed in a CO₂ incubator for 24h. The metabolites were then extracted, evaporated and subjected to thin layer chromatography for their separation. The thin layer plates were scanned in a Berthold's linear analyser for quantification of radioactive metabolites. There were 87.2%, 50.6% and 6.3% increases in DHT synthesis by human gingival tissue, in response to the culture supernatants of Pi, Pg and Aa respectively over control incubations ($P<0.05$, $P<0.025$, respectively). It is relevant that the less destructive pathogen Pi stimulated DHT synthesis to a greater extent.

Hence in the environment of plaque-associated inflammation bacterial steroidogenic enzymes may influence matrix tissue turnover via the C19 steroid synthetic pathway.

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K Takahashi¹, I Poole² & DF Kinane¹ (¹Infection & Immunity Group, Univ. Glasgow; ²MC Human Genetics, Edinburgh): Detection of interleukin-1 β mRNA-expressing cells in human gingival crevicular fluid by *in situ* hybridization.

The aim of this study was to detect and enumerate interleukin-1 β (IL-1 β) mRNA-expressing cells in human gingival crevicular washings (GCW) obtained from patients with periodontitis and healthy controls by *in situ* hybridization. GCW were performed at 15 diseased sites (G1a1, PDa5 mm) from 5 patients with adult periodontitis (AP) and 8 clinically periodontally healthy sites from 3 volunteers (G1a1, PDa3 mm). *In situ* hybridization using digoxigenin-labelled oligonucleotide probe complementary to human IL-1 β mRNA showed IL-1 transcripts in both polymorphonuclear leucocytes (PMN) and mononuclear cells (MNC) but not in epithelial cells in all samples. PMN were the predominant leucocytes in diseased and healthy sites, averaging $91.7 \pm 4.6\%$ and $77.0 \pm 10.3\%$, respectively. The percentage of IL-1 β mRNA-positive PMN in GCW samples from diseased and healthy sites were $92.3 \pm 4.7\%$ and $80.9 \pm 10.3\%$, respectively. We quantified the IL-1 β gene signals in individual cells in 5 samples (2 healthy; 3 diseased sites). The mean amount of IL-1 β mRNA expression in PMN was higher than that of MNC in all samples and there was a heterogeneity within the population of PMN and MNC in their ability to express the IL-1 β gene.

These findings indicate that IL-1 β is predominantly produced by PMN in the gingival crevice of patients with AP and periodontally healthy controls.

This study was partially supported by SERC.

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R MOSELEY*, RJ WADDINGTON and G EMBERY. (Dept. of Basic Dental Science, Dental School, Cardiff, UK): The degradation of gingival and alveolar bone proteoglycans by reactive oxygen species *in vitro*.

Reactive oxygen species (ROS), such as O₂⁻, H₂O₂, OH \cdot , generated by active phagocytes during the inflammatory response, have been implicated in the pathogenesis of periodontal disease. Previous studies by ourselves have demonstrated that ROS are capable of the degradation of the glycosaminoglycan (GAG) constituents of proteoglycans, in addition to the non-sulphated GAG, hyaluronan. This present study, concentrates on the effects of ROS upon the proteoglycan components of the periodontal extracellular matrix. Proteoglycans were isolated from ovine gingiva and alveolar bone by guanidinium chloride extraction, followed by a two-step anion exchange chromatography procedure. The proteoglycans were subjected to either H₂O₂ alone or H₂O₂ plus FeCl₃, in order to generate a hydroxyl radical (OH \cdot) flux, and incubated at 37 °C for 1h or 24 h. Controls included proteoglycan only, or proteoglycan plus the hydroxyl radical scavenger, thiourea. The extent of degradation was examined by SDS-PAGE, cellulose acetate electrophoresis and gel filtration chromatography. The results suggest that the proteoglycans were partially degraded by the addition of H₂O₂, compared to controls and complete degradation was achieved following exposure to the OH \cdot flux. Both gingival and alveolar bone proteoglycans were degraded to the same extent when subjected to the ROS. In conclusion, we have demonstrated that ROS, in particular the hydroxyl radical species, can effectively depolymerize periodontal proteoglycans *in vitro*. These observations may be a contributory factor in considering the pathogenesis of periodontal disease.

153 R. LABELLA (Department of Biomaterials, Eastman Dental Institute, University of London, UK): Characterisation of Bis-GMA based resins containing mono or dimethacrylate diluents.

Bis-GMA is normally mixed with low viscosity dimethacrylate diluents (DD) to reduce its high viscosity. In this study monomethacrylate diluents (MD) were compared to DD for a range of physical properties of the diluted resins. Bis-GMA was added with 0.544 molar fractions of ethyleneglycol dimethacrylate (EGDMA), triethyleneglycol dimethacrylate (TEGDMA), hydroxypropyl methacrylate (HPMA), tetrahydrofurfuryl methacrylate (THFMA) and iso-boronyl methacrylate (IBMA). All monomer mixes contained benzoyl peroxide as an initiator (1% w/w) and were heat cured at 70 °C for 8 hours. Polymerisation shrinkage (PS), degree of conversion (DC), Young's modulus (YM), flexural strength (FS) and water uptake at equilibrium (WU) were evaluated. PS and DC were calculated from the gravimetrically determined densities of the monomer mixes and of the corresponding polymers. Eight 2x10x30 mm specimens were subjected to three point bending test for YM and FS at 5mm/min cross head speed. Three 1x10x20 mm specimens were stored in distilled water at 37°C for WU determination. Results: PS: TEGDMA(6.81%) > THFMA(7.7%) > HPMA(6.9%) > IBMA(5.3%) > DC: THFMA(68%) > TEGDMA(63.6%) > HPMA(55.5%) > IBMA(53.5%). YM: EGDMA(4.1 GPa) > THFMA-IBMA-HPMA (3.8, 3.8, 3.8 GPa) > TEGDMA (3.3 GPa). FS: HPMA(99.5 MPa) > TEGDMA-THFMA(75.6, 75.1 MPa) > EGDMA-IBMA (53.5, 48.5 MPa). WU: HPMA(6.2%) > TEGDMA(3.5%) > THFMA(3.0%) > EGDMA(2.0%) > IBMA(1.5%). MD can give bis-GMA based resins with equivalent or superior physical properties when compared with DD. IBMA produced a resin with noticeably low PS and WU, while HPMA conferred high FS. The Bis-GMA/THFMA resin showed the best balance of properties.

154 S. PATEL*, R.W. WASSILL, T. BUTLER, A.W.G. WALLS (The Dental School, Newcastle upon Tyne): In vivo 3 year wear in composite inlays and conventionally placed composites.

The aim was to determine whether tempered composite inlays had better wear resistance than conventionally placed composites and whether tooth location, preparation features or occlusal factors affected wear. A paired clinical trial comparing the two types of restorations (using a fine particle hybrid composite) was started in 1989. The exposed preparation margins around individual cusps of each restoration were assessed. Wear was measured using an indirect optical comparison technique by 2 observers under standardised conditions on stone casts of 69 pairs of restorations. A stone replica of the Ivoclar-Vivadent (IV) standard was used (pilot study results indicated that plastic standards produced dissimilar wear values compared to replica stone standards). Minimum, maximum and mean wear values were calculated. On average inlays wore 101µm (mean SD=2.3) whilst conventionally placed restorations wore 110µm (mean SD=2.5). The difference in wear was small but significant ($p < 0.001$). Individual measurements ranged from 25µm-400µm for inlays and 25µm-362.5µm for conventional restorations (mean of four repeated measurements). Using ANOVA, various factors were shown to have statistically significant effects, however only factors with differences in wear values greater than measurement reproducibility (14µm) were considered clinically significant. Restorations in premolars wore less than those placed in molars ($p < 0.007$). Patients with anterior faceting had significantly less wear of their restorations than those who did not ($p < 0.001$). Restorations in contact with opposing teeth in occlusal movements wore more than those that were not ($p < 0.004$). Also there was a significant trend towards more wear on functional sides of restorations than on nonfunctional sides ($p < 0.004$).

Although the overall difference in wear of inlays compared to conventionally placed composites was statistically significant, it may not have been clinically significant.

155 L.H. MAIR* (Department of Clinical Dental Sciences, The University of Liverpool, UK): The clinical performance of three posterior composites and two amalgams after 10 years service: preliminary results.

A preliminary sample of 24 posterior composites (Clearfil-Posterior¹, Occlusin² and P-30³) and 16 amalgams (New True Dentalloy⁴ and Solita Nova⁵) were reviewed within two months of their 10 year anniversary. The protocol for the placement and review have been previously described (Cunningham *et al.*, *Br Dent J* 169: 319-323, 1990). None of the restorations involved extensive cuspal coverage. The following statistics were recorded: colour match, the presence of marginal or general stain and the gingival condition. Impressions were taken and used to fabricate epoxy resin and stone models. Marginal wear was measured using the step wedge technique (Mair L.H. *Dent Mater* 6: 271-275, 1981).

In this sample many of the Occlusin restorations had stain all around the margin. Although some of the restorations appeared dull there was no significant change in the colour match. Marginal wear was ranked Occlusin > P-30 > Clearfil = Amalgams. However, the mean marginal wear did not exceed 250 µm. There was no difference between the composites and amalgams in terms of gingival response. None of the restorations warranted replacement.

It is concluded that all the materials had provided adequate posterior restorations for ten years.

¹ Cavex, ² ICI, ³ 3M Company, ⁴ SS Whites, ⁵ De Trey.

156 A.A.A. RAZAK* and A. HARRISON (Restorative Dentistry, Dental School, Bristol, U.K.): Effect of the filler content on some physical and mechanical properties of a composite inlay material.

Considerable interest has been generated in the use of composite inlay materials and they are now being recommended for use in posterior teeth. It is recognised that the filler components will influence the properties of the material (Hosoda *et al.*, *J Prosthet Dent* 64:669-674, 1990) and the purpose of this investigation is to evaluate the effect of varying the filler contents on transverse strength, modulus of elasticity, notched impact strength and linear coefficient of thermal expansion. Batches of Prisma AP.H composite inlay material¹ containing 79%, 65% and 50% fillers were prepared by the manufacturer. Specimens were cured using the manufacturer's recommended cycle and stored in water for 30 days before testing. The mean transverse strengths showed only a probably significant difference ($P < 0.05$) between the three filler concentrations. However, the modulus of elasticity exhibited a highly significant difference ($P < 0.001$) between each of them. There were no significant differences in the notched impact strengths. The test of linear coefficient of thermal expansion demonstrated highly significant differences between each of the three filler concentrations ($P < 0.001$).

It is concluded that filler concentration plays an important role in defining the optimum properties and will influence the success or failure of the restoration in situ.

¹ DeTrey/Dentsply, Germany.

157 R.W. WASSILL*, J.F. McCABE, A.W.G. WALLS & P.J. KELLY* (Restorative Dentistry, Medical Statistics¹, University, Newcastle upon Tyne): Temperature and pressure sensitivity from direct composite inlays.

One reason for the introduction of composite inlays was that the reduction of polymerization contraction effects should reduce post operative sensitivity. The aim of the study was to test the hypothesis that composite inlays produce less sensitivity than conventionally placed composites. 100 pairs of restorations (a direct composite inlay and a conventionally placed composite) were placed in 73 patients. Both restorations were made from Coltene Brilliant Dentin; the inlay (I) was tempered in the D500 oven and the conventional composite (C) placed incrementally. Patients were recalled at baseline and at 6m intervals. After three years, 79 pairs of restorations remained in the trial. At each recall, patients were asked if either their tooth was sensitive to temperature or biting pressure or both. Patients were asked to confirm the affected tooth by pointing to it. Teeth affected by dentine sensitivity, as determined by applying a jet of air to exposed cervical dentine, were excluded from the results. At baseline, similar numbers of both types of restorations showed temperature sensitivity (61, 5C) and pressure sensitivity (31, 3C). Most of the sensitivity was mild to moderate in nature, but in 5 restorations (4I, 1C) pressure sensitivity eventually resulted in removal of the restoration. Several restorations developed sensitivity during the trial this being most marked at 2.5 and 3y for I. Treating each recall as a separate event, there were 792 observations of the trial teeth over 3 years. Sensitivity events were recorded as follows: temperature I = 24, C = 7, both = 2; pressure I = 16, C = 2, both = 2. McNemars test showed significantly more sensitivity events for I (temperature $p = 0.002$, pressure $p = 0.001$) although there was no significant difference in the number of teeth affected.

Composite inlays can result in significantly more sensitivity than conventionally placed composites.

Supported by Coltene AG

158 A.C. SHORTALL*, E. HARRINGTON (School of Dentistry, The University of Birmingham, U.K.): Influence of light intensity on polymerisation of three light cured composites.

Light activated composites rely on adequate intensity of the light source to cause polymerisation through the bulk of material. Material factors such as filler size and loading, resin composition and shade are influential in determining the effective depth of cure. This study compared upper and lower surface microhardness of 2mm thick and 4mm diameter samples of three light cured composites 1-3 cured with twelve light curing units. Composite samples were irradiated for 40s at zero distance from the light guide tips. Light intensity was measured with a commercially available dental radiometer.⁴ Mean light intensity for the light activation units tested ranged from 101 to 725 (mW/cm²) at full optic diameter. Upper surface microhardness was little affected by changes in light intensity. Lower surface microhardness was related to light intensity and the correlation between lower surface microhardness and light intensity was most pronounced with the microfilled composite³.

Material composition is a very important variable at low light intensity in determining adequate polymerisation in bulk.

1. Herculite XR, Kerr
2. Z100, 3M Dental
3. Silux Plus, 3M Dental
4. Cure-Rite, Efos Inc

159 R.S. HOBSON*, J.F. McCABE, S.D. HOGG (Dental School, University of Newcastle upon Tyne): Degradation of dental composite by *Pseudomonas aeruginosa*

Previous work by Matsuda (Orthodontic und Kieferorthopädie 3, 269-283 1993) and Hobson *et al.* (J Dent Res 73: 801, Abstr. 118 1994) has indicated that oral bacteria can degrade dental composites.

This study investigated the effect of *Ps. aeruginosa* on two commercially available dental composites (Silux¹ and Heliomolar²) using the method described in the ASTM standard G22-76 for determining the resistance of plastics to bacteria. Discs (10mmx2mm) were prepared according to the manufacturers' instructions. These were sterilised by autoclaving at 121°C, 15 mins, and placed in either distilled water (control), or on the surface of Nutrient Agar (NA control) alone, or Nutrient Agar inoculated with 5x10⁸ viable cells/ml¹ of *Ps. aeruginosa* [ATCC 13388] (NA + *Ps. aer.*). The specimens were then incubated at 37°C and 85% humidity for 3 months.

The discs were removed, disinfected with glutaraldehyde (2% V/V, 1 hour) and tested for microhardness using a Knoop microhardness indenter at loads of 15g, 25g, 50g and 100g for 15 seconds. The results revealed that there was no significant difference between the controls and NA + *Ps. aer.* with test loads of 25g, 50g, or 100g ($p > 0.05$), but there was a significant difference with the lightest test load of 15g ($p < 0.01$).

The results indicate that microbial degradation of composite occurs and affects the superficial layer of the material.

¹3M Co, Loughborough, England, ²Vivadent, Lancaster, England

160 N.H. ABU KASIM* and J.F. McCABE (The Dental School, University of Newcastle upon Tyne, UK): The effect of thermocycling on the fatigue behaviour of dental composites.

The effect of thermocycling on the fatigue behaviour of 2 microfilled composites; Silux Plus¹ and Heliomolar² and 3 hybrid composites; P50³, Clearfil Photo Posterior⁴ (both light-activated) and Clearfil Posterior⁵ (chemically-activated) was investigated using a ball mill. Ninety specimens (2 x 2 x 25mm) of each material were used. The specimens were divided into 3 groups of 30 and were stored for 24 h in water at 37°C (Group A), subjected to 10⁵ thermal cycles over 45 days (Group B) and stored in water at 37°C for 45 days (Group C). Ten specimens of each material were tested in a 0.5 litre capacity ceramic ball mill⁶ which was rotated at 100 rev/min. This procedure was repeated 3 times. The mill was charged with 470g stainless balls and 250ml distilled water. At 30 minute intervals the number of fractured specimens was noted. All fractured and intact specimens were returned to the ball mill and the process repeated up to 5 hours. The mean survival time (mins) for P50 were >300(A), 85(B) and 130(C); Silux >300(A), 76(B) and 110(C); Heliomolar >300(A), 240(B) and >300(A); Clearfil Photo >300(A), >300(B), >300(C) and Clearfil Posterior >300(A), 165(B) and 160(C). Group A is significantly different ($P < .05$) from Groups B and C for all materials except Clearfil Photo Posterior. However, significant differences ($P < .05$) between Group B and C was only observed in P50 and Silux. The fatigue behaviour of P50 and Silux is affected by thermocycling.

¹3M, St. Paul, USA; ²Vivadent, Liechtenstein; ³Kuraray, Japan; ⁴Pascal Engineering, UK.

161 EAM KIDD*, DNU RICKETTS* and D BEIGHTON* (Dept of Conservative Dentistry, UMDS, Oral Microbiology, KCSMD, London, UK): Rational criteria for caries removal at the enamel-dentine junction.

In UK cavity preparation at the enamel-dentine junction (EDJ) is considered complete when all soft and/or stained tissue is removed. In other European schools excavation is terminated once hard tissue is removed, irrespective of its colour. The aim of the present studies was to investigate the microbiological basis of these two approaches and provide rational criteria for caries removal at the EDJ. Cavities were prepared in 570 teeth (161 primary caries; 403 replacement restorations). Under rubber dam, access to the EDJ was gained and sample sites selected (n=449). Their consistency was noted as hard or soft and wet or dry when probed. The colour of the site was noted as stain-free or stained. Dentine was sampled using a round bur and the sample placed in broth. Samples were vortexed, diluted and cultured for total anaerobic counts, mutans streptococci and lactobacilli. Counts were expressed as log₁₀ (colony forming units per sample). Soft and wet sites harboured significantly more total bacteria, mutans streptococci and lactobacilli than soft and dry sample sites which in turn harboured significantly more bacteria than hard and dry sites. Stained and soft sites harboured significantly (p<0.001) more bacteria than other sites. Stained and hard sites harboured more bacteria than stain-free and hard sites (p<0.05) but this difference was not considered to be clinically significant. The relatively simple clinical criteria used here correlated significantly with the microbial findings. If minimal infection of the residual dentine at the enamel-dentine junction is important it is necessary to continue cavity preparation until the area is hard. Further work with samples collected at the end of cavity preparation is required to test this hypothesis.

162 DW BARTLETT*, D F EVANS*, A ANGLANSAN, BGN SMITH* (Dept of Cons Dental Surgery and Surgery, UMDS, 'GH Research Unit, London Hospital Medical College): The role of gastro-oesophageal reflux (GOR) in palatal erosion.

GOR has been associated with palatal dental erosion. To test this hypothesis 26 subjects presenting with palatal erosion were investigated for GOR by recording the oesophageal and oral pH over 24 hours. Distal and proximal oesophageal pH were measured using a dual channel portable pH monitor. Palatal pH was measured with a pH sensitive radiocapsule (RTC) and signals were detected by an aerial worn around the head. A control group with no previous history of GOR or palatal erosion were monitored in a similar manner. Each control subject underwent two separate tests, first a curry meal taken with lager and secondly with a bland meal. GOR was analysed for % time <pH 4 occurring in the distal and proximal oesophagus. Oral reflux was analysed for the number and the % time <pH 5.5 and 6. The results showed that 17 of the erosion group (65%) were found to have pathological GOR using standard criteria. There was a significant increase in acid exposure < pH 4 between the erosion and control (bland) meal in the distal and proximal oesophagus (p < 0.001), but no difference in distal acid exposure in the curry meal. However, proximal oesophageal reflux was significantly increased for GOR in the curry (p < 0.05) and the bland meal (p < 0.001). There was similar significant difference in palatal pH between the erosion patients and the bland meal (p < 0.05) but not in the curry meal. It is concluded that pathological GOR was present in 65% of the patients presenting with palatal erosion. The curry meal provoked GOR in the distal oesophagus in all control subjects, but despite this little reflux migrated to the proximal oesophagus. These results emphasise the importance of the presence of GOR in the proximal oesophagus in patients with palatal erosion.

163 C ROBINSON*, H BULBULIA*, R C SHORE, C C YOUNGSON* and J KIRKHAM* (Div. Oral Biology, Div. Restorative Dentistry, Leeds Dental Inst., Univ. of Leeds, UK): Filling without Drilling: Application of infiltrative technology to dental defects.

Destruction of enamel by carious attack generates micro-porosities which ultimately enlarge until the tissue collapses, requiring conventional restoration. It has been shown previously (Robinson C et al, J Dent Res 55: 812-818, 1976) that it is possible to infiltrate materials into such micro-porosities effectively occluding space, providing mechanical support and reducing further acid attack. Efforts over the past 10 years to minimise restorative dentistry have highlighted this approach to treatment. With new adhesive materials, the possibility of treating not only carious enamel but also healthy or exposed carious dentine has taken on a new dimension. With this in mind a series of contemporary adhesive materials already in clinical use were selected (Scotchbond, Aditebond, Amalgambond-plus and a Cynoscyte). Panels of enamel on sound teeth delineated by acid resistant varnish were exposed to gelatine gels at pH 4.5 for 6 weeks. This produced areas of porosity similar to white spot carious lesions. The porosity was thoroughly imbedded with 2-chloronaphthalene to ensure complete occlusion of all of the space. After wiping the surface of the tooth, the chloronaphthalene was subsequently removed from the porosity by incubation in spectrophotometrically pure hexane. Determination of the chloronaphthalene from each lesion gave a direct measure of the volume of porosity. The porosities were treated with each of the selected adhesives and after polymerisation, the volume of accessible porosity was again determined. In all cases the adhesive had reduced the accessible pore volume by about 50%. Further treatment reduced this volume by up to 90%. The results indicated that occlusion of porosity in early caries lesions can be achieved using contemporary bonding agents. These may offer a non-destructive alternative to early treatment.

164 MORGANSTEIN S.I.* Department of Conservative Dentistry, The London Hospital Medical College Dental School, UK: The fluoride uptake by human enamel from 21 European toothpastes.

The fluoride active in most dentifrices available in retail outlets in Europe is either Sodium Fluoride, Sodium Monofluorophosphate or a combination of both. They are formulated with either a silica, calcium carbonate or DCPD abrasive. This experiment was designed to examine the fluoride uptake into human enamel samples from 21 'over the counter' dentifrices. The enamel samples were produced from extracted teeth (White, D J, Caries Res 21: 228-242, 1987) and were exposed to a demineralisation solution for 96 hours to create an artificial caries lesion prior to cycling through pooled human saliva, a slurry made from toothpaste and saliva and the demineralisation solution. After six days of treatment the enamel specimens were analysed for fluoride content using the microdrill biopsy method.

Results showed that dentifrices containing Sodium Fluoride when formulated with a silica abrasive demonstrated significantly higher fluoride uptake into enamel than the other formulations.

165 L V FOSTER* (Department of Oral and Dental Science, University of Bristol, UK): Variations in stimulated saliva secretion rate and the buffering capacity of saliva

This investigation aims to assess the variation in stimulated saliva secretion rate and the buffering capacity of saliva over an eight month period. Sixty two patients (32 male and 30 female) were selected, who had at least one smooth surface primary carious lesion which extended into the dentine which was considered to be 'active'. The stimulated salivary secretion rate and the buffering capacity of the saliva were determined for each patient using the Dentobuff[®] system at baseline, four months and eight months. Considerable variations were found. Overall, the stimulated secretion rate of the saliva remained static for 41 (66%) patients. At baseline 33 (53%) patients had a mean stimulated saliva secretion rate of more than 1.0 ml/min. This increased to 42 (68%) at four months but then fell back to 40 at eight months. When the values obtained were averaged then 38 (61%) patients had a mean stimulated saliva secretion rate of more than 1.0 ml/min whilst 24 (29%) had less than 1.0 ml/min. The buffering capacity remained static for 38 (61%) patients. On average, 10 (16%) patients had a low buffering capacity, whilst 28 (45%) and 24 (39%) patients had medium and high buffering capacities respectively. Patients with lower stimulated saliva secretion rates tended to have lower buffering capacity whilst those with higher stimulated saliva secretion rates had higher buffering capacities (Chi square = 12.4, D of F = 2, p = 0.002).

It is concluded that there is considerable variation in both the stimulated secretion rate and the buffering capacity of saliva over time. It is therefore recommended that these tests are repeated on at least one other occasion particularly for those patients exhibiting low values.

Dentobuff, Vivadent, Schaan, Liechtenstein.

166 S M STRAFFORD*, C ROBINSON, J KIRKHAM, M STRONG and R C SHORE (Division of Oral Biology, Leeds Dental Institute, University of Leeds, Leeds, UK): Use of sorbate to determine oral clearance of foodstuffs following normal consumption.

Clearance patterns for fluoride and glucose in the oral cavity after rinsing have been shown to be site-specific (Weatherill J A et al, Caries Res 20: 111-119, 1986). Clearance rates for foodstuffs following normal consumption have received less attention, however, due to the unavailability of a suitable marker molecule. A marker must be non-toxic, tasteless, non-metabolisable and easily detectable at low levels. The aim of this study was to evaluate sorbate as such a marker and so compare the clearance patterns of sorbate containing yoghurt from specific sites in the oral cavity following (a) a yoghurt mouthrinse and (b) normal consumption of a pot of yoghurt. Subjects were asked to rinse their mouth for 15 seconds with 10 mL of yoghurt. Alternatively, subjects were asked to consume a single (150g) portion of yoghurt according to their normal eating habit. Samples of saliva were collected from sites in both anterior and posterior upper and lower sulci using filter paper points. Both fluoride ion and sorbate were used as markers in the rinsing study. Sorbate alone was used in the consumption experiments. After elution from the paper points, fluoride was measured using a micro-fluoride electrode and sorbate determined by HPLC. The results indicated that while rinsing with yoghurt produced site specific clearance curves similar to those reported for fluoride and glucose (ie. most rapid clearance from the posterior sulci), these patterns were not the same after normal consumption. The results highlight the need for further studies using normal consumption of foodstuffs to provide meaningful data in respect of availability of substrate in relation to dental caries. Sorbate may be a useful marker molecule in this respect.

167 E J KAY* and D LOCKER (Universities of Manchester and Toronto): A meta-analysis of dental health education research.

In order that health services resources are allocated in the way which most benefits the population, systematic review of the available evidence regarding the effectiveness of programmes and interventions are required. This study examined papers relating to dental health interventions, published between 1980 and 1992 (N = 143). Each was scored by two researchers according to a set of predetermined validity criteria. For each paper which achieved a score of more than 60% (N = 37), data concerning the objectives of the intervention, the types and numbers of participants, and the outcomes, were extracted from the article. Where sufficient data were provided, they were pooled to give an overall intervention effect with confidence intervals. The results showed that DHE has a small positive, but temporary effect on plaque accumulation (i.e. = -0.37 95% CI -0.29-0.59); no discernible effect on caries increment and a consistent positive effect on knowledge levels. This analysis demonstrates that the quality of evidence supporting the effectiveness of DHE is not good and that the research suffers from a lack of valid reliable outcome measures.

Dental health education urgently requires well designed randomised controlled trials, which are reported in sufficient detail, before conclusions regarding their efficacy and cost effectiveness can be drawn.

168 B HIKMAT*, N SOUKOS, I OLSEN, SS PRIME*, PM SPEIGHT (Dept Oral Pathology, Eastman Dental Institute and LHMC, and University of Bristol, UK): Alterations in epithelial-fibroblast interactions in malignant oral epithelial cells.

Epithelial-fibroblast interactions are important in development and wound healing and may be mediated via the extracellular matrix, via cytokines or by direct cell contact. Such interactions are altered in malignancy but the role of direct cell contact in modulating the behaviour of epithelial cells is not known. The purpose of this study was to determine if keratinocytes (KC) can adhere to fibroblasts (FB) and to determine if adhesion is altered in malignancy.

Cell lines derived from oral squamous cell carcinomas, H357 and H376, which are E-cadherin positive and negative respectively, and a normal KC cell line (UP) were allowed to adhere to glass or to monolayers of normal mucosal FB for 30 min. After washing to remove non-adherent cells, adhesion was measured as the number of bound KC/mm². Assays were carried out in plain medium, in the presence of RGD peptide, which blocks integrins, or with an anti-E-cadherin blocking antibody (DECMA-1). H357 and UP cells adhered preferentially to FB and adhesion of H357 was greater than UP or H376 (p < 0.001). Adhesion of H376 to glass was greater than H357 or UP (p < 0.05). Adhesion of H357 to FB was inhibited by RGD and by DECMA-1 (p < 0.05). The results show that KC can adhere to FB. This interaction is altered in malignant cells and is dependent on E-cadherin. Since E-cadherin is a homotypic cell adhesion molecule, which is not expressed by FB, our finding that RGD inhibited binding of H357 suggests that KC-FB adhesion is mediated by integrins and regulated by E-cadherin. The loss of E-cadherin in carcinomas may therefore be important in regulating the invasive behaviour of malignant cells.

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W THOSAPORN & G T CRAIG* (Dept of Oral Pathology, University of Sheffield, UK): Immunohistochemistry (IHC) in the differential diagnosis of minor salivary gland neoplasms.

Occasionally, the histological appearances in biopsies from minor salivary gland neoplasms present diagnostic problems. For example, the "cribriform" pattern typical of adenoid cystic carcinoma (ACC) may also feature as a component in monomorphic and pleomorphic adenomas (MA & PA) and in the polymorphous low-grade adenocarcinoma (PLGA). Given their widely differing biological behaviour, it is essential to distinguish reliably between these possibilities prior to definitive surgery. The role of IHC as an aid to differential diagnosis in such cases forms the basis for the present study. Representative numbers of paraffin-embedded, trypsinised (10 mins) sections from 5 cases each of typical examples of MA, PA, PLGA and ACC were evaluated for the expression of glial fibrillary acidic protein (GFAP¹ - 1:300 - 60 mins), S-100 protein (S-100¹ - 1:500 - 60 mins) and muscle specific actin (MSA¹ - 1:200 overnight at 4°C) using the Avidin-Biotin-Complex² peroxidase method; negative and positive controls were included. Immunoreactivity for each antibody was assessed subjectively. The results showed that the different patterns of GFAP expression alone clearly discriminated between ACC (-ve epithelium & -ve stroma), PLGA (-ve epithelium & +ve stroma), PA (+ve epithelium & +ve stroma) and MA (+/- epithelium & stroma). S-100¹ & MSA immunoprofiles also separated ACC from PLGA.

Immunohistochemistry is of potential value in the differential diagnosis of minor salivary gland neoplasms.

1. Dako Ltd, UK. 2. Vector Labs, UK.

170

AJC POTTS*, MR BRICKLEY, J HAMBURGER & JB MATTHEWS (Dental Schools, University of Wales College of Medicine & University of Birmingham): Can a neural network predict focal lymphocytic sialadenitis from clinical and serological data?

While a number of clinical, serological and imaging investigations are used to support a diagnosis of Sjögren's syndrome (SS) many patients require labial gland biopsy to confirm the diagnosis. This study investigates the ability of a neural network to predict the histological features of SS.

Information was available from 164 patients referred to an Oral Medicine Clinic for investigation of suspected SS, comprising 134 with underlying connective tissue disease and 26 suspected of having primary SS. Data from 84 cases was used to train a neural network (Neudeck 1.2, Neural Computer Sciences, Southampton) which was subsequently tested with data from 80 cases. Variables examined included sex, age, symptoms and signs of xerostomia, parotid flow rate, serum immunoglobulin levels, rheumatoid factor, anti-nuclear factor, anti-Ro and anti-La antibodies. The model used was a fully connected multilayer perceptron trained using stochastic back propagation to a validation error minimum using internal cross validation. Test data were dichotomised and analysed in SPSS. Network decisions, dichotomised with a threshold of 0.61, placed 30 of 43 cases with focal lymphocytic sialadenitis (FLS) and 28 of 37 without FLS in the correct groups, an agreement of 71.6% with a sensitivity of 0.70 and a specificity of 0.76, even though there was missing data from one or more variables in 70% of the cases.

This study suggests that a neural network can predict the presence of focal lymphocytic sialadenitis from clinical and serological data and may eliminate the need for labial gland biopsy in some patients.

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J GIBSON*, M RIGGIO, D G MacDONALD and D WRAY, (Glasgow Dental Hospital and School, Glasgow, U.K.): Identification of *Mycobacterium paratuberculosis* by PCR in orofacial granulomatosis.

Orofacial granulomatosis (OFG) is a condition characterised by oedema variably involving lip, buccal mucosa and gingivae. The condition is defined by clinical presentation and the histological presence of lymphoedema and non-caseating epithelioid granulomata. After full medical investigation, a proportion of cases of OFG can be redesignated as Crohn's disease with gastro-intestinal manifestations or sarcoidosis and a significant number of cases have identifiable dietary and environmental allergens (Patton D W, Ferguson M M, Forsyth A and James J. *Brit J Oral Maxillofac Surg* 23: 235-242, 1985). In view of evidence linking *Mycobacterium paratuberculosis* and Crohn's disease (Sunderland J D, Moss M T, Tizard M L V and Hermon-Taylor J. *Gut* 33: 890-896, 1992), mucosal biopsies from 10 patients with OFG, but no clinical evidence of extra-oral gut Crohn's disease or sarcoidosis, were subjected to polymerase chain reaction (PCR) analysis to detect the presence of IS900 DNA sequences specific to *Mycobacterium paratuberculosis*. Two biopsies suggested positivity by this technique.

*It is concluded that *Mycobacterium paratuberculosis* may be present in some cases of OFG as an aetiological agent.*

172

S SMITH*, S WARNAKULASURIYA, NW JOHNSON (RCS Dental Sciences, King's College, London): Baseline knowledge of oral cancer and the effect of training on diagnostic accuracy for oral mucosal lesions, amongst 58 General Dental Practitioners (GDPs).

The rising incidence and high mortality of oral cancer in the UK have generated much interest in screening. The UK Working Group (Comm Dent Health 10, Suppl 1; 1993) has recommended opportunistic screening by all dental practitioners, creating a demand for training. This study assessed background knowledge of 58 GDPs concerning oral malignancy and assessed their diagnostic skills on clinical photographs of a range of mucosal lesions before, and immediately after, a half day lecture/seminar course. Over half (57%) had accurate knowledge of UK incidence rates, and most (61-90%) answered most questions on risk factors and natural history correctly; however only 52% recognised high alcohol consumption as a marker of individuals at risk and the importance of dietary micronutrients was not widely appreciated. Only 22% routinely enquired about tobacco habits of their patients and only 9% enquired about alcohol use. When asked to indicate whether 20 mucosal lesions were benign, potentially - or overtly - malignant, only 2 were correctly judged by 75% of the GDPs before teaching, and only 9 after teaching (mean correct answers 11/20 pre and 12/20 post-teaching). Of the 20 lesions there was a swing from incorrect to correct judgements by 2 or more participants in 11 cases, but a comparable swing to incorrect judgements in 6 cases. Speckled leukoplakia was an unknown entity before training and knowledge of oral hairy leukoplakia was limited.

We conclude that there are important gaps in GDP knowledge of oral cancer; that diagnostic confidence and accuracy is poor, and that the latter is not markedly improved by short didactic teaching. If we aspire to diagnostic accuracy using GDPs as oral mucosal screeners, more intensive/ appropriate training is necessary, perhaps involving formal calibration.

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CG COWAN*, TA GREGG and F KEE (School of Clinical Dentistry, The Queen's University Belfast and Dept. Public Health Medicine): Experience and management of oral cancer and pre-cancer in Primary Dental Care.

Previous studies in N. Ireland have shown that epithelial dysplasia is diagnosed infrequently compared with squamous carcinoma (Gregg et al, *Br Dent J*, 173:234-236, 1992). This is in contrast to cervical carcinoma where the occurrence of dysplasia far out numbers carcinoma. This may indicate that potentially malignant lesions are not being detected and/or referred from primary care.

Aim: To determine the experience, usual management and referral strategies of primary care dentists in N. Ireland for oral cancer and pre-cancer.

Method: A previously piloted questionnaire was sent out to 635 primary care dentists.

Results: Final response was 67% (428/635). Mucosal examination was routine for 94% (402). White lesions (presumptive leukoplakia or lichen planus) were the most common with a median of 11 per year for all types. Virtually all dentists referred such cases within six months of presentation. Only 12% (53) dentists offered biopsy. Overall experience in referring patients with suspected cancer was low (median 3) and only 25% (107) of respondents had referred more than six. Of these less than one third were subsequently diagnosed as malignant.

Summary: Mucosal examination is routine but overall experience of carcinoma and potentially malignant lesions is low. Suspicious lesions are referred early. These findings do not explain the perceived low incidence of dysplastic lesions and questions the duration and/or existence of a clinically detectable pre malignant phase in all cases of intra oral squamous carcinoma.

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C K HARRIS*, S WARNAKULASURIYA, N W JOHNSON, S GELBER and T J PETERS (RCS / King's College School of Med. & Dent., London): Oral health in alcohol misusers.

107 alcohol misusers (mean age 42.9 yrs, range 21-65; 80 males) attending 4 centres in South London were interviewed on alcohol consumption (units/week and their total audit scores). Nutritional status (body mass index, BMI and mid arm muscle circumference) was recorded. Each subject completed a dental and oral mucosal examination. 94% consumed greater than 50 units per week and 80% greater than 100 units per week. Smoking and alcohol misuse were found to be related (83% reporting both habits). Plaque index scores and mean subject pocket depths were not correlated to alcohol consumption but both smoking frequency (R=0.2; P<0.04) and duration (R=0.34; P<0.001) were. Overall mean DMFT was 15.4; age specific mean DMFT and tooth loss of the sample were closely similar to the UK Adult National Survey data (1988). The prevalence and severity of tooth wear and attrition were greater in the sample than levels described in the literature and these dental features may prove useful markers to the practitioner. Trauma to teeth and oral mucosae was noted in 25% of the sample. 8 oral mucosal lesions (3 candidiasis, 2 keratosis, 1 traumatic ulcer, 1 papilloma and 1 treated carcinoma) were detected (all in smokers); mucosal trauma could have acted as a co-factor. Furthermore, 19% of the alcoholics were mildly under-nourished (BMI<20) which may also influence mucosal health and disease. *We conclude that, unlike several reports from the USA, dental health in this sample of alcoholics is not compromised; however mucosal health is of concern.*

175

ROCHE S.A. (Unit of Oral Medicine, School of Dentistry, The University of Birmingham): Oral Health Needs of a Frail Elderly Population.

Birmingham's elderly population aged 75 years or more is estimated to be 64,500 (OPCS 1991), 34% of whom are registered with a general dental practitioner (GDP) for continuing care, (General Dental Service Quarterly return figures, April-June 1994).

The aim of this study was to assess the prevalence of oral mucosal disease in a frail elderly population. All the participants were residents in nursing homes in North and West Birmingham. A total of 415 elderly people were screened by the same clinician for dental and oral disease. At the time of the survey, none were registered with a GDP under a continuing care contract.

34% (n=142) of those surveyed had conditions requiring treatment or regular monitoring. 9.4% (n=39) required new dentures. 25% (n=103), had clinically obvious oral mucosal disease. The most frequently presenting conditions were xerostomia (9.4% n=39) and oral candidiasis (5.3% n=22). 5 patients had ulcerative lesions, one of which was a carcinoma in situ.

Results suggest that the Normative Need for oral health provision is high among the frail elderly population of Birmingham, despite a low expressed need.

Screening programmes should be extended to identify unmet need amongst the frail elderly and access to appropriate oral health care provided.

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J A BEELEY*, L MOORE AND F NEWMAN (Dept of Oral Sciences, University of Glasgow Dental School, UK): Interaction of buccal epithelial cells with salivary basic proline-rich protein Ps1.

Although proline-rich proteins (PRPs) comprise some two-thirds of the total protein in duct saliva, the levels in mixed (whole) saliva are very low. As it has been shown that some PRPs interact specifically with certain oral bacteria (Newman F et al, *Electrophoresis* 13: 1322-1327, 1993), the possibility that PRPs might also interact with buccal epithelial cells (BECs) was investigated.

Parotid saliva was incubated at 37°C for 50 mins with suspensions of either exfoliated BECs or oral carcinoma BECs (KB cells) grown in culture. The cells were removed by centrifugation and parotid salivary protein profiles analysed by SDS-PAGE (Beeley J A et al, *Electrophoresis* 12: 1032-1041, 1991).

Incubation with either cell suspension resulted in the specific removal of the basic PRP Ps1. There was little or no effect on the levels of the other major PRPs. SDS-PAGE of proteins extracted with 10% SDS from KB cells after incubation with parotid saliva revealed an additional protein corresponding to Ps1 as compared with KB cell controls.

Basic PRP Ps1 interacts specifically with buccal epithelial cells but the nature of the process and its role have yet to be characterised.

177 G.H. CARPENTER*, G.B. PROCTOR and C.L. PANKHURST (Oral Pathology & Oral Microbiology, K.C.S.M.D., London): Expression of blood group antigens on human parotid salivary glycoproteins.

Expression of the oligosaccharide A, B and H blood group antigens (Ags) in saliva has been attributed to mucins, glycoproteins which are not secreted by parotid glands. The aim of the present study was to assess whether particular parotid secretory glycoproteins express these Ags. Parotid and whole saliva were collected under controlled conditions from healthy individuals (age 20 to 40 years) of different blood groups; the former using a Lashley suction cup placed over the orifice of Stenson's duct. The salivary protein content of different samples was assayed by absorbance at 215nm then loaded by vacuum onto nitrocellulose membranes. The presence of blood group Ags was then determined by probing the blots with biotinylated lectins (*Ulex europaeus* agglutinin-I, UEA-I, for H Ag; *Dolichos biflorus* agglutinin for A Ag; *Griffonia simplicifolia* agglutinin-IB₂ for B Ag) or monoclonal antibodies to different blood group Ags followed by biotinylated secondary antibodies. The presence of binding was detected using the avidin-biotin-horseradish peroxidase complex and the chemiluminescent substrate luminol (ECL, Amersham). To characterize which parotid proteins expressed blood group Ags, Western blots of SDS gels were similarly probed. UEA-I binding appeared to be quite specific for blood group H Ag, large amounts of which were associated with the major proline-rich glycoprotein(s). Lesser amounts of the A and B Ags may be present on other unidentified glycoproteins of low molecular weight. **It is concluded that H Ag is the major blood group Ag in human parotid saliva and that it is expressed mainly on the major parotid proline-rich glycoprotein.** (Supported by KMRT)

179 X.S. ZHANG*, L.C. ANDERSON, J.R. GARRETT, G.B. PROCTOR and D.K. SHORI (Oral Pathology, K.C.S.M.D.): Protein secretion from rat submandibular ducts requires different sympathetic impulse formation from acini.

We have used male adult Wistar rats to study the secretion of acinar peroxidase and granular ductal kallikrein (rK1) from submandibular glands during graded sympathetic stimulations on a background of parasympathetic stimulation. Rats (n=6) weighing 350-500 g were fasted overnight and anaesthetized by chloralose (80 mg/kg i.v.) after induction with pentobarbitone (36 mg/kg i.p.). The right submandibular duct was cannulated, its chorda lingual nerve was stimulated continuously on the duct at 4 Hz, 5 V, 2 ms. The right cervical sympathetic trunk was stimulated at 5 V, 2 ms, 0.1 - 2 Hz continuously or 10 and 20 Hz in bursts (1s every 10s). Dual stimulations were applied for 5 min periods, submandibular saliva was collected, weighed and assayed for peroxidase, using 2',7'-dichlorofluorescein diacetate and rK1 with 7-amino-4-trifluoromethylcoumarin. Results (means \pm S.E.M.) were expressed as outputs per stimulation period per gram gland wet weight. Peroxidase secretion during parasympathetic stimulation was 0.23 ± 0.04 U. Addition of sympathetic stimulation caused increases from 1.55 ± 0.16 U at 0.1 Hz to 6.60 ± 0.59 U at 2 Hz. Burst stimulation at 10 and 20 Hz caused no further increase. Secretion of ductal rK1 was 17.3 ± 2.4 U² in parasympathetic saliva and showed no change with additional sympathetic stimulations at 0.1 - 2 Hz (14.7 ± 2.5 U), but when applied in bursts sympathetic stimulation caused highly significant increases in secreted rK1 reaching 2265.8 ± 950.4 U at 20 Hz (1:10). **The results suggest that separate nerve populations may be responsible for the secretion of acinar peroxidase and ductal kallikrein.** (Supported by KMRT).

¹ 1U activity= 1nmole dichlorofluorescein/min;
² 1U activity= 1nmole 7-amino-4-trifluoromethylcoumarin/min.

181 J.R. GARRETT*, X.S. ZHANG, G.B. PROCTOR, L.C. ANDERSON and D.K. SHORI (Oral Pathology, K.C.S.M.D., London): Luminal secretion of rat submandibular kallikrein continues under resting conditions.

It is customary to reject first drops of saliva for they contain pre-existing material. We have now examined first samples of submandibular parasympathetic saliva for comparison with ensuing samples and for the effects of preceding rest pauses. Male Wistar rats (300 - 450 g) were anaesthetized by chloralose (80 mg/kg i.v.) after induction with pentobarbitone (36 mg/kg i.p.). A submandibular duct was cannulated and its chorda lingual nerve was stimulated by a bipolar electrode using 2 ms impulses at 6v. Saliva from the cannula was analysed for kallikrein activity fluorogenically using D-Val-Leu-Arg-Tamino-4-trifluoromethyl-coumarin in the presence and absence of soya bean trypsin inhibitor (SBTI) (as in Shori et al., 1992, Biochem. Pharmacol. 43: 1209-1217). In 3 animals the first 10 drops of saliva at 1 Hz stimulation were analysed. Tissue kallikrein activity in the first drop was 10.285 ± 2.045 nmol AFC/ml/min (mean \pm S.E.M.), but fell to 428 ± 114 in the ensuing 9 drops. In 11 animals saliva was collected in 5 sequential 30 s samples at 5Hz. The mean flow rate was 167 ± 32 μ l/gm. Activity in first samples was 158 ± 37 nmol AFC/gm and ensuing samples 17.3 ± 2.4 . The same pattern emerged after rest pauses but activity in first samples increased from 37 ± 3 after 10 min (n=4) to 301 ± 21 after 90 min (n=11). Throughout, activity in saliva had a parasympathetic, non-granular, pattern of $80 \pm 1\%$ resistance to SBTI compared to $54 \pm 2\%$ resistance in granule containing extracts (p<0.001).

It is concluded that, even in the absence of stimulation, a constitutive (vesicular) secretion of kallikrein continues into lumina, reflecting ongoing synthesis.

183 C.A. FRANCIS* and M.P. HECTOR (The London Hospital Medical College, El 2AD, UK): A universal device for collecting submandibular/sublingual saliva.

Collection of submandibular/sublingual (SM/L) saliva has always been more difficult than parotid. The aims of this study were: 1) to develop a universal, reusable collecting device for SM/L saliva; and 2) to determine its validity while collecting saliva at rest and in response to a gustatory stimulus. The device was made of a polyethylmethacrylate soft lining material¹ to a design based on a description by Truelove et al. (*J Dent Res* 46: 1400-1403, 1967) and was held in situ in the floor of the mouth by means of suction applied to bilateral chambers on the under surface. Saliva drains through flexible vinyl tubing from a central chamber positioned over the SM/L duct openings. The device was easily fitted to dentate and edentate subjects and was comfortable for periods in excess of 30 minutes. The collecting device was fitted and following a 10 minute period of acclimatization, resting saliva was collected over 10 minutes. Stimulated saliva was collected in response to 1.0 ml of 1% w/v citric acid solution, placed on the dorsum of the tongue per minute for 5 minutes. Blue food dye was added to the citric acid to confirm that there were no leaks of the stimulant into the collected saliva samples. Preliminary results from 5 subjects compare well with previously reported data. Mean flows at rest and during citric acid stimulation were 0.20 ml/min (range 0.06-0.42 ml/min) and 1.04 ml/min (range 0.65-1.13 ml/min) respectively. **In conclusion, submandibular/sublingual saliva can be collected reliably using this simple, versatile and universal device.**

¹ Softex, Zhermack, Italy.

178 B DOUBLEDAY*, S HANDLEY*, G P REYNOLDS*, R CHESS-WILLIAMS* (Depts. of Orthodontics, Leeds Dental Institute* and Biomedical Science, University of Sheffield, UK): Clozapine-induced hypersalivation: are M₂ receptors involved?

A common side effect of classical antipsychotic drugs (e.g. chlorpromazine) is dry mouth, which appears to be related to their anticholinergic effects. The atypical antipsychotic drug clozapine, however, induces hypersalivation, which can be severe in up to one fifth of cases (Alpha LD et al., *Atypical antipsychotics: clinical advantages* [Symposium] 7-8, 1990). It was proposed that this could be due to agonist activity of clozapine at the post-ganglionic parasympathetic (muscarinic acetylcholine) receptors in the salivary glands. The predominant muscarinic receptor in human salivary glands is the M₃ subtype. This study examined the actions of three antipsychotic drugs (clozapine¹, chlorpromazine² and thioridazine³) at a functional M₃ receptor in an *in vitro* tissue preparation.

The M₃-induced contractions of rat ileum to carbachol were measured in the presence and absence of clozapine, chlorpromazine and thioridazine. None of the drugs exhibited any agonist activity at these receptors, but all three behaved as antagonists with a high affinity for the M₃ receptor. Clozapine had the highest affinity (K_D = 36.8nM) thioridazine (K_D = 120nM) and chlorpromazine (K_D = 270nM). **These results confirm the antagonist activity of antipsychotic drugs at M₃ receptors which probably induces hypersalivation, however, the hypersalivation caused by clozapine is not related to any agonist effect at these receptors. The functional activity of clozapine at other autonomic receptors in the salivary glands needs to be investigated.**

¹Sandoz, ²Sigma.

180 G.B. PROCTOR*, X.S. ZHANG, L.C. ANDERSON and J.R. GARRETT (Oral Pathology, K.C.S.M.D., London): Protein secretion from rat submandibular acini and granular ducts on parasympathetic stimulation.

Parasympathetically evoked protein secretion from submandibular acini and ducts has been compared. Five fasted, male Wistar rats (350 - 500 g) were anaesthetized with chloralose (80 mg/kg i.v.) following pentobarbitone (36 mg/kg i.p.). The chorda lingual nerve was exposed and stimulated with a bipolar electrode. Saliva elicited with graded stimuli ranging in frequency from 1 to 40 Hz (4-6v) was collected from the cannulated submandibular duct into weighed tubes then frozen for later analyses. Salivary enzyme activities were assayed and expressed as follows: acinar-derived peroxidase as nmols of dichlorofluorescein/min (=1U) released from the substrate 2',7'-dichlorofluorescein; ductal-derived tissue kallikrein (rK1) and tonin (rK2) as nmols of 7-amino-4-trifluoromethylcoumarin (APC)/min released from the substrate D-Val-Leu-Arg-APC in the presence of 0.2mg/ml soya bean trypsin inhibitor and Z-Val-Lys-Lys-Arg-APC in the presence of 1 μ M aprotinin, respectively. Results were expressed as means \pm S.E.M. and compared using Student's t-test following ANOVA. Peroxidase secretion increased with frequency of stimulation reflecting both increases in salivary concentration (0.06 \pm 0.02 U/ml at 1 Hz and 1.95 \pm 0.34 U/ml at 40 Hz) and salivary flow. Salivary rK1 and rK2 concentrations changed insignificantly with increasing frequency. The rK2:rK1 ratio differed from that seen previously in glandular extracts and sympathetic saliva (Shori et al., 1992, Biochem. Pharmacol. 43: 1209) suggesting a non-granular origin. **The results suggest that protein secretion from acinar cells responds differently to higher frequencies of parasympathetic stimulation compared to ductal cells.** (Supported by KMRT)

182 D B FERGUSON (School of Biological Sciences, University of Manchester, UK): Proteins of labial gland saliva.

The concentration of protein in labial gland saliva is 1-3g/L (Ferguson D B, *J dent Res* 70: 670, 1991). The proteins of palatal gland saliva have been separated and some identification attempted (Shiba A, Sano K, Nakao M, Kobayashi K, Igarashi Y, *Arch. Oral Biol* 28: 363-364, 1983) but little is known about labial saliva proteins. A micro-disc-electrophoretic method has been developed using 1 mm diameter acrylamide gels. Parotid saliva (3.75 μ l; 3.5-7 μ g) was applied to the gels and a 5M sucrose solution used to maintain it in position for isoelectric focusing. Single gland drops of labial saliva were collected in capillary tubes and applied directly to the gel surface before covering with sucrose. Staining with Coomassie blue showed two major bands similar to minor components of parotid saliva. Silver staining showed some further weak bands. The major bands may be the same as the two major bands reported in palatal gland saliva by Shiba et al. (1983).

It is concluded that single drops of labial saliva can be analyzed by this micro-modification of disc-electrophoresis to demonstrate the major protein components.

184 S A MITCHELL*, G M HUMPHRIS and G T R LEE (Departments of Clinical Psychology and Clinical Dental Sciences, The University of Liverpool, UK): The development of dental anxiety in child dental patients: A Q-methodological study.

Previous research appears to have shed little light on how dental anxiety develops in children. This study aimed to address socio-cultural factors in child patients' discourses about dental anxiety, using Q-methodology. Three hundred children aged 10-16 years attending two Liverpool inner city schools were screened for dental anxiety and demographic factors. Seven children of various ages (males and females) with high or low dental anxiety were interviewed in semi-structured format. Interviews were all tape recorded, lasted 20-30 min and were transcribed. Two Q-studies focusing on representation and treatment issues were conducted, reflecting the range of views expressed at interview. Various accounts emerged, including one where children express high anxiety on questionnaire but ambivalence at interview (ie a defensive account). Another account shows that strong feelings of shame or embarrassment can be evoked by the dentist's questioning.

It is concluded that children adopt a range of accounts of their dental anxiety or potential to become anxious. It is considered that these accounts may have important implications in later life.

- 185** G. HUMPHRIS (Clinical Psychology Dept., Liverpool University), T. MORRISON (URPCL Ltd., Wirral) and S. J. E. LINDSAY* (Institute of Psychiatry, London): An improved Dental Anxiety Scale.

The investigation aimed to produce a Dental Anxiety Scale with high validity. Our modified version (MDAS) of the Corah Dental Anxiety Scale (CDAS) added a question about oral injections. New multiple choice answers, in clear order of anxiety and the same for each question, were provided. Twenty-five dental personnel all independently confirmed the order of the answers for the MDAS. On the CDAS seven subjects gave one sequence for the answers denoting intermediate anxiety. The other subjects placed them in a different order. Of 1392 dental patients, 13% expressed extreme anxiety about injections on the MDAS but were only "fairly" or less anxious about drilling.

Therefore, the CDAS, unlike the MDAS, could reliably discriminate only extremely high or low dental anxiety and would overlook patients who are very afraid of injections only.

- 186** A N CRAWFORD* and M A LENNON (Department of Clinical Dental Sciences, School of Dentistry, The University of Liverpool, UK): Evaluation of a community based dental service for anxious adults.

Anxiety is a major barrier to attending a dental practice; therefore, a dental support group and anxiety clinic were established within the Community Dental Service in a deprived area of South Manchester. The management regime included behavioural, cognitive and sedative techniques. 48 adults (mean age 36 years) were enrolled for treatment in the clinic, and 41 (85%) completed a course of treatment. Half the participants attended the support group. The mean time since last attendance at the dentist was 7.8 years, and most participants postponed a visit to the dentist even when in pain. The subjects perceived needs at baseline were greater than their normative needs, particularly for extractions and general anaesthesia.

A mean of 4.5 visits were made for each course of treatment, which included on average, 2.9 restorations, one extraction, a scale and polish and preventive advice. Inhalation sedation using N₂O/O₂ was used for 81% of participants. The Corah Dental Anxiety Scale recorded a mean reduction in anxiety score from 16.7 at baseline to 11.6 following treatment ($p < 0.01$). The Dental Beliefs Survey reduced from 49.6 to 28.8. The greatest improvement in attitudes occurred in the areas of behaviour, control and communication.

This service for anxious and non-attending adults in a socially deprived area, resulted in a high rate of completed treatment, a reduction in dental anxiety, and an improvement in attitudes to receiving treatment. The relationship between perceived and normative needs in this group may be the reverse of that in the general population.

- 187** J.G. WHITTLE* and K.W. WHITTLE (Salford & Trafford Health Authority and Salford Community Healthcare NHS Trust, UK): Dental caries in the same schoolchildren at ages 5, 7 and 9.

Three hundred and ten children, who were examined at school in Salford when they were aged 5, were re-examined at ages 7 and 9 using BASCO criteria. The aims of the study were to find out how dental health changed over these four years and how dental services in the city were coping. The mean dmft at 5 was 3.15. At the age of 7 the dmft was 3.74 and the DMFT 0.25 (total 3.99). At the age of 9 the dmft was 3.43 and the DMFT 0.70 (total 4.13). The mean number of decayed teeth, deciduous and permanent, fell from 2.51 at age 5 to 2.43 at age 7 and 1.80 at age 9. When the city was divided geographically similar changes were recorded in all four sections. The Care Index rose from 5% at age 5 to 11% at age 7 and to 17% at age 9. It is concluded that the level of dental disease increases most between ages 5 and 7. However the dental services had reduced the level of active disease and provided more restorative care.

- 188** McCOMBES W* and PINE C (Department of Dental Health, University of Dundee, Scotland UK): Health Education for the Prevention of Recession and Root Caries in Middle-Age.

Increasing numbers of adults are retaining their teeth and wish to have a natural dentition for life (Todd JE and Lader D: *Adult dental health* 1988 UK, HMSO). Recession and root caries become an increasing problem with age, however, to-date most caries-preventive material is targeted at children. Therefore, the overall aim of this study is to develop an appropriate health education package in relation to the prevention of root caries to be used by general dental practitioners in Scotland. In the first part of the study reported here 10 vocational trainees and 2 experienced general dental practitioners from the east of Scotland participated. The knowledge and attitudes of the 12 GDPs was determined using a series of projected slides, questions and discussion. Subsequently, 227 patients aged 35-54 years were examined in the practice surgeries within the same 10 day period. The presence of gingival recession, root surface caries and restorations was recorded. Patients were questioned on their knowledge and attitudes to caries, gingival recession, oral hygiene practices and the presence of sensitivity. Gingival recession was present on at least one tooth in nearly all the patients examined. 34% of those aged 35-44 had untreated caries on root surfaces, with 43% in those aged 45-54. Similarly, restored root surfaces were found for 66% and 72%. Root caries prevalence in patients exhibiting good oral hygiene was 44%, with fair oral hygiene 51% and for those with poor oral hygiene 62%.

In conclusion, the majority of adults were aware of gingival recession but uncertain of appropriate preventive techniques. A high level of gingival recession and history of root lesions was recorded.

- 189** E J KAY and A S BLINKHORN* (University of Manchester): Acceptance of fuzzy logic in restorative treatment decision making.

"Fuzzy logic" is a theory which assists in the acceptance of uncertainties, and their integration into day to day thinking. Fuzzy logic questions the positivist belief in dichotomies which may be expressed as a concept such as "healthy-unhealthy". It postulates that most events, particularly in living organisms, fall into a so-called "grey area". If this multivalent universe is accepted, then treatment decision making becomes an extremely complex process. Good decision making will depend not only upon knowledge but also on how a practitioner integrates experience and information, thereby bringing many other influences into the process. A qualitative study is described which sought to identify issues, other than the extent of pathology, which impinge on a dentist's restorative treatment decision making. Content analysis of in-depth interviews with 20 randomly selected practitioners delineated ten themes of influence on restorative decisions. These were: self-esteem; patient preferences; reputation with colleagues; benefits to patients; benefits to society; benefits to profession; professional responsibility; professional fees/time; ethical conscience. These issues were divided into a taxonomy which relates to patient, profession and practitioner characteristics. The dentists felt that 'ethical conscience' and 'reputation with patients' most influenced their decision making, although self-esteem was highlighted as having a profound influence on the avoidance of false positive decisions.

The study indicates that an understanding of disease processes and available treatment options is an insufficient basis for treatment decision making.

- 190** I G CHESTNUTT* and V I BIRNIE (Department of Adult Dental Care, University of Glasgow, UK): Smoking cessation counselling - a role for the dental profession?

Whilst several studies have investigated the views of North American dentists on providing advice to patients on stopping smoking, the role of British dentists in this area is uncertain. Thus this study aimed: (i) to examine dentists' awareness of the effects of smoking on general and oral health; (ii) to determine their views on counselling patients to give up smoking; (iii) to investigate the extent to which they currently engage in this activity; and (iv) to survey barriers to providing such advice. Data were collected via a postal questionnaire mailed to 587 Scottish dentists, of which 448 (76.3%) were completed and returned. The importance of smoking as a cause of ill health and death was universally acknowledged, and most were aware of the adverse consequences of smoking on the oral cavity. Over half the respondents (245/54.7%) thought dentists had a role in counselling patients to give up smoking and whilst 107 (23.9%) were uncertain, the remaining 95 (21.2%) felt this was outside their remit. Nonetheless, 384 (85.6%) reported that, at least occasionally, they advised patients to quit. Views on counselling were significantly ($p < 0.05$) related to the dentists' experience, recently qualified graduates having a more positive view. Provision of cessation advice was also significantly ($p < 0.001$) influenced by the dentist's own smoking habit. Lack of time was seen as an important barrier to tobacco counselling, as was lack of training. Whilst dentists have positive views on providing smoking cessation advice, further studies are required to determine the most useful strategies or approaches, and to evaluate their effectiveness.

Supported by SOHSD Grant No. K/OPR/15/6/F10

- 191** I D M MACGREGOR (Department of Restorative Dentistry, University of Newcastle upon Tyne, UK): Dental health instruction as an aid to reducing cigarette consumption.

This investigation aims to determine the efficacy of counselling dental patients who smoke to reduce their tobacco consumption on account of its adverse effects on periodontal health. 98 habitual cigarette smokers attending the Newcastle Dental Hospital, who expressed a wish to reduce their cigarette smoking, received smoking reduction counselling with dental health advice and periodontal care. Results showed that those counselled reported a greater reduction in cigarette consumption during treatment and follow-up, compared with 44 control subjects: smokers receiving dental health advice and periodontal care, but no counselling against smoking ($P < 0.001$). 50% of counselled subjects reduced their cigarette consumption by at least one half, compared with 23% of control subjects who spontaneously reduced to this level. The cessation rate in those counselled was 13.4%, compared with 4.5% in control subjects.

It is concluded that advice against smoking combined with dental health instruction can be an effective aid to reducing smoking in dental patients.

- 192** C N KENNETT*, S W COX and B M ELEY (Department of Periodontology, King's College School of Medicine and Dentistry, London, UK): Histochemical, immunocytochemical and biochemical studies of dipeptidyl peptidases in human gingival tissue.

Our earlier biochemical studies using synthetic peptide substrates with the AFC leaving group have shown that gingival tissue homogenates contain dipeptidyl peptidases (DPP) II and IV activities (Cox, S W et al., *Arch Oral Biol* 37: 167-173, 1992). The purpose of the present study was to examine tissue sections for enzyme-containing cells by histochemistry with analogous MNA substrates (and by immunocytochemistry for DPP IV) and also to confirm enzyme identities by biochemical analysis of tissue section extracts. Unfixed cryostat sections of inflamed human gingival tissue were incubated with MNA substrates and Fast Blue B in either acid or alkaline buffers. Adjacent sections were reacted with mouse monoclonal anti-human DPP IV, followed by biotinylated rabbit anti-mouse antibody, streptavidin-biotin-alkaline phosphatase complex and new fuchsin substrate solution. The identity of inflammatory cells containing enzyme was established using the same procedure with mouse monoclonal antibodies directed against CD antigens. Enzymes were extracted from tissue sections with 100 μ l portions of buffer and activities were characterized using fluorometric assays with AFC-linked substrates. Lys-Ala-MNA and Ala-Pro-MNA staining in 0.05 M cacodylate, pH 5.5, was only found in a few fibroblasts. Staining with Gly-Pro-MNA and Ala-Pro-MNA in 0.1 M Tris-HCl, pH 7.7, was localised in a small proportion of CD4 and CD8 positive T cells, CD68 positive monocytes/macrophages, and fibroblasts and this matched staining with the DPP IV antibody. The acid staining could be extracted with 0.1 M MES, pH 6.0, and Lys-Ala-AFC and Ala-Pro-AFC activity in the extracts had a pH optimum of 6.0 and was inhibited strongly by PMSF, puromycin and diisopropyl A. By contrast, extraction of the alkaline staining required 0.1 M Tris-HCl, pH 8.0, containing 0.1 M NaCl and 0.5% Triton and Gly-Pro-AFC and Ala-Pro-AFC activity in the extracts had a pH optimum of 7.0 and was less sensitive to puromycin and PMSF but still reduced significantly by diisopropyl A. The properties of the acid and alkaline extracts were consistent with our previous results with tissue homogenates for DPP II and IV respectively. Thus DPP II was localised in fibroblasts and DPP IV in T cells, monocytes/macrophages and fibroblasts.

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S W COX*, C N KENNETH* and B M ELEY* (Department of Periodontology, King's College School of Medicine and Dentistry, London, UK): Evaluation of the cellular contribution to protease activities in gingival crevicular fluid.

Gingival crevicular fluid (GCF) has been shown to contain several proteases derived from host tissue (Cox S W & Eley B M, *J Period Res* 34: 353-361, 1999; Cox S W et al., *Arch Oral Biol* 37: 187-193, 1992). The aim of this study was to assess the contribution of cell-bound enzymes to measured GCF activity. GCF was collected from chronic periodontitis patients using plastic micropipettes and smeared on to coverslips. The smears were examined for inflammatory cells using antibodies directed against CD4 (T4 lymphocytes), CD8 (T8 lymphocytes), CD28 (B lymphocytes), CD15 (granulocytes), and CD68 (monocytes/macrophages) and by Toluidine Blue staining for mast cells. Trypsase, cathepsin B, elastase, and dipeptidyl peptidases (DPPs) II and IV were localized by immunostaining using antibodies to the human enzymes and by cytochemistry using papainyl DNA substrates with selective uridine acid sequences. GCF was also collected on filter paper strips, eluted into buffer, and protease activities determined biochemically with analogous AFC-linked substrates. Detergent (Triton X-100), sonication, freeze-thawing and centrifugation were tested for their effects on the measured activities. The proportions of different cell types in the GCF smears were 70-80% granulocytes, 10-20% monocytes/macrophages, 5% mast cells and <5% lymphocytes. Trypsase was detected in mast cells, elastase in granulocytes, cathepsin B in macrophages, DPPs II and IV in a small proportion of macrophages, and DPP IV in a few T lymphocytes. Similar numbers of cells were stained with antibody and substrate, except for elastase which was frequently seen by immunofluorescence but rarely by cytochemistry. Biochemically, neither freeze-thawing nor sonication had much influence on protease activities in GCF eluates. However, the addition of Triton increased activities to 140-240% of control values, depending on the enzyme. Centrifugation reduced measured activities to 1-30% of original figures; this effect was less if samples were pre-treated with Triton. Centrifugation presumably removed whole cells, while Triton caused cell lysis. Thus it is concluded that the presence of inflammatory cells in the gingival crevicular fluid makes a significant contribution to measured protease activities in GCF.

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B M ELEY* and S W COX (Periodontal Department, King's College School of Medicine and Dentistry, London, UK): Preliminary comparison of GCF elastase levels and attachment loss in chronic periodontitis.

We have previously shown that elastase is present in gingival crevicular fluid (GCF) and gingival tissue and that GCF elastase levels correlate with clinical indices in untreated chronic periodontitis patients and reduce after treatment. The aim of this study was to find whether GCF elastase correlates with progressive loss of attachment. 25 chronic periodontitis patients received basic periodontal treatment prior to baseline. 30 sec. GCF samples were collected on paper strips from the mesio-buccal site of 16 molar and premolar teeth at baseline and at 3 monthly intervals to 12 months. Following this the attachment level from the occlusal surface and the probing depth was measured with a Florida probe at each site. Sites were assessed as progressive when the attachment level increased 1.5mm or more and this was confirmed at a subsequent visit. GCF was eluted into buffer and frozen prior to analysis. Elastase activity was assessed by fluorometric assay with the substrate MeOSuc-Ala-Ala-Pro-Val-AFC in 0.1 M Tris HCl, pH 7.5 with 1.0 M NaCl. By 12 months, 6 sites in 5 patients progressed rapidly (RP) and 3 sites in 3 patients progressed gradually (GP). Elastase levels at RP sites were compared with those at control sites in the same patient matched at baseline for similar clinical parameters with a paired t-test. The diagnostic sensitivity and specificity were calculated with a critical value of 250µUnits/30sec. During the period of progression, the GCF elastase at RP sites ranged from 254-326µUnits/30sec and at non progressive control sites from 5-66µUnits/30sec. These differences were statistically significant ($p < 0.0005$). GCF elastase levels at GP sites ranged from 137-306µUnits/30sec. The sensitivity was 76% and the specificity 97%.

GCF elastase levels may have diagnostic value in the progression of periodontitis.

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M I GAZI*, S W COX and B M ELEY (Department of Periodontology, King's College School of Medicine and Dentistry, London, UK): Characterization of the proteases produced by periodontopathic bacteria.

Bacterial proteases are thought to play a role in the pathogenesis of periodontal disease. A number of these proteases have been described but only a few fully characterized (Suido H et al., *J Dent Res* 65: 1835-1840, 1986; Pike R et al., *J Biol Chem* 269: 406-411, 1994). The aim of this study was to examine the proteases from some suspected periodontal pathogens and to determine their pH optima, substrate specificities and inhibitor responses in biochemical assays, as well as their isoelectric focusing points by electrophoresis. Molecular weights were also determined for some enzymes by gel filtration chromatography. Bacterial cells were obtained from cultured reference strains and protease activities in sonicates were examined with selective synthetic peptide substrates linked to a fluorogenic detecting group (AFC). Dipeptidyl peptidase activities were found in *Porphyromonas gingivalis*, *Prevotella intermedia*, *Campylobacter* spp. and *Treponema denticola*. *Porphyromonas gingivalis* and *Campylobacter* spp. had separate arginine and lysine endopeptidases, whilst only arginine activities were found in *Prevotella intermedia* and *Treponema denticola*. *Actinobacillus actinomycetemcomitans* had a unique lysylalanine specific activity which differed in some respect from other bacterial proteases. Elastase-like activity was found only in *Campylobacter* spp. whilst chymotrypsin-like activities were found in both *Campylobacter* spp. and *Treponema denticola*. The *Actinobacillus actinomycetemcomitans* enzyme, like the *Campylobacter* elastase, could not be resolved by isoelectric focusing. The *Campylobacter* elastase was partially purified by gel filtration chromatography and its protein pattern was further examined by SDS electrophoresis and silver staining. The characteristics of these bacterial proteases varied between species and differed from corresponding enzymes previously reported in human tissue.

In conclusion, the use of sensitive and selective synthetic peptide substrates allowed the identification and characterization of several proteases from periodontopathic bacteria and this may enable them to be distinguished from human tissue proteases.

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S DIEDERICH*, FP ASHLEY, PY COWARD and RF WILSON (Guy's Hospital Dental School, UMDS, London): *Porphyromonas gingivalis* in subgingival plaque of subjects susceptible or resistant to periodontitis.

The aim of the study was to evaluate, in the presence of equivalent plaque-induced gingival inflammation, any differences in the level of *P. gingivalis* in the subgingival plaque of subjects categorised as susceptible or resistant to chronic periodontitis. Patients were required to be over 40 years old, have at least 20 teeth and bleeding on probing from at least 50% of sites. Eight patients with radiographic bone loss of at least 50% at 75% or more of sites were included in the susceptible group, and eight patients with minimal bone loss were classified as resistant. A pooled subgingival sample was collected on paper points from four sites with gingival inflammation in each subject. In susceptible subjects, the four sites all had minimum probing depths of 6mm. Total counts were determined by dark field microscopy and numbers of *P. gingivalis* by indirect immunofluorescence using a monoclonal antibody. With the exception of one resistant subject, *P. gingivalis* was present in all samples. The mean number of *P. gingivalis* in samples from susceptible and resistant groups was 72.9 (SD = 45.5) and 26.1 (16.0) respectively ($p < 0.05$). The mean percentage of *P. gingivalis* was 4.9 (1.8) in susceptible and 3.1 (1.1) in resistant patients ($p < 0.05$). The results are consistent with *P. gingivalis* having a role in the aetiology of chronic periodontitis but do not rule out the possibility that they are secondary to the destructive disease process.

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F-M EGGERT* and M H McLEOD (Department of Stomatology, University of Alberta, Edmonton, Alberta, Canada T6G 2N8): Detection of *Porphyromonas gingivalis* by immunosensory versus BANA Testing.

Bacteria associated with periodontal lesions can be demonstrated by non-selective monitoring of proteolytic activity via the hydrolysis of BANA (N-benzy-DL-arginine-sulphamide; PerioScan®, Oral B Inc, Canada) or by highly selective monitoring of bacterial antigens via an immunosensory (Evalue®; Kodak Inc, Canada). We wished to compare these two methods of demonstrating bacteria in samples from periodontal lesions affecting patients in private dental practices. We obtained both PerioScan® and Evalue® samples for 41 sites in 19 patients in general practice (30, 10m, aged 14-77), each of these sites was sampled on two separate occasions; for 16 sites in 8 patients in a periodontal practice (46, 4m, aged 36-64) and for 66 sites for 32 patients in another periodontal practice (20, 12m, aged 16-64). We had a total of 164 samples for which results were available using both methods of testing. Our results showed that 25% of sites were strongly positive for proteolysis (detected by PerioScan®) but negative for *Porphyromonas gingivalis* (detected by Evalue®). This result was the major reason why we could not demonstrate a statistically-significant association between the presence of *P. gingivalis* and the presence of proteolysis at a site. No sites that were positive for *P. gingivalis* were negative for proteolysis. Approximately 10% of sites were negative for both assays and approximately 14% of sites were strongly positive for both assays.

We conclude that: 1) *P. gingivalis* is not the predominant proteolytic organism at many periodontal sites. 2) The presence of *P. gingivalis* at a site is accompanied by proteolysis. 3) An immunosensory such as Evalue® provides selective microbiological information about bacterial species at a site, while a proteolytic assay such as PerioScan® provides non-selective microbiological information. 4) Interpretation of a proteolytic assay requires additional microbiological information.

Supported in part by the Faculty of Dentistry, University of Alberta.
PerioScan® provided by Oral B Inc, Canada.

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M A KERR*, S MCKENZIE* and A D GILBERT* (Dept Dental Surgery and Periodontology, Dundee Pathology, University of Dundee, UK): Measurement of Respiratory Burst Induced in Murine Polymorphonuclear Neutrophils by Murine IgA.

This investigation aims at determining firstly whether murine polymorphonuclear neutrophils (PMNs) have an immunoglobulin class A (IgA) receptor and whether such a receptor is the same as or closely related to the human IgA receptor.

Murine PMNs were obtained from a ten week old BALB/c male mouse according to the method of Gallin et al (Gallin J I, Patten E, Woff H S M, *Blood* 43:201-206, 1994). The animal was sacrificed by carbon dioxide inhalation. Murine PMNs were isolated from heparinized blood from healthy volunteers by density centrifugation. Microtitre plates were coated in murine and human IgA and human IgG (150µl of 1 in 200 dilution in Phosphate Buffered Saline). After plate washing luciferin or luminol (100µl of 0.1mg/ml in Hanks Balanced Salt Solution/Bovine Serum Albumin) was added to each well, followed by addition of either mouse or human PMN's (50µl of 10⁶/ml). The resultant chemiluminescence was read every five minutes.

Results showed that murine IgA stimulated a respiratory burst in mouse neutrophils only and that the magnitude of the burst was greater using luciferin than luminol.

It is concluded that mouse PMN's have an IgA receptor which, though not identical to the human IgA receptor, triggers similar events. It is also concluded that O₂ was produced as a result of the murine respiratory burst due to the greater effect of luciferin.

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J HOLT¹*, C ALLISON¹, C L WILLIS² and G R GIBSON² (Unilever Dental Res. Belington, UK ¹ MRC Dunn Clin. Nutr. Cent. Cambridge²): Identification of disassimilatory sulphate reduction in the human oral cavity.

A study was undertaken to enumerate sulphate reducing bacteria (SRB) and rates of sulphate reduction in bacterial populations isolated from different regions of the human oral cavity. Twelve healthy subjects were sampled for plaque at six different sites; posterior tongue, anterior tongue, lower buccal mucosa, upper vestibular mucosa, supragingival plaque and subgingival plaque. Samples were subjected to analysis for the presence and number of SRB as well as the rate of sulphate reduction in the presence of ³⁵S radiolabelled sulphate. Results showed that 83% (10/12) of subjects harboured SRB and consequently significant levels of sulphate reduction were detected in these individuals. SRB counts in different regions of the mouth of SRB-positive individuals showed that these bacteria were present throughout the oral cavity with highest numbers generally being found in supragingival and tongue samples (range 10⁴-10⁶ cfu/g wet wt. plaque). Sulphate reduction rates were also relatively high in these areas for most individuals. The numerically predominant SRB species cultured in this study were identified as *Desulfovibrio* spp.

These data indicate the presence of populations of hydrogen utilising bacteria in the oral cavity which may play an important role in plaque physiology.

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B. SHEARER*, M. GOUGH and D. SETCHELL (Department of Conservative Dentistry, Eastman Dental Institute, University of London UK) Fit of In-Ceram crowns: Influence of margin type and porcelain firing.

This investigation aimed to assess the fit of In-Ceram¹ crowns made upon either chamfer or shoulder margins, before and after porcelain addition. An upper central incisor [Columbia Demoform] was prepared with a 120° chamfer and subsequently with a shoulder margin. Twenty dies were made from this using a poly vinyl siloxane impression for each preparation type. Forty crowns cemented with a glass ionomer cement were sectioned on their respective silver dies. In addition eighty poly vinyl siloxane cement analogues were taken before and after porcelain addition, embedded and sectioned. All samples were cemented under a 5Kg load and measurements were made at the absolute margin with the Reflex Microscope² in X and Y co-ordinates. Marginal fit was found to range from 1 µm to 63 µm with an overall mean of 19 µm. No significant differences were found between margin types or before and after porcelain addition.

It was concluded that Inceram crowns fit well and that marginal type and porcelain addition did not alter the fit.

¹In-Ceram, Vita Zahnfabrik, Germany

²Reflex Microscope, Reflex Measurement, Somerset UK

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M. ABOUR BASHIR, P. O'NEILLY*, D.J. SETCHELL (Department of Conservative Dentistry, Eastman Dental Institute, UK): Accuracy, detail and hardness of casts from alginate disinfected with 4.65% NaOCl.

The dimensional accuracy, surface hardness and reproduction of surface detail of stone casts produced from alginate impressions treated with 4.65% sodium hypochlorite disinfectant solution were investigated. To test dimensional accuracy, a cast of a maxillary dentate arch served as a master model. A Reflex Microscope¹ was used to compare casts from 10 untreated impressions with casts from 10 impressions immersed in the solution for 30 mins. Surface detail and hardness were investigated on casts produced from impressions of a master stainless steel plate 4 cm in diameter with 10 micro-indentations of increasing size. 30 alginate impressions were divided into 6 groups of 5. One group was not treated (control) and one group of the remaining 5 groups immersed for 5, 10, 15 and 20 mins respectively. Surface detail was assessed by observation and surface hardness by a Vickers Hardness Machine. The dimensional accuracy test showed no significant differences between the experimental and control groups. Surface detail was unaffected after immersion of up to 10 mins. Surface hardness decreased linearly with respect to immersion time, when this was greater than 5 mins, there was a statistically significant difference in hardness with respect to the control.

It is concluded that the immersion of alginate impressions in 4.65% sodium hypochlorite for up to 5 mins (consistent with disinfection) had no detrimental effect on the accuracy, surface detail or surface hardness of the resultant cast.

¹Reflex Microscope, Reflex Measurement Ltd, Somerset, BA6 86P.

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J G ROBINSON* and J F MCCABE (Dental School, University of Newcastle, U.K.): Displacement rheometry of acrylic resin denture base materials.

Current methods for determining the doughing and working times of acrylic resin denture base materials are empirical. The dough time is often described as "when the mix leaves the mixing pot cleanly" or "when the dough has reached the snappy stage". Displacement rheometry (Abuasi, H A et al J Dent 21:360-366) can be used to follow the development of elastic properties in denture base material and this effect can be displayed as the % recovery in the dough following the application of a measured displacement (0.25mm). Two readily available denture resins were investigated, standard heat cure¹ and impact modified heat cure². Five trials were carried out on each material and it was found that the dough time, when 10% recovery had developed, was in the range 24 - 27 mins. The time taken to reach the end of the working time, when 50% recovery had developed, was in the range 32 - 35 mins giving a working time of 5 - 10 mins. *It is concluded that displacement rheometry may be used to determine the doughing and working times of acrylic denture base materials.*

¹Acron standard, Austenal Dental Products

²Lucitone 199, Dentsply

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A C MURPHY* and R G HILL (Dept. of Materials Science and Technology, University of Limerick, Ireland): Fracture Toughness of Tooth Acrylic.

Presently, acrylic teeth are prone to abrasive wear and fracture as manifested by chipping of the incisal edges. The optimization of the fracture toughness of tooth acrylics, as used in the construction of artificial teeth, is an important method in improving the wear resistance. In this work, the influence of variations in the crosslinking agent content and curing conditions on the fracture toughness of tooth acrylic was examined. In addition, the effect of using a polymer bead, based on an interpenetrating polymer network (IPN), was investigated. The crosslinking agent, ethylene glycol dimethacrylate (EGDM), percentage was increased from 0% to 20%, the curing time was varied between 15 min and 240 min and the curing temperature varied between 70°C and 150°C. Double torsion tests were used to determine the fracture toughness. Prolonged cure time, increased crosslinking content and cure temperature all resulted in a slight increase in the fracture toughness although significant results were only obtained when 20% EGDM was used, here the crack moved to stick slip failure mode. The IPN material exhibited an increased fracture toughness due to crack deflection around the beads.

Curing time and temperature have minimal effect on the fracture toughness and the EGDM content has little influence except at high concentrations. IPN acrylics exhibit a small but significant increase in fracture toughness.

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PETER KURER, Private Practitioner, Hale, Cheshire, U.K.: "Posts rarely fall out or split roots, what else can go wrong?"

Now that retention and stress distribution of posts is considerably improved, (Reumpling D.R. J.P.D. 41:2 159-167 1979) (Standlee J.P. O.O.O. 33:8 959-960 1972) posts coming loose or splitting roots is much diminished. The aim of this study was to discover the remaining causes of post failures. Using the records of every cylindrical post¹ placed by the author over a period exceeding 25 years, with radiographic and clinical verifications and photographic confirmation, a league table of the remaining failures evolve. Results: When this post system is used, secondary caries is the greatest failure of the post crown restorations in excess of the other 4 definable groups.

Over stress from loss of other teeth, over stress from differential wear of porcelain and enamel, denture stabilising post restorations and change of use from a single post crown to a bridge abutment are the other definable failure categories. ¹Kurer K4, Prestige Dental. *It is concluded that secondary caries is now the greatest cause of failure of post restorations, when using this cylindrical threaded post system. This cause of failure is greater than the other 4 groups specified. Awareness of these enables the practitioner with co-operation of the patient, to reduce the failure rate still further.*

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G. WOOLLEY, S.E. NORTHEAST* and R. VAN NOORT (Department of Restorative Dentistry, University of Sheffield, Sheffield S10 2TA) Torsional and tensile resistance of cemented vs resin bonded cast NiCr posts.

This study compared the resistance to torsional and tensile loading of cemented or resin bonded cast post-retained cores. Post holes 9mm deep x 1.5mm diameter were prepared in 100 single rooted extracted human premolar teeth and randomly assigned to 5 groups of 20. An anti rotation keyway 2mm x 1mm wide was cut with a no 701 bur in two of the groups. Identical casting patterns were prepared using parallel-sided, serrated plastic posts and cores¹ and cast in NiCr alloy². Castings were grit blasted with 50µm alumina and immediately cemented with: Group 1, Zinc phosphate cement³; 2, glass-ionomer cement⁴; 3, dentine bonded⁵ resin composite⁶; Groups 4 and 5 as for 1 and 3 respectively but with an anti rotation keyway. Half the samples in each group were tested in pure tensile mode at a crosshead speed of 3mm/min. Torsional loads were applied 150mm from the long axis to the remaining samples using a beam secured perpendicular to the core. Torsional data is normalised to a lever arm distance of 3.5mm representing the average radius of premolar tooth crowns. Mean torsion at failure (Newtons) was 30.4±6.3 (Gr1), 51.3*±14.9 (2), 75.9*±8.0 (3), 177*±29.4 (4), 231*±16.5 (* significant differences p<0.05, Tukey's test). Mean tensile load at failure (N) was 424±56.9 (1), 284±79 (2), 625±66.3 (3), 511±57 (4), 579±49 (5) (* significance p<0.001, Tukey's test). *Resin composite dentine bonded posts produced significantly more resistance to displacement under either of the loading conditions.* ¹OPO posts Optident West Yorkshire. ²Talladum V Talladum UK. ³De Trey Zinc Dentistry, UK. ⁴Aqua Cent Dentistry, UK. ⁵Permagen Ultradent products, US. ⁶Paravita Hex Kuraray, Japan.

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L J HENDERSON (Dental School, University of Western Australia): Dentine adhesion - a comparison of different testing methods

The aim of this study was to compare a destructive (shear bond testing) and a non-destructive method (acoustic microscopy) of testing the dentine/material bond. Ten molar teeth were sectioned to expose the maximum amount of occlusal dentine. With the dentine surface exposed the teeth were embedded in epoxy resin and highly polished. Polycarbonate rings (internal diameter 6.6mm) were positioned on the exposed dentine and 10 dentine adhesive/composite¹ restorations were placed inside the rings. With 5 specimens maximum adhesion was attempted and with 5 specimens maximum adhesion was not attempted. The bonded area was tested with a modified acoustic microscope to give values for the quantity of bonding (expressed as a percentage of total area available) and the quality of bonding [measured on a scale of O (high) - 125 (low)] prior to shear testing (Shimadzu Corporation - Japan). A wide range of results was obtained: Force (0.43-5.07 MPa), bonded area (15.4-99.9%) and bond quality (17-108). Multiple linear regression was used to relate shear force to the quality/quantity of the bond ($R^2 = 0.80$).

It is concluded that a high correlation exists between shear force (bond strength) and the bond quality and quantity readings obtained using the modified acoustic microscope.

¹Pentac Universal Bond/Pentac Hybrid - Espe, Germany (Supported by the Rankine Bequest)

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P BAIDYA*, J G MEECHAN and J F MCCABE (The Dental School, University of Newcastle upon Tyne, UK): Bonding of composite to bone: durability and effect of drying time.

This study investigated the effect of bone drying time on the shear bond strength obtained between composite and bone at 1 day, 1 week and 6 weeks, using All-Bond 2¹ dentine-bonding agent. Sixty samples of fresh pig calvarium were embedded in polyester resin with the cortical surface uppermost and lapped with 100 grit carborundum paper under water irrigation. Samples were divided into 6 groups of 10. After 24 hours storage, at 37°C in 0.5% aqueous chloramine solution, the bone was roughened with a surgical bur before drying (for 1 second or 60 seconds) and application of the adhesive. P50² was bonded to the samples using All-Bond 2 following the manufacturer's instructions for use on dentine with the exception that acid-conditioning was omitted. Samples were stored in solution (saline 0.9% and chloramine 0.5%) at 37°C for 24 hours (Group A), 1 week (Group B) and 6 weeks (Group C) before shear bond testing on a Universal Testing Machine at a crosshead speed of 1mm/min. The mean (±s.d) shear bond strengths (MPa) were as follows: Group A(1) 9.6±5.8, A(60) 10.1±4.4, Group B(1) 9.2±5.4, B(60) 10.5±4.0, and Group C(1) 6.9±6.3, C(60) 5.8±2.8 respectively, where number in brackets is drying time in seconds. Data were subjected to ANOVA; for the 1 second drying time F=0.62, p=0.54; for the 60 seconds drying time F=4.67, p<0.02. *It is concluded that drying time significantly affects the durability of the bond between composite and bone.*

¹Bisco, Itasca, Illinois. ²3M, St. Paul, MN, USA.

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M RIGGIO*, D MacKENZIE, A LENNON, T W MacFARLANE and D KINANE (University of Glasgow Dental School, Glasgow, U.K.): Identification of periodontal pathogens in subgingival plaque by PCR and culture methods.

In this study the major periodontal pathogens *Actinobacillus actinomycetemcomitans* (Aa) and *Porphyromonas gingivalis* (Pg) were identified in subgingival plaque samples by polymerase chain reaction (PCR) and conventional culture methods. The PCR primers for detecting Aa and Pg were derived from the leukotoxin A and fimbria genes, respectively, and have previously been shown to be highly specific (Goncharoff P, Figurski D.H, Stevens R.H and Fine D.H, *Oral Microbiol Immunol* 8: 105-110, 1993; Watanabe K and Frommel T.O, *J Dent Res* 72: 1040-1044, 1993). Of 170 samples analysed, PCR identified Aa in 40 (24%) and Pg in 40 (24%) of the samples. By comparison, conventional culture methods identified Aa in only 25 (15%) and Pg in only 19 (11%) of the samples. Additionally, only 5 samples harboured both Aa and Pg.

It is concluded that PCR is more sensitive than conventional culture methods for identifying Aa and Pg in subgingival plaque samples.

- 209** BPPA GOMES¹, JD LILLEY and DB DRUCKER (University Dental Hospital of Manchester, UK): Recovery of *Porphyromonas endodontalis* and *P. gingivalis* by two different sampling techniques.

Different sampling techniques for bacterial recovery from root canals and periodontal tissues have been used over the years. The paper point has proved to be a reliable and reproducible means of sampling. The aim of this study was to compare the recovery of *P. endodontalis* (*P.e.*) and *P. gingivalis* (*P.g.*) using the direct inoculation of the paper point a) onto pre-reduced solid media with b) into a reduced transport fluid (RTF). Dilutions of *P.e.* and *P.g.* suspensions in 0.85% sterile saline were sampled anaerobically. Paper points were introduced into each of the dilutions for 1 min. and then inoculated onto the pre-reduced FAA agar plates. Testing the RTF was by inoculating separate paper points in each dilution for 1 min. and transferring to tubes with 1 ml of RTF. These tubes were agitated ultrasonically for 10 sec. Serial dilutions were also performed using reduced RTF, and 0.05 ml of each was inoculated onto pre-reduced FAA agar plates. Bacterial growth was obtained after 5-7 days. *P.e.* was recovered from the standard suspension and from 10^1 dilution using the direct inoculation of the plates, but only from non-diluted suspension using RTF. *P.g.* was recovered from the standard suspension up to 10^3 dilution using the direct inoculation, and from standard suspension and 10^1 dilution using the RTF. It is concluded that recovery of *P.e.* and *P.g.* is possible by either technique. However, the concentration of these organisms in the sample is critical to their isolation and hence to their recognition in the clinical situation.

- 211** K REDDI^{1,2*}, S POOLE³, S NAIR^{1,2}, S MEGHJI¹, B HENDERSON³ and WILSON¹ (Depts. Microbiology, Maxillofacial Surgery and Endocrinology, ¹EDI, ²NIBSC, London): IL-6 inducing activity of periodontopathogenic Lipid-A-associated proteins.

The aim of this study was to determine whether Lipid-A-associated proteins (LAP) from *Porphyromonas gingivalis* W30 and *Prevotella intermedia* NCTC 9336 could stimulate human gingival fibroblasts (hgf) or a human myelomonocytic cell line (Mono-Mac-6) to release Interleukin-6 (IL-6). Endotoxin was extracted from each of the bacteria by butanol extraction (Morrison and Leive, *J. Biol. Chem.* 258:2911-2919) and the LAPs and lipopolysaccharides (LPS) extracted from these by the hot phenol-water method of Westphal and Jann (*Meth Carbohydr Chem.* 5:83-91). Release of IL-6 in response to LAP or LPS was determined as follows. Cells were seeded in microtitre plates, cultured to confluency and incubated overnight with various concentrations of LAP or LPS (10ng-10µg/ml). The IL-6 released into the media was measured by an ELISA. Both LAP and LPS from *Por. gingivalis* stimulated IL-6 release from hgf in a dose-dependent manner over the concentration range 10ng-10µg/ml. LPS was less potent than LAP at stimulating release of IL-6. Both LPS and LAP from *Pr. intermedia* failed to stimulate hgf to release IL-6. LAPs and LPSs from both organisms stimulated Mono-Mac-6 cells to release IL-6 in a concentration-dependent manner. Heat and trypsin-treated LAPs failed to stimulate cells to release IL-6, confirming that the active components were proteinaceous.

In conclusion LAPs from Por. gingivalis and Pr. intermedia were able to stimulate Mono-Mac-6 cells to release IL-6, whereas only LAP from Por. gingivalis was able to stimulate hgf to release IL-6. LPSs from the two organisms were less potent stimulators of IL-6 release. Stimulation of cytokine release by LAPs may be relevant to the pathogenesis of chronic periodontitis.

- 213** JM RANGARAJAN^{*}, S SMITH and MA CURTIS (MRC Molecular Pathogenesis Group, Department of Oral Microbiology, LHM, London E1 2AA, UK): Three isoforms of protease specific for arg-x bonds from *Porphyromonas gingivalis* W50.

Proteases of *P. gingivalis* are thought to be important virulence determinants in adult periodontal disease through their ability to subvert host defences and deregulate the inflammatory response. We have purified and characterized three proteases with arg-x cleaving activity from culture supernatants of *P. gingivalis* W50, denoted as ArgI, ArgIA and ArgIB which appear to be products of a single gene. The aim of this study was to determine the structural and kinetic properties of the three forms by chromatographic, N-terminal sequence analysis of the purified proteins, and immunochromatographic techniques. ArgI is a heterodimer which is composed of the protease monomer (α) and a hemagglutinin monomer (β) whereas ArgIA and ArgIB are monomers containing only the α subunit with different amounts of covalently attached LPS^{*}. ArgI has the unique ability to bind to arginine affinity resins whereas IA and IB are not retained by these resins. The ability of ArgI to bind to affinity gels is abolished if the hemagglutinin (β) moiety undergoes proteolysis. The behaviour of the three enzymes on ion-exchange resins suggests that the dimer has a much higher pI than IA and IB. The two latter enzymes appear to have comparable pIs in spite of the presence of LPS in ArgIB which requires the presence of detergents to maintain solubility during the course of its purification. Protease activity is associated only with the α subunit.

However, despite these major structural differences, the three enzymes show almost identical enzymatic properties, kinetic parameters and substrate specificity.

*LPS = Lipopolysaccharide.

- 215** MA CURTIS^{*}, J ADUSE-OPOKU and S K WOODWARD^{*} (MRC Mol. Path. Group, Dept Oral Micro, LHM, London & ^{*}Physical Biochemistry, NIMR, Mill Hill, London): Baculovirus-mediated expression of the adhesin domain of the ArgI protease of *P. gingivalis*.

ArgI of *P. gingivalis* is composed of an α chain which carries the protease active site and a β chain which resembles adhesins of other micro-organisms at the primary sequence level. The α and β chains are contiguous on the initial translation product of the ArgI gene, *pprRI*. Studies using monoclonal antibodies have demonstrated that the β chain contains epitopes which are involved in the hemagglutination reaction of *P. gingivalis* whole cells and also that proteins bearing these epitopes are expressed by the bacterium *in vivo* and are targets of the host's specific immune response. The aim of this work was the development of an expression system for the production of recombinant β chain of ArgI to examine the biochemical and immunological properties of this protein. An internal fragment of *pprRI*, corresponding to residues 784-1132, within the β region, was originally cloned in pUC18 in *E. coli* and was then transferred into baculovirus transfer vectors, pBacPAK9 and pAcGT1, which would permit the expression of the β chain either alone or as a glutathione-S-transferase fusion protein incorporating a Factor Xa cleavage site. *Spodoptera frugiperda* (SF9) insect cells were then co-transfected with each transfer plasmid and *Autographa californica* viral DNA. Recombinant viruses were isolated via plaque assays of the co-transfection culture supernatants and screened via Western blotting of infected SF9 cells. Yields of recombinant protein in the mg/litre range were generated by both viruses.

Baculovirus-mediated protein expression in SF9 cells provides a suitable system for the production of recombinant β chain of ArgI of *P. gingivalis*.

- 210** J MOONEY, E ADONOGIANAKI, K TAKAHASHI, A HAERIAN, DF KINANE (Infection & Immunity Group, Univ. of Glasgow, UK): Initial serum antibody titre to *Porphyromonas gingivalis* influences therapy outcome in periodontitis.

The aim of this study was to assess the effect of periodontal therapy on specific serum antibody concentration, expressed as titre, and antibody binding strength, expressed as relative avidity. The immune responses to *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* were investigated. Titre of antibody was assayed by ELISA and relative avidity was measured by thiocyanate elution in seventeen adult periodontitis patients before and after therapy. IgG avidities (expressed as thiocyanate molarity) to *Porphyromonas gingivalis* increased from 1.01M to 1.38M ($p=0.05$) and IgA titres (expressed as ELISA units) increased from 80EU to 237EU ($p=0.012$). More specifically, when patients were dichotomized into sub-groups which had originally been either IgG seropositive (i.e. having an IgG titre to this organism > 2 X control median) or seronegative for *Porphyromonas gingivalis*, only patients who were initially seropositive showed a significant increase in antibody avidity ($p=0.028$, mean difference=0.09M). Patients who were originally seropositive in terms of IgG and IgA titre to *Porphyromonas gingivalis* had demonstrably better treatment outcomes in terms of a reduced number of deep pockets and sites which bled on probing ($p=0.05$).

These findings suggest that periodontal therapy affects the magnitude and quality of the humoral immune response to suspected periodontopathogens, that this effect is dependent on initial seropositivity, and that initial seropositivity may have a bearing on treatment outcome.

- 212** J M FLETCHER^{1*}, B HENDERSON² and M WILSON¹ (Depts. of Microbiology and Maxillofacial Surgery, EDI, London, UK): Tyrosine phosphatase activity of oral bacteria.

Several oral bacteria have been shown to produce acid phosphatases. In addition to a role in nutrition, it has been suggested that these enzymes may also contribute to the pathogenesis of disease. In eukaryotic cells tyrosine phosphorylation is a key signal in cellular activation by many agonists. Phosphorylation is tightly controlled by protein kinases and protein phosphatases. The aim of this study was to determine whether the phosphatases produced by certain oral bacteria could dephosphorylate phosphotyrosine both as a free amino acid and when incorporated into short peptides. 7 oral bacterial species were assayed for their ability to hydrolyse free phosphotyrosine. Saline suspensions of the organisms were incubated with phosphotyrosine at pH 4.8 for 3h at 37°C. Release of free phosphate was measured colorimetrically. Dephosphorylation of tyrosine residues on short peptides from human gastrin by *Pr. nigrescens* was determined by ELISA^{*}. Only *Pr. nigrescens* and *A. actinomycetemcomitans* demonstrated hydrolysis of free phosphotyrosine releasing 4.09 ± 0.07 and 1.61 ± 0.08 nmoles of phosphate/min/mg bacterial protein respectively. *Pr. nigrescens* was found to hydrolyse 6.69 ± 1.55 pmoles of phosphorylated peptide/min/mg bacterial protein.

In conclusion Pr. nigrescens and A. actinomycetemcomitans were shown to produce significant amounts of acid phosphatase which could hydrolyse free phosphotyrosine. In addition the phosphatase of Pr. nigrescens was able to dephosphorylate tyrosine residues within short peptides.

¹Boehringer Mannheim

- 214** J ADUSE-OPOKU^{*}, J M SLANEY and MA CURTIS (MRC Mol. Path. Group, Dept Oral Micro, LHM, London E1 2AA): ArgI protease of *P. gingivalis* W50 contains an adhesin domain which is common to three serotype products.

P. gingivalis W50 produces an extracellular arginine-specific protease, ArgI, which is synthesised as a precursor, PprRI (1526aa), with large N- and C-terminal flanking regions. Processing of PprRI generates not only the free monomeric protease (α region) but also a heterodimeric form in which the protease is associated with a 50kDa polypeptide derived from the C-terminus of the precursor (β region). DNA sequence analysis and monoclonal antibody studies suggest that the β region is involved in adhesion/binding processes. In the present report, Southern hybridisation analyses and recombinant protein immunoreactivity studies demonstrated that the β region of PprRI shares considerable antigenic and sequence similarity to at least two other gene products of *P. gingivalis*. One of these homologous genes was cloned on a 6kb insert in pUC18 (pJM7). Nucleotide sequence analysis of pJM7 revealed a complete open reading frame of 3219bp with coding potential for a protein of M_r 118k (1097aa) which was in close agreement with the experimentally determined size of the recombinant product. Residues 305-766 of the translated sequence were 97.9% identical to residues 918-1383 within the β region of PprRI. In contrast to PprRI however, the N-terminus of the pJM7 translated sequence showed significant similarity to iron/barn receptors of other micro-organisms. We have consequently named this gene *barn binding*, *gibbsin* (*hbo*).

On the basis of their antigenic and sequence relatedness we propose that these gene products comprise a family of *P. gingivalis* proteins with common biochemical and physiological properties relating to binding and which are likely to have been derived from a common ancestor.

- 216** M HAYES (Department of Biochemistry, University of Bristol, U.K.): The effects of anticalculus dentifrices on seeded crystal growth of calculus.

This work was to test the effectiveness of anticalculus dentifrices using human supragingival calculus. This had an inorganic content of 45% (w/w) and a Ca:P molar ratio of 1.45. Crushed calculus (<150µm) was suspended in a calcium-phosphate-fluoride-KCl solution, pH 7.4, and crystal growth measured as H⁺ ion production in a pH-stat using KOH as the titrant.

Crystal growth was stimulated by fluoride and inhibited by pyrophosphate and by water extracts of fluoride dentifrices containing either pyrophosphate or zinc citrate. Prior removal of organic material from calculus with 1M NaOH also inhibited. In contrast, crystal growth was stimulated by pretreatment of calculus with whole saliva, followed by washing to remove unbound material. Similar pretreatments with the dentifrice extracts showed that NaF but not mono-fluorophosphate stimulated growth and that pyrophosphate was a more effective inhibitor than was zinc citrate. However, pretreatment of calculus with saliva-dentifrice mixtures collected during toothbrushing showed equal inhibition in the presence of saliva. It is concluded that crystal growth was enhanced by an organic surface layer and that pyrophosphate and zinc citrate were retained and inhibited at this surface.

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J. OKAZAKI*, G. EMBERY, R.C. HALL, D.T. HUGHES-WASSALL, R.J. WADDINGTON (Dept. Basic Dental Science, Dental School, Cardiff): Adsorption of glycosaminoglycans to hydroxyapatite

Proteoglycans are known to play an important role in the mineralisation process, acting either as promoters or inhibitors. In this study the binding affinity of a variety of constituent glycosaminoglycan (GAG) to hydroxyapatite (HAP) was studied. GAG (10-1000 µg/ml) in 0.02M sodium acetate (pH 6.8) were constantly circulated through a HAP column (Bio-Rad) for 1 hr. Unbound GAG was removed using the same sodium acetate buffer and the total amount of GAG bound was determined by dimethyl blue assay. The relative affinities of the different GAG remaining bound to HAP was investigated by examining their release in a 0-1M sodium phosphate gradient. Binding isotherms were constructed for chondroitin 4 sulphate (C4S), dermatan sulphate (DS) and chondroitin 6 sulphate (C6S) and were indicative of Langmuir type adsorption. Differences were noted between the desorption profiles of C4S, DS and C6S. With each GAG a number of elution peaks were identified. A higher ionic strength was required to elute the DS species.

These findings suggest the presence of a variety of binding forms of each GAG or the differing orientation of these forms to yield different complexes with HAP. The Ca^{2+} co-ordinates of the GAGs are known to vary and may in part explain these findings.

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SG REES*, RJ WADDINGTON, G EMBERY (Dept. of Basic Dental Science, Cardiff Dental School, UK): Characterisation of proteoglycans from sheep alveolar bone.

The role of alveolar bone proteoglycans (PG) has not been described in detail although their importance as markers of tissue activity in crevicular fluid and in the mineralisation process has been implicated. To obtain sufficient PG for domain-recognition, Ca^{2+} binding and apatite interaction, sheep alveolar bone has been used as a model system. PG were extracted from EDTA demineralised sheep alveolar bone under dissociative conditions using 4M guanidinium chloride in the presence of protease inhibitors. The extracts were purified by anion-exchange chromatography on Q-Sepharose, using a step-wise salt gradient and again on a Resource-Q column with FPLC. PG rich fractions were analysed for GAG, protein, amino acid composition and molecular size. Chondroitin sulphate was found to be the predominant GAG, representing $16.5 \pm 4.1\%$ of the PG molecule. Examination by SDS-PAGE identified two chondroitin sulphate species with the molecular weights of 76 and 59 kDa respectively. The core proteins had molecular weights of 49 kDa for both PG. Western blotting with monoclonal antibody CS-56 confirmed the presence of chondroitin sulphate. Fourier-transformed infra-red spectroscopy identified the sulphate isomers of chondroitin 4- and 6- sulphate, the 4-sulphate isomer predominating. Amino acid analysis showed the PG to be rich in aspartate, glutamate, glycine and leucine, but significantly low in cysteine and methionine. An isoelectric point of 3.9-4.3 was confirmed for the protein core. Similar biochemical data has previously been obtained for human alveolar bone PG and confirms the value of a sheep model in future determination of the biological roles played by PG within extracellular matrices, in particular mineralisation.

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M. J. SHERRATT, C. M. KIELTY, C. A. SHUTTLEWORTH* (School of Biological Sciences, University of Manchester): Fibrillin synthesis by foetal bovine skeletal cell lines.

Marfan syndrome, a heritable connective tissue disorder characterised by skeletal, cardiovascular and ocular abnormalities, has been shown to be linked to mutations in the glycoprotein fibrillin. Despite the skeletal abnormalities found in this condition no biological role for fibrillin in bone has been found. Our investigations are concerned with elucidating the function of fibrillin in skeletal tissues, and this report examines the ability of a variety of skeletal cells to synthesise and secrete fibrillin. Bone-derived and cartilage-derived cells exhibited characteristic morphologies and different mineralising capacities. Both osteoblastic and chondrocytic cells expressed fibrillin 15, although Northern blot analysis indicated more in the former. In osteoblastic cells there appeared to be an increase through foetal development. The ability of cells to synthesise and secrete fibrillin was studied by radioactive labelling and immunoprecipitation. All cell types synthesised fibrillin, evident as a 300 kDa component on discontinuous SDS-PAGE gels. The majority of the fibrillin was associated with the cell layer, and rotary shadowing showed that this had polymerised and was present as characteristic 10-14 nm beaded microfibrils. These experiments show that osteoblasts and chondrocytes can synthesise and assemble fibrillin microfibrils, and that this synthesis takes place during bone formation and highlights the potential importance of this molecule to skeletal development.

This work was supported by the Arthritis and Rheumatism Council.

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F S L WONG*, J C ELLIOTT, P ANDERSON and G R DAVIS (Dept of Child Dental Health, Lond Hosp Med Coll, UK): Three dimensional mineral distribution in the dentine of a rat incisor measured by x-ray microtomography.

The aim was to non-destructively determine the mineral concentration (C_m) in dentine along a developing rat incisor using laboratory x-ray microtomography (XMT). From a position where enamel was initially formed towards the incisal edge, fifty-five serial transverse XMT slices (100 µm apart) were taken along the lower left incisor from a 12 week old rat. The C_m in dentine (assumed to be $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ and protein) was determined from the measured linear attenuation coefficients ($\text{AgK}\alpha$, 22.1 keV) in the XMT image. In each slice, the dentine on the enamel side was more mineralized than the dentine on the cementum side. A band of peripheral dentine (extending ~0.16 mm from the amelodentinal and ~0.02 mm from cementodentinal junctions) had a higher C_m than the remaining dentine. Unlike the enamel, the C_m for the peripheral band and the remaining dentine (1.5 and 1.3 g cm⁻³ respectively) varied very little along the length of the incisor. The area of dentine increased 1.8-fold on the enamel side and 2.8-fold on the cementum side from the first to the last slice, giving a total increase in area from 0.69 to 1.38 mm².

Conclusions: (1) dentine has a rapid initial mineralisation phase which is close to its maximum C_m ; (2) the mantle dentine in the periphery has a higher C_m than the remaining secondary dentine and (3) secondary dentine is laid down more rapidly on the cementum side of the rat incisor.

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C E MERCER* and P ANDERSON (The London Hospital Medical College, Turner St. London E1 2AD, UK): X-ray microtomographic quantification of the effects on enamel following CO_2 laser application.

The aim of this study was to investigate the possibilities of using X-ray microtomography (XMT) to quantify the mineral density changes resulting from laser application to enamel. A rod containing enamel and dentine of cross section 2 mm x 2 mm was cut from a premolar. 20 XMT transverse sections were taken at 100 µm intervals along the tooth rod using $\text{AgK}\alpha$ radiation at a resolution of ~50 µm, with a section thickness of ~15 µm. A series of laser impacts were made on the natural surface of the enamel using a Satelec CO_2 CW laser (Dayray Lighting, Beds, UK). A second set of XMT sections were taken at the same positions along the tooth rod as before. The extent of the effect of the laser on the mineral concentration at each pixel forming the image was calculated by comparison of the initial and final XMT images.

In conclusion, XMT can provide a quantitative non-destructive measure of the change in mineral concentration of dental hard tissues following laser application.

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R WADGE*, T VANDERNOOT* and M LEVINKIND (London Hospital Medical College, London E1 2AD, QMW College, London UK): In vitro demineralisation of human enamel assessed with alternating current impedance spectroscopy.

This study aims to use alternating current (AC) impedance spectroscopy to evaluate alterations in ion motion due to structural changes caused by artificial demineralisation of human enamel. Healthy enamel sections were prepared from freshly extracted human permanent and deciduous molars from patients 23 months to 27 years of age. The sections were equilibrated in unbuffered 10^{-3} M NaCl for one week. Impedance measurements were made by the application of a 200 mV alternating potential across each specimen mounted in an electrochemical cell at 37°C over a frequency range of 10 Hz to 1 MHz. After characterising the healthy enamel, the natural enamel surface was exposed to a demineralising solution. Following demineralisation, each section was re-equilibrated in unbuffered 10^{-3} M NaCl for at least 24 hours before being recharacterised. Each section was demineralised for a total of 77 hours. There was a general trend for the resistance values of healthy permanent enamel sections to be greater than sections obtained from deciduous teeth. For all specimens there was a marked reduction in resistance in the first five hours of demineralisation and this was followed by a n increase in resistance which in some cases exceeded the resistance values recorded for healthy enamel.

We conclude that AC impedance measurements can be used to investigate the effects of exposing permanent and deciduous enamel sections to demineralising solutions in vitro. However, further experiments are needed to interpret our findings.

We gratefully acknowledge financial support from The Wellcome Trust (Grant # 036689).

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W A COULTER*, F J T BURKE and S W CHEUNG (School of Clinical Dentistry, Queen's University Belfast and University of Manchester): An audit of autoclave performance in general dental practice.

The use of a steam autoclave has been advised as a means of sterilisation of instruments in dental surgery. A number of factors such as improper wrapping, inadequate pre-sterilisation cleaning, or lack of maintenance of equipment may interfere with the sterilisation process and failure rates of 15 % have been reported in the USA (Palenik C J et al., J Endom 12: 206-209, 1986). Four hundred dentists in Northern Ireland were offered the opportunity to test their autoclaves on two occasions using Attest biological spore ampoules¹ and requested to complete a questionnaire on training and sterilisation practice.

There was a 52% response rate with 4% showing autoclave failure. Autoclaves were used on average 10 times per day. Of the 70% of dentists who checked their autoclave performance, 36% checked them every cycle, 26% once per day, and 38% once per week. Those who made checks every cycle did so mostly (64%), by observing dials and 36% by chemical indicator strips. Seventy seven percent of GDPs and 68% of DSAs have had formal training in cross-infection control. A monthly update on cross-infection would be considered beneficial by 80% of respondents.

It is concluded that periodic testing of autoclaves using biological indicators in general dental practice has an important role to play in assuring the effective sterilisation of instruments. The need for in-service training of GDP and DSA is highlighted and a regular update on cross-infection issues is seen as desirable by a large majority of dentists.

This work was supported by N. Ireland Health Board, DHSS and 3M UK. Loughborough, UK.

1 3M, St Paul, MN, USA.

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R. K. ROSE*, A. R. LEE AND R. P. SHELLEY (MRC Dental Group, Dental School, University of Bristol, UK). Investigation of the role of cation bridging in plaque fluoride binding.

Topically applied fluoride is related to the recent dramatic fall in the prevalence of dental caries. However, the mode of action is not fully understood and there has been considerable debate over the role of loosely and firmly bound fluoride in caries protection. The loose fluoride binding model proposed by Rella and Bowen (Scand J Dent Res 85: 148-151, 1977), in which fluoride acts as a counterion to calcium ions bound by fixed acidic groups, has become widely accepted but has never been investigated experimentally. During a low pH episode, approximately half of total plaque calcium may be released (Rose et al J Dent Res 72: 78-84, 1993), which if the model is valid, implies a release of a similar quantity of fluoride at exactly the sites where it is most useful. In order to investigate the role of cations in fluoride binding to Strep. mutans R9, microequilibrium dialysis was used here with up to 40 mmol/L KF in 0.05 mol/L PIPES buffer (pH 7.0) containing 5 mmol/L calcium, magnesium or zinc. Free fluoride was measured at equilibrium using combination ion-selective electrodes and non-specific binding was accounted for by control experiments. Dissociation constants of 12.2 ± 3.8 mmol/L (in the presence of Ca^{2+}), 9.9 ± 0.4 (Mg^{2+}) and 14.4 ± 0.5 (Zn^{2+}) were found. Total fluoride binding capacity was found to be 119 ± 26 µmol/g wet wt. This work demonstrates that divalent cations are important in plaque fluoride binding and that there is a possibility that fluoride increases plaque cation binding by breaking intramolecular calcium bridges.

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A G WALTON*, P J MOYNIHAN and W G WRIGHT (The Dental School, University of Newcastle upon Tyne, UK): The effect of soya infant formula on the pH of dental plaque.

Infant soya milk formulae offer an essential substitute for standard baby milk formulae for children who are cow's milk intolerant. Although the carbohydrate content of both formulae are the same, there has been concern that soya milks containing glucose syrups may cause dental caries. The aim of this study was to compare the effect of soya (Infasoy) and standard (Premium¹) infant formulae on the pH of dental plaque. Twenty adult volunteers refrained from oral hygiene for 48 hours. Pooled plaque samples were obtained and pH measured before and at timed intervals after rinsing the mouth with the infant formulae. Stephan curves were compiled and the pH areas calculated. The mean pH area for Infasoy was $13.2 (\pm 11.4)$, that of Premium was $10.5 (\pm 8.7)$ the difference was not statistically significant. Mean minimum pH values obtained were $6.37 (\pm 0.49)$ and $6.32 (\pm 0.45)$ for Infasoy and Premium respectively.

In conclusion: soya infant formulae and standard infant formulae show equal acidogenic potential in adults. It is possible that the mode of consumption is the most important determinant of cariogenic potential of infant formulae.

¹Cow & Gate Nutricia Ltd.

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E LYNCH*, N D JOHNSON*, D P NAUGHTON² and M GROOTVELD² (Department of Conservative Dentistry¹, and Inflammation Research Group², LHM, UK): Proton ¹H nuclear magnetic resonance investigations of an oxidizing chlorine dioxide rinse.

A multicomponent evaluation of the oxidative consumption of salivary biomolecules by a commercially available oral rinse¹ preparation containing an admixture of the stable free radical species chlorine dioxide (ClO_2) with chlorine anion (ClO_2^-) has been investigated using high resolution proton (¹H) Nuclear Magnetic Resonance (NMR) spectroscopy. Unstimulated human saliva samples were collected from ten patients. To some samples rotar DEX oral rinse was added. To further samples, doubly-distilled H_2O was added as a control. Aqueous solutions containing 1.00×10^{-3} mol dm⁻³ sodium pyruvate, L-cysteine or L-methionine were prepared in phosphate buffer (pH 7.00). Samples of these solutions were treated with rotar DEX and additional aliquots of each solution treated with an equivalent volume of doubly-distilled H_2O served as controls. The samples were subject to (1) ¹H NMR measurements, (2) spectrophotometric and (3) ESR (electron spin resonance) analysis. The results obtained demonstrated that ClO_2 and/or ClO_2^- present in this preparation effected the oxidative decarboxylation of salivary pyruvate (to acetate and CO_2). Experiments conducted on chemical model systems confirmed the oxidative decarboxylation of pyruvate by this oral rinse, and also demonstrated that the amino acids cysteine and methionine, (precursors to volatile sulphur compounds responsible for oral malodour), were oxidatively consumed.

High resolution ¹H NMR spectroscopy offers a unique opportunity for the in vitro testing of oral rinses and dentifrices. It enables the studies of the molecular mechanisms of actions underlying therapeutic agents present in oral rinses and dentifrices.

¹ rotar DEX, Rowper Pharmaceuticals, Arizona USA

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D PINKERTON* and N PENDER (School of Dentistry, The University of Liverpool): The perception of non-accidental injury: a pilot study.

Dentists are requested to define whether particular injuries, frequently delivered to young children, have occurred as the result of biting or other non-accidental injury. This small study was designed to examine the abilities of members of the medical and dental professions to recognise non-accidental injuries from photographs. These same photographs were also shown to an unselected group of lay people. Sixteen photographs were presented to each of 40 individuals. Twelve of these photographs were of injuries caused by dental biting. The types of injury varied from clear arcades of teeth arranged as bite marks to patterns of bruising conformed to the shape of a dental arch. The remainder were all photographs of non-accidental injury of known cause but not of a dental aetiology. The 19 lay people were, mean \pm sem, $46.7 \pm 5.3\%$ successful at identifying the cause of the injuries. Those with medical training were $63.1 \pm 3.9\%$ successful. In the whole sample 13 people scored more than 65%, only 2 of these were lay people ($P < 0.02$). Those with medical training included 6 dental students and 7 qualified dentists (D). When compared with 5 Paediatric Consultants (P), those with dental training produced similar overall scores. However, when only the 12 bite mark photographs were considered, whilst D were $56.4 \pm 5.9\%$ successful; P were $36.7 \pm 10.2\%$ successful. This pilot study suggests that there may be scope for improvement amongst non-dentally trained medical professionals in the identification of bite marks as a cause of non-accidental injury.

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R BURN-MURDOCH (Physiology Division, St. Thomas's Hospital, London SE1 7EH): The shortening of rats' impeded incisors when the adjacent incisor is unimpeded.

When a lower incisor is unimpeded, rats shorten the adjacent incisor by 1mm within a day and the impeded rate over the day is higher than normal; also the variance of the eruption is significantly greater than the variance of the amount of tooth worn away and the intercept on the eruption axis of the correlation of eruption rate against length change is more than the unimpeded rate. Mathematical modelling has previously shown that this is not compatible with a simple slowing of eruption by wear, but it could be produced if wear slows long teeth more than short ones. The following have now been studied in the model: the shortening taking less than one day; the shortening taking longer in longer teeth; the relationship between wear and eruption being non-linear; the presence of circadian rhythms and rounding the results to the nearest 100µm, to simulate eyepiece readings. None of them improved the fit of the model to the results when the wear was equally effective at all lengths, making it more likely that it is wear being more effective in long teeth that makes the results inconsistent with simply slowing eruption. Hourly rates were calculated from the model, with wear being more potent in longer teeth; eruption at the beginning of the shortening was much slower than normal impeded rates and sometimes stopped. Therefore the shortening of an impeded incisor when its neighbour is unimpeded is a more sensitive model for the effect of function on eruption than the difference between impeded and unimpeded incisors.

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S.HADJ-BAGHERI* (Department of Orthodontics, King's College, London, U.K.): A comparison of bite force in a group of Caucasian and West-Indian children.

Bite force was measured in a group of Caucasian and West Indians by a device constructed at King's College School of Medicine and Dentistry. The sample consisted of 20 in each ethnic group, with equal distribution of sex and age range between 1-16 years. Prior to measurement of bite force the validity and reliability of the bite force measuring device was tested and established against a conventional machine. The results show that the measurement of bite force by the constructed device was close to that of the tensile machine.

Although the cephalometric measurements between the two groups showed expected differences, no statistically significant difference was found in the maximum bite force between the groups. The male subjects in the sample showed higher bite force than the female.

On examining the relation between the skeletal variables and bite force, the measurement describing the vertical skeletal relationship (e.g. the maxillary-mandibular planes and gonial angles) showed weak and negative correlation with the bite force.

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F J BAGGETT*, I C MACKIE and H V WORTHINGTON (University of Manchester, UK): An investigation into the measurement of the working length of non-vital incisor teeth in children.

There is a growing concern in dentistry about patient exposure to radiation and recently there has been a significant revision of the estimates of the risks from exposure to ionising radiation.

The object of this study was to validate a tactile technique involving paper points for measuring the working length of non-vital incisor teeth and compare it with the traditional diagnostic radiographic technique.

The method was to measure non-vital incisor teeth of children attending the Trauma Clinic in the Department of Oral Health and Development. Two operators were involved in measuring 35 incisor teeth. The results showed that in 93% of cases the estimated working length was within 1mm of the radiographic diagnostic length.

It is concluded that diagnostic radiographs are no longer necessary in the majority of cases involving endodontic treatment of incisor teeth in children. However, if a tooth has been on open drainage a diagnostic radiograph is still required.

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S S GHREBI*, I C MACKIE and H V WORTHINGTON (University of Manchester, U.K.): The Periotest for measuring tooth mobility in children.

This study aimed to assess the potential of the Periotest to quantify tooth mobility in children and to obtain baseline values for normal tooth mobility in healthy children. One hundred and sixty children, with equal numbers of boys and girls aged between 9 and 16 years took part in this study.

The four upper permanent incisor teeth were tested and 2 Periotest readings were taken at different times. In all 1,280 measurements were collected and analysed.

The results showed that there was evidence of some systematic error as the first Periotest readings were significantly higher than the second for three of the teeth (paired t test) ($p < 0.05$). Although these differences were statistically significant the actual differences were small amounting to no more than 0.21 of a Periotest unit. Periotest readings were lower for girls than boys of the same age (paired t test). A negative correlation existed between the Periotest reading and the age of the child. As the child gets older the Periotest reading decreases.

It is concluded that the Periotest has potential as a special test for use in paediatric dentistry. The initial steps for producing reliable baseline figures have been achieved. However, further research is required before the Periotest becomes an accepted part of the paedodontist's armamentarium.

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M A M ASFOUR*, B J MILLAR, P B SMITH. (King's College Dental School, London): An assessment of the reliability of pulp testing in 7-10 year olds.

The validity of pulp testing deciduous teeth is uncertain. This study tested the reliability of pulp testing deciduous canines with at least two-thirds of root intact. One hundred children aged between 7 and 11 years attending the Department of Paediatric Dentistry were tested using two pulp testing methods: ethyl chloride (EC) and electric pulp testing (EPT) and compared with sham tests. Children were allocated by a minimisation technique to either an EC or EPT group. Each child scored their response to the EC, or EPT test, and to the appropriate sham test from 1-10 on a visual analogue scale (Abu-Saad, H, Pain, 13 163-171, 1984). The results showed a significant difference between the EC test (3.5 ± 2.5) and sham EC test (0.5 ± 1.5) scores (Paired t-test, $P < 0.001$) and between the EPT test (3.5 ± 2.4) and sham EPT test (0.7 ± 1.5) scores (Paired t-test, $P < 0.001$). To eliminate the possibility that stimuli were being conducted via the gingivae the EC, or EPT, and sham tests were repeated following the topical application of a 5% lignocaine local anaesthetic paste. The results obtained were not significantly different from those obtained before the topical anaesthetic application.

In conclusion, pulp testing of deciduous canines in 7-10 year-olds is a valid technique.

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K.M. BLAIN*, F.J. HILL (Department of Oral Health and Development, University of Manchester, UK): Inhalation Sedation and Local Anaesthesia as an Alternative to General Anaesthesia for Extractions in Children

This study aimed to assess the extent to which patients referred for extraction under general anaesthesia could be treated with inhalation sedation and local anaesthesia. Fifty-four patients mean age 7 years 7 months were included. After explanation, the children were sedated with inhalation sedation to a standard technique (Crawford A.N., *BDJ* 168: 395-398, 1990). Local anaesthetic administration and extraction were completed in a routine manner. Patient details (including referral source and previous dental history), time taken, number of extractions and reasons for failure were recorded. In addition, the parent was asked to complete a questionnaire to assess the child's and parent's reaction to treatment. Treatment was completed for forty patients (75.5% of the total). Thirty-eight of the patients were referred from general dental practitioners and forty-eight had constant or occasional pain from their teeth. Results showed that a mean of 1.4 extractions per patient were completed at 53 of 64 visits in a mean time of 36.34 minutes. 79.7% of parents indicated that they would opt for this form of treatment if their child required further extractions in the future.

It is concluded that inhalation sedation and local anaesthesia is a feasible alternative to general anaesthesia for extractions in children and that parents found it an acceptable form of treatment.

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E M BENTLEY*, R P ELLWOOD and R M DAVIES (Dental Health Unit, University of Manchester, UK): Factors influencing the amount of toothpaste dispensed by mothers of young children

It has recently been suggested that a 'smear' of toothpaste should be used by pre-school children rather than the commonly recommended pea-sized amount (Rock W P, *BDJ* 177: 17-20, 1994). The aim of this study was to investigate the influence of instruction (pea or smear), nozzle shape (star or round) and type of toothpaste (clear gel or opaque paste) on the amount of toothpaste dispensed for pre-school children. Fifty-three mothers of pre-school children took part. Each undertook eight tests, combinations of instruction, nozzle and toothpaste type in random order, and were asked to place the stated amount on pre-weighed child-size brushes. Results showed that the instruction to use a pea-sized amount produced a mean weight of 0.30g (SD 0.13), whilst the mean for 'smear' was 0.22g (SD 0.21). A four way analysis of variance was carried out with subjects, instruction, nozzle shape and toothpaste type as factors. Significant differences were found between subjects, instruction and toothpaste type ($p < 0.05$). A 'smear' was generally interpreted as a thin layer covering the bristles.

It is concluded that the 'smear' instruction will result in less toothpaste applied than 'pea' and that the type of toothpaste influences the amount dispensed.

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R CROUCHER*, M C B M TORRES, F HUGHES & W MARCENES (Joint Dept of Dental Public Health, UCL/LHMC; Dept of Periodontology, LHMC, UK): Life-events and periodontitis: a case-control study.

Previous studies linking life-events to oral disease have suffered methodological limitations such as small sample size, unsuitable sample selection criteria, use of self-report disease measures and limited numbers of life-events. This case-control study ($n=100$ dental patients, matched for age and gender) investigated the role of life-events in periodontitis. Criteria for selection into test group was the presence of at least one site with a pocket depth of 5.5mm or more. Control patients had no periodontal pockets. The main data collected were: life events, tobacco and alcohol use, dental behaviours and socio-demographics. Logistic regression analysis results showed periodontal disease associated with negative life-events ($p < 0.01$), dental plaque levels ($p < 0.01$), tobacco smoking ($p < 0.01$), level of education ($p < 0.05$) and the number of missing teeth ($p < 0.05$). Smoking was related to dental plaque levels ($p < 0.01$). Those reporting higher numbers of negative life-events were more likely to smoke ($p < 0.05$) and had higher levels of dental plaque ($p < 0.05$). It was hypothesised that the statistically significant association between negative life-events and periodontitis, which remained significant after adjusting for the other variables, was due not only to the risk-related behaviours studied but also through other pathways involving the host-defence mechanism. *It was concluded that negative life events may play an important role in the determination of periodontitis.*

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R ABDEIA*, V CLEREHUGH*, P S HULL and H V WORTHINGTON (University of Manchester and Leeds Dental Institute): Periodontal condition of elderly Libyans

The aims of the study were to assess the periodontal condition and to determine the prevalence, extent and severity of periodontitis in a group of elderly subjects in Benghazi, Libya as part of a larger epidemiological investigation. 100 dentate subjects were examined for probing depth (PD), clinical attachment level (AL), recession, mobility, plaque, supragingival calculus and bleeding on probing. An oral questionnaire was completed for each subject. There were 59 males and 41 females with a mean age of 69.4 years (range 65-99 years). The majority of the sample (90%) were symptomatic dental attenders; the remaining 10% reporting that they had never visited a dentist. 49% reported using a toothbrush to clean their teeth with 20% indicating that they brushed at least once a day. The mean PD was 2.1 mm and mean AL was 4.0 mm on mesio-buccal sites. Mean buccal recession was 2.2 mm. 26% of the study group had at least one tooth with a shallow pocket (PD 4-5 mm), while 15% had at least one deep pocket (PD ≥ 6 mm). 33% of the subjects had moderate disease (AL 4-6 mm), while 59% had severe AL (AL ≥ 7 mm). *It is concluded that use of pocket depth alone would have greatly underestimated the prevalence and severity of periodontitis in this study group.*

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I C MACKIE*, E M BENTLEY (University of Manchester, U.K.): Trends in prescribing of sugar-free paracetamol for children.

The most commonly prescribed paediatric medicine is paracetamol. Sugar-containing and sugar-free versions which have identical therapeutic actions have been available for many years. Doctors have the option of prescribing generic or brand named varieties.

The object of this study was to investigate trends in prescribing by general medical practitioners in the North West of England.

The method was to study the Prescribing Analysis and Cost (PACT) data held by the Regional Health Authority.

The results showed that the proportion of paracetamol prescribed which was sugar-free was slowly increasing over the years 1991, 92 and 93. In 1991 on average 29 per cent was sugar-free, this had risen to 38 per cent in 1993. Generic prescriptions accounted for 19 per cent in 1991 and 25 per cent in 1993 but of these less than 4 per cent were sugar-free.

It is concluded that many general medical practitioners do not routinely prescribe sugar-free paediatric medicine whenever possible.

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G KIDNER*, R T LEE and M LEVINKIND (Dept Child Dental Health, Lond Hosp Med Coll, London E1 2AD, UK): Study of psychometric factors affecting pain in relation to orthodontic treatment

This study aims to establish the incidence of pain experienced during and after visits to the orthodontist for routine fixed appliance therapy and to determine the relationship between reported pain and a psychometric assessment of the patients' anxiety before the treatment.

Ethics committee approval was obtained for this study from the Royal London Hospital Trust. Prior to treatment patients completed the State-Trait Anxiety Inventory for Children (Spielberger et al., Consulting Psychologist Press, STAIC Manual 1973) and the Child Manifest Anxiety Scale (Reynolds and Richmond, *J Ab Psych* 6:271-280, 1978). Visual analogue scales were used to assess the pain experienced during treatment sessions. Pain for seven days after each treatment session was recorded in a diary using an Intensity Rating Scale (Keele K D, *Lancet* ii: 1127-1131, 1948).

Preliminary analysis of our data shows that those patients with the highest anxiety scores recorded greater levels of pain experienced during and after orthodontic treatment.

We conclude that pre-treatment anxiety scores may be a useful screening tool to predict which patients are more likely to experience pain during and after orthodontic procedures.

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W MARCENES*, R CROUCHER, F HUGHES & M C B M TORRES (Joint Dept of Dental Public Health, UCL/LHMC; Dept of Periodontology, LHMC, UK): Tobacco smoking and periodontitis: a case-control study.

Whether or not smokers have more dental plaque than non-smokers is controversial. This case-control study, carried out on 100 patients matched for age and gender, aimed to elucidate the relationship between tobacco smoking, levels of dental plaque and periodontitis. Criteria for selection into test group was the presence of at least one site with a pocket depth of 5.5mm or more. Control patients had no periodontal pockets. Logistic regression analysis showed that periodontitis was associated with tobacco smoking ($p < 0.01$), dental plaque levels ($p < 0.01$), negative life-events ($p < 0.01$), level of education ($p < 0.05$) and the number of missing teeth ($p < 0.05$). There was no significant difference in the periodontal health status and levels of dental plaque of those who smoked in the past and those who have never smoked. However, smokers had higher levels of dental plaque than both past smokers and those who never smoked ($p < 0.01$). The association between tobacco smoking and periodontitis remained significant after adjusting for social class and educational level ($p < 0.05$), but not dental plaque levels. These results corroborate the theory that tobacco smokers have a greater amount of dental plaque than non-smokers and that stopping smoking may improve oral hygiene.

It was concluded that tobacco smoking may play an important role in the occurrence of periodontitis.

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RM PALMER*, TLP WATTS, RF WILSON (Department of Periodontology, UMDS, London): Effect of adjunctive tetracycline in the treatment of early onset periodontitis.

The aim of the present study was to evaluate the adjunctive effect of systemic tetracycline (250mg qds for 14 days) following both a root planing and surgical phase of treatment in a double blind study. 26 patients (13 test/tetracycline, 13 control/placebo) from a previously reported non-surgical study (Palmer et al, *J Dent Res* 72:696, 1993) completed both phases of treatment and probing depths (PD) and attachment level (AL) were recorded. Data were analysed, with transformations where appropriate, by repeated measures ANOVA and ANCOVA using baseline covariates. Both groups responded well to root planing. At 3 months PD was less in the test group (Baseline: Test(T) mean=4.59 [SD=1.19], Control(C)=4.57 [0.84]; 3 months: T=3.22 [0.85], C=3.79 [1.01]; $p < 0.05$) whereas AL changes were not significantly different (Baseline: T=3.29 [2.05], C=2.62 [0.90]; 3 months: T=2.49 [1.90], C=2.31 [1.07]). All teeth with residual pockets had periodontal surgery and were evaluated after a further 3 and 12 months. Further improvements in probing depth were noted in both groups at 3 months ($p < 0.01$) and maintained at 12 months, but the difference between groups was no longer significant (PD 3 months: T=1.89 [1.18], C=2.62 [1.35]; 9 months: T=2.00 [1.28], C=2.55 [1.08]; AL 3 months: T=1.96 [1.72], C=1.86 [1.31]; 9 months: T=2.07 [1.65], C=1.83 [0.98]). *The results indicate that systemic tetracycline offers a significant adjunctive effect following a course of root planing but not following a subsequent course of surgery in early onset periodontitis.*

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M RADVAR*, TW MACFARLANE, D MCKENZIE, CJ WHITTERTS, AP PAYNE and DF KINANE (University of Glasgow, UK): An evaluation of the Nd:YAG laser in periodontal pocket therapy.

The aim of this study was to determine whether the Nd:YAG laser energies of 50 and 80 mJ at 10 pulses per second (pps) were capable of improving the clinical parameters associated with periodontal disease. These energy settings were chosen as previous works indicated that higher values would damage root surfaces and that 80 mJ had an *in vitro* bactericidal effect. Eighty periodontally affected sites in teeth scheduled for extraction from 11 patients with adult periodontitis were randomly placed in one of the following 4 treatment groups: 1) laser treatment at 50 mJ, 10 pps for 3 minutes; 2) laser treatment at 80 mJ, 10 pps for 3 minutes; 3) scaling and 4) untreated control. Probing depth, bleeding on probing (BOP), plaque index, gingival index and gingival crevicular fluid (GCF) volume were measured at baseline and week 6. Baseline subgingival microbiological samples were collected, then repeated immediately after treatment and at week 6 to assess the total anaerobic counts (CFU). Only the scaling group showed a significant reduction in pocket depth and BOP ($p < 0.001$). The microbial samples taken immediately after scaling and laser at 80 mJ and 10 pps treatments showed a significant reduction in total CFU compared to the baseline ($p < 0.01$), which was sustained only in the scaling group until week 6. Electron microscopy demonstrated that most lasered teeth still had calculus present, and no heat damage was observed on root surfaces.

This study demonstrated that application of Nd:YAG laser pulses of 50 mJ and 80 mJ failed to improve the clinical and microbiological parameters of periodontal disease.

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D S JONES*, A D WOOLFSOFT*, W A COULTER* and G J LINDEN* (School of Pharmacy and *School of Dentistry, Queen's University of Belfast): *In vitro* release of Tetracycline from novel, bioadhesive gels.

Recently, the treatment of Periodontal Disease has increasingly involved the use of intra-pocket delivery systems, which release antimicrobial agents (e.g. tetracycline, TC) at a controlled rate. This study reports the *in vitro* release of TC from novel, bioadhesive, syringable periodontal gels. Nine aqueous gels were formulated containing hydroxyethylcellulose (HEC, 5, 10, 20% w/w), polyvinylpyrrolidone (PVP K90, 5, 10, 20% w/w) and Polycarbophil (PCP, 1% w/w) at pH 6.8 (Phosphate buffer), into which TC hydrochloride ($< 63 \mu\text{m}$) was suspended. *In vitro* release of TC at 37°C into Phosphate Buffered Saline at pH 6.8 was determined using a Cava dissolution apparatus (paddle stirring at 100rpm). Samples of dissolution fluid were removed at pre-determined intervals and their TC content analysed using ultra-violet spectroscopy at 353nm. TC release were statistically analysed using a two-way Analysis of Variance ($P < 0.05$ denoting significance). For all formulations the release of TC was proportional to time, i.e. zero-order release, for time periods ranging from 24-54 hours, dependent on formulation. Observed release rates ranged from $1.59 \pm 0.22 \text{ mg h}^{-1}$ to $15.77 \pm 0.51 \text{ mg h}^{-1}$. Increasing concentrations of HEC significantly decreased TC release rates. PVP (20% w/w) significantly increased TC release rates in comparison to gels containing 5% and 10% w/w, however, the usefulness of higher concentrations of PVP (circa 20% w/w) is limited due to increased gel viscosity.

*In conclusion, a controlled, zero-order, release of tetracycline was obtained *in vitro*. This rate was primarily controlled by the HEC concentration within the gel. It is anticipated, however, that the *in vivo* release rate of TC from such gels will be lower than observed in this study due to the limited fluid volume within the periodontal pocket. Selection of candidate formulations for periodontal use will be dependent on both gel syringability and the required rate of TC release.*

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HM ANSTICE* and JW NICHOLSON* (Eastman Dental Institute, University of London, UK; * KCSMD, University of London): Investigation of a turntable mixing device for the preparation of polyelectrolyte cements.

The properties of a glass-ionomer cement (GIC) are mixing operator dependent (Watson EW & Nicholson JW, *Clin. Mater.* 15, 169-172, 1994) and encapsulation may compromise the final physical properties of the cement. This investigation describes the effect of an alternative mixing technique, the turntable mixer, on compressive strength. Five polyelectrolyte cements, varying between easy to hand-mix and difficult to hand-mix were chosen. The cements were mixed by hand (spatulation on a glass block) or by hand (for the initial wetting of the powder) followed by turntable mixing. The resulting pastes were used to pack compressive strength moulds (12mm high x 6mm diameter). The specimens were stored at 37°C, 1h in mould and then 23h in water. The load at failure in compression was then determined. The strength of a material was enhanced if it had a reasonable powder/liquid ratio and reasonable setting speed (IE, P). In this situation turntable mixing made a significant improvement to the dispersion of the powder in the paste. If the material was very easy to mix (A, F), then hand-mixing was sufficient to achieve a good dispersion and if the material was very hard to mix (IS) then wetting of the powder was all that could be achieved in the working time. In these two situations there was no difference in measured compressive strength between hand-mixed and turntable mixed specimens.

Cements prepared using the turntable mixer were at least as strong (and in some cases stronger) in compression than those prepared by hand-mixing.

A Aquakent, P Polykint, IB Ionexpress & IS Ionosilver (Kent Dental) - F Fuji IX (GC Corp.)
* - Automixer AM1, GC Corp.

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N E CARTER* and J F MCCABE (Department of Child Dental Health and Dental Materials Science Unit, University of Newcastle upon Tyne, UK): Laboratory studies of a dual-cured glass-ionomer cement as an orthodontic bonding agent.

This study compares the performance of a dual-cured glass-ionomer cement as an orthodontic bonding agent with a 'no-mix' orthodontic composite resin. Four groups of twenty premolar teeth each had orthodontic brackets bonded using: 1) glass-ionomer without enamel pre-treatment, 2) glass-ionomer after enamel pre-treatment with 'conditioner' (polyacrylic acid) for 10 seconds, 3) glass-ionomer after enamel pre-treatment by etching with 37% phosphoric acid for 20 seconds, 4) 'no-mix' composite after etching with 37% phosphoric acid for 20 seconds. Shear bond strengths were tested after 24 hours' storage in distilled water at 37°C, using a cross-head speed of 10mm/min. Weibull analysis showed that the characteristic failure values were: Group 1, 58.2N; Group 2, 62.7N; Group 3, 74.1N; Group 4, 110.4N; and that pre-treating by etching with 37% phosphoric acid reduced the probability of failure of the glass-ionomer at 50N by 60% but this was still double the probability of failure of the 'no-mix' composite. Four further groups of ten specimens each were prepared in the same way for fatigue life testing using a ball mill (Abu Kasim *et al.*, *J. Dentistry*, in press). All glass-ionomer specimens without pre-treatment failed in the ball mill within 16 hours, but all specimens pre-treated with either polyacrylic acid or 37% phosphoric acid and all 'no-mix' composite specimens survived in excess of 36 hours.

It is concluded that the performance of the dual-cured glass-ionomer cement as an orthodontic bonding agent is significantly poorer than the 'no-mix' orthodontic composite resin, but may be improved by enamel pre-treatments.

*Photac-Fil, ESPE *Right-On, TP Laboratories

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RM BILLINGTON*, YEY ABOSH (Eastman Dental Institute, London & University of Bristol Dental School): Effect of time on glass ionomer adhesion to enamel and dentine.

The elastic and viscoelastic properties of glass ionomer cements change significantly with maturation times longer than 24 hours. Literature adhesion results are not reported at these longer times. This study compared adhesion after 24 hours with that at 3 months. Two materials were selected, a powder/liquid restorative (A) and a encapsulated metal-reinforced restorative (B). Test procedure followed that of Aboush & Jenkins Br Dent J 161, 179, 1986. The substrates used were buccal enamel (E) and occlusal dentine (D) of extracted human 3rd molars. All surfaces were pre-treated using 25% polyacrylic acid in accordance with manufacturers directions. Specimen preparation was modified so B, which had a stiff consistency, could be packed as in clinical use. Results (Mean:MPa, (s.d.), N=20) show time effects differ for the material/substrate combinations: A/E24hr 7.6(1.3); 3m 10.0(2.2); B/E24hr 8.3(2.1); 3m 9.3(3.0); A/D24hr 3.5(0.8); 3m 2.7(0.9); B/D 24hr 2.8(0.9); 3m 2.6(1.1). Statistical analysis (2-tailed 't'-test) showed maturation significantly increased adhesion of A to enamel but reduced it to dentine. The changes shown by B, though in the same direction, were not significant. *It is concluded that glass ionomer adhesion to enamel and to dentine differs in its response to maturation changes in the cement.*

Materials: A Hi-P1, B Hi-Dense, Shofu Dental Products.

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DC WATTS* and AJ CASE (The University of Manchester, UK): Morphology of resin /ionomer restoratives after conditioning and abrasion.

Resin-modified glass-ionomer or ionomer-modified resin 'hybrid' materials are currently available in several formulations with indications for Class V restorations. The aim of this study was an *in vitro* evaluation of equilibrium surface finish for two such biomaterials (Dyrac-Composers* & F.L.*), with controls of glass-ionomer (CHF*) and resin-composite (TFH*). Three of these products^{1,2,3} were from the same manufacturer. For each material, three disk specimens: 5 mm diameter x 2 mm, were prepared. All were finished by a single operator according to Manufacturers' instructions to a final lustre. We distinguish morphologically between initial finish (R_i) (roughness of initially polished surface) and equilibrium finish (R_e) (roughness of surface after chemical/mechanical wear). Perthometer equipment was used for 2-D surface mapping and determination of roughness parameters, especially R_a and R_z. These parameters were measured before and after 1 month storage in isotonic saline at 37°C. Specimens were then subject to three body abrasion using a rotary bi-directional tooth brush machine plus a dentifrice. For each material the resulting surface changes were monitored at intervals until a steady state was reached; typically after 4 h. The 2-D surface maps of the steady-state showed that Dyrac* and TFH* both exhibited smoothness-persistence, with R_a = 0.2 µm. CHF* developed much rougher surfaces, with mean R_a = 1.5 µm and 2.2 µm, respectively. Considerable differences were apparent between the two 'hybrid' systems with the Composites* formulation exhibiting optimal durability of finish. This relative equilibrium finish provides some means for prediction of texture changes for products included in the study under clinical conditions.¹ Dyrac² - Fuji II LC, GC Corporation, Tokyo. ³ Chemfil Sup. ⁴ TPH. ⁵ Dentsply, Germany.

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E L BOYLE*, C W BARCLAY* and R L WILLIAMS* (University of Liverpool; *University of Birmingham): Tensile strength of bonds between five cements, and four surfaces, following thermal aging.

This study aimed to compare the tensile strength of the cement / metal interface for a selection of materials (Aquacem, Compspan, Panavia EX, C & B Metabond and Biomer). Four metal surface finishes (sandblasted, tinplated, silicoated and etched) of semi-precious alloy were used, and three aging regimes employed (24 h ambient, 37°C 7 days in saline and Thermocycling (5-22-55-22°C)). Testing was with a Nene M5 using specially designed jigs. Results showed that after 24 h C & B metabond had clearly the highest strength, an advantage accentuated by the use of silicoating. This was still true after aging at 37°C, however after thermocycling variation in surface finish had only a small effect on strength and the advantage of 4-Meta chemistry faded.

*In conclusion, this laboratory-based study may indicate that the advantages of some dental adhesives are eroded by thermocycling *in vitro*. This may have important clinical consequences.*

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J Williams* and R Billington (Dept of Biomaterials, Eastman Dental Institute, 256 Grays Inn Rd., London, UK): Influence of supporting structure on glass ionomer biaxial tensile strength.

Biaxial tensile strength (shell) has been measured using either a knife edge or 3 ball support. The aim was to study the effect of varying the number of supports compared to a ring. One material, a hand mix, anterior glass ionomer restorative (1) was used to make specimens 1mm thick, 3mm ball bearings were evenly spaced around a diameter of a) 11, b) 20 and c) 30mm. For each condition the strengths of 6 specimens were measured after storage in water for 24h at 37°C. A Hounsfield tester was used at a crosshead speed of 1µm/min. Maximum crosshead travel at fracture was <0.2mm. Average strengths (MPa) obtained follow, with number of supports given in parentheses.

a) 50.4 (3), 56.6 (4)*, 52.1 (6), 52.5 (12)
b) 46.6 (3), 59.7 (4)*, 53.9 (6), 48.8 (12), 57.3 (12)*
c) 43.9 (3), 50.6 (4), 44.4 (6), 49.3 (9), 48.7 (18), 45.8 (30)
Those marked * differed significantly (Wilcoxon) from the value of 48.8 MPa obtained on a 15mm ring. None were significantly lower. Although condition c) gave the lower values no systematic effect on shell strength was found for number of supports, distance between supports or disc size.

It was concluded that under these conditions the biaxial tensile strength of a glass ionomer was little affected by the type of supports used.

1-HiFi, Shofu Inc.

249 S GRIFFIN* AND R HILL (Department of Materials Science and Technology, University of Limerick, IRELAND): Glass composition influence on Glass Polyalkenoate Cement Mechanical Properties.

In the present study single phase homogeneous glasses have been synthesised with negligible fluorine weight loss, during the firing procedure. In some of the glass compositions, the phosphate and fluorine contents have been varied, while in others the silicon:aluminium ratio has been altered. The silicon:aluminium ratio within the glass network has long been regarded as parameter controlling cement formation and properties. Cement mechanical properties have been evaluated as a function of glass composition. The phosphate species was found to have a significant effect on the compressive strength (σ_c) and on the setting characteristics of the cements formed. Increasing the phosphate content by only 4 wt%, reduced the 24 hour compressive strength values from 130MPa to 8MPa. Increasing the fluoride content across a series of glasses resulted in the compressive strengths going through a maximum, with a further increase leading to a slight reduction in strength. Altering the silicon:aluminium ratio was found not to be as significant as varying either the phosphate or fluoride content of the glass composition.

It is concluded that the high fluoride containing glasses yielded the highest compressive strength, with values ranging between 170-190MPa after 24 hours.

This study was funded under the Brite-EuRam Scheme Project No. BE6062 Contract BRE2-349.

251 M WILSON*, H PATEL and J FLETCHER (Dept of Microbiology, Eastman Dental Institute, London): Susceptibility of biofilms of *Streptococcus sanguis* to oral antiseptics.

Bacteria in biofilms are known to be less susceptible to antimicrobial agents than their planktonic counterparts. The purpose of this study was to determine the susceptibility of biofilms of *Streptococcus sanguis* to two commonly used oral antiseptics. A constant depth film fermentor (CDF) was used to grow biofilms of *S. sanguis* in an aerobic atmosphere at 37°C using a mucin-containing artificial saliva as the nutrient source. Biofilms, as well as planktonic cells, of the organism were exposed to 0.2% (w/v) chlorhexidine gluconate (CHG) and 0.05% (w/v) cetylpyridinium chloride (CPC) and survivors enumerated. Biofilm-grown cells of *S. sanguis* exhibited a lower susceptibility to both antiseptics than planktonic cells. No viable bacteria were detectable after 5 mins exposure of planktonic cells to either antiseptic whereas bacteria in biofilms survived a 4 hour exposure. CHG achieved kills corresponding to a 2 log₁₀ reduction in the viable count of biofilms containing 10⁷ cfu after 5 mins, the corresponding kills for CPC amounted to approximately a 1 log₁₀ reduction. However, on a molar basis, CPC was the more effective of the two antiseptics. In contrast, MIC values showed CHG to be more effective than CPC against *S. sanguis*.

The result of this study have revealed that biofilm-grown cells of *S. sanguis* are less susceptible to CHG and CPC than planktonic cells of the organism and that MICs are not reliable predictors of the relative effectiveness of CHG and CPC against biofilms of the organism.

253 M A O LEWIS*, M J WILSON and P A BISHOP (Department of Oral Surgery, Medicine and Pathology, UWCM, Cardiff, UK): Isolation of "*Streptococcus milleri*" group from orofacial infections and association with *Prevotella* species.

It has been proposed that the pathogenicity of the "*Streptococcus milleri*" group is enhanced in pulmonary infection by the presence of gram-negative strict anaerobes, in particular *Prevotella intermedia*. However, there would not appear to be any information concerning the occurrence of a similar synergistic relationship between these bacterial species within the polymicrobial flora of orofacial infections. The microbiological reports of 198 consecutive suppurative infections processed in the oral microbiology unit were examined. Specimens had been obtained predominantly by needle aspiration and all samples were processed using laboratory techniques capable of isolating slow-growing strict anaerobes. Routine identification methods, including the rapid ID 32 STREP system, had been employed. Strains of SMG were cultured from 43 of the infections studied (21/146 acute dentoalveolar abscesses, 6/11 infected cysts, 1/11 cases of suppurative sialadenitis, 0/11 periodontal abscesses and 15/20 miscellaneous infections). The SMG isolates comprised of 19 strains of *S. intermedius*, 16 strains of *S. constellatus* and 8 strains *S. anginosus*. Determination of average odds ratio (Soorankat et al. Oral Microbiol Immunol 3: 1-7, 1988) failed to reveal an association for either *S. anginosus* or *S. constellatus* with *Prevotella* species. A negative association was demonstrated for *Prevotella* species both with *S. intermedius* alone and the SMG overall.

It is concluded that members of SMG are frequently present in the microbial flora of a variety of orofacial infections. However, isolation of SMG is not associated with presence of *Prevotella* species.

255 P A WHITE^{1,2*}, M WILSON¹, S NAIR¹, A KIRBY¹ and B HENDERSON² (Depts of ¹Microbiology & ²Maxillofacial Surgery, EDI, London): *Actinobacillus actinomycetemcomitans* anti-proliferative protein is neutralized by juvenile periodontitis patients sera.

Surface-associated material (SAM) from *A. actinomycetemcomitans* NCTC 9710, obtained by saline extraction, produces a dose dependent inhibition of [³H]-thymidine incorporation by a number of cell types *in vitro*. Anion exchange and size exclusion HPLC of the SAM have shown that the activity elutes as a low molecular weight component (<20kDa), which is susceptible to heat or trypsin. The purpose of this study was to determine whether this anti-proliferative activity could be neutralized by sera from patients with localised juvenile periodontitis (LJP). Dilutions of sera from patients with LJP, and from healthy controls with no signs of periodontitis, were incubated with MG63 cells in the presence of SAM (500ng/ml), and the proliferation assessed by measuring the incorporation of [³H]-thymidine. The osteoblast-like cell line MG63 was used in these studies, being sensitive to low concentrations of SAM (IC₅₀=200ng/ml). Antibodies to the constituents of the SAM were found in patients with LJP. Sera from 9 of 16 patients with LJP significantly neutralized the anti-proliferative activity of the SAM, whilst sera from 15 controls were unable to neutralize this activity. Neutralization was not directly related to the antibody titre.

In conclusion SAM from *A. actinomycetemcomitans* contains a low molecular weight anti-proliferative protein whose activity can be neutralized by a proportion of sera from patients with LJP.

250 G K WATSON*, D HALLIDAY, L ALBISTON, S SINGLETON, C ALLISON (Unilever Dental Research, Bebbington, UK): An *in vitro* biofilm system for study of plaque ecology and physiology.

A Continuous Culture Plaque (CCP) biofilm system was developed, utilising a 9-member oral bacterial consortium grown on a mucin-based medium in an anaerobic primary chemostat. The chemostat culture was used to continuously inoculate flow cells for growth of biofilm populations on hydroxyapatite surfaces. Flow rates of inoculum and fresh growth medium were varied to determine the effects of dilution rate on biofilm development. Results indicated that at high dilution rates the facultatively anaerobic species (streptococci, *Lactobacillus rhamnosus*) predominated, whereas the obligate anaerobes (*Fusobacterium nucleatum*, *Prevotella nigrescens*, *Porphyromonas gingivalis*) were increasingly selected at lower growth rates. Flow cell cultures and the primary chemostat grown at low dilution rates produced high levels of products of amino acid metabolism (e.g. phenols, indoles). In contrast, at high dilution rates concentrations of aromatic fermentation products were low whereas increased levels of electron sink products (succinate, lactate) and acetate were detected. Sucrose pulsing to the system produced characteristic increases in biofilm populations of aciduric species (*S. mutans*, *Lactobacillus*) and *Veillonella parvula*, and lactate production increased, concomitantly with this ecological shift. These data demonstrate that biofilms produced in the CCP system respond physiologically in a similar way to plaque bacteria *in vivo*, and that the system may be useful for future studies on plaque ecology and physiology.

252 DJ BRADSHAW¹, PD MARSH¹, GK WATSON², and C ALLISON² (ICAMR, Salisbury¹ and Unilever Dental Research, Bebbington², UK): Effect of Oxygen and Flow Rate on Bacterial Survival and Biofilm Development

Previously, we reported the development of mixed culture biofilms of oral bacteria on hydroxyapatite (HA) discs. Biofilms developed in this conventional chemostat were dominated by anaerobes, even after a few hours. Later experiments using a two-stage model system, in which the second stage was aerated (5% CO₂ in air), allowed prolongation of biofilm development, and experiments revealed differences between cultures grown with or without an obligate aerobe, *Neisseria subflava*. In both experiments, anaerobes persisted in high numbers in planktonic cultures, and succession led to anaerobes predominating in older biofilms. This study examines the effect of reducing the flow-rate from the first (conventional) stage (without *N. subflava*), from 50 ml/h to 5 ml/h, to establish whether anaerobic bacteria were capable of growth in the aerated phase. Thus, if the anaerobic bacteria were simply surviving without growth, their numbers should be reduced 10-fold in the aerated second stage. The planktonic cultures and biofilms in this experiment were predominated by streptococci, *F. nucleatum*, *P. nigrescens* and *A. naesslundii*. The planktonic population was reduced by only 2-fold compared with the study in which the full 50 ml/h of conventional culture was added. In biofilms, there was a clear increase in the proportions of anaerobes with time, particularly after 4 days and 7 days. The data indicate that the anaerobic bacteria in the mixed culture can grow in the aerated phase, despite a steady-state dissolved oxygen tension of 25-50%, and an E_h of +90 mV to +140 mV. Future studies will examine the mechanisms by which the anaerobic bacteria might survive such conditions. Supported by Unilever Dental Research.

254 P. BARBER*, M. WILSON*, B. HENDERSON, A. KIRBY¹ (E.M. Unit*, Dept. of Microbiol., Max-fac. Surg. Res. unit, Eastman Dental Institute, UK): Localization of the molecular chaperone GroEL on the surface of *Actinobacillus actinomycetemcomitans*.

A. actinomycetemcomitans, a Gram-negative bacterium implicated in the pathology of periodontal diseases releases surface-associated material (SAM) *in vitro* which contains potent bone resorbing activity. We have now isolated the active constituent in the SAM and have identified it as the molecular chaperone GroEL. The aim of this study was to determine if this protein was indeed present on the surface of this bacterium.

SAM was prepared from a range of bacteria known to stimulate calvarial bone resorption run on SDS-PAGE and immunoblotted with a monoclonal antibody (P2) which recognizes *A. actinomycetemcomitans* GroEL. Pure cultures of *A. actinomycetemcomitans* were fixed in 0.5% glutaraldehyde, dehydrated and embedded in LR White acrylic resin. The distribution of the SAM and the GroEL were disclosed by use of a post-embedding immunogold labelling technique using a rabbit polyclonal antiserum and Mab P2.

Only the SAM from *A. actinomycetemcomitans* but not from *Porphyromonas gingivalis*, *Eikenella corrodens* or *Staphylococcus aureus* contained GroEL assessed by Western immunoblotting. Gold labelling showed the presence of the GroEL in the cytoplasm, as expected, but also within the cell wall complex and in the SAM surrounding the outer cell wall. All controls were negative.

These findings confirm the biochemical studies showing that GroEL is present in material external to the outer membrane.

256 CW DOUGLAS¹, I FORD² and FE PRESTON² (Oral Pathology¹ and Haematology², University of Sheffield, UK): The aggregation of platelets by *Streptococcus sanguis* NCTC 7863 requires specific antibody.

We have previously reported that many strains of *S. sanguis* cause human platelets to aggregate *in vitro* and that this process is dependent upon the activation of complement by the alternative pathway. We recently observed that plasma absorbed with NCTC 7863 no longer supported platelet aggregation by that organism but it would support aggregation by other strains. This led us to investigate the role of specific antibody in platelet aggregation by *S. sanguis*. Plasma depleted of total IgG by passage through protein A Sepharose or of specific IgG by absorption at 0°C with NCTC 7863 cells did not support platelet aggregation but addition of IgG purified by protein A Sepharose restored aggregating activity to the depleted plasma. However, IgG alone failed to support aggregation. Also, bacteria coated with complement and IgG, by incubation in plasma at 37°C, were prevented from aggregating platelets by treatment of the organisms with antibody to human IgG but not by antibody to C9. No correlation was found between the level of IgG to NCTC 7863 determined by ELISA and the time to onset of platelet aggregation in different subjects' plasmas. Antibody binding to NCTC 7863 was maximal if added prior to or at the same time as depleted plasma but binding was markedly reduced and platelets were not aggregated if IgG was added after interaction of bacteria with depleted plasma. Treatment of platelets with antibody to FcγRII (IV.3) inhibited platelet aggregation by bacteria but not by ADP or collagen.

These data indicate that specific IgG is required for aggregation of platelets by *S. sanguis* NCTC 7863 and cell bound antibody interacts with platelets via the FcγRII.

Supported by The British Heart Foundation.

- 257** JIM FEARNE*, SJ JONES and A BOYDE (Child Dental Health, The London Hospital Medical College and Anatomy, University College London, UK): Mineralisation of enamel in infantile hypercalcaemia.

The aim of this study was to determine whether hypercalcaemia during infancy has any influence on mineralisation of the deciduous enamel. Five exfoliated incisors were collected from children with Williams Syndrome. Features with the syndrome include an elfin-like face, mental retardation, cardiac anomalies and infantile hypercalcaemia. Five teeth, macroscopically and microscopically normal, from low birthweight (LBW) children were also studied as a reference group. The teeth were embedded in PMMA, the blocks cut longitudinally, highly polished, and coated with carbon. Quantitative backscattered electron analysis (Zeiss DSM962, 20kV) was used to evaluate mineral density in the enamel. On an arbitrary grey level scale where the mean value for normal coronal dentine was 134.8, the mean grey level for the IHC group enamel was 209.4 (S.D. ± 3.7) and for the reference enamel was 210.5 (S.D. ± 3.27). Comparison using T-Test showed the difference was not significant. There was no evidence of areas of hypoplasia and hypomineralisation, as found in some hypoplastic LBW teeth in a previous study (Fearne J M et al, *Anat Embryol* 189:375-381, 1994), in any of the IHC or reference teeth studied.

These findings suggest either that hypercalcaemia during infancy does not affect mineralisation of the deciduous enamel or that the hypercalcaemia occurs after the deciduous incisors have completed mineralisation.

- 258** C ROBINSON*, S J BROOKES, J KIRKHAM, R C SHORE and W A BONASS (Division of Oral Biology, Leeds Dental Institute, University of Leeds, Leeds, UK): Epiphyseal plate mineralisation: A role for albumin?

Recent studies on enamel mineralisation have indicated that serum albumin is present as an endogenous component of the matrix and its ability to bind to apatite and inhibit crystal growth has raised the possibility that it may act as a crystal growth modulator. Albumin degradation prior to maturation when apatite crystals are growing rapidly supports this view. Albumin has also been reported in the matrix of developing bone (Triffitt J T and Owen M, *Calcif Tiss Res* 23: 303-305, 1977). Much of this was not easily extracted from the tissue (57%) and was presumed to be bound to bone mineral, raising the question as to whether albumin in bone participates in mineralisation. The aim of this study was to investigate the mineralising epiphyseal plate for the presence of albumin, to determine whether it might be mineral bound and whether, like enamel, there was evidence of breakdown before mineralisation. Young rats (age 3 weeks) were killed by anaesthetic overdose. The femurs were quickly removed, freeze-dried and sectioned in their long axis to produce ~1mm thick sections. A 1mm wide strip was cut at right angles to the epiphyseal plate and this was divided into approximately 1mm² samples. Each sample was weighed and extracted with 0.1M phosphate buffer to remove mineral bound proteins. The residue was extracted after demineralisation. Both extracts were subjected to SDS PAGE and Western blotting with polyclonal antibodies to serum albumin. In all cases the phosphate extract produced material at Mr = 66K which cross-reacted with the antibodies. Smaller Mr material was also present indicating some breakdown. Albumin is present in the epiphyseal plate and shows evidence of degradation. The metabolism of albumin may play a role in epiphyseal plate mineralisation.

- 259** C L GODFREY*, C ROBINSON, J KIRKHAM, S J BROOKES AND R C SHORE (Division of Oral Biology, Leeds Dental Institute, University of Leeds, Leeds, UK): The role of albumin in the aetiology of white-spot hypoplasias.

Recent data has demonstrated that serum albumin is a major non-amelogenin component in developing enamel. Albumin is a known inhibitor of hydroxyapatite crystal growth and studies have demonstrated that it must be degraded and removed from the tissue prior to secondary crystal growth. Failure to do so could conceivably result in impaired maturation and the subsequent eruption of white hypoplastic tissue. The aim of this study was to investigate the presence of albumin in idiopathic white spot hypoplasias of human erupted permanent teeth. Human molars with obvious white spots were obtained from the Dundee Dental Hospital. Control teeth were obtained from the Leeds Dental Institute. Bucco-lingual sections were prepared and either a) lightly etched using 35% phosphoric acid for 15 secs, blocked using soya bean flour and reacted with polyclonal antibodies to human serum albumin followed by a second antibody conjugated to 1µm polystyrene spheres (PolySciences UK Ltd) for viewing in the SEM or b) white spots were microdissected, extracted in 0.1M phosphate buffer, demineralised and proteins investigated using SDS PAGE and Western Blotting. The results revealed immunolocalisation of albumin to areas of white spots. Western Blotting using the same antibody probe showed cross-reactivity at Mr = 66K (presumably intact albumin) and Mr = 55 K (presumably an albumin degradation product). Little cross-reactivity was seen in sound enamel using either technique. The results indicate a possible role for albumin in the aetiology of idiopathic white spot hypoplasia. This may result from a failure of the normal mechanisms operating to remove albumin prior to secondary crystal growth or due to ingress of albumin into the tissue during the maturation stage.

- 260** R C SHORE*, J KIRKHAM, S J BROOKES, W A BONASS AND C ROBINSON (Division of Oral Biology, Leeds Dental Institute, University of Leeds, Leeds, UK): Effects of fluoride on the distribution of albumin in the rat incisor.

Many of the non-amelogenin proteins isolated from developing enamel appear to be serum derived such as albumin. Indeed, albumin may be an important modulator of crystal growth during enamel secretion and maturation (Robinson et al, *Calcif Tissue Int* in press) and its ingress from extraneous sources such as adjacent dentine may be restricted (Shore et al, *Conn Tissue Res* in press). High levels of fluoride produce enamel in which final crystal growth appears to have been inhibited and the tissue retains a relatively high protein content. One possible cause of this may be the uncontrolled ingress of albumin from the dentine during this critical phase of enamel development. An immunohistochemical investigation was carried out of the distribution of albumin within the incisor of rats given 75ppm fluoride in the drinking water for 3 weeks. The right mandible of each rat was dissected out, immersion fixed in formal saline, decalcified in acid, embedded in wax and sectioned parallel to the long axis of the incisor. 4µm sections were taken, the wax removed and following thorough washing, reacted with a polyclonal anti-rat albumin antibody (Organon UK Ltd). Localisation of cross-reactivity was determined by the use of a peroxidase conjugated second antibody and diaminobenzidine as substrate. The results showed that whereas in the control animals there appeared to be little albumin present in the dentine adjacent to secretory enamel, in the animals given fluoride many tubules within this region of dentine were labelled. These results suggest that inhibition of final crystal maturation occurring as a result of high fluoride levels may be in part due to abnormal ingress of albumin from the adjacent dentine during the secretory stage of enamel development.

- 261** J KIRKHAM*, S J BROOKES, R C SHORE, W A BONASS AND C ROBINSON (Division of Oral Biology, Leeds Dental Institute, University of Leeds, Leeds, UK): Enamel crystal growth modulation by specific components of the amelogenin degradation pathway.

Control of crystal growth during enamel development is presumed to be mediated by the extracellular organic matrix. During enamel development, the principal matrix protein, amelogenin, undergoes a highly orchestrated series of proteolytic cleavages generating a range of specific smaller molecules. Previous investigations have suggested that the parent amelogenin (Mr = 25K, SDS PAGE data) can be selectively bound by synthetic hydroxyapatite and may thus modulate crystal growth. The aim of the present study was to use enamel hydroxyapatite crystals and simulated enamel fluid to determine the relative binding properties of a range of enamel matrix proteins in vitro. Enamel matrix proteins were extracted from developing rat incisors using acetic acid and were freeze-dried prior to resuspending in "enamel fluid" (Aoba T & Moreno EC, *Calcif Tiss Int* 41: 86-94, 1987). Enamel crystals from secretory stage rat incisors were rendered protein free and were added to this solution. Proteins which bound to the crystals were released by subsequent dissolution of the crystals and were separated using SDS PAGE. N-terminal sequencing of bound proteins indicated that 5 components (Mr ~ 25-18K), each with amelogenin N-termini, were selectively bound to the crystals. However, there was no preferential binding of the parent 25K amelogenin. Unbound components with Mr < 11K were found in the supernatant. The results suggest that specific amelogenin breakdown products, as well as the parent molecule, may be modulators of enamel crystal growth. Inhibition of growth in width and thickness could encourage preferential growth in the C axis. Controlled degradation of these fragments with increasing developmental age would permit secondary crystal growth in these dimensions.

- 262** W A BONASS*, C ROBINSON, J KIRKHAM AND S J BROOKES (Division of Oral Biology, Leeds Dental Institute, University of Leeds, Leeds, UK): Cloning and expression of rat enamel protein cDNA in *Escherichia coli*.

The extracellular matrix in which tooth enamel forms is composed primarily of the secreted protein amelogenin and its breakdown products. The enzymatic processing of amelogenin generates a number of specific degradation products, some of which play a key role in the mineralisation process. In addition to the heterogeneity produced by proteolytic processing, alternative forms of amelogenin are generated by alternative mRNA splicing (eg the Leucine Rich Amelogenin Peptide - LRAP). The various roles for these multiple forms of polypeptide and the mechanism by which they are generated from a single gene remains to be discovered. As part of an ongoing study on the mechanism of amelogenesis in the developing rat incisor, experiments have been designed to identify the enzymes involved in the proteolytic processing of amelogenin and to determine their temporal and spatial expression. In order to generate sufficient quantities of amelogenin for use as a substrate, cDNA clones of amelogenin and the alternatively spliced LRAP, have been cloned into the eukaryotic expression vector pVL1392. In order to identify the products of expression, the generation of anti-amelogenin anti-serum is required. To produce an antigen for this purpose cDNA clones of amelogenin and LRAP have been constructed in the prokaryotic fusion protein expression vector pGEX-2T. *Escherichia coli* cells containing the recombinant pGEX-2T plasmids have been grown in LB medium and shown, on induction with IPTG, to synthesise recombinant fusion proteins. A 33KDa glutathione S-transferase-LRAP fusion protein has now been synthesised and purified and used as an antigen for raising a polyclonal antiserum against rat amelogenin.

- 263** L GIBBS*, G CRAIG & A CHAMBERLAIN (University Depts of Archaeology & Prehistory, & Oral Pathology, Sheffield): Extraction of sex-specific DNA from teeth

Specific sequences of DNA derived from bone have been used to determine the sex of ancient human remains (Hummel & Herrmann, *Ancient DNA*, 1993). To date, little is known about the nature of DNA derived from human teeth which are less susceptible to post-mortem degradation and survive in the ground longer than other parts of the bony skeleton. This study reports on the extraction and characterization of sex-specific DNA sequences derived from 41 modern and 14 forensic human teeth. Extraction and characterization of dental DNA was performed using the conventional phenol-chloroform procedure followed by PCR-based sex-typing. Quantity and quality of the DNA was assessed by UV-spectroscopy and gel electrophoresis. All modern and forensic adult and juvenile teeth assessed by UV-spectroscopy produced quantifiable DNA. Visualization of the DNA by gel electrophoresis was unpredictable and possibly individual-specific. The DNA recovered from modern permanent teeth was highly variable within tooth type ranging from 25.1 to 70 µg/g original tissue. Adult dental DNA was also substantially lower than the DNA recovered from juvenile deciduous teeth which ranged from 69.0 to 117.0 µg/g dry weight. Despite this variation, the results confirm the potential of using dental DNA for identifying the sex of ancient human skeletal remains.

- 264** E A EALES*, M L JONES, C NEWTON, A SUGAR (U.W.C.M, Cardiff, and Morriston Hospital, Swansea): Computerised prediction of soft tissue changes following Le Fort 1 osteotomies.

A study was performed to compare soft tissue movements resulting from a series Le Fort 1 osteotomies with those predicted for the same cases by a computerised software package (CG3). The source of material was serial lateral cephalometric radiographs for 25 consecutive patients that had received similar Le Fort 1 osteotomies primarily to correct an antero-posterior skeletal discrepancy.

Many of the digitised points on the facial profile were well predicted. The chin and profile changes resulting from mandibular autorotation were reasonably well predicted. However in some cases prediction was less consistent particularly in the region of the nose and lips, and in those cases initial size, thickness and morphology of the soft tissues and the tendency for the upper and lower lip plots to cross over appeared to be important factors.

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MA BAMBER*, Z ABANG and M HARRIS. (Dept. Maxillofacial Surgery, Eastman Institute of Oral Health Sciences, London): Investigation into the effect of posture and anaesthesia on occlusal registrations for orthognathic surgery planning.

Aims were to investigate the differences in centric occlusion (intercuspal-CO) and centric relation contact position (CR) in: a) upright and supine postures when awake, and b) supine under general anaesthesia, for orthognathic surgery planning.

Records:- 6 patients; 1 male and 5 females, 4 skeletal CI.II div.1, 1 CI.II div.2 and 1 CI.III, were included in this study. Awake records per patient were 1 CO and 3 CR, upright and supine. Anaesthetised records per patient were 3 CR, supine. All were recorded with Moyco' X-Hard Beauty wax. Casts were mounted in the Densar Mark II articulator and transferred to the Vericheck for measurements.

Results:- mean Co-CR (mm) for awake upright was 2.2; SD:1.1, awake supine 2.6; SD:1.00, and anaesthetised supine 3.6; SD:1.4. No significant difference between the two sides, so data from both sides were integrated. The differences between postures were significant; awake upright v awake supine $p=0.0048$, awake supine v anaesthetised supine $p=0.00002$, and awake upright v anaesthetised supine (positions normally used in orthognathic planning) $p=0.00001$.

79% of the CR were registered posterior to CO in awake upright, and 90% in awake supine and anaesthetised supine. In skeletal CI.III, CR was recorded anterior to CO.

Conclusion: for orthognathic surgery, centric relation should be recorded in the supine patient and overcorrection built into the planning.

MOYCO Industries, Inc. Philadelphia, PA 19132, USA.

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M. HARRISON* and J. SHEPHERD. An investigation of potential facial protection conferred by cycle safety helmets. Department of Oral Surgery, Medicine and Pathology, UWCM, Cardiff, UK.

Cycle safety helmets are designed to prevent head injury, but they may also protect the facial structures. A non-destructive method was developed for assessing the potential of helmets to protect the facial structures. Computerised digital imaging was used to measure the extent of the shadow cast onto the facial surface of a mannequin headform by a helmet when illuminated by a plane light source. Measurements were taken over a range of angles of incident light in the vertical plane, and in rotation about the central vertical axis of the headform. For each combination of angles, the shaded area represented the zone of potential facial protection conferred by the helmet in an impact with a plane surface. Twelve helmets were tested and ranked in order of their potential to protect the facial structures. Wide variation was found in the contour of the facial aperture; ranging from the minimum inferior extension demanded by international standards, to well-defined inferior extensions - particularly over the temporal area and zygomatic arches. Bulk of the helmet at the aperture was found to contribute greatly to the degree of protection conferred. Bulky helmets with positive inferior projections provided potential protection for a larger area of the face over a wider range of angles of illumination. Whilst the current trend is towards smaller 'microshell' helmets of minimal extent, these findings suggest that the international standards for cycle helmets should be changed to incorporate this potential protective effect. Supported by The Welsh Scheme for the Development of Health and Social Research, and the Royal College of Surgeons of Edinburgh.

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BRICKLEY M. R., SHEPHERD J. P., (Oral Surgery, Medicine and Pathology, UWCM, Heath Park, CARDIFF CF4 4XY): ROC validity assessment of neural network and oral surgeon's treatment planning.

A fully connected back propagation neural network was trained to make third molar treatment decisions utilising a training set of clinical information for 119 patients (79 female, 40 male). Training was stopped when a previously obtained validation error minimum was reached. Clinical information from 178 patients (with 348 lower third molars) was then used to test the network on a toothwise basis. Network outputs were then categorised into 10 uncertainty threshold levels. At each uncertainty threshold, true positive and false positive values were obtained by comparison with NIH gold standard treatment decisions. These values were used to derive an ROC curve for the system which was then compared to those obtained from 3 consultant oral and maxillofacial surgeons. The network achieved an ROC curve area of 0.893 compared to values ranging from 0.570 to 0.943 for the consultants. The areas beneath the ROC curves were assessed statistically to determine whether performance between the network and each consultant differed. The network performed as well as one consultant and significantly better than the two others making third molar planning decisions using the NIH criteria as a gold standard. It was concluded that neural networks have an important role in informing clinical decision making in this area.

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RA ARMSTRONG*, MR BRICKLEY, ML JONES. Dept. of Oral Surgery, Medicine and Pathology, University of Wales College of Medicine, Heath Park, CARDIFF CF4 4XY, UK): Treatment strategies for lower third molars following orthodontic care.

Impaction of the lower incisor teeth has been advocated as a reason for the removal of lower third molars. Crucial to making decisions to remove teeth on this basis is the opinion of the referring orthodontist. Despite this, the criteria that this group employs are unknown. The study was undertaken to investigate this issue.

Data from 15 completed orthodontic cases were presented to orthodontic specialists who were requested to choose a management strategy for the lower third molars present, to predict the final position for each lower third molar and to indicate on a visual analogue scale the strength of their reasons for referral. These data were analysed by speciality. The data show that orthodontists do not routinely refer patients to oral surgery departments for an opinion regarding third molar management upon completion of their orthodontic treatment. Hospital orthodontists tended to delegate review of these teeth to the general dental practitioner. Orthodontic specialist practitioners were more likely to review patients routinely at a specific age (16-21 years) or to refer the patients to their own practitioner for third molar management. Orthodontists within the Community Dental Service were divided between review at a specific age and referral for a specialist oral surgery opinion. Impaction of the lower incisor teeth was rarely (1.14%) cited as the reason for referral to a specialist oral surgery department across all specialities. In conclusion there is little evidence that orthodontists see the need for an oral surgery opinion concerning third molar management following orthodontic treatment within the sample population in this study.

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M WILLIAMS*, S PARMAR, M MCGURK (Departments of Oral and Maxillofacial Surgery, Nottingham, & Guy's Hospital, London): A Study of Gortex Assisted Osseous Regeneration in Apicectomised teeth.

Current research indicates that Gortex guided tissue regeneration may significantly improve bone healing. (1) Periapical bone cavities were used to establish its application in the management of apicectomised teeth. The success of the apicectomy procedure will be significantly increased if complete bony healing can be achieved.

The study involves a randomised sample of 100 apicected teeth, 50 in the control, 50 in test arms. All patients had radiographs at set time points pre and post surgery, and bone cavity size measured both from the radiograph, and directly at the time of surgery. In the test group, a Gortex membrane was placed over the periapical defect and removed one year post-operatively. To date the control group comprises 41 apicected teeth in 31 patients. The test group comprises 25 apicected teeth in 22 patients. The success rate in the control group is currently 88%. The success rate in the test group using Gortex is currently 100%.

These preliminary results suggest that Gortex can be used successfully in the management of apicectomised teeth and the bone repair improves results of treatment.

(1) Dehlin C, Lindar A, Gottlow J, Nyman S: Healing of bone defects by guided tissue regeneration. *Plast Reconstr Surg* 81:672-676

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P J THOMSON* (Department of Dental Medicine and Surgery, University of Manchester UK): Double labelling of oral mucosa - a quantitative assessment of epithelial cell activity.

Epithelial dysplasia in oral lesions is classified histologically into mild, moderate or severe, but these criteria are not always reliable and carcinoma may develop in lesions where previous biopsy revealed minimal dysplasia. In this investigation a new, double labelling technique was carried out, utilising tritiated thymidine and bromodeoxyuridine to facilitate autoradiographic and immunocytochemical examination of excised oral mucosa. Labelling of epithelial cells occurs during the phase of DNA synthesis and sequential labelling allows classification and counting of cells within different stages of the cell cycle; this enables a quantitative assessment of epithelial cell activity. Results showed consistently reliable labelling and scoring of biopsy material for both normal and abnormal oral epithelium, with enhanced scores in malignant tissue.

It is concluded that double labelling allows quantitative assessment of epithelial cell activity; the use of labelling profiles of premalignancy or malignancy offers an alternative method of assessing malignant potential in oral lesions.

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C LONGBOTTOM* and K ROBOTI (Department of Dental Health, University of Dundee, UK): Comparison of panoramic tomography and radiovisiography for assessing dental development

The aim of this study was to compare dental panoramic tomography (DPT) and full-mouth radiovisiography (RVG) for the dental developmental assessment of children in the mixed dentition stage. The areas of comparison were: a) clinical utility of the radiographic information; b) the radiation exposure dosage; c) the acceptability of the procedures, and d) the total image acquisition time. Of the 75 study children (aged six to 12 years) 50 were examined using DPT and 25 using RVG. It was found that DPT was superior to RVG in terms of: clinical utility ($p < 0.001$ - chi-square test), acceptability to patients and operators ($p < 0.01$) and total image acquisition time (7.5 vs 37 mins), but the calculated radiation dosage of full-mouth RVG was 27% of the DPT dose.

It is concluded that a full-mouth radiovisiography technique, using currently available equipment, cannot yet replace panoramic tomography for dental developmental assessment of children in the mixed dentition stage.

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J H NUNN*, N HUNTLEY, A LAD and P H GORDON (Department of Child Dental Health, Newcastle University, UK): Assessment of dental maturity from radiographs in children with renal disease and a control group.

It has been a common observation that children with chronic renal disease suffer delay in skeletal maturation such that they lag behind their peers in height.

This study aimed to assess dental maturity using the Demirjian index in comparison with chronological age for a group of children with chronic renal failure and a control group. The latter were matched for age (within a month) and sex. The index is generated by staging the development of seven mandibular teeth from an orthopantomogram, taking into account missing teeth. The total maturity score is converted to an 'age' score using percentile charts for the sex. Two of the authors read the 31 pairs of radiographs 'blind' and arrived at a joint score. Intra- and inter-examiner variability were assessed from a repeat examination of five radiographs. The data were entered onto magnetic media and analyzed using paired T-tests and coefficients of correlation. The results from this study indicate that for the renal children their dental maturity as assessed using the Demirjian index was significantly behind their chronological age (20 months) and consistently so across the group. This was not the case for the control group of children where the Demirjian age was 14 months different from the chronological age but with only poor correlation between the Demirjian age and the chronological age.

Comparing the Demirjian ages between the renal and control children did not reveal any significant difference (0.7 months). It can be concluded that children with renal disease do not suffer any significant delay in their dental maturation.

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R.A.E. BRYAN* and G.J. ROBERTS. (Dept of Orthodontics and Paediatric Dentistry, UMDS, London): The dental status and health attitudes of children with Epidermolysis Bullosa.

Objectives: The dental status and health attitudes of children with Epidermolysis Bullosa (EB) was investigated to identify levels of dental caries, plaque, gingival inflammation and developmental dental anomalies.

Subjects and Methods: Oral examinations of 31 children attending The Great Ormond Street Hospital for Children, London were performed. A record was made of decayed, missing and filled teeth, plaque, gingivitis and developmental dental anomalies. The parents completed a questionnaire assessing attitudes to general and dental health, and a diet diary for their child over the following three days. The data were compared to that from healthy controls.

Results: The mean dmft(permanent) of the control and EB groups were 1.77 and 4.19 ($p < 0.05$), and 0.67 and 2.22 for deciduous teeth. There was a large "missing" component for the EB group (mean difference of 8.5 surfaces). The filled components were similar (mean difference 1.0 surfaces). The oral hygiene and gingival condition of the children with EB were significantly worse than the controls ($p < 0.0001$). Only one child with recessive dystrophic EB had any developmental dental anomalies. The questionnaires and diet diaries showed very little difference in the health attitudes or diets of the two groups.

Conclusions: It is concluded that this sample of children with EB have significantly poorer oral health than non-EB children.

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D WARING, M S DUGGAL*, T M WRIGHT¹. Depts Paediatric Dentistry, Leeds Dental Institute, Leeds & University N Carolina¹, USA
Mineral Composition and Ultra-structure of dentinogenesis imperfecta

Dentinogenesis Imperfecta (DI) is an inherited autosomal dominant condition involving 1:8000 individuals. Most studies of this condition have concentrated on the treatment aspects with the mineralisation defect and ultrastructure still remaining poorly defined. The aim of this study was to characterise the mineralisation defect and analyse the ultrastructural appearance of the enamel and dentine of a tooth with DI.

An extracted first permanent molar was obtained from a male patient affected with type 2 DI with a normal extracted premolar serving as a control. The teeth were sectioned and hand ground to 100µm thickness samples for light microscopy and photomicrography. SEM was also performed using field emission EM. Mineral per volume was determined using the method of Robinson et al (1971).

Light microscopy revealed hypomineralisation of dentine with lack of dentinal tubules in the circumcuspular dentine in the tooth with DI. SEM also showed fewer and variably sized tubules. Interestingly, the ADJ showed normal scalloping. The DI enamel appeared to have 7% and dentine 8% less mineral per volume as compared with control. Similar results were obtained for the phosphorus content of DI teeth. The DI enamel and dentine also had 33% and 19% less Mg respectively as compared with control.

It was concluded that the tooth with DI clearly had an altered histological ultrastructure and an abnormal mineral content as compared with normal control.

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KINIRONS MJ* and FLEMING P (School of Dentistry, Queens University Belfast): Dental Caries experience in children with lymphoblastic leukaemia related to the duration of treatment and remission.

The aim of this study was to examine possible relationships between their dental caries experience and the durations of their medical treatments. Their dentitions were examined for dental caries (Palmer JD et al Comm Dent Health 1 55-56, 1984) and the timing and duration of chemotherapy, prophylactic treatments and remissions were recorded. There were significantly more decayed deciduous teeth in the leukaemia group than in matched controls (means 2.2 and 1.2, $P < 0.05$) and similarly for the permanent dentitions (means 3.4 and 1.5, $P < 0.05$). Those in remission for more than 3 years had more decayed teeth than those in shorter remission (means 4.6 and 2.1, $P < 0.05$). An increased number of these lesions were seen in patients whose oral Nystatin prophylactic treatment was longest.

It is concluded that early caries in these patients is associated with increased remission time and use of sugar containing medication and is not related to their chemotherapy.

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P J WATERHOUSE* and J H NUNN (Department of Child Dental Health, University of Newcastle upon Tyne, UK): The chemical bleaching of non-vital teeth and its sequelae.

The chemical bleaching of discoloured non-vital teeth is an acceptable alternative producing aesthetic improvement, although long-term results are equivocal. The present study was undertaken to investigate a chemical technique for the bleaching of non-vital permanent teeth, by assessment of outcomes and the variables affecting this.

20 patients (21 teeth) with discoloured central incisors were treated by the walking bleach technique and reviewed clinically and radiographically for 18 months following completion of bleaching.

The modal duration of bleaching was 2 months, with a mean of 4.9 applications of bleaching agent. At the end of treatment 61.9% of patients expressed satisfaction with the result. All teeth bleached lightened in colour to varying degrees. Utilising a porcelain shade guide as a means of reference, the percentage of teeth prior to bleaching which were deemed 'off the shade guide', grey-shade or reddish-grey was 96.6%. Immediately post bleaching the percent had reduced to 57.2 with relatively more teeth being at the lighter end of the scale for each colour band. By 18 months 3 teeth had been veneered and 33.4% of the teeth remaining were seen as 'off the shade guide', grey-shade or red-grey. 81% of teeth were classed as healthy at 18 months and there was no evidence of cervical resorption or progressive apical resorption.

It is concluded that at 18 months post-bleaching no teeth re-discoloured to a degree which matched the original discolouration.

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PJM CRAWFORD, DC BABB* AND IM HARVEY². (Division of Child Dental Health and ²Department of Epidemiology and Public Health Medicine, University of Bristol UK): A Peer Assessment Rating for Paediatric Dentistry (PARPD).

It may not be necessary in paediatric dentistry always to restore decayed teeth. In a conventional assessment of dental findings, the outcome of such operative inactivity would still be recorded as "disease", despite being an acceptable outcome in total patient care.

In an attempt to define "acceptable care", the present work attempts to establish a paediatric dental equivalent to the Peer Assessment Rating (PAR) in orthodontics (Richmond S, PhD Thesis, University of Manchester, UK). 35 dental practitioners completed an anonymous questionnaire covering items of dental care for children which, when occurring in a regular dental attendance, could indicate failure of clinical activity. 8 items were used in each of 4 "case histories" for 6 year old children.

The practitioners rated all items most indicative when occurring in the context of a caries-free child and least indicative when occurring in a child with high caries experience. In all "cases" the need for a general anaesthetic was rated as the most indicative measure of failure of treatment (Score 4.24/5), followed in order by unexpected pulp therapy (3.38), enforced tooth loss (3.36), an episode of dental pain (3.28), cervical decalcification in a deciduous molar (3.16), new occlusal caries in a permanent molar (3.15), new caries in deciduous teeth (2.58) and, finally, the loss of a restoration (2.49).

The present study should form a foundation for the development of monitoring systems for paediatric dental restorative care.

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MELLOR A C*, BLINKHORN A S, HASSALL D, HOLLOWAY P J, WORTHINGTON H V. (University Dental Hospital of Manchester, UK.): Dental health of 14/15 year olds under capitation and fee-for-item.

The aim of this study was to compare the mean DMFT indices and components of samples of 14/15 year old subjects registered with general dental practitioners under capitation for at least a year in 1994 in Doncaster (Don), Wycombe (Wyc) and Hereford/Worcester (H/W), with the same indices collected in the same areas on a similar age group of regular attenders in 1989 when the dentists were paid under a fee-for-item system. The same examining technique and diagnostic criteria were used on both occasions.

A total of 891 regularly attending subjects were included in the study. The 1994 samples were, on average, 2-5 mths older than those of 1989. Between 1989 and 1994 the mean DMFT indices in all three districts had statistically significantly reduced ($p < 0.01$) by 30-35% (Don 2.97 to 1.82; Wyc 1.86 to 1.29; H/W 2.60 to 1.83). The FT indices had also significantly reduced by 25-42% (Don 2.71 to 1.55; Wyc 1.59 to 0.87; H/W 1.92 to 1.12). The DI indices were all greater in 1994 but only in Wycombe was the increase from 0.26 to 0.37 significant ($p < 0.01$). The WT indices did not differ significantly over the five year period.

There has been a continuing improvement in the dental health of 14/15 year olds since the introduction of a capitation system of remuneration for general dental practitioners.

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HASSALL D C*, BLINKHORN A S, HOLLOWAY P J, MELLOR A C, WORTHINGTON H V. (Turner Dental School, Manchester, UK.): Sealant and Preventive Resin Restoration in general dental practice under capitation.

This study examined the use of fissure sealants and preventive resin restorations (PRR) by general dental practitioners working under a capitation payment system in Doncaster (n=24), Wycombe (n=25) and Hereford/Worcester (n=24). This random sample of dentists in each area provided data on treatment carried out on a sample of 6-12 and 13-15 year old children between 1.10.92 and 30.9.93. The number of dentists who placed sealants during the study period was 52 (71.2%). Of the 4250 children whose treatment data were collected, 284 (6.7%) had at least one tooth sealed during the study period, the mean number of sealants placed per child for those children with sealants being 3.3 (range 1-16). There was no gender difference in sealant provision, however the mean number placed per child was significantly higher ($p < 0.001$) in the 13-15 year age group (4.0) than in the 6-12 year age group (2.7). The number of practitioners who had placed a PRR was 32 (43.8%), but only 59 children (1.4%) had this type of restoration, the mean per child of those children with PRR's being 1.6.

A substantial majority of general dental practitioners in the three areas placed fissure sealants under capitation arrangements but the percentages of children receiving sealants or preventive resin restorations were low.

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J A DAVIES*, P A SMITH and N B PITTS (Dental Health Services Research Unit, University of Dundee, UK): Monitoring the capitation scheme in Scotland - how useful is the data collected?

Data on the dental care of children collected via form GP17C should serve a dual purpose - allowing payment to dentists and monitoring of the capitation scheme. To date, any monitoring has largely reported costs and not patterns of treatment or dental health. This study looks at possible extensions to monitoring which are feasible with currently collected information. Data relating to dental treatment in 1992 and 1993 were collected via the Scottish Dental Practice Board for a sample of children born in 1977. Mean number of visits between registrations was recorded as 2.4 (range 1 to 19), but a visit is not easily interpreted and counts may include repeated orthodontic assessment. Mean D, M and F figures recorded for these children at re-registration were 1.21, 0.92 and 5.22, but criteria for assessing DMF in dental practice have not been specified. Comparative figures from an epidemiological survey of Scottish 14 year olds were D=0.59, M=0.38 and F=2.59 in 1990. On average, 13% of GP17C forms used for capitation patients also involved item of service payments.

Data collected via form GP17C, whilst less extensive than that previously collected, can give some insight into patterns of treatment and dental health of attenders. However, there are problems of interpretation which must be borne in mind in the utilisation of this data and in the development of this, or any future, capitation scheme.

Supported by the Scottish Office Home and Health Department

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LMD MACPHERSON* and VI BINNIE (Department of Adult Dental Care, University of Glasgow, UK): A survey of general anaesthesia, sedation and resuscitation in general dental practice.

The Poswillo Report, published in 1990, made recommendations with regard to general anaesthesia (GA), sedation and resuscitation in dentistry. The aims of the present study were to examine the level of provision of GA and sedation by general dental practitioners (GDPs) a few years after the publication of the Report, and to estimate the degree of compliance of GDPs with a number of its principal recommendations.

A questionnaire was sent to 268 GDPs in two Health Boards in Scotland, and a response rate of 80% was achieved. Thirteen percent of respondents had treated GA cases during 1993-94, while 18% indicated that they had stopped providing a GA service since the immediate pre-Poswillo years. However, 91% had referred some patients elsewhere for treatment under GA during 1993-94. There was very little evidence of a replacement of GA by sedative techniques. The percentages of respondents using inhalation or intravenous sedation during the previous year were 9% and 7%, respectively. Almost all of the respondents reported that they had been trained in cardio-pulmonary resuscitation and 60% stated that resuscitation exercises were practised at least once yearly. Whilst over 80% of the practitioners indicated that they stocked the emergency drugs recommended in the Poswillo Report, 12% expressed concern regarding the length and content of the drug list.

If the recommendation that sedative techniques be used as an alternative to GA is to be realised, improvements in training at both undergraduate and postgraduate levels will be required. Additionally, the concern of some respondents regarding the emergency drug list is in accordance with opinion expressed elsewhere. Further consideration is perhaps required to determine whether a curtailed list of "essential drugs" would be adequate.

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M J PRENDERGAST*, J F BEAL and S A WILLIAMS (Dental Public Health Unit, Leeds Healthcare and Leeds Dental Institute): Deprivation and dental health in 5 year olds in Leeds, UK.

This study investigated the relationship between material deprivation, dental health and related behaviour in 5 year old children from the Leeds health district in 1994. Stratified cluster sampling of primary schools was used to obtain a sample of one third of the 5 year old population. Examinations were carried out in school by four trained and calibrated examiners using standard criteria.

2677 children were examined and 1881 (70%) parental questionnaires returned. Children were assigned to one of 5 bands according to the ranking of the Townsend deprivation index for the enumeration district in which they lived. Caries experience increased significantly with deprivation. Mean dmft in each band was 1.29, 1.89, 2.31, 2.93 and 3.24 ($P < 0.001$). Deprivation gradients were found in reported use of the Community Dental Service (1-10%) and parental regular dental attendance (88-57%). Findings were similar in both White and Asian ethnic groups but Asian children from the most deprived districts had a higher mean dmft than their white counterparts (4.80 vs 3.21, $P < 0.001$). In Asian children deprivation was confounded by religious and cultural differences. Muslim Asians had higher caries experience (dmft 4.63) than non-Muslims (dmft 2.08, $P < 0.001$) and were more likely to live in deprived areas.

This study suggests that the Townsend index of deprivation could be used as an indicator of dental health and related behaviour in planning services and targeting dental health promotion.

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C M JONES* and C O TAYLOR (North West Regional Health Authority): Paediatric Dental General Anaesthesia rates, the relationship to caries prevalence.

The use of General Anaesthesia in the three branches of the NHS dental services (Hospital Dental Service, Community Dental Service and the General Dental Service), was investigated. The statistical relationship of the rates of dental general anaesthesia to caries prevalence, as shown by the RASCO co-ordinated epidemiology programme was examined.

In only one of the last five years did the correlation reach statistical significance. There are many problems associated with collection and interpretation of the data sources used.

It is concluded that the majority of GAs are carried out in the GDS and that treatment need, as shown by caries prevalence, plays only a small part in influencing dental general anaesthesia rates.

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N B PITTS* and Z NUGENT (Dental Health Services Research Unit, University of Dundee, Scotland, UK): Capitation registration of Scottish 5-year-olds related to caries prevalence and deprivation scores

This investigation aims to explore the relationships between capitation registration of Scottish 5-year-olds and (1) their dental caries experience and (2) postcode-based scores of deprivation. A list of the children examined in the Scottish Health Boards' Dental Epidemiological Programme survey of 1993/94 was supplied to the Scottish Dental Practice Board who advised on their registration status. Postcode information collected at the survey was then used to derive "DEPCAT" and "Carstairs" deprivation scores (Carstairs V and Morris R. Deprivation and Health in Scotland, Aberdeen, Aberdeen University Press, 1991). The 5,915 5-year-old children were classified into 3 groups according to capitation status: 2,855 (48.3%) were registered ("present"); 2,628 (44.4%) had no record of registration ("never"); while 432 (7.3%) had been registered previously ("past"). The mean dmft for the total sample was 3.21, for "present" the mean (2.96) was significantly lower than for "never" (3.29, $p < 0.01$) which in turn was lower than for "past" (4.30, $p < 0.001$). Similarly, the % of the "present" group who had a dmft=0 was 59.7%, significantly lower than the "never" group (62.9%, $p < 0.05$) and, in turn, the "past" group (69.0%, $p < 0.05$). There were no significant area / registration interactions when assessed by log linear analysis, thus the relationships between the capitation groups held constant in spite of the differing levels of caries across individual Health Boards. Rank correlations showed that low frequency of the "present" group was associated with high DEPCAT and Carstairs scores ($p < 0.001$). More than half of this national sample of 5-year-olds were not registered under capitation; those not registered had significantly higher levels of disease experience while those previously registered had the worst dental health of all. Higher deprivation scores were associated with a lower frequency of registration. Moves to improve the dental health of 5-year-olds should address the needs of the non-registered and previously registered groups.

Supported by the Scottish Office Home and Health Department.

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L SEOW*, J WICKENS, A. McDONALD, B. DAVIES, G. PEARSON (Conservation Department, Eastman Dental Institute, UK): An investigation of the flexural strength of IPS-Empress, In-Ceram and Vitadur Alpha porcelain.

This study aimed to compare the flexural strength of 1.25mm thick discs of IPS-Empress¹ (a leucite-reinforced glass-ceramic) and In-Ceram² (a high alumina porcelain) with that of conventional feldspathic porcelain, Vitadur Alpha³. The effect of layering veneer porcelain on various core thicknesses of In-Ceram and IPS-Empress was also evaluated. Core thicknesses investigated were 0.50 mm, 0.75 mm and 1.00 mm layered to a final thickness of 1.25 mm. The flexural strength was assessed using the shell test (Timoshenko 1959). The Vitadur Alpha, IPS-Empress and In-Ceram of 1.25 mm demonstrated mean flexural strengths of 90 MPa, 114 MPa and 578 MPa respectively. The mean flexural strength of In-Ceram was found to be significantly higher than IPS-Empress and Vitadur Alpha whilst there was no significant difference between IPS-Empress and Vitadur Alpha ($p < 0.05$). As the core thickness was reduced, the flexural strength of the veneered specimens decreased. This weakening effect was more significant for IPS-Empress. The 1.00 mm veneered specimens of IPS-Empress showed a significantly lower mean flexural strength (78 MPa) than the 1.25 mm non-veneered specimens, whilst the 1.00 mm veneered specimens of In-Ceram (524 MPa) were not statistically different from the 1.25 mm non veneered specimens.

It is concluded that IPS-Empress did not offer any strength advantage over conventional feldspathic porcelain and In-Ceram was stronger than either material.

¹Ivoclar-Vivadent UK Ltd. ²Vita Zahnfabrik, Germany. ³Vita Zahnfabrik, Germany.

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M. O'SULLIVAN*, A. McDONALD, D.J. SETCHELL and R. GLOVER (Conservation Department, Eastman Dental Institute, UK): Porcelain veneers: Marginal opening by various clinical/laboratory techniques (on refractory dies)

This study compared the marginal opening produced by two operators using three different techniques when fabricating porcelain veneers for six maxillary anterior teeth by refractory die method. The first technique involved fabrication of six veneers on an unsectioned cast, the units being separated only before glazing. The second involved sectioning the refractory cast into individual dies in the laboratory. The third utilised a plastic strip placed between the teeth during the impression stage, to create individual dies on pouring of the cast. Thirty six veneers were fabricated, thus producing 72 surfaces (mesial and distal) for measurement. A Reflex Microscope was used to measure the marginal gaps using silver powder for visual contrast. Measurements were made at the contact area, (11 sites) and along the remaining mesial and distal surfaces (8 sites). Smallest mean marginal openings were recorded when the plastic strips were utilised, both in the contact zone and the other sites (86.3 and 77.5 μ m respectively). The greatest mean marginal opening was recorded on veneers fabricated on the single refractory cast, for both areas (164.8 and 129.5 μ m). The laboratory sectioned model ranked second (105.5 and 97.3 μ m). The difference between the mean results obtained from the two operators was greatest for the single model method, and least for the plastic strip method.

It is concluded that the interdental placement of a plastic strip at the impression stage may improve marginal accuracy of porcelain veneers.

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RAR SHARP, V PIDDOCK and A JE QUALTROUGH* (Restorative Dentistry, University of Manchester, UK): An evaluation of the fit of porcelain inlays.

This investigation compared two methods of assessment of the fit of porcelain inlays. Simulated mesio-occlusal-distal cavities were prepared in ten perspex blocks using a computer-controlled milling machine. Twelve porcelain inlays were produced per block. The following variables were investigated: a) the use of die spacer, b) porcelain build-up techniques and c) the refractory material. Restoration fitting accuracy was assessed by the impression wash method and by image analysis of sectioned replicas. Results indicated that the impression wash technique resulted in a mean misfit of just over 100 μ m with a range of 57.4 μ m to 168 μ m. Image analysis showed a smaller mean fit of less than 70 μ m for all samples. The use of die spacer created a greater internal misfit at the 1% level of significance. There were no significant differences between different methods of porcelain application nor refractory materials.

It is concluded that the use of die spacer resulted in a poorer fit of porcelain inlays. No statistically significant differences in fit were detected between two investment materials, nor between different build-up methods. The impression wash technique did not prove to be sensitive enough to detect differences in processing parameters.

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N J A GREY*, V PIDDOCK and M A WILSON (Dept of Restorative Dentistry, University of Manchester, UK) Strength of ceramic crowns determined on brass dies - effect of luting material

Traditionally, acid-base reaction cements have been used to lute metal and ceramic crowns. However, cements based on zinc oxide are necessarily opaque due to residual powder and may have a very low pH. Resin based composites are now used for their aesthetic and retentive potential. The compressive strength of all-ceramic crowns (Empress¹) of controlled dimensions and fixed on brass dies with different luting media, were determined *in vitro*. Groups of at least ten specimens were loaded to failure after luting with zinc phosphate² (Gp1) and a resin based composite³ (Gp2). The mean compressive loads to failure were 1603 \pm 394 N and 1811 \pm 362 N respectively, with no significant difference at the 1% level using a Student-Newman-Keuls multiple range test. It is concluded that when Empress crowns were loaded to failure on brass dies, no significant effect on strength due to the luting material was determined. Further studies are required using substrates with bonding characteristics similar to natural tooth substance.

¹ Empress, Ivoclar-Vivadent, Schaan, Liechtenstein

² De Trey Zinc, De Trey-Dentsply, Weybridge, UK

³ Variolink, Vivadent, Schaan, Liechtenstein

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R. GLOVER*, R. IBRETSON and A. OLUSILE (Conservation Department, Eastman Dental Institute, UK): The Stability of three removable die systems.

Accuracy in cast restorations demands a stable removable die system. This study compared the performance of three commercial die systems the Ney-Pin¹, Pindex² and Bi-Pin³. A master cast containing four milled metal dies was constructed, the inner dies represented prepared teeth and the outer dies unprepared teeth. Each die had datum points scribed on its occlusal surface. Thirty identical working casts for each pin system were made in Type IV stone. The datum points on each die were measured using a Reflex Microscope⁴ under the following conditions a) casts unsectioned, b) casts sectioned but die not removed, c) casts sectioned, dies removed and replaced 30 times, d) casts sectioned, dies removed and replaced 30 times, followed by lateral loading. Sectioning the casts did not affect the position of the dies. Removal and replacement of the dies thirty times produced an occlusal displacement of all dies. Values were [mean (SD)] in micrometres: Ney-Pin 70 (49), Bi-Pin 135 (150) and Pindex 77 (90). The effect of lateral loading affected the Ney-Pin and the Bi-Pin more than the Pindex pins.

It is concluded that the process of separation and repeated removal of the dies produced an occlusal displacement but with no visible trend in terms of comparative performance of any one system. All three die systems should be considered incapable of maintaining the position of the dies through our laboratory procedures.

¹ Ney-Pin, Chaparrin and Jacobs Ltd, UK ² Pindex, Whaledent International, New York, USA.

³ Bi-Pin, Orthomax Dental Ltd, UK ⁴ Suprastone, Kerr U.K. Ltd.

⁵ Reflex Microscope, Reflex Measurement Ltd, Somerset, UK.

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B C M PATEL*, P BARBER, R LAWS, S M DUNNE (Medical Laser Centre UCL, 185, Rutherford Appleton Labs, Kings College Dental School): Micro-mechanical surface engineering: An alternative to acid etching?

The mechanism underlying retention of plastic restoratives and adhesives is concerned with micro-mechanical interlocking. Fissure sealants, orthodontic brackets, enamel and dentine bonding agents depend entirely on micro-interlocking with the tooth surface. It is possible to significantly improve restorative/adhesive properties by creating micro-engineered geometric structures. The technique of ultra-violet laser photobleaching and image projection was investigated with the view to machining banks of 20-100 µm sq (separation of 20-100 µm) geometric structures with a height of 10-50 µm. A 248nm excimer laser operating at 30ns pulse duration was used at pulse frequency of 20Hz, coenergy density of 2 J/cm² with a pulse range of 100-10,000. A stable portion of the laser beam was isolated and projected through a precision engineered mask (1cm²) (consisting of squares (400µm-200µm) with a separation of 100-200µm) through a complex set of optics (x5 reduction). The image was directed onto 10 roots of extracted human premolar teeth, on an X-Y translation stage. A total of 70 different sites were ablated. LM/SEM examination revealed micro-engineered grids of 'perfectly' square (1-2 µm tolerance) cubic units of 40-100µm sq; 5-50µm high with a separation distance (channel) of 20-40µm. The floor of these channels (the ablation site) consisted of tightly packed, perforated, structures in the form of cones (5-10µm diameter). These are the remains of ablation debris and solidified hydroxyapatite. The surface interface between a low viscosity resin and the engineered root surface showed excellent wetting/covering. The resin penetrated into the channels to create a 'dovetail-like' interlock.

The surfaces of root dentine can be micro-engineered to create discrete series of geometric micro-interlocking units. This may be an alternative means of surface conditioning prior to the placement of a restorative. However, the bonding strength of these surfaces requires further investigation to realise the potential of micro-engineered surfaces.

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F M BLAIR*, J WHITWORTH and JF MCCABE (Dental School, University of Newcastle Upon Tyne): The physical properties of a gallium alloy restorative material.

The purpose of this study was to compare the physical properties and handling characteristics of a gallium alloy (G) restorative material¹ and a widely used dental amalgam (A)². ISO (ISO 1559: 1986) tests were carried out for both materials. The dimensional change on setting for G was +0.39 (0.04)% whilst that for A was -0.05 (0.01)%. Compressive strength values at 1h and 24h were G (1h) 128 (10) MPa, A (1h) 105 (15), G (24h) 345 (38) MPa, A (24h) 365 (35) MPa. Differences between G and A were investigated using t-tests.

G has greater setting expansion (P<.05) and less creep (P<.05) than A. There was no significant difference in strength. The handling characteristics of G were considered unacceptable.

¹Gallium Alloy GF (Tokuriki Honien Co., Japan). ²Dispersalloy dental amalgam (Johnson and Johnson Co., NJ, USA)

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A.J. IRELAND*, M. SHEKRIFF (RUM BATH, UK, UNDS GUY'S HOSPITAL, LONDON UK): THE EFFECT OF STATIC LOADING IN ORTHODONTIC BONDING

The aim of this investigation was to determine the effect of static loading on the measured shear bond strength of steel attachments bonded to enamel. 60 steel attachments were bonded to extracted and prepared premolar teeth with Orthodontic Concise¹ and allowed bench cure for 2 weeks. Half the specimens had 78g weights suspended from the attachment, close to the bondline, prior to testing. All specimens were then shear tested to failure. The mean shear bond strength for the prestressed samples was 127-0N and for the unstressed was 86-1N. The 95% confidence intervals for the difference between the means was 22-3 to 59-2N indicating that static loading prior to shear testing significantly increases shear bond strength.

To conclude, prestressing of steel attachments bonded to human enamel prior to shear testing significantly increases bond strength and should be considered an important experimental variable in the evaluation of orthodontic bonding systems.

1. Orthodontic Concise, 3M.

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A HOUGHTON*, B OYAJOB, A FRAZER, RM GRAVELEY, RGG RUSSELL and BMJ STRINGER (Dept of Oral Pathology and HMCB, University of Sheffield): Immortalised human osteoblast precursor cells.

It is well recognised that tooth loss in periodontitis results from bone resorption that occurs during the course of the disease process. The reasons for bone loss remain obscure although cytokines and growth factors released locally from inflammatory and other cells are likely to play important roles (M Alexander et al., Curr Opin in Periodontol. 39-53: 1994). Also, the bacteria found in periodontal pockets may produce substances that have direct effects on bone cells, leading to bone erosion and loss of tooth support (PM Loomer et al., Infect and Immunity 62: 1289-1297, 1994). In order to study the role of cytokines and growth factors, and also, the potential direct role of bacterial agents on bone cell growth and function, we have produced differentiating human osteoblast precursor cell lines by retroviral temperature-sensitive oncogene transduction, a method that was reported to the BSRD in 1994. Preliminary characterisation of clones which have been grown for over a year in culture, show the cells to retain features expected of an osteoblast precursor lineage. In the presence of the differentiating agents dexamethasone and vitamin D₃, they substantially upregulate their alkaline phosphatase activity. Also, our cytokine-growth factor studies show cells in the undifferentiated state express IL-1a and IL-18 along with IL-6, IL-8, GM-CSF and TNFα, as well as the matrix protein collagen type I. After 7 days of dexamethasone, IL-1a, IL-6 expression is lost along with GM-CSF, TNFα and collagen type I. IL-6 and IL-8 expression, however, is maintained. Furthermore, no expression of IL-3 or IL-4 is seen at any stage. Interestingly, the cytokine/growth factor profile seen after treatment with differentiating agents reflects the profile we see of differentiated human osteoblast-like cells in primary culture.

We conclude that we have been able to develop human osteoblast precursor cell lines that are capable of differentiating to provide features of mature osteoblasts. Our intention is to use these cells as tools to study the molecular and cellular basis of the bone loss seen in periodontal disease.

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R GRAVELEY, B OYAJOB, A FRAZER*, A HOUGHTON, RGG RUSSELL and BMJ STRINGER (Depts of Oral Pathology and HMCB, University of Sheffield, UK): In-vitro studies of human articular chondrocyte cells.

A major problem in the study of human bone and cartilage cell biology is the inability to obtain a constant and abundant supply of human tissue. In 1994, we reported to the BSRD, a method of providing large homogeneous populations of human bone and cartilage cells by retroviral temperature-sensitive oncogene transduction. Results of our preliminary investigations presented at this meeting showed that immortalised chondrocytes derived from biopsies of patients of over 65 years of age, expressed specific markers for differentiated articular chondrocytes. We have now further characterised these cells using reverse transcriptase (rt) PCR to determine their cytokine/growth factor profiles. Cells were incubated either in medium containing 10% heat inactivated foetal bovine serum, or in a serum free medium at the immortalising oncogene's permissive and non-permissive temperatures. To determine the presence or absence of: IL-1a; IL-18; IL-3-6 and IL-8; GM-CSF; M-CSF; TNF-α; TNF-β; TGFβ1-3; PDGF-A and PDGF-B chains; rt-PCR was performed. Little or no difference in cytokine/growth factor expression was seen between immortalised chondrocytes grown at the permissive and non-permissive temperatures. Apart from the lack of expression of TGFβ3 in cells grown in nutrient medium, no difference was seen in cytokine/growth factor expression between cells grown in serum containing and serum free medium. The immortalised chondrocytes were found to express: IL-1a; IL-6 and IL-8; M-CSF and TGFβ-3. A synonymous cytokine/growth factor profile was seen for human articular chondrocytes in primary culture.

In conclusion, we have further characterised our immortalised human chondrocyte clones and shown them to have virtually identical cytokine/growth factor profiles to those of human articular chondrocytes in primary culture. We are presently applying our cells to the study of cartilage biology and cartilage diseases such as osteoarthritis.

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B M J STRINGER* and George FOSTER (Department of Oral Pathology, and MOMED, University of Wales): Immortalisation of raphe nuclei neural cells by transduction with a modulatable oncogene

Serotonin neurones from the medulla oblongata are known to be involved in attenuating the transmission of nociceptive signals from the periphery. In order to study these neurones as a homogeneous population, neural cells from the embryonic rat raphe were transduced with a retrovirally-packaged modulatable oncogene (SV40-T), linked to geneticin resistance marker. After incubation for 8-10 days at 33°C, the transduced cells were selected for by application of geneticin. Individual clones were picked and expanded (in some cases from single cells). After allowing differentiation to occur, by raising the temperature to 39°C and thus switching off the SV40-T active product, the characteristics of the homogeneous neural precursor cell clones were analyzed. Several of the clones bore many features in common. These included expression of SV40-T, expression of 5HTP and 5HT uptake (K_m = 16µM), synthesis and release of 5HT, and expression of neurone specific enolase (NSE) and neurofilament triplet, but not of glial fibrillary acidic protein (GFAP). Interestingly, the choice of neuronal phenotype could be switched off, and a glial phenotype switched on by incubation of the cells with foetal calf serum, or retinoic acid. Under these conditions NSE is no longer apparent, whereas GFAP is observed. Electrophysiological measurements revealed a membrane potential of -40 to -60 mV and evidence of voltage-sensitive K⁺ channels and delayed rectification.

In conclusion, we have produced several cell lines exhibiting most of the known characteristics of serotonergic neurones. These can be used in future studies into the antinociceptive mechanisms of the spinal cord.

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ND CROSTHWAITE*, D TEALE, CD FRANKLIN and BMJ STRINGER (Dept. Oral Pathology, University of Sheffield, UK): Role of p53 in the pathogenesis of lip cancer.

It is well recognised that p53 plays a major role in the pathogenesis of many cancers. In oral cancer in particular, p53 has been shown to be associated with squamous cell carcinomas, verrucous carcinomas and adenocarcinomas. Additionally, the involvement of p53 has also been demonstrated in premalignant lesions of the mouth such as dorsal lingual hyperkeratosis, leukoplakia and epithelial dysplasia. However, little is known of the role of this tumour suppressor gene in the pathogenesis of lip cancer. To our knowledge only one publication has considered this lesion (Bernier A et al., Anticancer Res. 13: 2421-2424, 1993), and in this article, only squamous cell carcinomas were studied. In order to provide further information on the role of p53 in the pathogenesis of this disease, we undertook an immunocytochemical study to determine the potential localisation of p53 in a variety of lip lesions: five each of squamous cell carcinoma, solar keratosis, chronic hyperplastic candidosis and lichen planus respectively. To support this, we also provide a series of positive and negative controls from intraoral (non-sun-exposed) and skin (sun-exposed) lesions. Our results demonstrate that in lip, p53 was localised to the tumour cells of all squamous cell carcinomas studied, most cases of solar keratosis, and even in several examples of chronic hyperplastic candidosis and lichen planus. With regard to the skin lesions taken from sun exposed sites, all were positive.

We conclude that the aberrant expression of p53 is associated with the pathogenesis of malignant lip lesions. In view of the results from premalignant lesions, this expression may be an early feature in the onset of the disease process. Furthermore, our observations on skin lesions would suggest that the overexpression of p53 in lip tumours is likely to be sunlight related.

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J.M. RISK¹*, J. WHITTAKER², D.T. BISHOP³, I.M. LEIGH⁴, A. ELLIS⁵, J.K. FIELD¹.
 (1)Department of Clinical Dental Sciences, The University of Liverpool. (2)CRF:
 Localisation of the tylosis oesophageal cancer gene (TOCG) to 17q.

Diffuse non-epidermolytic palmoplantar keratoderma (NEPK or tylosis) is associated with oesophageal cancer in a large Liverpool family (Howell-Evans syndrome). There is evidence that mutations in keratin genes underlie multiple diseases characterised by palmoplantar keratoderma with or without epidermolysis. Owing to the sites of hyperkeratotic lesions, keratins 6, 9, 16 and 17 are implicated in this family and are coded for in the keratin gene clusters located on 12q11-q13 and 17q12-21. This investigation aimed to determine the chromosomal location of the tylosis oesophageal cancer gene (TOCG). PCR amplification of microsatellite markers from chromosomes 12 and 17 followed by the detection of alleles on silver stained non-denaturing polyacrylamide gels was used to undertake linkage analysis and mapped the TOCG to chromosome 17 at 17q23-qter, telomeric to the type I (acidic) keratin gene cluster. Keratins 6, 9, 16 and 17 are therefore excluded as candidate genes. Significant lod scores were obtained for the following markers: D17S15 (Z_{max} : 7.43 at $\theta=0.12$), D17S929 (Z_{max} : 4.13 at $\theta=0.08$), D17S801 (Z_{max} : 4.67 at $\theta=0.075$), D17S785 (Z_{max} : 5.41 at $\theta=0.09$) and D17S939 (Z_{max} : 3.26 at $\theta=0.11$). Haplotype analysis mapped the TOCG between D17S929 and D17S937, a map distance of 6cM.

The tylosis oesophageal cancer gene is located in a 6cM region of the q arm of chromosome 17, situated telomeric to the known keratin gene cluster.

Supported by the North West Cancer Research Fund.

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H. KLIARIS, T. LILOGLU, D.A. SPANDIDOS, E.D. VAUGHAN, A.S. JONES, J.K. FIELD. (Dept of Clinical Dental Sciences, University of Liverpool L693BX, UK) Genomic instability, mutations and expression of the H-ras gene in head and neck cancer.

Mutations and overexpression are the main mechanisms of activation for the ras family genes in human tumors. A variable tandem repeat (VTR) at the 3' end of the H-ras gene has been proposed to be associated with the risk of tumour development. In squamous cell carcinomas of the head and neck (SCCHN), ras genes seem to be implicated in aberrant expression. In the present study, we have analysed the relative levels of H-ras mRNA in 27 samples from SCCHN, using a competitive reverse transcription PCR technique. We also investigated the possible correlation between ras expression and the presence of alterations in the VTR region of the gene, in 15 SCCHN samples. In addition, we screened 120 SCCHN samples for the presence of point mutations in codon 12 for H-ras, codons 12 and 13 of K-ras and codon 61 of N-ras genes, using a PCR-RFLP technique. Only two samples were found to carry mutations in codon 12 of K-ras, while no mutations were detected in H- and N-ras genes. However, increased levels of H-ras mRNA were detected in 13/17 (45%) SCCHN cases and were found to correlate with favourable prognosis: 1/15 patients of the H-ras overexpressing group had died while 6/12 patients of the non-overexpressing group had died. A further analysis of the above data relatively to previous data on microsatellite instability and LOH of a marker positioned within the H-ras gene, showed no correlation between the presence of overexpression of H-ras with the genomic instability of the locus. We conclude that ras genes are implicated in the development of SCCHN rather by quantitative than qualitative changes.

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J.K. FIELD¹*, H. KLIARIS^{1,2}, P. HOWARD³, E.D. VAUGHAN⁴, D. SPANDIDOS⁵, A.S. JONES⁵. (1)Clinical Dental Sciences, The University of Liverpool, UK. (2)Athens Greece. (3)Microsatellite instability in squamous cell carcinoma of the head and neck.

Microsatellite instability (MI) or genomic instability has been recognised in carcinoma of the colon and also in a number of other carcinomas where it has been associated with mutations in DNA repair genes. Although a number of reports exist on MI in various tumours, its real significance in tumour progression is unknown.

We have investigated 34 microsatellite markers in squamous cell carcinomas of the head and neck (SCCHN). Fifty six tumours have been studied of which 25 have been investigated with ten or more microsatellite markers. In this study we considered two or more microsatellites as diagnostic of MI. 7/25 (28%) of the tumours had MI at two or more loci and 3 of these patients had evidence of 20 or more loci with MI. No correlations were found between MI and previously untreated/previously treated patients, site, histological differentiation, positive nodes at pathology, survival or a history of alcohol intake. MI was demonstrated in T1N0 stage tumours indicating that these changes may occur early in the disease. Two or more markers of MI were found in 3 of 4 non-smokers compared to 1 of 13 in the smoking group of patients which suggests a novel mechanism of carcinogenesis in non-smokers.

Microsatellite instability is a detectable phenomenon in SCCHN and correlates with non-smokers

Supported by the North West Cancer Research Fund.

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A. SCHOLES¹, P. SNUDETS², R. ADAMSON³, J. WOOLGAR¹, CA. HART⁴ & J. FIELD¹. (1)Clinical Dental Sciences, Univ. Liverpool, (2)Free University Hospital, Amsterdam; (3)Human Papillomaviruses and genetic alterations in head and neck cancer.

Mutation of the p53 tumour suppressor gene (TSG) is the most common genetic abnormality identified so far in human cancer. Alteration of normal p53 TSG function may also occur as a result of binding of human papillomavirus (HPV) E6 and p53 TSG proteins. The objective of this study was to determine the prevalence of HPV and p53 TSG alterations in squamous cell carcinomas of the head and neck (SCCHN).

The prevalence of HPV in 64 SCCHN was determined using HPV general and specific primer-mediated PCR. HPV 16 alone was detected in 13/64 (20%) SCCHN. The presence of HPV did not correlate with clinicopathological parameters. Genetic alterations in exons 4-9 of the p53 TSG were examined using single strand conformational polymorphism analysis (SSCP) in 29 SCCHN, 7 of which were HPV 16-positive. Two of 7 (29%) HPV-positive and 3/22 (14%) HPV-negative SCCHN contained alterations in the p53 TSG. Initial studies of the p53 TSG in dysplastic oral lesions have been carried out immunohistochemically, using the monoclonal antibody DO-1. Overexpression of the p53 TSG was detected in 24/40 (60%) dysplasias with highest levels of expression in severe dysplasia.

The presence of HPV 16 and genetic alterations in the p53 TSG do not appear to be mutually exclusive and may be important in the pathogenesis of a subset of SCCHN.

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T. BURNS¹*, M. WILSON², G.J. PEARSON³. (Depts of 1)Microbiology and 2)Biomedical, Eastman Dental Institute, London; 3)Mechanisms of lethal photosensitisation of *Streptococcus mutans*.

The purpose of this study was to determine the mechanisms by which *Streptococcus mutans* is inactivated by toluidine blue O (TBO) and laser light. To determine whether cell membrane damage occurred, suspensions of TBO-sensitized *S. mutans* were exposed to light from a 7.3 mW HeNe laser for 30 minutes and samples removed every 5 minutes. Survivors were enumerated and cell-free filtrates were assayed for intracellular material. Lipid peroxidation was assessed by assaying for malondialdehyde (MDA). The role of oxygen was studied by exposing sensitized bacteria to laser light (i) under different atmospheric conditions (ii) in the presence of deuterium oxide and (iii) in the presence of inhibitors of reactive oxygen species. Following exposure of TBO-sensitized *S. mutans* to 13.2 J laser light, 0.76 micromoles phosphate was detected in the cell-free filtrate, 0.3 micromoles MDA was also detected. When the sensitized bacteria were exposed to laser light under anaerobic conditions there was no significant decrease in the viable count. In the presence of the singlet oxygen enhancer D₂O there was a 15 fold increase in the numbers of *S. mutans* killed. 0.1 M methionine and 0.5 M sodium azide both afforded 98 % protection from lethal photosensitisation.

These results imply that lipid peroxidation and membrane damage play a role in lethal photosensitisation of *S. mutans* and that reactive oxygen species are mediators of the process.

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J. PRATTEN¹ and M. WILSON². (Department of Microbiology, Eastman Dental Institute, London): Effect of environmental factors on the photolysis of methicillin-resistant *Staphylococcus aureus*.

Previous studies in our laboratories have shown that *S. aureus* can be sensitised by aluminium disulphonated phthalocyanine (AlPcS₂) to killing by light from a gallium aluminium arsenide (GaAs) laser. The purpose of this study was to determine the effect of pre-irradiation time (PIT), serum and the physiological state of the organism on the kills achieved. Saline suspensions of methicillin-resistant *S. aureus* (MRSA) were incubated in the dark with 12.5 µg/ml of AlPcS₂ for 60 s or 300 s and then exposed to 1.2 J (1.8 J/sq.cm.) of GaAs laser light (pulse frequency = 20kHz) and the survivors enumerated. The experiments were repeated using bacteria suspended in horse serum, a PIT of 300 s and light doses of 0.6 J and 1.2 J. The susceptibility of MRSA in the lag, logarithmic and stationary phases of growth was determined using a PIT of 300 s and 1.2 J of laser light. For both PITs, 0.8 x 10⁶ cfu/ml (99.9%) of MRSA were killed. 5.8 x 10⁶ cfu/ml of lag-phase MRSA, 5.9 x 10⁶ cfu/ml (99.9%) of stationary-phase MRSA and 5.5 x 10⁶ cfu/ml (99.8%) of log-phase cells were killed by 1.2 J of laser light. In the presence of serum, 24 x 10⁶ cfu/ml (99.6%) were killed by a light dose of 1.2 J.

In conclusion, MRSA were rapidly (60 s) sensitised by AlPcS₂ to killing by GaAs laser light and cells in all three growth phases had similar susceptibilities. Considerable kills were achieved in the presence of serum. This approach, if effective in vivo, may be useful for treating topical infections due to MRSA.

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D. DRUCKER¹ and D. HARTY². (Turner Dental School, Manchester and Institute of Dental Research, Sydney): *Lactobacillus* phospholipid profiles analyzed by FAB-MS.

The major phospholipid of *Lactobacillus* is known to be phosphatidylglycerol yet nothing is known concerning the particular analogues present. The aim of this study was to examine in detail the phospholipid profiles of strains of *Lactobacillus* by extracting lipids and analyzing them using fast atom bombardment mass spectrometry (FAB MS). In addition to simple FAB MS which provided data on molecular weight of analogues, tandem MS was used to provide unequivocal information on molecular structure. The major phospholipid anions found were of m/z 733, 747, 759, 761, 773 and 787 which are consistent with the presence of the following phospholipids: PG(33:1), PG(34:1), PG(35:2), PG(35:1), PG(36:2) and PG(37:2). Major peaks consistent with the presence of carboxylate anions associated with phospholipid were of m/z 295, 281, 267, 255, 253, 227 and 211 which were consistent with the expected presence of cyc-C₁₉, C_{18:1}, cyc-C₁₇, C_{16:0}, C_{18:1}, C_{18:0} and C_{17:1} respectively. Many minor peaks were also recorded. Quantitative differences were noted between species. The profile of phospholipids displayed by *Lactobacillus* is unique to the genus.

It is concluded that the combination of anions found in this study is diagnostic for *Lactobacillus*.

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G. CONRAD¹*, SE. GHARBIA¹, F. LAMPERT² & HN. SHAH¹. (Dept. Microbiology, Eastman Dental Institute, UK; 2)Conservative, Preventive & Periodontology Clinic, Technical Univ. Germany): Ribosomal RNA signatures, selection and sensitivity in detection and quantitation of putative oral pathogens

Elucidating the role of bacteria in mixed oral infections necessitates accurate detection of specific species. Despite the advances made in selective and reduced culture media and sampling techniques, cultural methods fail to detect several of these fastidious organisms. Within the last few years molecular analytical methods have been employed to identify bacteria in clinical samples without the need for their isolation. In this study we report the use of two genetic techniques for the identification and quantitation of 15 putative pathogens from oral infections including gingivitis, rapid progressive periodontitis and pus from infected root canals. DNA probes directed against 16S rRNA specific sequences were constructed from comparative alignment of rRNA encoding genes of the target bacteria. The efficacy and sensitivity of each probe was initially assessed against reference strains of all species. The selected probes were used to detect the presence of each pathogen. In dot blots of nucleic acid extracted from clinical samples. The results showed 10²-10³ fold increase in detection compared to cultural techniques. Furthermore, serial dilutions of the extracted nucleic acid (50-0.5 ng) were used to quantitate specific species. To increase the detection threshold we employed PCR amplification of 16S ribosomal nucleic acids from clinical samples using universal eubacterial ribosomal primers to amplify 1400 bp. Amplicons were hybridised with species-specific probes to detect their corresponding bacteria. This resulted in a ten-fold increase in detection sensitivity, however the practicability to quantify the pathogenic bacteria in the samples by PCR was limited.

These studies provide substantial information on the sensitivity and specificity of ribosomal RNA signatures for the detection of putative pathogens from oral clinical samples. Furthermore, comparison between direct hybridisation and selective amplification of bacterial genes were established.

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DMA ANDREWS*, HN SHAH & SE GHARBIA (Department of Microbiology, Eastman Dental Institute, London): Characterisation of an infective bacteriophage from *Pseudomonas varians* and its potential as a cloning vector.

Present systems for cloning genes of oral, anaerobic bacteria have relied on the use of aerobic/facultative anaerobic bacteria such as *E. coli* that are phylogenetically distant related from the test organisms. Consequently numerous problems are encountered such as deletions, rearrangements, lack of expression or lack of *Ci*-subunit recognition. It is desirable to develop an anaerobic system for such microorganisms. Several cryptic plasmids have been reported from members of the genus *Pseudomonas*. However, little is known about their function and mechanism of mobilisation. In the present study we report preliminary studies on the characterisation of a phage from *F. varians*. The phage was induced from one of the isolates by incubation at 45°C and formed a circular structure prior to entry into the lytic cycle. The phage residues were observed using transmission electron microscopy and were released from the infected cells by budding rather than cell lysis. Phage particles were separated from the culture supernatant by high speed centrifugation. The resulting fraction was precipitated by the addition of chloroform and the collected phage particles were used to purify phage DNA (ca. 23kb). Restriction digest profiles confirmed its AT rich DNA content and a restriction map of the phage was constructed. Infectious particles were added to 10 different *F. varians* and *F. mortiferum* strains to determine its specificity. The phage did not infect *F. mortiferum* but lysed all *F. varians* strains tested.

The results of this study confirm the presence of phages infecting *Pseudomonas* species which probably account for the description of large cryptic extrachromosomal DNA. The isolated phage provides a potential vehicle for DNA cloning and transfer into *Pseudomonas* species.

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VJ HILL*, SE GHARBIA*, DR CLARK* and HN SHAH* (Department of Microbiology, Eastman Dental Institute, London; *GeneSys Ltd, UK): Construction of a dual origin cosmid for cloning and expression of virulence genes from putative periodontal pathogens.

Expression of virulence genes of putative periodontal pathogens has presented considerable problems and some such as the proteinase, gingipain encoding gene, reduces host viability. In other studies the products of the test organism are expressed in minute quantities which hampers detection and purification and therefore subcloning in high expression systems is essential. This often results in genetic recombination and rearrangement and disrupts the encoding genes. We have designed a broad host range cloning vector to clone partial fragments (20-25kb) from bacterial genomic DNA. This was carried out by the digestion of pUC12 using *Bcl*II and insertion of pAFRI (a derivative of RP4). The new dual-origin cosmid vector pMAX 3 allows direct plasmid transfer from *polA* to streptomycin resistant wild type strain via conjugation. The *polA* strain maintains the plasmid at 1-2 copies per cell under the RP4 ori control, while the wild type strain allows replication of the vector at high copy number (100-200 copies per cell) using the pMB1 ori. This novel cosmid was used to construct genomic libraries from *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia* and *T. denticola*. The resulting clones were maintained in low copy number in C2110. Direct plasmid transfer on selective agar plates via conjugation permitted the transfer of the cloned fragment into wild type *E. coli* strains allowing high expression and screening without disrupting the original clones.

This study has so far revealed that the present system allows rapid identification of copy number lethal fragments without the requirement for plasmid acquisition and transformation and, therefore provides a useful tool for manipulating virulence encoding genes of periodontal pathogens.

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HN SHAH* and SE GHARBIA (Department of Microbiology, Eastman Dental Institute, London WC1X 8LD): Biochemical and molecular analysis of glutamate dehydrogenases of putative periodontal pathogens.

We have shown previously that anionic amino acids represent a major substrate for energy assimilation among key periodontal pathogens, the favoured type being a distinctive feature of each species. Glutamate, a prominent substrate of the free amino acid pool of this ecosystem is utilised differently by species. Previously, we have studied possible degradative mechanisms, especially in *Pseudomonas*. In the present study we focus on the regulatory enzyme glutamate dehydrogenase (GDH) using enzymic and genetic techniques to study some of its properties and functions. GDH was present in *P. gingivalis*, *P. intermedia*, *P. nigrescens*, *F. nucleatum* and *T. denticola* and found to be sufficiently polymorphic to be used as a stable species marker. Primers flanking the *gdh* open reading frame were used for amplification and sequencing. Sequence alignments using the Higgins and Waterman Algorithms indicated high homology between *P. intermedia* and *P. nigrescens* and to a lesser extent between *P. gingivalis* and *F. nucleatum*. These results were consistent with the properties of species. For example, the GDH of *F. nucleatum* is intracellular while the enzyme of *P. gingivalis* is surface-associated. Unlike previous reports, we have confirmed that GDH of *P. gingivalis* is NAD-dependent and is a hexameric enzyme of Mr 49,275. Nucleic acid sequence analysis revealed a mature peptide of 445 amino acids that shared 43.5% homology with the GDH produced by *C. symbiosum* and less than 10% with that produced by *C. difficile*.

High optimum codon usage, high activities, low K_{ms} and efficient energy usage suggest a major role for this enzyme in the ecology of subgingival plaque.

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SE GHARBIA* and HN SHAH (Department of Microbiology, Eastman Dental Institute, London WC1X 8LD): Nucleotide sequence and alignment of the gene encoding gingipain of *Porphyromonas gingivalis*.

The extracellular cysteine proteinase of *Porphyromonas gingivalis* has been implicated in destructive periodontitis. We were the first to characterise its cysteine/unkindase catalytic site using chemical reactivity probes, designated the enzyme "gingipain" and demonstrated its P₁-endopeptidase characteristics. This was later confirmed by other workers, through enzyme purification, substrate kinetics and cysteine-specific inhibitors. Genetic evidence of its size and characteristics are confusing due largely to the deposition in databases of various sequences of a "thiol-proteinase" without critical evaluation. We have cloned gingipain in a low expression vector and used cysteine proteinase-specific oligonucleotides to localise the gene responsible for its expression. The gene was subcloned and its proteolytic activity confirmed using BAPNA assay and reactivity to cysteine-specific inhibitors. Nucleic and amino acid sequences of the cloned enzyme revealed 3 overlapping reading frames encoding a mature peptide of Mr 52,591. Analysis revealed 12 arginine and 7 cysteine residues which were used to identify the active site. Higgins's algorithm was used to align the amino acid sequence of gingipain to the 4 complete codon sequences of the proteinase genes referred to as pta, prtr, prtc and tpr. 93.3% homology was obtained with prtr while the pta sequence was found to be 100 amino acids shorter than gingipain, but C-terminal alignment revealed 96% homology within the 65 amino acids analysed. The prtc was 247 amino acids shorter and had 26% homology with gingipain. Within the aligned region, both peptides shared 61.4%. Gingipain shared 4.7% homology with streptopain and 3.5% with chymotrypsin. However, less than 3% association was detected with papain, chymotrypsin and cathepsin B. The results of this study confirm the cysteine proteinase nature of the endopeptidase activity associated with *P. gingivalis*. Comparative analysis of amino acid sequence data indicated that despite differences in size, source and designation, all sequences of the proteinase deposited to date share >80% homology.

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F E SMITH* and N PENDER (School of Dentistry, The University of Liverpool): Features associated with fixed appliance treatment outcome.

The aim of this study is to investigate features of treatment with fixed appliances which may be associated with the treatment outcome. A cohort of 252 consecutive patients treated with dual arch fixed appliances (FA) with or without upper removable appliances functional appliances (RA), or orthognathic surgery, were assessed from study casts at the start and end of treatment using the Peer Assessment Rating / PAR Index (Richmond *et al*, Eur J Orthod 14: 125-139, 1992). Reproducibility was assessed using Kappa at 0.86. From clinical notes several features of treatment were recorded: patient age, sex, treatment time, number of visits, number of failed appointments, appliance types, reason for termination of treatment.

Two groups were defined from the 252 treated cases on the basis of the PAR score at the end of treatment. Group A, 23 patients, and Group B, 31 patients had final PAR scores of no more than 2 or greater than 14 respectively. The pre-treatment PAR, mean \pm sem, was 29.3 \pm 2.3 for A and 33.0 \pm 1.9 for B ($P=0.2$). The percentage reduction in PAR was 92.6% \pm 1.1 for A and 34.9% \pm 6.4 for B ($P<0.001$). Treatment time, 24.2 \pm 1.3 months for A and 26.5 \pm 1.9 months for B ($P=0.4$), did not differ. In 52% of Group B treatment was terminated before completion (TBC) compared with 9% of Group A ($P<0.01$). 14% of A and 40% of B had FA plus RA. Those cases from B treated with FA plus RA and TBC had an increased treatment time of 35.9 \pm 3.8 ($P<0.03$).

These data suggest that the result obtained by fixed appliance therapy may be associated with additional appliances and extended treatment time.

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S RABIN, P BENINGTON* and E N HORROCKS (Department of Orthodontics, Eastman Dental Hospital and Institute, London): Cephalometric comparison of soft tissue changes between two non-extraction treatment modalities.

Functional appliances are often used in the treatment of class II malocclusion with the aim of improving the profile. This retrospective cephalometric study compared the soft tissue changes in class II division 1 cases, treated on a non-extraction basis using either fixed appliances with extra oral traction or a combined functional/fixed approach. Preadjusted edgewise brackets were used in all cases. Each group of 30 patients were matched for age, sex and malocclusion. 9 soft tissue points were digitised from lateral skull radiographs and 9 angular and 8 linear measurements made at the pre-treatment, post-functional (where applicable) and post-treatment stages for all subjects. There was a tendency for the nasolabial angle to increase in the fixed-only group and decrease in the functional/fixed group, but not significantly. Lower lip prominence was significantly increased within both groups, with a tendency to a greater increase in the functional/fixed group. The difference between the groups was not significant. Profile convexity significantly decreased in both groups, particularly the functional/fixed group, but there was no significant difference between the groups.

In conclusion, functional/fixed appliance therapy tended to decrease profile convexity to a greater extent than fixed appliance therapy alone, but not at a statistically significant level. Non-extraction treatment had no significant effect on the nasolabial angle.

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S RABIN* and E N HORROCKS (Department of Orthodontics, Eastman Dental Hospital and Institute, London): Cephalometric comparison of non-extraction treatments of Class II division 1 cases.

The use of functional appliances in the management of Class II malocclusions is intended to encourage favourable mandibular growth thereby aiding sagittal correction. The aim of this study was to compare the skeletal and dental changes occurring in Class II division 1 cases treated using two different non-extraction treatment approaches.

The records of 30 patients treated using a pre-adjusted Edgewise appliance incorporating extra-oral traction were matched for age, sex and malocclusion with those of 30 patients treated using a functional/fixed appliance combination. Skeletal and dental landmarks were digitised from lateral skull cephalometric radiographs taken before treatment, at the end of the functional phase where appropriate and at the end of active treatment.

The results showed significant differences between the groups. There was a reduction in angle SNA with downward tipping of the anterior part of the palatal plane in the fixed only group, whereas the functional/fixed group had a greater increase in mandibular body length. Significant incisor tipping was common to both groups. In the vertical dimension, there was minimal posterior rotation of the mandible in both groups, however, there was a significantly greater increase in lower anterior face height in the functional/fixed group.

In conclusion, the two treatment approaches showed similar reductions in the sagittal relationships of the mandible to the maxilla, however the means by which was achieved varied between the groups.

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J C HUNTER*, S D SPRINGATE and N P HUNT (Department of Orthodontics, Eastman Dental Institute and Hospital): A comparison of the dental archform and facial morphology of two professional brass and wind instrument groups.

This investigation examined the arch dimensions, incisor relationships and facial morphologies of two groups of professional brass or wind instrumentalists with contrasting embouchures. Two study groups, comprising 21 French horn players (mean age 32, range 24-50 years) and 21 clarinetists (mean age 36 years, range 24-58 years), were selected from a total of 78 players. The mean length of playing experience was 21 years (range 13-35 years) and 25 years (range 14-45 years) respectively. Data obtained from clinical examination and study casts were compared between the two groups. In addition, information was obtained as to the number of hours played per day, age on commencing playing and experience with pupils undergoing fixed appliance orthodontic treatment. The most striking finding was the morphological similarity of the two groups. No statistically significant differences were found between the arch dimensions, incisor relationships or facial morphology. Although trends were evident in the data suggesting an increase in overjet and greater frequency of Class II division 1 malocclusions in the clarinet group and a greater frequency of Class II division 2 malocclusions in the horn players, large intra-sample variation existed for all arch-dimensions. Thus any actual differences that may have existed were not detected as significant between the samples.

It is possible for people with varying malocclusions and facial morphologies to compensate for morphological deviations and successfully play the French horn or clarinet to the highest level.

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N P HUNT* (Department of Orthodontics, Eastman Dental Institute and Hospital, London): The effect of orthognathic surgery on the generation of maximum occlusal force.

One of the many goals of orthognathic surgery is to provide an aesthetic, balanced, stable and functionally efficient occlusion. However, studies to measure the effects of surgery on occlusal performance are few in number. The aim of this investigation is to study the response of masticatory muscle function, as measured by the generation of maximum occlusal force, in relation to the surgical correction of vertical facial deformities.

Occlusal force was measured in 42 patients (25 long faces, 17 short faces) prior to any treatment, one month following operation and again at six and 12 months later. Recordings were made using a customized bite force transducer, bilaterally, in the first molar region, giving a molar separation of 9 mm.

The results showed a significant lower level of force generation prior to treatment in the long face group compared to the short faces. A significant reduction in force was noted in both groups following operation before a gradual increase over the subsequent review period. However, even after 12 months, the short face group could not achieve their pre-operative level, whereas the long face group increased their force to a level, on average, 130% greater than their initial values.

It is concluded that orthognathic surgery is successful in improving bite force in long face patients but that correction of short face deformity can lead to a reduced maximal occlusal force for at least one year following operation.

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HJ NEVARD*, N P HUNT and S D SPRINGATE (Department of Orthodontics, Eastman Dental Institute and Hospital, London): A comparison of growth changes occurring within the mandible and cervical vertebrae.

Changes in pattern of incremental linear dimensions of the mandible and second and third cervical vertebrae were investigated using lateral cephalometric radiographs of a sample of 22 subjects (12 males and 10 females) involved in the Leighton Growth Study. Serial radiographs were traced and digitised in order to measure linear dimensions. The results were analysed according to sex using Time Series Analysis involving cross correlation.

Correlations were significant ($p \leq 0.05$) and greatest at zero time lag with total mandibular length and all cervical variables for both sexes excluding third cervical vertebral height for males. In males, the increase in incremental width of both second and third cervical vertebrae was correlated ($p \leq 0.05$) with the pattern of incremental growth in mandibular body length occurring at a lag of two years, that is, peak increases in incremental width of both cervical vertebrae happened before those of mandibular body length. Correlation was statistically significant ($p \leq 0.05$) with mandibular body length and second cervical vertebral height in females with a lag of one year, mandibular body length having an increase in incremental growth ahead of that of second cervical vertebral height. Similar associations were found with mandibular length and third cervical vertebral width in males, but at a time lag of two years, again with growth events in the mandible occurring before those of the cervical vertebrae.

This study indicates that potential indicators of growth exist, which may allow prediction of the timing of growth changes in cervical vertebrae from the mandible and vice versa in males.

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S J CUNNINGHAM*, N P HUNT* and C FEINMANN* (Departments of Orthodontics and Oral Surgery, Eastman Dental Institute and Hospital): Patient satisfaction and changes in quality of life following orthognathic surgery.

The assurance of quality of health care is becoming increasingly important with the move towards purchaser/provider mode. This questionnaire based study investigated patient satisfaction and changes in quality of life following orthognathic surgery for the correction of facial deformity. Questionnaires were distributed to 83 pre-operative patients and 100 post-operative patients. All post-operative patients were at least 9 months post-surgery and had completed active orthodontic treatment.

Analysis of the data involved the use of the calculated Standard Normal Deviate, Fisher's Exact Test and Wilcoxon's Two-Sample Rank Test to compare pre- and post-operative mood states and opinions about various aspects of appearance and personality. Following surgery there was found to be significant improvement in dental, facial and general appearance, as well as in self-confidence, the ability to mix socially, performance at work/college and in overall mood states. The majority of patients felt that the technical aspects of surgery had been well explained to them but almost a quarter felt that the effects following surgery were badly explained. Pre-operative counselling, therefore, needs to be improved.

It was concluded that the majority of patients were happy with the outcome of their surgery and quality of life appeared to improve significantly. However, attention must be paid to pre-operative counselling.

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M IDE*, FP ASHLEY and RF WILSON. Reproducibility of new and conventional methods for assessing dentine sensitivity. Guy's Hospital Dental School, UMDS, London

Methods used in clinical trials to assess dentine sensitivity have not been tested extensively for reproducibility. Previous and new controlled methods were compared in 23 subjects. One sensitive tooth was tested using tactile (pressure probe), controlled cold fluid and controlled air flow stimuli applied in random order followed by a 1 sec air blast from a 3-in-1 syringe. Tests were separated by a 10 min rest period. Pain induced was recorded by the subject using a Visual Analogue Scale (VAS) and a short form McGill Questionnaire, except for the tactile stimulus where threshold pressure replaced a VAS. The number of teeth sensitive to air blast was recorded. The procedure was repeated exactly two days later. Reproducibility was assessed using proportional 'limits of agreement' (LA) within which 95% of the repeated measurements were distributed (Bland JM & Altman DG, *Lancet* 1986 1:307). The tactile stimulus (LA: -68% to +47%) appeared more reproducible than other single tooth stimuli, with controlled fluid (LA: -91% to +80%) and air flow (LA: -69% to +80%) better than air blast (LA: -146% to +119%). The number of teeth sensitive to air blast showed absolute reproducibility. All methods used on single teeth showed relatively poor reproducibility and potential power of test, and the results suggest that assessing multiple teeth may be a more powerful statistical approach in clinical trials.

This work was supported by Unilever Dental Research

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D K WHITTAKER*, M M BAKRI and E ANDERSON (Department of Basic Dental Science, Dental School, Cardiff, UK): Ultrastructural changes in odontoblasts associated with apical sclerotic dentine.

This investigation aims at characterising intra-cellular changes in odontoblasts from apical regions of teeth from elderly persons. Most previous studies on the fine structure of human odontoblasts have been on cells at the stage of dentinogenesis (Frank R M, Malband J, *J Dent Res*, 42: 422-437) or young mature cells (Frank R M, *Archs Oral Biol*, 11: 179-199). In the human tooth changes have been observed in the life cycle of the odontoblast but only up to the age of ten years. Ten teeth from donors aged 9 to 75 years were collected fresh, split and fixed immediately in 2.5% glutaraldehyde. Decalcification in EDTA was followed by epoxy embedding and sectioning transversely through the apical region. Results showed that marked intra-cellular changes occur as age progresses and these are associated with production of an abnormal dentine matrix and are associated with apical sclerosis.

It is concluded that apical odontoblast ultrastructure and probably function change markedly as age progresses and these changes may be associated with the formation of sclerotic apical dentine which is an age related phenomenon.

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D EVANS*, R STRANG* and J REID* (* Department of Dental Health, University of Dundee, UK and *Depts of Oral Biology and Child Dental Care, Glasgow Dental School, UK): Non-pulpal signals and Laser Doppler Flowmetry of the dental pulp.

The aim of the study was to compare the non-pulpal component of laser doppler flowmetry (L.D.F.) recordings of dental pulp blood flow made using five different recording methods reported in the literature. L.D.F. recordings were taken from permanent maxillary incisors classified as non-vital by standard diagnostic methods and from vital maxillary incisors, using a 633 nm laser doppler flowmeter². The L.D.F. probe was held against the tooth by hand (nine non-vital (NV), five vital (V) teeth), by orthodontic tubing (five NV, seven V teeth), by a vinyl mouthguard (six NV, nine V teeth) and by elastomeric impression material covering only the labial surface of the crown (seven NV, six V teeth). An intra-tooth control recording was taken for every tooth using a full coverage elastomeric impression jig. The non-pulpal/pulpal blood flow signal ratio for each recording method was calculated from the mean blood flow signals from the non-vital and vital teeth in each recording group. The non-pulpal/pulpal signal ratios were 0.46 (Hand held), 0.38 (Vinyl mouthguard), 0.29 (Orthodontic tubing), 0.25 (Labial impression) and 0.10 (Full impression).

Non-pulpal signals are an inevitable component of laser doppler flowmetry signals from the dental pulp. All dental pulp studies using laser doppler flowmetry should state the non-pulpal/pulpal blood flow signal ratio for the recording method used.

²Pf2b (Perimed, Sweden) Purchased with a grant from Greater Glasgow Health Board

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C LOUCA*, S W CADDEN & R W A LINDEN (King's College London & Dundee University, UK.): The mechanical threshold of an inhibitory jaw reflex in man.

We have reported that applying 'ramp-and-hold' mechanical stimuli to teeth results in a single short-latency reflex inhibition of activity in jaw closing muscles (Louca C *et al.*, *J Dent Res*, 73: 791, 1994). Since such stimuli are likely to excite exclusively periodontal ligament mechanoreceptors (PdLMs), it may be concluded that these nerves play a role in evoking the reflex. The aim of this study is to determine the threshold force for evoking this reflex and to compare this with forces which are known to excite PdLMs. Experiments were performed on 9 volunteer subjects. EMG recordings were made from a masseter muscle while the subject clenched at a constant level with the aid of visual feedback, and ramp and hold stimuli were applied to an upper central incisor tooth. The ramps had a constant rate of rise of 0.2 N ms⁻¹ and a target force of up to 2 N. The EMG recordings were full-wave rectified, averaged and analysed by computer. Down-going waves in the recordings were designated as inhibitory reflexes when analysis of pooled data (ANOVA with post-hoc Scheffé tests) showed them to be significantly larger ($P < 0.05$) than those obtained from control records when no stimuli were applied. It was found that significant short latency responses were produced by all the stimuli with target forces of 0.25 N or more. There is evidence that human PdLMs would be activated, albeit submaximally, by a 0.25 N force (Thullman M & Johansson R S, *J Neurophysiol*, 72: 1734-1744, 1994). However this threshold for an inhibitory jaw reflex is much lower than many of the forces which occur during mastication (Anderson D J, *J Dent Res*, 35: 664-673, 1956). Supported by the Wellcome Trust.

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A LONG*, U BONGENHIELM, K FRIED*, F M BOISSONADE & P P ROBINSON (Dept Oral & Max Surg, Univ of Sheff, UK & *Dept Neurosci, Karolinska Inst, Swe.): Changes in neuropeptide expression in inferior alveolar nerve neuromas in the ferret.

Patients who sustain inferior alveolar nerve injuries sometimes develop dysesthesia. Since changes in the expression of neuropeptides may be associated with this disorder, the presence of immunoreactivity to the neuropeptides substance P (SP), calcitonin gene-related peptide (CGRP) and vasointestinal polypeptide (VIP) at the injury site were investigated, in an animal model. In five anaesthetised adult male ferrets the left inferior alveolar nerve was sectioned and ligated in the region of the third premolar tooth. The animals were allowed to recover for periods of 3 days (2 animals), 8 days, 3 weeks and 12 weeks. An unoperated animal and the contralateral sides of the operated animals were used as controls. Under sodium pentobarbitone anaesthesia (42mg/kg i.p.) the animals were perfusion-fixed and the neuroma on the experimental side and the nerve on the contralateral side were removed. Frozen, longitudinal, serial sections, 14µm-thick, were cut and incubated with antisera to SP, CGRP, and VIP using an indirect method of immunofluorescence. After 3 days, immunoreactivity against all three peptides was present close to the ligation. After 8 days the expression was reduced; after 3 weeks, only CGRP immunoreactivity was present and after 12 weeks, there was little or no evidence of peptide expression. The presence of neuropeptide immunoreactivity at the early stage after injury coincides with the highest levels of spontaneous activity and mechanical sensitivity recorded electrophysiologically.

Thus the neuropeptides SP, CGRP and VIP may play a part in the abnormal activity recorded following inferior alveolar nerve injury. Supported by the Wellcome Trust.

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P STEPHENS*, DW THOMAS and JP SHEPHERD (Department of Oral Surgery, Medicine & Pathology, Dental School, UWCM, Cardiff, UK): *An in vitro comparison of the contractile ability of oral & dermal fibroblasts.*

Previous studies have suggested that the privileged nature of foetal wound healing in some animals is reflected *in vitro* in a differential ability to induce fibroblast populated collagen lattice (FPCL) contraction (Ritsenberg T *et al*, *J Cell Physiol* 149: 444-50, 1991). We investigated whether the privileged nature of intraoral wound healing is related to differential contractile phenotypes of fibroblasts by studying the ability of intra- and extraoral fibroblasts to re-organise an FPCL *in vitro*. Oral mucosal or dermal fibroblasts, derived from child or adult tissues (1.5×10^5) were seeded into type I collagen lattices in 60mm bacteriological grade plates at passages (P) 1 to 5. Lattice contraction was measured over 14 days by recording lattice diameters. Cell number and DNA synthesis were assessed by direct cell counting and [3 H] thymidine incorporation. At P5, oral mucosal fibroblasts demonstrated a significantly greater contractile ability than dermal fibroblasts ($p < 0.01$). These differences were not reflected by differences in DNA synthesis or cell number between intra- and extraoral fibroblasts. Child fibroblasts exhibited significantly greater contractile abilities than adult fibroblasts ($p < 0.01$). Contractile ability increased with increasing P number as at P1, irrespective of donor age or tissue of origin, fibroblasts demonstrated no differences in their ability to contract a collagen lattice ($p > 0.05$). *It was concluded that specific phenotypic differences exist between intraoral and extraoral fibroblasts. Intraoral fibroblasts demonstrated a greater ability to re-organise their surrounding extracellular matrix: a factor which may be important in wound healing.*

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B SPENCER-DONE, *P THOROGOOD, B HENDERSON* (Maxillofacial Surgery Department, Eastman Dental Institute and Developmental Biology Unit, Institute of Child Health, UK): *Membrane metalloendopeptidases in craniofacial development.*

Membrane metalloendopeptidases (MMPs) are cell surface enzymes which inactivate bioactive peptides and we have proposed that they act to inactivate peptide morphogens, thus constituting a previously unrecognized level of developmental control. The presence of enzymes EC24.11 and EC24.18 in embryos was determined by Western blotting and immunohistochemistry and the transcription product of the EC24.11 gene was determined by *in situ* hybridization and RT-PCR. The effect of inhibition of EC24.11 in embryo culture was also determined. Both enzymes were temporospatially regulated as assessed by Western blotting and immunohistochemistry being found in various sites in the craniofacial region of embryos. The distribution of EC24.11 mRNA was confirmed by *in situ* hybridization. Using RT-PCR, mRNA for EC24.11 was detected as early as E 8.5. RT-PCR also identified a second band with 111 additional nucleotides which was present only in embryo and not in adult tissues suggesting the presence of a putative embryo-specific MMP. Inhibitors of EC24.11 induced an asymmetric swelling and craniofacial dysmorphogenesis resembling hemifacial microsomia. *Membrane metalloendopeptidases appear to be involved in craniofacial development.*

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JW BRECKON*, RM HEMBRY*, JJ REYNOLDS* and MC MEIKLE (Department of Orthodontics, UMDS of Guy's and St Thomas's Hospitals, London and *Strangeways Research Laboratory, Cambridge): *Neural crest cells synthesize gelatinases in culture.*

Gelatinases (type IV collagenases) are neutral matrix metalloproteinases that specifically cleave denatured interstitial collagens (gelatins), basement membrane collagen types IV and V, fibronectin, elastin and to an extent proteoglycan. Two forms have been identified, gelatinase-A (72-kDa) and gelatinase-B (92-kDa); their extracellular activity is inhibited by TIMPs (tissue inhibitor of metalloproteinases). This investigation aims to show gelatinase synthesis by neural crest cells (NCCs) *in vitro* during early migration. 8.5 day post coitus (p.c.) cranial fold and 9.5 day p.c. neural tube explants from mouse embryos were used to prepare NCC cultures. Using indirect immunofluorescence microscopy, gelatinase-AB synthesis was demonstrated as faint intracellular staining in both migrating NCCs and cells of the neural tube. SDS-gelatin gel zymography documented gelatinolytic activity in supernatants from short term NCC cultures; both gelatinase-A and gelatinase-B were constitutively synthesized. Enzymes were detected as latent and active forms and as complexes with TIMP-1 (28-kDa) and TIMP-2 (22-kDa). NCC migration and gelatinase synthesis were modified by cell culture substrates and interleukin-1 α .

These results suggest cells of the neural tube synthesize gelatinases capable of degrading adjacent basement membrane at the time of initial NCC migration. NCC cultures also produce gelatinase-A and gelatinase-B and their inhibitors TIMP-1 and TIMP-2 during migration in vitro.

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JW BRECKON*, RM HEMBRY*, JJ REYNOLDS* and MC MEIKLE (Department of Orthodontics, UMDS of Guy's and St Thomas's Hospitals, London and *Strangeways Research Laboratory, Cambridge): *Neural crest cells synthesize gelatinases in culture.*

Gelatinases (type IV collagenases) are neutral matrix metalloproteinases that specifically cleave denatured interstitial collagens (gelatins), basement membrane collagen types IV and V, fibronectin, elastin and to an extent proteoglycan. Two forms have been identified, gelatinase-A (72-kDa) and gelatinase-B (92-kDa); their extracellular activity is inhibited by TIMPs (tissue inhibitor of metalloproteinases). This investigation aims to show gelatinase synthesis by neural crest cells (NCCs) *in vitro* during early migration. 8.5 day post coitus (p.c.) cranial fold and 9.5 day p.c. neural tube explants from mouse embryos were used to prepare NCC cultures. Using indirect immunofluorescence microscopy, gelatinase-AB synthesis was demonstrated as faint intracellular staining in both migrating NCCs and cells of the neural tube. SDS-gelatin gel zymography documented gelatinolytic activity in supernatants from short term NCC cultures; both gelatinase-A and gelatinase-B were constitutively synthesized. Enzymes were detected as latent and active forms and as complexes with TIMP-1 (28-kDa) and TIMP-2 (22-kDa). NCC migration and gelatinase synthesis were modified by cell culture substrates and interleukin-1 α .

These results suggest cells of the neural tube synthesize gelatinases capable of degrading adjacent basement membrane at the time of initial NCC migration. NCC cultures also produce gelatinase-A and gelatinase-B and their inhibitors TIMP-1 and TIMP-2 during migration in vitro.

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S GELBIER*, L ZOITOPOLOUS, SR BRAILSFORD, S MARCHANT & D BEIGHTON. (Faculty of Clinical Dentistry, KCSMD, London): *Relationships between dental caries, oral hygiene and microflora in pre-school children.*

A population of 648 pre-school children [mean age (std) = 45.7 \pm 8.9 months] was examined to determine the caries prevalence, oral hygiene status and the carriage of mutans streptococci (m-s) and lactobacilli (lb). Caries was recorded using BASCD criteria, oral hygiene was scored using a debris index and bacteria was determined in saliva and plaque samples (detection limits were $\geq 10^3$ cfu per ml of saliva and ≥ 10 cfu per plaque sample). The data were analysed using non-parametric methods. The mean dmft was 0.82 \pm 2.16 with dt accounting for 79.6 per cent of this score. Dmft was significantly related to the age of the children ($r=0.174$; $p<0.05$). The dmft scores were significantly ($p<0.0001$) greater in children with m-s or lb in plaque or saliva. Children with a positive debris score harboured significantly ($p<0.001$) more m-s and lb. The mean dmft of children ($n=54$) with a positive debris score was 3.24 \pm 3.80 compared with 0.61 \pm 1.81 ($p<0.001$) for those ($n=630$) with a score of 0. The mean dmft of children ($n=385$) with undetectable levels of m-s and lb in saliva was 0.25 \pm 1.00 compared with 3.46 \pm 3.79 for those ($n=67$) detectable levels of both taxa in their saliva ($p<0.001$). The children with m-s or lb detected had mean dmft scores of 1.06 \pm 2.45 ($n=218$) and 0.78 \pm 1.40 ($n=18$), respectively. *These data demonstrate significant interactions between oral hygiene, carriage of m-s and lb and caries in pre-school children. The role of good oral hygiene instruction as a means for controlling oral colonisation and subsequent caries development merits further study.* This project was supported by Action Research.

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PA SMITH* and C LONGBOTTOM (Dental Health Services Research Unit & Department of Dental Health, University of Dundee, UK): *A study of referrals to Dundee Dental Hospital and School.*

The aim of this study was to determine the characteristics and location of dental and medical practitioners referring patients to Dundee Dental Hospital. The types of patients and dental problems most frequently referred for treatment and advice were also investigated. The study started on 1st January 1994 and of the first 838 referrals made there have been 236 referral sources of which 197 were dental practitioners and 39 medical practitioners. These were based in 103 dental practices, 4 hospitals and 23 medical practices. Of the dentists, most (52.1%) graduated between 1970 and 1990, with 42.8% having attended Dundee Dental School. The majority of the referrals came from Tayside (62.6%) and Fife (26.3%) and the remaining 11.1% derived from elsewhere in Scotland. The treatments most often provided were extractions under general anaesthetic for children (29.0%), examination and advice to patient and/or dentist (28.2%) and surgical procedures (19.8%) with the Department of Oral Surgery and the Preventive and Children's Section of the Department of Dental Health being the clinics most often receiving referrals.

This study has shown that the majority of referring dentists were from within Tayside and that extraction under general anaesthetic for children accounted for nearly a third of referrals.

Supported by the Scottish Office Home and Health Department

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ZJ NUGENT* and NB PITTS (Dental Health Services Research Unit, University of Dundee, UK): *Patterns of dmft and care index in British 5-year-olds 1987-93.*

The deciduous teeth of five year olds, by their ephemeral nature, reflect changes in the environment more quickly than would be observed in older children or adults. The British Association for the Study of Community Dentistry (BASCD) data for 5 year olds is available for 1987, 1989, 1991 and 1993 (new data) for 157 health districts/boards across Great Britain, allowing the study of patterns of change in dmft and its components. Between 1987 and 89, dmft improved by 1%, but then deteriorated by 11% between 1989 and 1993. The dt component increased by 30%. The care index (ti/dmft) reflects the prevalence of restorative treatment. As it only provides information about those who have suffered disease, it should always be viewed with an indicator of general caries experience such as dmft. The care index has fallen by 39% through the period 1987-93, with some evidence of accelerating decline in more recent surveys.

The continuing Great Britain-wide surveys of caries prevalence indicate that the deciduous teeth of five year olds may be heralding a problem in the delivery of dental resources, as reflected by changes in both care index and dmft.

Supported by the Scottish Office Home and Health Department

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K.W. WHITTLE* and J.G. WHITTLE (Salford Community Healthcare NHS Trust and Trafford Healthcare NHS Trust, UK): *Changes in dental health behaviours between 1989-90 and 1993-94.*

During the 1989-90 5-year-old BASCD survey, a questionnaire was sent home to parents to enquire about dental health behaviours. This was repeated in 1993-94 with the aim of finding out how these behaviours had changed. In 1989-90 2390 children were examined in Salford and 2098 in Trafford. Questionnaires were returned by 1858 (response rate 78%) and 1691 (81%) respectively. In 1993-94 2794 Salford children and 2343 Trafford children were examined and 2094 (75%) and 1707 (73%) questionnaires returned. In Salford the proportion of children who were having their teeth brushed with a fluoride toothpaste by the time they were aged one rose from 46% to 80% ($p<0.001$) and in Trafford from 47% to 62% ($p<0.001$). The proportion who had visited a dentist by the time they were aged two rose from 44% to 80% in Salford ($p<0.001$) and from 39% to 55% in Trafford ($p<0.001$). The percentage of 5-year-olds whose most frequent drink contained sugar fell from 52% to 39% in Salford ($p<0.001$) but remained the same (37%) in Trafford. *It is concluded that there have been significant improvements in dental health behaviours over the four years.*

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A H FORGIE*, C M PINE and N B PITTS (Department of Dental Health, University of Dundee, Scotland, UK): Dip-slide test derived Streptococcus mutans levels in 11-13 year olds in Tayside.

As the apparent level of dental decay reduces in developed countries, the salivary levels of *S. mutans* can be used to help in the identification of high/low caries risk children (Klock B and Krasse B, *J Dent Res*, 87:129-139, 1979). Although relatively common in Scandinavia and some parts of North America this method has not been used so frequently in Britain. The following procedure was used to identify high caries risk individuals as a preliminary stage of a large trial of a caries preventive agent being carried out in Tayside. A multi-disciplinary dental team comprising a dentist, hygienist, therapist and dental nurses visited 30 schools in Tayside over a two month period in pairs. A stimulated whole saliva sample was taken from all the available 1st year secondary school children aged 11-13 years (n=4029). The saliva was tested for *S. mutans* levels using a dip-slide test (Jordan H.V. et al, *J Dent Res*, 66 (1): 57-61, 1987) and the distribution of different *S. mutans* levels was recorded. The results were as follows: 4.6% had no readable *S. mutans* levels, 33.2% had dip-slide level 1 (<10,000 CFU/ml), 27.7% had levels 2 or 3 (>10,000 and <250,000 CFU/ml) and 34.5% had levels 4, 5 or 6 (>250,000 CFU/ml).

In conclusion: 34.3% (1388) of the children investigated had a dip-slide test level of 4 or more i.e. a *S. mutans* level of >250,000 CFU/ml. These children were judged to be at higher caries risk than their peers and were invited to participate in a 3 year clinical trial of a chlorhexidine varnish.

Supported by Knowell Therapeutics, Toronto, Canada.
¹Cariescreen® and ²Chlorocoin®, Knowell Therapeutics.

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C DEERY*, HE FYFFE, Z NUGENT, NM NUTTALL and NB PITTS (Dental Health Services Research Unit, University of Dundee, UK): An assessment of the diagnosis of occlusal caries in fissure sealed surfaces.

The aim of this investigation was to assess the clinical diagnosis of occlusal caries associated with fissure sealed occlusal surfaces in a sample of regularly attending adolescents. 529 subjects (mean age 12.5 years) of 41 volunteer General Dental Practitioners in Scotland, were examined under dental surgery conditions by a trained and calibrated examiner, at the D1 threshold (enamel and dentine caries). Blewing radiographs were exposed for each child (E-speed film, 65 KVP, 0.2 s) and examined under standardised conditions (X2 magnification). Overall, there were 1,810 sealed occlusal surfaces of which 152 (8.4%) were judged clinically to be sealant restorations; these were excluded from the analysis. Approximately half of the occlusal surfaces were sealed by a clear material (53%). Of 251 surfaces exhibiting occlusal dental radiolucencies 107 (43%) were assessed clinically as sound at the D1 threshold. Considering clear and opaque/tinted sealants separately no statistically significant difference was found in the radiographically detected prevalence of caries. However a significantly greater number of the lesions present (detected clinically) were associated with clear sealants rather than opaque/tinted sealants ($\chi^2=17.44$, df=1, $p<0.001$).

It is concluded that, even when sealed surfaces are examined clinically under optimal conditions, undetected occlusal dental lesions represent a meaningful problem for both clinicians and epidemiologists.

Supported by the Scottish Office Home and Health Department

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R KULASEGARAM, MC DOWNER*, JA JULLIEN, JM ZAKRZEWSKA and PM SPEIGHT (Oral Cancer Screening Gp, Eastman Dent. Inst. London, UK): Case control study of oral dysplasia and risk habits.

Several studies have investigated risk factors for oral cancer, fewer have considered precancer. Records accumulated from 1975 to 1993 of dental hospital patients with histologically confirmed oral dysplasia provided the opportunity for a retrospective case-control study of the association between oral precancer and smoking tobacco and drinking alcohol. Seventy sets of case notes were available and each case was matched with records of a control subject, known to be free from dysplasia from another study, for birth date, gender and presumed ethnicity. The relative risk (OR) of having a dysplastic lesion for smokers compared with non-smokers, or ex-smokers for > 10 years, was 7.00. Logistic multiple regression revealed a dose-response relationship for tobacco dependent upon level of cigarette consumption. Also subjects with moderate or severe dysplasia included a higher proportion of smokers than those with mild dysplasia. No overall increased risk from alcohol was found. However, the proportion of subjects who drank spirits was significantly higher among cases than controls. The study reaffirms the role of dental practitioners in identifying individuals at risk of mucosal disease, the importance of public education about the risk factors, and the necessity for counselling patients with precancerous lesions on avoiding further risk.

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J STEER*, J WHITTAKER (SmithKline Beecham Consumer Healthcare, UK): The effect of toothbrush design on the force generated during brushing.

An in vitro method has been developed which simulates the force generated on teeth and gums by toothbrushes during use. This enables ranking for possible soft tissue trauma due to excess brushing pressure.

The method utilises a cantilever beam with differential strain gauges attached. These are connected in a full Wheatstone bridge arrangement to a data recording instrument set to measure peak force. A simulated tooth shape is screwed onto the beam, centre 5cm from the free end. The bristle face of the toothbrush is set parallel to the beam. It brushes across the simulated tooth at predetermined loads. The resultant peak force generated on the beam after 30 sec brushing is measured. Toothbrushes of various bristle profile and handle composition have been evaluated as the test is a comparison of the total brush as used. The influence of bristle contact and/or handle interaction has not yet been evaluated separately. Data has been analysed by using paired "t" tests. These show that toothbrushes with special flexible parameters generate significantly less force on the beam when loaded to between 10-500g (250g-500g is considered normal brushing loads) compared with Blend a Dent Professional, Colgate Preclean and Oral B40.

These laboratory results indicate that the Aquafresh/Dr. Best flexible toothbrush design has a significant effect in reducing the forces produced during brushing. This suggests that in use such a toothbrush would reduce the possible risk of trauma due to excess brushing pressure.

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TF WALSH, E UNSAL and PV CARROTTE* (Departments of Restorative Dentistry, Sheffield University, UK and Periodontology, Ankara University, Turkey): The effect of subgingival 2% minocycline gel on pocket probing depths.

The aim of the present study was to assess the effects of 2% minocycline dental gel together with mechanical treatment on pocket probing depths of subjects with adult periodontitis. A split mouth study design was utilised with a minimum of two pairs of sites identified on contralateral teeth in each subject. Treatment by mechanical therapy as described by Rawlinson & Walsh (*B Dent J*, 174, 161-166, 1993) with or without a subgingival application of 2% minocycline gel, was randomly applied to the contralateral sites. Probing pocket depths were monitored at baseline, one month and three months. A total of 15 patients entered the study, but one was excluded because of tooth extraction and another had periodontal flap surgery, leaving 13 for analysis. No statistically significant differences in probing pocket depths were found between experimental and control sites at any time point ($p=0.26$). However the reduction in probing pocket depths was greater in the minocycline treated sites (mean reduction of $2.47\text{mm} \pm 1.27$ than the control sites (mean $1.83\text{mm} \pm 1.28$). By 3 months these differences had lessened, but the reduction was still greater in the minocycline treated sites ($2.47\text{mm} \pm 1.63$ vs $2.09\text{mm} \pm 1.55$).

It was concluded that a single application of topical subgingival 2% minocycline dental gel together with mechanical treatment, did not statistically improve probing pocket depths over a 3 month period.

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SA BHATTI*, TF WALSH and CA McQUAID (Charles Clifford Dental Hospital, Sheffield and Department of Restorative Dentistry, University of Sheffield, UK): The effect of a scaling gel on shear forces required to remove subgingival calculus.

This study measured the shear force required to remove subgingival calculus from the root surfaces of extracted teeth, with and without the application of a calculus scaling gel. Six extracted teeth with linear deposits of subgingival calculus on the root surfaces were prepared for analysis with a Lloyd MSK tester operated at 0.5mm per minute. Each section of root was divided in two at 90° through the line of calculus to give two equal samples. 12 matched samples were mounted in epoxy resin. A chisel was placed against the top edge of the calculus and the shear force increased until the calculus was dislodged from the root. One sample was tested after the calculus had the scaling gel (SoftScale™) applied for two minutes according to the manufacturers instructions, and the matched sample had a placebo inactive gel applied. The results were analysed using a paired t-test. The results showed that the force required to remove the calculus was less for the samples treated with the active periodontal scaling gel (mean $9.77\text{N} \pm 6.27$) than the placebo gel (mean $11.28\text{N} \pm 7.77$) but this difference was not significant at the 5% level ($p=0.458$, DF=5, $t=2.58$).

It was concluded that periodontal scaling gel did not significantly reduce the shear force required to remove calculus in the in-vitro situation.

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TF WALSH, E UNSAL and CA McQUAID* (Department of Restorative Dentistry, Sheffield University, UK and Department of Periodontology, Ankara University, Turkey): The validity of digitised plaque vitality measurement.

Plaque samples from 12 patients suffering from adult periodontitis (8 male, 4 female), aged 32-58 (mean age 44.3 years) were stained using the technique of vital fluorescence described by Neuschil et al. (*J Clin Periodontol* 1989 16, 484-488). The proportions of vital plaque was then assessed by two techniques: the visual methods previously described by Walsh et al. 1994 (*J Clin Periodontol* 21, 1994, in press), and by an image capture system linked to a computerised image programme. A total of 94 plaque samples were included in the study. Data analysis of the two sets showed that there was no significant difference between the two methods ($p=0.26$). However the digitised procedure was less liable to error than the multiple estimation required by the visual evaluation method, but the former technique showed a greater standard deviation than the visual method. The digitised technique was more precise when estimating samples at the two extremes of the distribution, whereas visual estimation was more likely to place samples towards the middle of the range. However, the computerised technique was considerably less time consuming and more objective, with less possibility of operator error.

It was concluded that the digitised plaque vitality measurement system was quicker to use and less liable to error.

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TF WALSH*, CA McQUAID and P I VARELA-CENTELLES (Department of Restorative Dentistry, University of Sheffield, UK): The effect of topical subgingival 25% metronidazole on plaque vitality.

The aim of the present study was to compare the effects of 25% metronidazole dental gel and mechanical treatment on the plaque vitality of subjects with adult periodontitis. A split mouth study was utilised with two pairs of sites identified on contralateral teeth in each subject. Treatment either by mechanical therapy as described by Rawlinson & Walsh (*B Dent J*, 174, 161-166, 1993) or by two subgingival applications of 25% metronidazole 7 days apart, was randomly applied to the contralateral sites. Plaque vitality was monitored at baseline, one week, one month and three months. A total of 15 patients entered the study. Subgingival plaque vitality was measured using the method described by Walsh et al. (*J Clin Periodontol* 21, 1994, in press). No statistically significant differences in plaque vitality were found between experimental and control sites at any time point although there was a trend for vitality to be less in the metronidazole treated sites ($p=0.06$).

It was concluded that topical subgingival application of 25% metronidazole gel was equally as effective as mechanical debridement at reducing subgingival plaque vitality over a 3 month period.

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R J JACKSON (SmithKline Beecham Consumer Healthcare, Weybridge): The effect of the addition of fluoride to antimicrobial agents on plaque pH.

Previous studies (Jackson et al. *J Dent Res* 71 711 1992) have demonstrated that antimicrobial agents are effective at inhibiting the fall in plaque pH following a sucrose challenge. The effect of a combination of antimicrobial agents and fluoride has not previously been reported. The aim of the present studies was to determine the effect of combinations of the antimicrobial agents CHX, TCN, CPC and fluoride on plaque pH. Plaque pH was measured following the technique described by Duke et al. (*Caries Res* 22 350 1988). Subjects abstained from oral hygiene for 24h and from all food and drink for 2h prior to plaque sampling. Thirty minutes following rinsing with the test agents the subjects rinsed with a 10% sucrose solution. Plaque was sampled 10m after the challenge and the pH measured with a micro combination electrode. The studies were double-blind and cross-over in design. Throughout the study the subjects used a low-fluoride toothpaste in place of their normal toothpaste. The test mouthwashes were presented in a balanced randomised order with a one week wash-out period between each treatment. The results demonstrated that the post challenge plaque pH was significantly higher ($p < 0.02$) after rinsing with the mouthwashes containing the antimicrobial agents or fluoride compared with rinsing with the placebo mouthwash. The plaque pH was further elevated following rinsing with combinations of the antimicrobial agents and fluoride compared with rinsing with the agents alone ($p < 0.05$). Since Mainwaring (*Caries Res* 15 206 1981) has demonstrated a negative correlation between caries increment and post challenge plaque pH it can be concluded that a combination of antimicrobial agents and fluoride may be more effective at reducing caries increment than fluoride alone.

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R J JACKSON, G SMITH & I SMITH* (SmithKline Beecham Consumer Healthcare, Weybridge, University of Edinburgh and ISC, Edinburgh): A comparison of mouthwashes containing antimicrobial agents on plaque regrowth.

Jenkins et al. (*J Clin Perio* 21 441 1994) have reported the effect of antimicrobial agents on the inhibition of dental plaque in the absence of toothbrushing. The current investigation compared the effect of twice daily use, after toothbrushing, of mouthwashes containing cetylpyridinium chloride (CPC 0.05% and 0.1%), triclosan (TCN 0.03%) and chlorhexidine gluconate (CHX 0.12%) on the formation of dental plaque. Healthy adult volunteers participated in two double-blind, cross-over studies. At the start of each treatment phase all plaque was removed and the volunteers instructed to use the allocated test product twice daily after toothbrushing for a period of five days. Twelve hours after the final use of the product the plaque was disclosed and the area on the buccal surfaces charted by two independent assessors. Photographs of disclosed plaque were taken after both clinical assessments had been completed. The plaque was assessed from the photographs using the Quigley Hein Index. The results demonstrated that there was excellent agreement between assessors and from the assessment of the plaque index from photographs. Both plaque area and plaque index were significantly less ($p < 0.05$) after use of the mouthwash containing 0.1% CPC compared with use of the control mouthwash, the product containing 0.05% CPC and the mouthwash containing 0.03% TCN. There was no significant difference in plaque levels after use of the mouthwashes containing 0.1% CPC or 0.12% CHX. The results indicate that the assessment of plaque area directly and plaque index from photographs are comparable.

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R J JACKSON, M J HUGHES* and I S SMITH (SmithKline Beecham Consumer Healthcare, Weybridge, Wotton Road, Dorking, ISC, Edinburgh): The effect of treatment frequency on the activity of mouthwashes containing CPC.

Previously reported studies (Lobene R et al. *Pharmacol. Therapy Dent* 4 33 1979) have demonstrated that use of mouthwashes containing cetylpyridinium chloride (CPC) will significantly inhibit the formation of dental plaque and reduce gingival inflammation. The effect of single and multiple treatments with the same mouthwash containing CPC has not previously been reported. The aim of the present study was to compare the effect of once daily, twice daily and four times daily use of a mouthwash containing 0.05% CPC, in addition to toothbrushing, on the inhibition of dental plaque. At the start of each treatment phase all plaque was removed and the volunteers instructed on the appropriate treatment regimen. Plaque area was assessed, following staining with erythrocin, using the technique described by Stem & Forward (*Comm. Dent Oral Epidemiol.* 8 420 1980). The results demonstrated that following a single rinse plaque regrowth was significantly reduced ($p < 0.004$) by 23%. This effect was increased following a twice daily regimen when the plaque area was reduced by 29% ($p < 0.02$) after one week and by 38% ($p < 0.03$) after two weeks. Use of the mouthwash four times daily resulted in a highly significant reduction ($p < 0.001$) in dental plaque of 63% after one week and 71% after two weeks use of the product. These studies indicate that whilst CPC has a moderate effect at inhibiting the regrowth of dental plaque following a single application increasing the frequency of use has a significant benefit in reducing dental plaque and probably gingival inflammation.

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D G GILLAM, J S BULMAN and H N NEWMAN (Eastman Dental Institute, University of London, UK): The influence of wellbeing in subjects participating in Cervical Dental Sensitivity (CDS) studies.

A previous study (Gillam et al. *J Dent Res* 73: 835 [Abstr. no. 390], 1994) demonstrated that the wellbeing of subjects had no significant effect on dentifrice efficacy. The current aim of the investigation was to determine whether the Hospital Anxiety and Depression Scale (HAD) (Zigmond & Snaith, *Acta Psychiatr Scand* 67: 361-370, 1983) would be suitable for assessing the influence of wellbeing on the reported efficacy of desensitizing products tested in alleviating CDS. 47 subjects (12 M/35 F, mean age 36.2 (SD 11.46) years) who participated in a 6-week double-blind clinical study completed a HAD form at 0, 2 & 6 weeks. Group data were analyzed with the 'A' (Anxiety) and 'D' (Depression) components treated as combined entities. Two series of comparisons were made, namely between-group (test & control groups) comparisons at 0, 2 & 6 weeks (Wilcoxon 2-sample rank) and within-group comparisons for 0-2 and 0-6 weeks (Wilcoxon matched pairs signed rank test). Between-group comparisons on total HAD scores at 0, 2, & 6 weeks, and within-group at 0 & 2, 0 & 6 weeks were clearly non-significant. The results confirmed the findings of a previous study which suggested that the wellbeing of subjects, irrespective of group, had no significant effect on the reported efficacy of desensitizing products tested in a CDS study. Two possible conclusions may be drawn: 1) the wellbeing of subjects, irrespective of group, had no significant effect on the subjects' response to either of the two desensitizing products tested, 2) The HAD scale as used in this investigation lacks the sensitivity required to assess the influence of wellbeing in studies of this nature.

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S HADDAD, S LESSER*, P DOWELL and R McANDREW (Periodontology, Dental School, Cardiff): A survey of mouthwash use in three different patient groups.

The aim of this study was to investigate the use of commercially available mouthwashes in three selected groups: I, 75 regular attenders to a periodontal department, II, 75 non regular dental attenders and III, 75 Health Care Workers. Calculated age range and male:female ratio was: Group I, 25-70 and 1:1.41, Group II, 18-70 and 1:1.5 and Group III, 18-64 and 1:9.7. History of mouthwash use per group was 90.6% (Group I), 90.6% (Group II) and 89.3% (Group III). No statistical difference was noted between groups. Of the 'past users' 63.9% (Group I), 50% (Group II) and 47.8% (Group III) reported continuing use of a mouthwash. Data collected concerning recommendation for mouthwash use showed that in Group I the main influence was the dentist (45.6%), in Groups II and III television was stated as the main influence, (32.35% and 42% respectively). Reported data on reason for use showed the main reason for Group I to be prevention of periodontal disease (48.3%), with Groups II (39.7%) and III (48.3%) reporting the main reason to be 'refreshment of the mouth'. For $\geq 50\%$ of all 'users' frequency was noted to be ≥ 2 once daily. Highest use was observed in Groups II and III.

Results showed there to be a considerable decline in the use of mouthwashes in all 3 groups. Each group had different influencing factors for mouthwash use.

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S HADDAD, T LOYNI*, R McANDREW, P DOWELL and J MORAN (Periodontology, Dental School, Cardiff and Restorative Dentistry, Dental School, Bristol): Stain inhibition properties of three detergents *in vitro*.

This investigation compared the ability of three detergents (Sodium lauryl sulphate (SLS), Tween 20 and Sodium Sarcosinate (SS)) to inhibit stain caused by chlorhexidine mouthwash (Conodyl). Perspex blocks, in triplicate, were exposed to saliva, chlorhexidine (Conodyl) and detergent, (water for the control blocks), for two minutes respectively and then immersed in tea for one hour. The blocks were allowed to air dry and the optical density after each soaking determined using an uv/vis spectrophotometer at the lambda maximum for tea (395 nm). This cycle of immersion and reading was continued over a period of 7 days (23 cycles), until the mean optical density (OD) of the accumulated stain reached a level of 2-2.5. Results showed that there was no significant difference between SS (OD = 2.4), Tween 20 (OD = 2.41) and the control (OD = 2.4). However, significantly less stain was found with SLS (OD = 0.317) compared to the other detergents and control.

It may be concluded that SLS has a good inhibitory effect on stain caused by chlorhexidine (Conodyl) and that the other two detergents did not have this property.

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P WARREN*, J MORAN, M ADDY, R NEWCOMBE (Bristol Dental School, UWCW, Cardiff): Plaque inhibition by chlorhexidine, essential oil/phenolic and anti-adhesive mouthrinses.

The inhibition of bacterial attachment to the tooth surface is one possible approach to plaque control. This study evaluated *in vivo* the plaque inhibitory action of a novel copolymer reported to have considerable anti-adhesive properties *in vitro*. The study was a single blind, 5-treatment, randomized Latin square crossover design, incorporating balance for carry-over effects. The rinses were the 1% anti-adhesive (A), the anti-adhesive with 0.02% chlorhexidine (B), a 0.2% chlorhexidine rinse product (C), an essential oil/phenolic rinse product (D) and water (E). 15 volunteers participated and on Day 1 of each study period were rendered plaque free, ceased toothcleaning and rinsed twice daily, under supervision, with the allocated formulation. On Day 5 plaque was scored by index and area. Washout periods were 2 1/2 days. Alone or combined with chlorhexidine the anti-adhesive agent showed no effects greater than water. The chlorhexidine rinse was significantly more effective than the essential oil/phenolic rinse (plaque area C = 0.54, D = 0.75, $p < 0.05$) which in turn was significantly more effective than the other rinses (plaque area A = 1.22, B = 1.30, E = 1.30, $p < 0.05$). It is concluded that the anti-adhesive mouthrinse would be of no value in inhibiting plaque regrowth.

Study sponsored by I.C.I. Pharmaceuticals, Macclesfield, U.K.

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M ADDY*, J MORAN, W G WADE, S MILSOM, R MACANDREW (Dental Schools, Bristol and Cardiff, UK): Effect of oxidizing rinses on salivary bacteria and plaque.

Despite use for many years in dentistry, little is known of the antimicrobial or plaque inhibitory properties of oxidizing agents. The aim of this study was to compare the effects of peroxyborate and peroxy-carbonate mouthrinses with saline and a chlorhexidine rinse for effects on plaque regrowth and salivary bacterial counts. The 4 day plaque regrowth study was a single blind, four treatment, randomised, crossover design balanced for residual effects and employing 16 healthy dentate volunteers. From a zero plaque baseline volunteers rinsed with allocated products for 60 seconds twice per day whilst omitting normal oral hygiene methods. On day 5 plaque was scored by area and index. Antibacterial effects were determined by recording salivary bacteria counts immediately before and up to 7 hours after single rinses. The order of effect on plaque was chlorhexidine > peroxy-carbonate > peroxyborate > saline with most differences reaching significance. Bacterial count reductions were significantly greater at all time points with chlorhexidine than all other rinses. The oxidizing agents showed a trend for bacterial count reductions but at no time point did this reach significance from saline. It is concluded that the oxidizing rinses have potential as plaque inhibitors but the mode of action may not be through a direct antibacterial effect.

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S. MILSOM*, M ADDY and WG WADE (Department of Oral and Dental Science, University of Bristol, Bristol, UK): French mouthrinses. 1. Staining and antimicrobial effects *in vitro*.

The large number of oral hygiene products available makes it difficult to prove efficacy in long-term studies. The aim of this study was to screen *in vitro* a number of French antiseptic mouthrinse products. Four products contained chlorhexidine (Eludril, Hibident, Parodex and Prexidine), one CPC (Alodent) and one hexidine (Hextril). The antibacterial activity of the chlorhexidine products was determined against a panel of 20 oral bacteria by an agar dilution method. The potential to produce tea staining on perspex specimens was determined for all products. The maximum inhibitory dilution (MID) values of the chlorhexidine products was essentially similar except for Eludril which showed activity against *Capnocytophaga sputigena* which is normally less sensitive to chlorhexidine. Tea staining was similar for Hibident and Prexidine but slightly lower for Parodex. Staining by Eludril and Alodent was similar to water. Hextril produced staining similar to that produced by the chlorhexidine rinses.

It was concluded that the similar results for three of the chlorhexidine rinses would support similar efficacy in vivo but that the non-staining chlorhexidine product would be predicted to have reduced substantivity and efficacy.

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P RENTON-HARPER*, S MILSOM, W G WADE, J MORAN and M ADDY (Dental School, Bristol, UK) French mouthrinses. 2. Effects on salivary bacteria and plaque regrowth.

A previous paper at this meeting (Milsom et al BSRD 1995) revealed differences in antimicrobial and staining activity of some French mouthrinses containing chlorhexidine. The aim of this study was to determine whether the data *in vitro* could be extrapolated *in vivo*. Of the 7 rinses 4 contained chlorhexidine (Eludril, Hibident, Parodex and Prexidine), one contained CPC (Alodent) and one hexidine (Hextril). Saline was used as the negative control. The first study assessed the persistence of action of the products by recording salivary bacterial counts before and up to 7 hours after single rinses. The second study measured inhibition of plaque regrowth from a zero baseline in the absence of toothbrushing over a 4 day period. Both studies used blind, randomised, crossover designs balance for residual effects and involved 21 subjects. Salivary bacterial count reductions were highly significantly different between products. Compared to saline decrements were significantly greater for Parodex to 5 hours and Hibident and Prexidine to 7 hours. The other 3 products were not significantly different from saline. Plaque inhibition was measured by area and index and was significantly different between products. Hibident, Parodex and Prexidine showed similar plaque inhibition and were significantly more effective than all other rinses. Eludril and Hextril were significantly more effective than saline but Alodent was not. *It is concluded that taken with the study in vivo the efficacy of a product cannot be assumed merely because it contains a known active plaque inhibitor.* Study supported by FRED, France

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A LEARD* and M ADDY (Restorative Dentistry, Dental School, Bristol, UK) The propensity of different brands of tea to cause staining associated with chlorhexidine

The mechanism by which chlorhexidine causes staining of teeth can be debated. However, chlorhexidine will precipitate dietary chromogens onto surfaces both *in vitro* and *in vivo*. The aim of this study was to determine whether different brands of tea and coffee vary in their propensity to cause staining on chlorhexidine treated acrylic surfaces. Brands of tea and coffee were obtained from supermarket outlets and infusions prepared as 1 gram in 100 ml of boiling water. For tea the solution was decanted from the leaves at 15 minutes. Acrylic specimens, with or without saliva pre treatment, were exposed to 0.2% chlorhexidine solution for 2 minutes, washed and placed into the respective beverages for 60 minutes, washed and the optical density read on a spectrophotometer. The cycle was then repeated until one group of specimens exceeded an optical density of 2. There was a marked difference in the staining produced by the different brands of tea, the least being noted with Earl Grey with approximately 50% of the optical density readings of a group of well known tea brands. Lapsang Souchong and Darjeeling lay intermediate in the range of staining potential. For coffee, of the instant brands tested, there was less variation in staining and overall the stain by coffee was less than that seen by most tea brands. Increasing the concentration of coffee increased the staining produced. *It is concluded that advice on beverage intake may help reduce staining during the short to medium term clinical usage of chlorhexidine.*

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J MORAN*, M ADDY, P WARREN, R NEWCOMBE (Bristol Dental School, UWCM, Cardiff) Comparative tea staining by chlorhexidine, essential oil/phenolic and anti-adhesive mouthrinses.

Staining of teeth and soft tissues is a well known side effect with chlorhexidine mouthrinses. The aim of this study was to determine (1) whether a co-polymer anti-adhesive agent would prevent staining by a low concentration chlorhexidine rinse and (2) if an essential oil/phenolic rinse could produce staining. The study was a single blind 5 treatment randomised Latin square cross over design, incorporating balance for carry-over effects. 15 volunteers rinsed randomly with the anti-adhesive alone (A), a 0.02% chlorhexidine and anti-adhesive rinse (B), a 0.2% chlorhexidine rinse (C), an essential oil/phenolic rinse (D), a negative control water rinse (E). On day 1 of each study period, subjects were rendered stain free, and whilst omitting all other oral hygiene procedures, rinsed eight times a day with the allocated rinse, followed by a rinse with warm black tea. On day 4 tooth and tongue were scored by area and intensity, followed by a washout of 3^{1/2} days. Tooth and tongue staining was significantly increased with the chlorhexidine compared to the essential oil/phenolic rinse (tooth stain area C = 0.40, D = 0.27, p<0.05), which in turn was significantly increased compared to the other 3 rinses (tooth stain area A = 0.04, B = 0.05, E = 0.10, p<0.05). The anti-adhesive/chlorhexidine rinse produced no more staining than the anti-adhesive or water rinse. *It is concluded that this inhibition of staining may be due to vitiation of chlorhexidine activity by the anti-adhesive.* Study sponsored by Imperial Chemical Industries, Macclesfield, U.K.

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N C A CLAYDON*, M ADDY, R J JACKSON and B O RIDGE (Dental School, Bristol and SmithKline Beecham Consumer Healthcare, Weybridge) Effect of toothbrushes on plaque removal.

Sharma et al (J Clin Dent III C13 1992) have reported a modification of the Navy Index to assess the effect of a single brushing with different toothbrushes on the removal of dental plaque. The aim of the current studies was to use both the modification of the Navy Index and the assessment of plaque area to determine the effect of different toothbrushes on the removal of dental plaque. Subjects who participated in these single-blind, cross-over studies refrained from all forms of oral hygiene for a period of 48 hours. Plaque was assessed and recorded on standard tooth charts both before and after brushing for 1 minute with one of the test toothbrushes. The results of the first study (n=30) demonstrated there was no significant difference in the removal of dental plaque after brushing with Aquafresh Flex, Oral B40 and Dr Best Floating Head toothbrushes. In the second study (n=38) there was a good correlation between the area of plaque and the plaque assessment using the modified Navy Index. There was however no significant difference in the removal of dental plaque using either the modified Navy Index or the assessment of plaque area, after brushing with Aquafresh Flex, Oral B40, Colgate Precision or Crest Complete toothbrushes. *It was concluded that no brush could be shown superior to another employing this single use methodology. If differences do exist longer term studies may be necessary.* Study supported by SmithKline Beecham

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A. BINNEY*, M. ADDY, S. MCKEOWN AND L. EVERATT (Department of Restorative Dentistry, Dental School Bristol, UK and Procter & Gamble Company, Egham, UK): The choice of controls in studies assessing commercially available toothpastes.

If any additional plaque inhibitory activity offered by a new toothpaste is to be meaningfully quantified then a comparison to a commercially available toothpaste control is necessary, such a standard toothpaste control can be argued to be more meaningful than simply water. The significance of any such comparison is highly dependent on the choice of control preparation, therefore this choice of the benchmark or standard toothpaste should be undertaken carefully. The aim of this 8 call crossover study was to compare the plaque inhibitory properties of 5 commercially available toothpastes and a rinse of water using a 4 days non brushing model (Addy et al 1983 *Journal of Clinical Periodontology* 10: 88-99 1983). The volunteers were rendered plaque free at each baseline visit, each then proceeded to rinse for 1 minute, twice a day with the allocated preparation. At the end of each 4 day test period the plaque was disclosed and measured by index and area. The plaque measurements indicated a significant difference between the toothpastes, the order of efficacy being Colgate Total*, Crest regular**, Crest Tartar Control**, Colgate regular*, Colgate (0-6) gel* and water. These results confirm previously reported data demonstrating the plaque inhibitory properties of commercially available toothpastes compared to water, and highlighted the differential activity of commercial toothpastes at inhibiting plaque growth *in vivo*. *In conclusion, these data indicate that the choice of control toothpaste with which to compare new toothpastes formulated for plaque control is important and could influence conclusions drawn from clinical trials of such products.* This work was kindly sponsored by Procter & Gamble (HC&B) UK, * Colgate-Palmolive Surrey, UK, ** Procter & Gamble UK.

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J. OWENS*, A. BINNEY, M. ADDY, AND L. EVERATT (Restorative Dentistry, Dental School, Bristol, UK and Procter & Gamble Company, Egham, UK) A 3 month home study investigating the antiplaque and anti-gingivitis effects of several toothpastes.

The study was single-blind, parallel design with a total of 128 healthy dentate volunteers (32 male, 97 female) who toothbrushed with one of 4 toothpastes, at home, twice a day over a period of 12 weeks. The volunteers all demonstrated a plaque score (Turesky et al *J. of Perio.* 41: 41-43, 1970) of at least 1.5 and a gingivitis score (Loe & Silness *Acta Odont. Scand.* 21 533-551, 1963) of at least 1.0. At the beginning of the trial each volunteer was scored for plaque and gingivitis and then received a thorough prophylaxis. Each volunteer was then allocated a toothpaste according to a predetermined randomisation. The volunteers were then re-examined after 6 and 12 weeks. The volunteers refrained from using any other oral hygiene products during the period of the trial. The toothpastes tested were Crest Regular**, Colgate regular*, Colgate Junior* and Colgate Total* (containing 0.3% triclosan). Results indicated that there were no overall treatment differences for either Plaque index (P>0.2) or Gingival index (P>0.2) and ordering of the toothpastes was not possible. All volunteers oral hygiene and gingivitis improved following the 6 week period and continued to improve to the 12th week. *This study indicated that none of the toothpastes tested demonstrated a measurable anti-plaque or anti-gingivitis action over a 12 week period of home use.* This work was kindly sponsored by Procter & Gamble HC&B UK.

* Colgate-Palmolive Surrey, UK, ** Procter & Gamble UK

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A. BINNEY, J. FAULKNER* AND N.H.H. CLAYDON (Restorative Dentistry, Dental School, Bristol, UK): Inter-examiner variability's occurring when using a plaque area index assessment.

Of the described Plaque Indices, Plaque Area is one commonly used for research purposes, all of these indices contain potential for scoring errors. Addy et al (*Journal of Clinical Periodontology* 10: 89-99 1983) described a plaque area index, which has been used in the assessment of oral hygiene products. Previously reported data has highlighted the variation between two non-clinical examiners (inter-examiner variation) measuring the same plaque area records using this index (Faulkner et al *Journal of Dental Research* Abstr. 326, 1994). The aim of this study was to quantify the variation between two experienced clinicians scoring the same volume of plaque, and the intra-examiner variability when the same non-clinical examiner measures the same plaque area records. The accumulated plaque on the tooth of a group of 42 volunteers (1008 individual readings) taking part in a trial assessing oral hygiene products, were represented on standard tooth forms by two independent clinicians, each clinician was blind to the others results. All the plaque records were then measured by an independent non-clinical examiner using a computer connected to a graphics package. The plaque records were then re-measured by the same examiner after a period of 3 months (a total of 672 readings). The results indicated there was an extremely significant difference between the two clinical examiners (p<0.0008) and a highly significant difference between the same non-clinical examiner measuring the same plaque records on two separate occasions (p<0.008). These results, and those previously presented, indicate that certain precautions should be taken when using this plaque area index if reproducible results are to be obtained. *It is concluded that during clinical trials assessing oral hygiene products, a single clinician, and a single non-clinical examiner, should always be used. Additionally all plaque records for a single visit/period should be measured promptly.*

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A SHALHOUB* and M ADDY (Restorative Dentistry, Dental School, Bristol, UK) Examiner accuracy and reproducibility of scoring plaque area using a model system.

Ideally, examiners should score plaque indices accurately and reproducibly. The latter can be determined from repeat measurements but the former requires modelling of the index *ex vivo*. The aim of this study was to determine how accurate and reproducible were examiners in recording plaque from charts and photographs. Study 1 involved 15 examiners who scored plaque by area on charts and by the Global Plaque Index from 8 standard charts or "patients". The recordings were repeated twice with 2 weeks separation. Study 2 involved 6 examiners recording plaque area and the Global Plaque Index from upper and lower central incisors on 8 projected transparencies. Planimetry measurements from the 8 charts and 8 photographs were taken as the "Gold" standard from which to determine accuracy. Intra and inter examiner reproducibility for plaque area and global plaque indices were high with a low standard deviation for differences for repeat scorings. Accuracy for both indices was also high and for the global plaque index reached 95% of judgements within 10% of true readings. Accuracy and reproducibility from photographs was markedly reduced compared to recordings from charts. It was concluded that examiners make accurate and reproducible assessments of plaque when plaque distribution is well defined but are much more variable and less accurate when the plaque is less clearly defined as in colour transparencies.

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N WEST*, J HUGHES and M ADDY. (Restorative Dentistry, Dental School, Bristol): Fluoride pre-treatment of the enamel surface prior to citric acid exposure.

The beneficial effect of fluoride in the enamel structure, particularly the surface layer, has been documented as reducing erosion (Sorvari R et al *Scandinavian Journal of Dental Research* 1988; 96:226-231). Once erupted it is thought the teeth obtain maximum protection from fluoride derived by topical application, probably in the form of sodium fluoride. Fluoride ions will exchange with hydroxyl ions in the hydroxyapatite for surface positions at low fluoride concentrations of less than 1000ppm. However, over this value calcium fluoride is thought to form instead (Gross P *Caries Research* 1977; 11:204). Sorvari R et al (*Caries Research* 1994; 28:227-232) showed that fluoride treatment *in vitro* on enamel, prior to exposure to acidic beverages, inhibited erosion. The aim of this investigation was to determine the effect of pre-treating human enamel with commercially available fluoride products, prior to 0.3% citric acid exposure, at pH 3.2 for three 10 minute intervals at 35°C. Various products were tested including toothpastes, mouthwashes and gels. Results were recorded by surfimetry. The study showed fluoride had a marked effect on reducing erosion of surface enamel with the mouthwash (2.78µm loss), gel (2.06µm loss) and the strontium acetate/fluoride toothpaste (2.58µm). The other toothpastes showed no statistically significant differences compared to exposure with no fluoride pre-treatment (3.37µm loss).

It is concluded that only one fluoride toothpaste investigated produced similar reduction in erosion to the fluoride mouthwash and gel.

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C MORRIS-CLAPP¹, S YEGANEH², E LYNCH³ and M GROOTVELD² (Department of Conservative Dentistry, and Inflammation Research Group², LHM, UK): An *in vitro* study to test the efficacy of five whitening agents.

Home bleaching systems are in use in both the UK and North America, the majority of which are based on carbamide peroxide. Many 'over-the-counter' brands are now available to whiten teeth. We tested the efficacy of the lightening capacity of five brands of these agents by rating and ranking their whitening effects on sectioned, freshly extracted teeth. Ten teeth were sectioned and tested in each group and results assessed blind by three operators. The agents tested were: (1) 2% sodium chlorite¹; (2) 0.3% carbamide peroxide, 0.5 sodium peroxodiphosphate, 0.5% bromelain and 0.125% papain (Ultrawhite Opal rinse¹); (3) 0.3% carbamide peroxide, 0.5% sodium peroxodiphosphate, 0.5% bromelain and 0.125% papain (Ultrawhite Opal toothpaste¹); (4) 0.1% chlorine dioxide mouthwash (retar DEX²); (5) 2% sodium chlorite¹, 0.125% papain (Dental White³). The results in descending order of whitening efficacy on intrinsic staining were: Ultrawhite Opal rinse¹; Ultrawhite Opal toothpaste¹; 2% sodium chlorite¹; retar DEX²; Dental White³. The control (H₂O, pH 7.0) had no effect. The results in descending order of whitening efficacy on extrinsic staining were: Ultrawhite Opal Rinse¹; Ultrawhite Opal toothpaste¹; 2% sodium chlorite¹. The other agents failed to remove extrinsic staining.

This study showed that Ultrawhite Opal spray and toothpaste were the most effective tooth whitening agents.

¹Janina International, UK. ²Rowpar Pharmaceuticals, USA. ³Medex, UK.

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M LEVINKIND¹, P KINCESH² and SCR WILLIAMS (The Lond Hosp Med Coll, London E1 2AD, ULJRS-MRI Dept Chemistry, QMW College, London, UK): Magnetic resonance imaging of proton movement through human root dentine.

The application of Magnetic Resonance (MR) imaging to study dental hard tissues is a comparatively recent innovation. The aim of this study was to evaluate proton movement through human root dentine. An access cavity was prepared and the pulp tissue extirpated from premolar teeth that were extracted for orthodontic reasons. Each tooth was mounted in a glass vial which contained water and was placed in a radiofrequency coil tuned to excite and detect proton resonances at 200MHz. The vial was positioned at the centre of a 30 cm bore, horizontal superconducting magnet operating at a magnetic field strength of 4.7 Tesla. Images were recorded of 300 µm thick serial sections through the tooth. The root canal was then filled with Magnavist (Schering AG, Germany) a paramagnetic contrast medium, replaced in the glass vial and further images were taken. The MR images were repeated after the tooth had been reequilibrated in water except when the pulp chamber was refilled with Magnavist a wax coating was placed on either just the apical 1.0 mm or all the root surface except for the apical 1.0 mm. It was found that the time taken for the Magnavist in the pulp space to be replaced with water was slowest when the wax coating covered most of the root surface. Further experiments are being carried out to determine diffusion parameters for protons and Magnavist through root dentine.

We conclude that site specific wax coating and a paramagnetic contrast medium permit Magnetic Resonance imaging to study of proton movement through human root dentine. We gratefully acknowledge support from The Royal Society (Research Grant 574005.G501)

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M LEVINKIND¹, R. WADGE and T VANDERNOOT² (London Hospital Medical College, London E1, 2AD, QMW College, London, UK): The effect of ion size and temperature on electrochemical impedance measurements on human enamel.

The aim of this study was to determine the effect of ion size and temperature on electrochemical impedance measurements on human enamel. Sections of healthy enamel were cut from the palatal aspect of five freshly extracted human third permanent molars. Impedance measurements were made with sections equilibrated in two different unbuffered electrolytes Sodium Chloride (NaCl) and TetraEthylAmmonium Chloride (TEACl) both with a concentration of 10⁻³M. After equilibrating the sections in NaCl for 72 hours, the impedance spectrum of each section was characterised at various temperatures between 10°C and 60°C. The sections were mounted in an electrochemical cell and an alternating potential of 500mV was applied over a range of frequencies from 10Hz to 100kHz. The sections were then rinsed thoroughly and immersed in TEACl solution for 72 hours and the impedance measurements were repeated with TEACl in the electrochemical cell. The measured impedance spectra behaved appropriately for variations in temperature with DC resistance values increasing as temperatures reduced. Typical DC resistance values recorded from equilibrating specimens in TEACl were larger (9.0 MΩ at 37 °C) compared to the DC resistances measured when the same specimens were equilibrated in NaCl (4.7 MΩ at 37 °C).

AC impedance spectroscopy is a useful technique to study the effects of ion size and electrolyte temperature on ion motion in healthy human enamel *in vitro*.

We gratefully acknowledge support from The Wellcome Trust (Grant #036689)

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T VANDERNOOT¹, R WADGE, and M LEVINKIND (London Hospital Medical College, London E1 2AD, QMW College, London UK): Alternating current impedance measurements used to quantify diffusion of ions through enamel.

The aim of this study was to use alternating current impedance measurements to study the effects of establishing a concentration gradient across sections of healthy mature and immature human dental enamel sections maintained at a constant temperature. Impedance measurements were made by the application of a 200 mV alternating potential across enamel sections via a potentiostat connected to a microprocessor controlled frequency response analyser. The resulting ionic current was recorded and the information used to determine the resistance of each section. The samples had been equilibrated fully in unbuffered 10⁻³ M NaCl prior to being characterised. The solution on the natural enamel surface was then changed to unbuffered 10⁻² M NaCl and measurements were made every 10 seconds for up to five hours. The specimen was then equilibrated fully in the unbuffered 10⁻² M NaCl solution and the impedance was remeasured. The initial and final characterisations provided upper and lower limits for calculation of diffusion coefficients. The change in resistance with time as the ions diffused down their concentration gradient was used to determine the diffusion coefficient for NaCl through dental enamel. In general, the diffusion coefficients determined were typically 3 x 10⁻⁶ cm² s⁻¹ and values for mature enamel were smaller than those for immature enamel.

We conclude that ac impedance measurements can provide an alternative technique to the use of radiolabelled ions for the study of diffusion of ions through sections of dental enamel. We gratefully acknowledge financial support from The Wellcome Trust (Grant # 036689)

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S R CHATFIELD* and G H DIBDIN (M R C Dental Group, The Dental School, University of Bristol, UK): Diffusion of water in dental enamel and its associated activation energy.

Dental enamel is known to be slightly porous with a complex pore structure (which is related to its histological structure). The fact that the initial caries process begins below a surface layer of intact enamel makes it important that we understand the pore system if sound enamel. There is a microporous component which, from various studies, may be associated with intercrystalline spaces within the body of each prism. We believe that this contributes in large measure to the 8% v/v of exchangeable water in enamel. We believe a much smaller but open and accessible pore system is associated with discontinuities at the prism boundaries, and contributes to the low porosity previously found by krypton sorption. Using ³H₂O clearance studies we have confirmed our earlier finding (G H Dibdin, *Caries Res* 27: 81-86, 1993) of anoseous thickness-dependent diffusion coefficients of the order of 2 x 10⁻⁷ cm² s⁻¹ which we explained in terms of a biphasic pore model. By varying temperatures over the range 0 - 70°C we have obtained activation energies for this process which are in the range 28 - 34 kJ mol⁻¹ (6.7 - 8.0 kcal mol⁻¹).

These energies are substantially above those expected for diffusion in water, giving evidence for an activated diffusion process, as expected for the inner intercrystalline spaces.

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PV HATTON¹, JE AARON², DH CARTER² (Dental Schools of Sheffield¹ and Manchester², Department of Anatomy, University of Leeds²): An ultrastructural and microanalytical study of mineralisation in rodents.

Optical studies have consistently suggested that the mineralisation event takes place within the osteocyte. However, there have been few attempts to reconcile this with transmission electron microscope (TEM) studies which continue to describe an extracellular event involving either epitaxial sites on collagen or specific vesicles. This study set out to investigate the nature of the bone mineral and its manner of formation using techniques designed to preserve the chemical integrity of the tissue. 4-day old mouse calvaria were 'slam frozen' at -170°C, freeze substituted at -70°C in acetone and embedded in resin at -20°C. Sections (250nm-1µm) were examined without staining in the TEM and by elemental analysis (single point and mapping). Mineralised microspheres were demonstrated both within the cytoplasm of osteocytes and in the extracellular matrix. The microspheres were composed of amorphous mineral and a transformation to a filamentous form was evident as they approached the calcifying front. In the more mature tissues, the microspheres were assembled into a complex microskelton and crystalline bone mineral was absent. X-ray microanalysis confirmed that the intracellular and extracellular microspheres were similar in composition to the mineralised bone matrix.

The evidence suggests that the mineralisation event takes place within the osteocyte, that the bone salt has the form of microspheres and that the microspheres are exported and assembled into a complex microskelton. This study was supported by Colgate Palmolive and the MRC.

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DH CARTER¹, JE AARON², PV HATTON² (Dental Schools of Manchester¹ and Sheffield², Department of Anatomy, University of Leeds²): A histological study of mineralisation in rodents.

The nature of the bone salt and its manner of formation remains the subject of debate. It is, however, generally recognised that the bone mineral is easily altered by histological preparation. This study set out to examine bone using techniques designed to preserve the *in vivo* character of the mineral. Fixed and unfixed cryosections of adult rat femora were stained by reliable techniques for calcium and phosphate and examined by the optical microscope. The mineralised matrix was stained in a fine granular pattern. At high magnification, the granularity was assigned to a complex microskelton of mineralised microspheres measuring up to 1 micron in diameter. At the same time, stain was not confined to the extracellular matrix and discrete mineralised microspheres, measuring up to 1 micron in diameter, were also evident in osteocytes.

The evidence suggests that the mineralisation event takes place within the osteocyte, that the bone salt has the form of microspheres and that the microspheres are exported and assembled into a complex microskelton.

This study was supported by Colgate Palmolive and the MRC.

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MS LANGLEY^{*}, RJ WADDINGTON and G EMBERY (Dept. of Basic Dental Science, Dental School, Cardiff, UK): The effect of fluoride on proteoglycan synthesis in rat bone cell cultures.

Fluoride can cause alterations of both the inorganic and organic matrix components of mineralised tissues, including bone and dentine. Proteoglycans (PG) have been implicated as important components in the mineralisation process. The present study investigates the *in vitro* effects of various fluoride concentrations on PG synthesis in rat bone cells. Bone cell cultures were established from bone marrow washes from femurs of 6-week old Wistar rats and maintained in α MEM supplemented with foetal calf serum, ascorbic acid, β -glycerolphosphate, dexamethasone and antibiotics. Fluoride was added to maintenance media to a final concentration of 0, 10^{-7} M, 10^{-5} M. Cells were cultured for between 1 and 10 days after which synthesis of the PG was determined by addition of 35 S-methionine pulse label for 30min. Cell layers were treated with 4M guanidinium chloride for 48 h, PG samples recovered from PD-10 desalting columns and lyophilised. Samples were analysed for 35 S by liquid scintillation counting, total protein content and glycosaminoglycan (GAG) content following protease release from PG and cellulose acetate electrophoresis. Sulphate incorporation into newly synthesised PG was maximum after 7 days of culture of the cells, with a corresponding increase in protein and sulphated GAG synthesised also evident at this time. The presence of fluoride appeared to delay the onset of synthesis of sulphated GAG. After 9 days sulphated GAG content within the cultures was reduced by 23% and 10% following incubation in 10^{-7} M and 10^{-5} M fluoride respectively compared to those grown in the absence of fluoride. The alteration in sulphated GAG and hence PG synthesis may be an influencing factor in the altered mineralisation witnessed following fluoride administration *in vivo*.

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R.C. HALL (Dept. Basic Dental Science, UWCM, Cardiff): Proteoglycan structure is altered in fluorotic rat dentine *in vivo*.

It has previously been established that the extracellular matrix components of rat dentine undergo structural modification *in vivo* and *in vitro*. This study reports on a more detailed purification and analysis on the changes to the structure of dentine proteoglycans (PG) during *in vivo* induced fluorosis. Rats were rendered fluorotic by the daily intake of F⁻ in the water (20ppm). Newly synthesised PG were labelled with 35 S-sulphate. Fluorotic and control dentine were demineralised in EDTA and PG extracted under dissociative conditions using 4M GuCl. Extracts were fractionated using anion exchange chromatography on Q-sepharose, and further purified on MONO-Q interfaced to FPLC. PG were detected in the eluent by A280 nm, DMB assay and 35 S-sulphate counting. The extracted PG were characterised by elution profile, glycosaminoglycan (GAG) composition, GAG chain length, intact and core protein weights. The results demonstrated a more diverse anionic profile for the fluorotic PG. The protein cores of both fluorotic and control PG was 45 kDa. However, there was a reduction in the GAG chains of the fluorotic dentine PG. In addition, the presence of dermatan sulphate was noted in the fluorotic PG but not the control PG where chondroitin 4 sulphate was the sole GAG identified.

The appearance of a more anionic PG fraction corroborates previous recognition of an oversulphated chondroitin epitope by Mob 704 (Hall R.C., J. Dent Res. 73:822, 1994). The shorter chain length and presence of dermatan sulphate may be implicated in the impairment of mineralisation seen in fluorotic dentine.

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AJ SMITH^{*}, RJ WADDINGTON and G EMBERY (Dept. Adult Dental Care, Glasgow Dental School; Dept. Basic Dental Science, Cardiff Dental School): Ultrastructural and immunocytochemical studies on chondroitin sulphate rich proteoglycans in human alveolar bone.

Chondroitin-4-sulphate rich proteoglycans have been described as having an important role in mineralisation (Fisher LW et al. *J Biol Chem* 258: 6588-6594, 1983). This study utilised immunocytochemical methods to characterise the chondroitin sulphate rich proteoglycans from human alveolar bone obtained from an oral source. Proteoglycans were extracted from bone by a sequential 4M guanidine HCl extraction process and purified by DEAE-ion exchange chromatography (Waddington RJ and Embury G, *Archs Oral Biol* 36: 859-866, 1991). SDS-PAGE and Western blot analysis, using CS-56 monoclonal antibody (Sigma) demonstrated proteoglycans with a core protein of 58kDa, GAG chains of 45-66kDa and proteoglycan species with a mean molecular weight of 205 kDa. Samples of alveolar bone were embedded in LRW resin (Newman GR et al. *Histochem J* 15: 543-555, 1983), labelled with CS-56 antibody and examined under the light and electron microscope. At the light level labelling was restricted to the osteocyte lacunae and canaliculi. Ultrastructural observations noted the labelling localised to fine filamentous material in the walls of the osteocytes and canaliculi. Sparse labelling associated with the collagen fibres immediately adjacent to the lamina limitans but no labelling of the mineralised matrix was observed. These results indicated subtle differences in the distribution of chondroitin sulphate from previously reported work (Bartold PM, *J Dent Res* 69: 7-19, 1990) which may indicate species or age differences in the samples used in this study. In conclusion, ultrastructural analysis confirmed and extended observations of glycosaminoglycan localisation at the osteocyte cell membrane of mature human alveolar bone.

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A J SLOAN^{*} and A J SMITH (School of Dentistry, University of Birmingham, Birmingham, UK): Culture of odontoblasts in human tooth slices.

We have previously reported the culture of the dentine-pulp complex of rat incisor teeth (Sloan et al. *J Dent Res* 73 838 [Abstr. 417] 1994) when embedded in a semi-solid agar medium. Few reports exist on the culture of human odontoblasts and the aim of this study was to investigate the culture of human dental tissues as a basis for development of a model of tertiary dentinogenesis. Deciduous and permanent human teeth were obtained immediately after extraction and thin transverse sections cut on a saw microtome prior to culture. Tooth slices were cultured on the base of a petri dish in Dulbecco's MEM medium containing vitamin C, glutamine, antibiotics and heat-inactivated foetal calf serum at 37°C in an atmosphere of 5% CO₂ in air for periods of up to 7 days prior to histological examination. The morphological appearance of the odontoblasts after the culture period was good with evidence of polarization of the nuclei in many cells, although other cells within the pulp showed varied and often poor morphology.

It is concluded that human odontoblasts from both permanent and deciduous teeth may be cultured in association with their extracellular matrices for periods of at least 7 days, which could provide the basis for the study of tertiary dentinogenesis within human dental tissues.

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VJ KINGSMILL^{*}, A BOYDE and SJ JONES (Department of Anatomy, University College London, UK): Does substrate collagen fibre orientation influence the size of osteoclastic resorption pits?

The aims of this study were to determine whether the shapes and sizes of resorption pits produced by osteoclasts in culture are influenced by the collagen fibre arrangement of the substrate and if this would need to be considered in an assay system. Sperm whale mandibular tooth cementum was selected as a highly anisotropic material. Flat slices were cut perpendicular (Group I) or parallel (Group II) to the extrinsic fibre axis. The slices were cultured for 24 hours with osteoclasts obtained from prechick chick long bones, then cleaned of cells and demineralised collagen, and dried from ethanol. Video-rate reflection confocal microscopic mapping (Lasertec) was used to measure resorption pit areas and volumes, and the mean depth per pit calculated as volume:area ratio. Two experiments were undertaken. In the first, Group I ($n=282$) had pits of a significantly ($p<0.02$, Mann-Whitney) smaller mean area and volume ($931\mu m^2 \pm 67\mu m^2$ SEM; $6072\mu m^3 \pm 500\mu m^3$ SEM), than Group II ($n=290$): $1506\mu m^2 \pm 100\mu m^2$ SEM, $8220\mu m^3 \pm 662\mu m^3$ SEM, and the mean depth ($I=5.76\mu m \pm 0.15\mu m$ SEM; $II=4.54\mu m \pm 0.10\mu m$ SEM) was significantly greater ($p<0.0001$). In the second experiment, the mean depth was again significantly greater ($p<0.005$) in Group I ($n=232$) than Group II ($n=326$): $I=6.11\mu m \pm 0.20\mu m$ SEM, $II=5.20\mu m \pm 0.12\mu m$ SEM, but there was no inter-group difference in mean area or volume. The results illustrate one variability that may occur in resorption assays.

This experiment indicates that collagen fibre anisotropy in a substrate may influence osteoclastic resorption *in vitro*, and that area measurements are unsafe to use as the sole index of osteoclastic activity.

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A R LEW and R P SHELLIS^{*} (MRC Dental Unit, Dental School, Bristol, UK): Studies on solubility of bone mineral.

Our aim was to study bone mineral solubility under conditions which minimised metastability artefacts as far as possible. Powdered, lipid-free ox bone was stirred at 2 g/200 mL in 100 mmol/L acetate buffer, pH 5.5, either plain or with Ca and P added to adjust the initial pH_{Ca} to 54, 55, 56, or 58 (temperature 37°C, atmosphere 5% CO₂). All solutions became saturated with respect to OCP after 2.5-40 min because of rising pH and, usually, Ca and P release, and remained so for a further 80-220 min. During this period, reprecipitation began and continued after the solutions became undersaturated with respect to OCP: pH_{Ca} increased to 55.0-55.9 by 16-20 d (apparent equilibrium). Further equilibration of samples previously treated with pH_{Ca} 55 solution, with the same solution then with plain buffer, resulted in equilibrium pH_{Ca} of 56.0 and 56.4. In a potential plot, points for all equilibrium solutions lay close to a line, the slope of which suggested that solubility was controlled by solid with a Ca/P ratio of 1.2-1.3. Mg was released in all equilibrations and all solutions became supersaturated with respect to whitlockite. By extrapolation, it appeared that 10-15% of Mg in the original mineral was loosely bound.

It appears that a small fraction of bone mineral is very soluble. The solubility of the major fraction can be represented by a pH_{Ca} of 55.5 ± 0.3 ($n=7$), but equilibrium solubility seems to be controlled in these experiments by a non-stoichiometric solid or surface phase.

- 368** J APPLETON¹, M COOKE² and K SZOSTEK¹ (Clinical Dental Sciences, The University of Liverpool, UK; ²Sheffield Hallam University, Jagiellonian University Cracow, Poland): Teeth as bioindicators of environmental exposures.

It is well known that the mineral phase of calcified tissues sequesters trace elements during their development. The objective of this study was to determine if the dentine of teeth can be used as a bioindicator of environmental exposure to trace elements. Teeth from residents of Cracow in Poland were compared with those of an 18th century nomadic Spitzbergen population. Teeth were sliced transversally beneath the cement enamel junction and the exposed surface polished and cleaned. A laser was used to ablate the dentine beneath the dentine enamel junction, mid point between the edj and the root surface and beneath the cement dentine junction. The ablated areas measured 100 µm in diameter and 30 µm in depth. After ablation the sample was entrained in an inert gas stream and transferred into the plasma source and a mass spectrometer as a detector. In contrast to the Cracow teeth the 18th century teeth revealed a spectrum including a range of lanthanide elements which occur naturally and uniquely in the Spitzbergen area. This would suggest that laser ablation ICPMS can be used to provide elemental fingerprints from teeth which incorporate elements from environmental exposure during their development.

- 369** D C ATTRILL¹, A S BLINKHORN¹, R M DAVIES², M R DICKINSON² and T A KING² (Dental Health Unit, Department of Laser Photonics, University of Manchester, UK): Erbium:YAG and Holmium:YAG Laser Ablation of Dentine.

Erbium:YAG ($\lambda = 2.94 \mu\text{m}$) and Holmium:YAG ($\lambda = 2.1 \mu\text{m}$) laser radiation have been suggested as being suitable for modification of enamel and dentine. The Er:YAG laser is of particular interest as its wavelength coincides with the peak of the tissue water absorption curve. The aim of this pilot study was to determine *in vitro* the latent heat of ablation of dentine for both lasers and to compare their efficiencies. Extracted teeth were prepared and irradiated at fluences of up to 750 J/cm². Radiation was focused using an 85mm CaF₂ lens. Latent heat data were obtained by cutting slots into blocks of dentine, and calculating the volume ablated for known energy inputs. Specimens were mounted on a translation stage, and traversed back and forth in the focal plane of the beam. The mean latent heats of ablation on dentine for Er:YAG and Ho:YAG were 5kJ/cm² and 55kJ/cm² respectively. The differences between the groups were significant ($p < 0.01$) using Mann-Whitney U test. It is concluded that *in vitro* Erbium:YAG laser radiation is more efficient than Holmium:YAG radiation for removal of dentine.

- 370** DEVLIN H¹, CASH A, GARLAND H, WATTS D (Department of Dental Medicine and Surgery and Division of Biological Science, University of Manchester, U.K.): Visco-elasticity of diabetic bone.

The aim of this study was to investigate the visco-elasticity of bone from diabetic rats under continuous compressive loading. We tested the hypothesis that stress applied to femoral bone from diabetic rats would be reduced faster than in control rats. 15 diabetic and 11 control animals were killed humanely with an overdose of nitrous oxide and carbon dioxide. 5mm long, mid-shaft sections of rat femur were removed and stored in formalin for 8 weeks. The cylindrically shaped diabetic bones were compressed to a peak mean cortical bone stress of 36.22 MPa, and control bones to 31.77 MPa. The stress was then measured, at constant strain, for 1000 s. A non-parametric independent sample test (Mann-Whitney test) was used to analyse the results. There was no statistically significant difference between the peak stress values ($p > 0.05$). The mean percentage reduction in stress for the diabetic rats (mean = 17.56, sd = 7.3) was not significantly different from the control rats (mean = 14.46, sd = 8.0). The mean time constant for the reduction of force in the diabetic group was 93.5 (sd = 26.67), which was significantly lower ($p < 0.05$) than for the control group (mean = 118.62, sd = 25.5).

A significantly lower time constant for the decrease in force was seen for the diabetic rats than for controls ($p < 0.05$). Visco-elastic changes in diabetic bone samples (involving molecular or structural flow) resulted in a faster reduction in stress than in control samples.

- 371** H MANNION¹, R BEDI (School of Dentistry, University of Birmingham, UK): Dental student debt - realities and perceptions.

This investigation aims at providing information on the financial status of dental students enrolled on the Bachelor of Dental Surgery degree course at the University of Birmingham. All undergraduate dental students enrolled during the academic year 1993/94 were asked to participate in the study. The pre-tested confidential and anonymous questionnaire was administered to all students. Domains explored in the questionnaire were: personal details, expenditure levels, income, loans, overdrafts, use of government schemes ("top-up loans"), etc. 115 dental students completed the questionnaire (response rate 47%). The study found that, on average, dental students debt increased each year throughout the course. 36% of students had taken the opportunity to take out a top-up loan (range £700-£850). 9% of the students surveyed had been eligible for awards from access funds, which meant their financial situations were severe enough to warrant assistance. Credit cards were possessed by 52% of the surveyed students; however, only half of these owed money, but it was disturbing to find that 22% owed between £500 and £2000. The majority of dental students (56%) have overdrafts rather than bank loans (18%). 17% of students engaged in weekly part-time employment. Dental students' estimates of the level of debt they are likely to incur are greater than the actual debt presently experienced by final year students. In conclusion, this pilot study showed that dental students invariably incur debt during their undergraduate course. This debt increases during the course and personal overdrafts are held by 56% of respondents.

- 372** M.BAHRAMI¹, G.PHILLIPS², and G.R. OGDEN¹ (Depts. of Dental Surgery & Periodontology¹ and Medical Microbiology², University of Dundee, UK): An audit of dental students infection control procedures.

The prevention of cross infection is an essential part of clinical practice. We have previously audited clinical dental students knowledge of infection control (Ogden et al, J Dent Res 73:840, 1994). In the light of these results a study was undertaken to audit what the students actually did. A proforma was completed for each patient episode observed. This detailed duration of handwashing, clothing worn, whether non sterile surfaces were touched during the operation, whether the student was assisted and preparation prior to treatment of the subsequent patient. Twenty treatment episodes were observed in each of 6 clinical areas for 3rd and 4th year students (i.e. 1st and 2nd clinical years). For final year students, 20 'treatments' were observed in two departments. A total of 200 treatment episodes were recorded. The students observed were not aware of the aims of the study. Handwashing was undertaken in over 95% of cases, although very rarely longer than one minute. Although the wearing of gloves was universal, eye protection and face masks were not. Non sterile surfaces were touched in over 60% of cases observed, with no marked difference observed between clinical years. In 60% of cases the students were not assisted. Correct post-operative disposal of waste and cleaning prior to the next patient was achieved in almost all cases. In conclusion adequate infection control was achieved. However the touching of non sterile objects must be avoided and would be expected to improve if assistance were available.

- 373** R C CRAVEN¹, A S BLINKHORN, C ROBERTS (Oral Health and Development Group, University of Manchester): Job stress and job satisfaction among dental nurses in North West England.

A postal survey was conducted among dental nurses in the former North Western Region. The aim was to study the prevalence and severity of reported job stress and job satisfaction, and their relationship with other variables. Responses were received from 82% of the practices approached and from 66% (370) of the individual nurses. The majority (68%) were "fairly well satisfied" with their job and 64% felt that their overall job stress was minimal. However, 47% felt moderate to severe stress about running behind time and 45% felt similarly about being undervalued by the dentist.

Job stress was most strongly associated with stress outside work. Those nurses with a regular salary review, staff meetings and a clear job description reported less job stress and higher job satisfaction.

These practice management techniques may help reduce stress among dental nurses.

- 374** R McANDREW, M McANDREW, K LAI, S ADAM¹ and A J C POTTS² (Periodontology and Oral Surgery, Medicine and Pathology¹, Dental School, Cardiff): Opinions of consultants on referral letters.

Referral letters are typically the sole method of communicating information for GPs and hospital specialists. There is evidence that clinicians are often dissatisfied with this aspect of their practice. (Doleman F, Family Practice 4: 176-182, 1987). The present study was designed to canvass the views of dental consultants on the relative importance of a range of information and data items that may be included in professional communications and how this procedure could be improved. 200 consultants selected at random from a list of oral and maxillofacial surgeons, orthodontists, restorative dentists and paediatric dentists were posted a questionnaire. No reminders were forwarded after initial contact. 144 replies (73% response) were received by the cut-off date:- 28.8% OMFS; 32.2% ORTHO; 26.7% REST; 9.6% PAED and 2.7% OTHER. Reported standard of referral letters was excellent 0%, good 24.5%, adequate 52.7%, poor 24.7% and appalling 3.0%. From the list of data items supplied 28.5% agreement within the specialities was only reached on the following points: practitioners name and address, patients name and address and suspected malignancy. Taking appropriateness of referral, 27.5% reported specialist investigation/treatment required (99.3%) and practitioner unable to carry out treatment (95.5%) as suitable reason for referral. Inappropriate referral reasons were: patient unable to pay for treatment (95.6%), practitioner unwilling to carry out treatment (76.5%) and patient unwilling to pay practitioner for treatment (95.6%). 68.2% thought a Section 63 course would be beneficial in improving referral letters.

This study suggests that that referrals from GPs to specialists could be improved.

- 375** R C CRAVEN, K D O'BRIEN¹ and E BENNETT (Universities of Manchester and Pittsburgh): Attitudes and reported practice towards HIV/AIDS patients among dentists.

This was a survey among dentists in the North West of England which sought to identify their attitudes towards patients with HIV or AIDS and their willingness to treat them. A mail survey achieved a response ratio of 63% (554/873). Some 7% had experience of HIV among their family, 2% among their colleagues. Seventy-one percent had never knowingly treated an HIV positive patient, but 47% indicated a definite willingness to treat them. Although 77% of respondents stated a definite agreement to the statement that the chances of them becoming occupationally infected were negligible, 64% said they would have chosen another profession because of the HIV risk.

Agreement with positive attitude statements and a willingness to treat HIV positive patients were more likely among salaried and among younger dentists.

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S E RIMMER* and A C MELLOR (Dept of Restorative Dentistry, University of Manchester, UK): Patients' perceptions of aesthetics in restorative dentistry.

The aims of this study were to compare patient perceptions of different types of fixed anterior restorations photographed in the same patient's mouth. Three comparisons were made: 1) metal ceramic v all ceramic, 2) NHS metal ceramic v private metal ceramic, 3) metal ceramic (normal buccal contour) v metal ceramic (overbuilt buccal contour). Two patients were used for the study, one requiring three upper anterior crowns, the other requiring a three-unit upper anterior bridge. Six restorations were fabricated for each patient and then each was photographed in situ. A total of 105 patients from three different locations viewed the photographs and completed a questionnaire. In comparison 1, more people thought the all ceramic crowns more natural and preferred these restorations. With the bridges, more people thought the metal ceramic bridge more natural and this was also their preference. In comparison 2, for both the crown and bridge comparisons, respondents thought the private work was more natural but the NHS work was their preference. In comparison 3, for both the crown and bridge comparisons, respondents thought the restorations with normal margins were of a higher technical standard and these were preferred.

Shade and colour of the restorations were the most important factor in the patients' assessment.

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BJ GIBSON*, R FREEMAN AND R EKINS¹ (Queen's University of Belfast, University of Ulster at Coleraine²): Housewife: clinical observations on the dentist-dental nurse interaction.

The aim of this study was to examine the interaction between dentists and their dental nurses using an ethnographic research technique. Clinical interactions of 10 dentists with their dental nurses were observed over a period of time. The subsequent observations were noted and in the vein of 'grounded theory' coded to develop a theoretical understanding centred around the dentist/dental nurse interaction. The results suggested that the work of the dental nurses could be described as one of housewifeing, since they were involved in a process of 'looking after' everyone in the dental surgery. The gender of the dentist affected the degree of housewifeing. Female dentists were less likely to include the dental nurse in any type of interaction which was comparable to housewifeing, they were more communicative and more procedurally active compared with their male counterparts.

Such findings as these require that the dental profession as a whole examine the role of the dental nurse as an active member of the dental team.

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R. FREEMAN*, E.K. ADAMS and BJ GIBSON (School of Clinical Dentistry, Queen's University of Belfast, UK): Provision of general dental services for special needs clients: facilities, treatments and decisions.

Locally sensitive purchasing, for special need clients, requires purchasers (DHA/FHSA) to engage in local consultation on service availability with providers (Trusts) so that dental services reflect patient need ensuring high quality of care and accessibility. The aim of this study is to assess the provision of general dental services for special need clients in the boroughs of Lambeth, Southwark and Lewisham. 126 general practices were invited to take part. The principal partner was contacted to complete a questionnaire. 107 practices responded giving a response rate of 85%. The majority of practices who were willing to accept new and to treat special needs patients were group practices with 96% of them accepting special needs patients who were exempt from NHS fees. The number of patients seen varied with the category of special need. 67 of the practices provided domiciliary care facilities with 92% visiting physically disabled clients but only 34% visiting the mentally ill. The majority of practices provided a range of treatment from extractions to crown and bridge but few provided any form of sedation or general anaesthesia. Over 50% of the practices were on the ground floor and had wheelchair access to waiting areas but less had access to lavatories (41%) and few had ramps (9%). Practices with high accessibility treated greater numbers of groups of special need patients.

The findings of this study suggest that the general dental service provides dental care for the majority of special need clients. The decision to treat a special needs client is based upon the type of practice, the category of special need, financial considerations and accessibility of the practice.

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L COOKE*, E S DAVENPORT and P ANDERSON (Dept of Child Dental Health, The London Hospital Medical College, E1 2AD, UK): Capitation: Effect on child referral patterns from General to Community Dental Services.

The aim of this study was to investigate the effect of Capitation on the criteria for, rate of, and pattern of, referral of child patients from the General Dental Services (GDS) to the Community Dental Services (CDS). A questionnaire to establish GDP referral patterns was distributed to 179 GDPs in two South West Thames Region districts. In addition, dental records of referred children, 403 before and 643 after the introduction of Capitation, were scrutinised for demographic details, reason for referral and treatment received. The response rate from the GDPs was 87% of whom 82% had referred child patients to the CDS. 65% indicated a preference for treatment of children under a fee per item system, 20% favoured Capitation. The majority believed that their treatment patterns had not changed following Capitation, whereas the others believed that recalls, timing of bitewing radiographs, placement of fissure sealants and referrals to the CDS had altered. The most important reasons for referral were for non co-operation or General Anaesthesia. Analysis of the referred children's dental records revealed that there had been an increase in the rate of referral from GDPs to CDS following Capitation. Also, an increased number of co-operative children had been referred and more routine restorative procedures ($p=0.01$), inhalation sedation ($p=0.003$) and deciduous extractions ($p=0.01$) were carried out post Capitation.

In conclusion: following Capitation there has been an increase in the rate of and change in the pattern of referral from GDS to CDS.

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B. BREISTEIN*, A. MCQUEEN, M. STEWART¹, R. FREEMAN² (North and West Belfast Health and Social Services Trust, Queen's University of Belfast¹): Fit for the future? The dental status of school entrants in North and West Belfast.

The study reported here is part of a larger study to obtain a health profile of primary school entrants, using key health indicators. A 9% random sample of children (240) in their 6th year was obtained. The mothers were asked for their consent and the response rate was 65%. Dental caries status and previous treatment experience was assessed using the WHO Pathfinder Oral Health and Treatment Assessment Methodology (WHO, Geneva: WHO: 1977). Each 10th child was re-examined to ensure intra-examiner agreement. The mothers completed a questionnaire which assessed the child's snacking behaviours, the mother's and child's dental attendance patterns, dental anxiety, toothbrushing and the child's use of fluoride supplements. Demographic information was obtained at this time. 64% of the children had experience of dental caries. The average dmft was 3.8 with the number of teeth affected ranging from 1 to 18. The mean number of decayed teeth was 2.36, missing teeth was 1.04 and filled 0.41. Surprisingly those children whose parents were employed had a significantly higher dmft compared with others. However larger proportions of children whose parents were unemployed had missing teeth and attended the dentist only when in pain. Maternal attendance pattern, dental anxiety status and mothers' attitudes towards toothbrushing, snacking and fluoride supplement use influenced the children's dental health behaviours.

These findings demonstrate the high prevalence of dental caries in North and West Belfast and suggest that employment in such high prevalence areas has unexpected influences upon caries experience. The importance of maternal attitudes has been shown and may be used to promote dental health in children.

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G T R LEE* and G M HUMPHRIS (Departments of Clinical Dental Sciences and Clinical Psychology, The University of Liverpool, UK): Dental information needs of adolescents from an Inner City area of Liverpool.

The aim of this study was to determine the dental information needs of adolescents and investigate the relationship of their desire for information with adolescents' opinion of who decides to make an appointment with the dentist (ie parent or child). 158 adolescents of similar age (14-16) attending a secondary school in Liverpool were invited to take part. A questionnaire was administered including questions about information needs and past experience. The results showed that adolescents are interested in finding out more information on different aspects of dental health which included especially: how to keep their teeth for life, about the best toothpaste, what to do in case they sustain dental injury and whether they required an orthodontic appliance. Another important finding was that more than half of the adolescents (57%) felt that they were responsible for taking decisions for their dental attendance.

These children expressed a desire to know more about certain dental matters. Information needs of young people are important because of evidence derived from this study suggesting that acquisition of dental information in this group, has possible implications for future patterns of seeking dental care.

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R BEDI* (1), MS GILTHORPE (2) (1. School of Dentistry, 2. Dept. of Public Health and Epidemiology, University of Birmingham, UK): The potential use of "ethnic mass media" in health education.

"Ethnic mass media" are forms of mass media where the primary audience are individuals from a minority ethnic background. The objectives of this study were to address, for the Bangladeshi community, firstly, how widely "ethnic mass-media" is utilised, and what are their preferences for the type of media that is used? Secondly, to record the personal and behavioural characteristics of individuals who utilise these media.

699 Bangladeshi households were identified, from the electoral register, by their surnames in the Ward that had the highest concentration of this population in the City of Birmingham. A systematic sampling procedure was used to select 127 households which formed the sample base for this study. All adults resident in the household were recruited into the study ($n=334$, response rate 71%) and interviewed by two bilingual interpreters.

"Ethnic mass media" was widely used and popular. Video programmes achieved the highest level of popularity, irrespective of age, gender or the ability to speak English. Only 9 adults had access to satellite television and therefore video cassettes were obtained from local retail outlets. Newspaper popularity declined with age and especially among females, who also had the poorest use of English. The study highlights the potential use for health promoters of "ethnic mass-media", and particularly video programmes in the communities' own languages, for this population. (Funded from a grant provided by the Department of Health).

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BEDI R (1), GILTHORPE MS (1), THOMAS DR* (2), JONES PA (3) (Univ. of Birmingham, 2 South Birmingham HA, 3 North Birmingham HA, UK): Barriers to Dental Care for Adults from a Minority Ethnic Background.

This investigation aims at providing information on the self-reported barriers to dental care by the five major minority ethnic groups resident in the City of Birmingham. Using a quota sample the communities; Black, Indian, Pakistani, Bangladeshi and Chinese were included in the study. 976 face to face interviews were completed (approx. 200 in each group). Street interviews were completed by a market research company in August 1994, using a pre-tested interviewing schedule. The quota sample was based upon: residency, ethnic group, age, and gender.

The availability of interpreters and/or translated information in dental practices was considered very important for all groups, with the exception of the Black community. Whereas the Chinese community thought that cultural and/or religious understanding from their dentist was of little importance, the Indian (94%) and Bangladeshi (79%) communities believed this to be very important. All the ethnic groups, with the exception of the Bangladeshi community, showed a high level of dental attendance ranging from 65% to 84% within the last two years. When these communities were examined separately using regression analysis, the ability of the individual to speak English and their gender were common explanatory variables for poor attendance. Gender variations were only apparent among the Pakistani and Bangladeshi communities. In conclusion this study highlights the diversity of responses showing the inappropriateness of using "ethnic minority" as a single entity. The ethnic variations observed are more strongly expressed by the Pakistani and Bangladeshi communities, in that gender variations are more important for these groups. (Funded by Birmingham Family Health Service Authority).

384 R P ELLWOOD* and D M O'MULLANE (Dept. Oral Health and Development, Manchester University, UK and *Oral Health Services Research Unit, Cork, Ireland): An Investigation of Tooth Brushing Behaviour and Dental Fluorosis in Premolars.

The aim of this study was to examine the relationship between tooth brushing behaviour and dental fluorosis in premolar teeth. The three year study was undertaken in North Wales (<0.1 ppm F) on 416 adolescents aged 15 years at the final examination. Dental fluorosis was recorded using the TF index from photographs. Subject scores were considered for both the 1st and 2nd premolars and based on the highest TF score recorded. At the baseline examination subjects provided information on their weekly brushing frequency, their rinsing method (with or without a beaker) and the amount of paste they used. Each subject was supplied with toothpaste and randomly allocated to formulations containing either 1000 or 1500 ppm F (NaF in silica base). Using a multivariate analysis (logistic regression) the relationship between the four tooth brushing variables and the presence or absence of dental fluorosis (dependent variable) was considered. For the 1st and 2nd premolars 5.6% and 12.4% of subjects had both teeth missing on the photographs. For subjects with one or more teeth present, for the 1st premolar ($n=402$) 33%, 4% and 1% of subjects had TF scores of 1, 2 or 3 respectively. For the 2nd premolar ($n=373$) the corresponding percentages were 33%, 3% and 1%. 102 subjects had 1st premolars erupting during the course of the study compared to 268 subjects with 2nd premolars. There was no association ($p>0.08$) between tooth brushing behaviour and dental fluorosis for teeth erupting during the study. *It may be concluded that the prevalence of dental fluorosis for premolar teeth was low. No relationship between use of fluoride toothpaste late in the developmental period of teeth and dental fluorosis could be demonstrated.*

386 P. PHILLIPS*, R. FREEMAN* (North Down and Ards Health and Care Trust, Queen's University of Belfast, UK): Measuring child dental anxiety: the validity of clinical observations.

For children there are few validated psychometric measures of dental anxiety. Those that exist may induce fear and so lead to inaccuracy with regard to the child's dental fear status. The study reported here aims to examine the validity of using clinical observations as a measure of child fear. 100 children consecutively attending a community clinic were asked to complete the validated Dental Fear Survey Schedule for Children. After the dental examination both dental nurse and dentist separately assessed the subject's dental fear using Scherer and Nakamura's checklist (Scherer MW, Nakamura CY, Behavioural Research and Therapy 6, 173-182, 1968). Factor analysis of the data from child, dentist and dental nurse demonstrated that for each separate group of data two groups of variables explained dental fear. These were a past treatment and immediate treatment factor. Furthermore using Cohen's Weighted Kappa high agreement was found between the child, the dentist and dental nurse respectively.

The findings of this study suggest that clinical observations are a valid means of assessing child dental anxiety.

388 C J RIVKIN* and C D STEPHENS (Division of Child Dental Health, University of Bristol Dental School, UK): Incisor edge-centroid relationship and incisor stability in treated Class II division I malocclusions.

A retrospective study was carried out to investigate the association between the post-treatment incisor edge-centroid relationship (Houston WJB, Eur J Orthod 11: 139-143, 1989) and overjet stability, and upper incisor to maxillary plane angle stability, respectively, following orthodontic treatment of Class II division I malocclusions. The sample consisted of 36 Class II division I malocclusion cases with study models and lateral cephalometric radiographs taken at pre-treatment, post-treatment and post-retention stages. The mean time out of retention was 10.77 months (SD 3.67). Overjet stability was measured as a linear change in overjet following treatment, from study models. The upper incisor to maxillary plane angle stability was measured as an angulation change from lateral cephalometric radiographs. The incisor edge-centroid relationship was determined cephalometrically. The random error for the incisor edge-centroid was 0.08mm. The method errors for the other variables were comparable with published studies. The post-treatment incisor edge-centroid relationship showed a significant correlation with overjet stability ($r=0.51$, $P=0.001$), and upper incisor to maxillary plane angle stability ($r=0.50$, $P=0.002$). Further statistical analysis using multiple regression showed that, for the study sample, the post-treatment incisor edge-centroid relationship was a true predictor for overjet stability ($P=0.015$).

It was concluded that a more stable overjet was achieved when the lower incisor edge was positioned in front of the upper incisor root centroid at the end of active orthodontic treatment.

390 A GUNASEKERA*, P ANDERSON and D DIBIASE (The Dept of Child Dental Health, The London Hospital Medical College, E1 2AD, UK): The perceived and normative need for orthodontic treatment of Bangladeshi children.

The aim of this study was to assess the suitability of the Index of Treatment Need (IOTN) for the perception and normative needs for orthodontic treatment for a group of Bangladeshi children ($n=38$) compared to a sex matched white Caucasian group ($n=28$) at age 15 years. A multiple choice questionnaire was designed to evaluate the acceptability and expectations of orthodontic treatment in relation to its importance, its acceptability and their awareness. The two components of IOTN (Dental Health Component, DHC, and Aesthetic Component, AC) were chosen to classify the level of treatment need. The results showed that the majority of the both groups rated having straight teeth as important, although a greater percentage of the Bangladeshi children did not. The Bangladeshis were less aware of orthodontic treatment regimes, and less prepared to consider wearing fixed appliances and extra-oral appliances. 43 % of the Bangladeshi children perceived a need for treatment, this being greater in females than males. However, using the AC of IOTN, 94 % of the Bangladeshi group rated themselves in the 'little or no need' category, whereas the DHC showed a better correlation to their perceived needs. It should be recognised that these apparent differences in the perception of orthodontic treatment need in the Bangladeshi will most likely change as more children from this ethnic group undergo orthodontic treatment.

In conclusion, the DHC of the IOTN is of obvious value in estimating treatment needs of ethnic groups, but the AC appears to be of limited value.

385 LMD MACPHERSON and DAM GEDDES* (University of Glasgow Dental School, UK): Comparison of patient non-attenders and attenders at a hospital periodontal department.

Failure of a patient to keep a hospital appointment interferes with the clinical management of the patient and results in an inefficient use of resources. It is therefore important that efforts be made to identify the reasons for non-attendance and to reduce their incidence.

A case-control survey by questionnaire was conducted among patient non-attenders and attenders in the Periodontal Department of Glasgow Dental Hospital. The purpose was to compare socio-demographic characteristics of the two groups of patients and to determine whether the incidence of administrative errors, identified in a survey conducted three years earlier, had been reduced.

Non-attenders and attenders were found to differ with regard to a number of socio-demographic characteristics. These included age and indicators of socio-economic status. The level of satisfaction with treatment in the clinic was high in both groups and there was no significant difference in the patients' perception of the seriousness of their periodontal condition or in their levels of anxiety in relation to dental treatment. Whilst errors in Master Patient Index entries had reduced from 92% of patients in 1991 to 17% in the present survey, administrative errors were still found to account for a large proportion (30%) of the recorded 'failed to attend'. The same percentage of patients indicated that the reason for their non-attendance was forgetfulness.

The results suggest that a large proportion of apparent broken appointments may be due to administrative errors. This has important implications with regard to the adoption of methods for reducing non-attendance and in relation to decision-making by clinicians regarding action taken following non-attendance.

387 J.P. McCANN* and D.J. BURDEN (School of Clinical Dentistry, Queen's University of Belfast, UK): Tooth size in the aetiology of bimaxillary dental proclination in Northern Irish people.

Bimaxillary dental proclination is a particular occlusal pattern wherein the upper and lower incisors are proclined and the molar relationship is usually normal. This malocclusion is predominant among black people but is also found among whites. The larger teeth found in black people are considered to play an important role in the aetiology of this malocclusion. In a previous pilot study (BSDR 1994 Abstract 427) it was demonstrated that tooth size may also play a role in the aetiology of bimaxillary proclination in whites. The present study further investigated this relationship. Record casts were obtained of 30 children who were diagnosed as having bimaxillary dental proclination and a control group of 30 patients randomly selected from the orthodontic department's records. All subjects included in the study had a full complement of permanent teeth and no restorations. The diagnosis of bimaxillary proclination was confirmed using cephalometric criteria (Keating, P.J. Brit. J. Orthod. 12, 193-201, 1985). The mesiodistal width of all teeth excluding the second and third molars were measured using a travelling microscope. The results of the study support the findings of the previous pilot study that tooth size may play a role in the aetiology of bimaxillary proclination in white people. For every tooth the mean mesiodistal distance was greater in subjects with bimaxillary dental proclination. For both males and females the sum of tooth sizes were 5.7% greater for the bimaxillary proclination group than for the control group ($p<0.001$).

It is concluded that tooth size may be a factor in the aetiology of bimaxillary dental proclination in whites and that this may have implications for clinical management of this malocclusion.

389 P RAO*, RT REED and F MCDONALD (Department of Orthodontics, Basingstoke and UMDS, London): An analysis of orthodontic treatment using the straight wire appliance.

The aim of this investigation was to compare the treatment results of four permanent first molar extraction cases, four first premolar extraction cases and non extraction cases treated with the straight wire appliance. A sample of 56 cases consisting of 16 first molar cases, 20 first premolar and 20 non extraction cases was examined. Numerical and percentage changes in PAR Scores were measured. Pre treatment and post treatment lateral cephalograms were traced and compared. Six angular and three linear measurements were taken. Using the Reflex metrograph, pre treatment and post treatment dental study models were examined to compare the arch widths. Of the angular measurements, the angle between upper incisors to the maxillary plane was reduced in the premolar group ($7.57 \pm 9.71^\circ$) compared to the first molar group ($3.78 \pm 12.56^\circ$). Lower incisors proclined in relation to the mandibular plane significantly in the non extraction group ($5.16 \pm 6.08^\circ$). Of the linear variables, the lower incisors moved forwards in relation to the A-Pog line in the molar group (1.18 ± 3.32 mm) and in the non extraction group (1.72 ± 1.73 mm). In the premolar extraction group the lower incisors moved backwards (0.4 ± 2.13 mm). The analysis of study model data demonstrated changes in the upper and lower arch widths in the three treated groups. The midline perpendicular distance was reduced more in the premolar group (5.15 ± 3.15 mm) for the upper arch. In the lower arch the reduction in the premolar group (4.44 ± 1.55 mm) and for the first molar group (3.84 ± 3.18 mm) was observed. *Varying extraction patterns, when used in conjunction with the SWA appliance, influences the final form of the dental arch.*

391 DH BOYD* and TA GREGG (The Queen's University of Belfast, Royal Belfast Hospital for Sick Children): A Prospective Study to Evaluate Treatment of Avulsed Teeth

This prospective study aims to evaluate a treatment policy for accidentally avulsed and subsequently replanted human teeth, and also to determine which factors discernable at presentation are the best indicators of prognosis. A data entry form is used to record information which is transferred to a computer database for analysis. Preliminary examination of the data has been carried out for the period 1990 - 1994. There were 41 teeth avulsed in 35 patients. The commonest cause of tooth avulsion was a cycling accident (45%), most teeth were avulsed after 4 p.m. (58%) and most teeth were avulsed in the summer (61%). An upper central incisor was the tooth most commonly avulsed (83%). Outcome was measured in terms of occurrence of root resorption and related to factors that have been suggested to exert an influence on prognosis. Results: 1) teeth with open apices suffered less resorption than those with closed apices ($p<0.05$), 2) more than five minutes of dry storage was critical regardless of subsequent medium into which the tooth was placed ($p<0.05$), 3) occurrence of root resorption increased when time out of the socket was in excess of one hour ($p<0.01$), 4) prescription of a systemic antibiotic at replantation reduced the occurrence of root resorption, 5) aetiology was not related to splinting time, 6) pulp extirpation within 21 days did not reduce the occurrence of root resorption in teeth with open apices, but did reduce this in teeth with closed apices. Conclusion: A) Results 1), 3), and 4) are not new findings and confirm reports found in the current literature. B) Results 2), 5), and 6) are new findings not previously reported in the literature. Result 2) shows statistical significance however further data collection is warranted to reveal whether the other trends found will also demonstrate statistical significance.

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PJM CRAWFORD* and MJ ALDRED. (Division of Child Dental Health, University of Bristol, UK, and The University of Queensland, Brisbane, Australia): A genomic classification for *amelogenesis imperfecta*.

Clinical and laboratory evidence has shown phenotypic variability in both the autosomal and X-linked forms of AI and locus heterogeneity in the X-linked form(s) (XAI) of AI. A classification based primarily upon phenotypic criteria, at whatever level, must therefore be unsatisfactory for the future. We propose an alternative classification based on molecular genetic findings together with the resulting biochemical changes and the mode of inheritance as the primary qualifiers. Phenotypic information can then be included, whilst accepting that variable expression will result in some ambiguity of description between family members. Applying the proposed classification to the AIH1 variant of XAI results in:

Genetic locus: AIH1, Xp22.2 - p22.3
Mutation: 1bp deletion, nonsense mutation
Biochemical outcome: premature termination of translation
Mode of inheritance: X-linked
Phenotype: Amelogenesis imperfecta, hypoplasia and / or hypomineralisation, vertical banding in females.

(Aldred et al., *Hum Genet* 1992; 90: 413-6.)

It is important that such a scheme, or a development of it, is agreed as the basis for the future definition of cases. This would eliminate the unhelpful and repetitive refinement of existing systems. It should be possible to extend this hierarchical concept to other inherited disorders.

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X IOANNIDES*, PJM CRAWFORD, K DUNCAN, RW VOWLES. (Division of Child Dental Health, University of Bristol, UK: Age changes in lingual frenae in British teenage children.

Children may be referred for the surgical removal of a band of tissue below the tongue binding it down to the floor of the mouth known as tongue-tie (ankyloglossia). This usually requires a general anaesthetic for it to be carried out in young children.

The present study was intended to investigate the changes in the lingual frenum with age in order to minimise the need for surgical intervention. 857 young people between the ages of 11 and 18 years were examined with consent. Using disposable dividers, measurement was made between the points O (mandibular alveolar insertion), A (sublingual papillae), B (ventral frenal insertion), and C (tongue tip - dorsal/ventral border). A clinical assessment of restricted tongue mobility (ankyloglossia) was also made.

The length of the tongue was seen to increase with age ($p = 0.01366$) in all three measurements. Tongue-tie was recorded in 21 males (4.6%) and 8 females (2.0%). There was no significant difference ($p = 0.3309$) between the age of children with ($N = 29$, Mean 13.31, SD 1.13) or without ($N = 828$, Mean 13.59, SD 1.49) ankyloglossia.

These preliminary results suggest that there may be no spontaneous reduction in ankyloglossia in the age group studied.

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DH BOYD* and TA GREGG (The Queen's University of Belfast, Royal Belfast Hospital for Sick Children): A Prospective Study to Evaluate Treatment of Avulsed Teeth

This prospective study aims to evaluate a treatment policy for accidentally avulsed and subsequently replanted human teeth, and also to determine which factors discernable at presentation are the best indicators of prognosis. A data entry form is used to record information which is transferred to a computer database for analysis. Preliminary examination of the data has been carried out for the period 1990 - 1994. There were 41 teeth avulsed in 35 patients. The commonest cause of tooth avulsion was a cycling accident (45%), most teeth were avulsed after 4 p.m. (58%) and most teeth were avulsed in the summer (61%). An upper central incisor was the tooth most commonly avulsed (83%). Outcome was measured in terms of occurrence of root resorption and related to factors that have been suggested to exert an influence on prognosis. Results: 1) teeth with open apices suffered less root resorption than those with closed apices ($p < 0.05$), 2) more than five minutes of dry storage was critical regardless of subsequent medium into which the tooth was placed ($p < 0.05$), 3) occurrence of root resorption increased when time out of the socket was in excess of one hour ($p < 0.01$), 4) prescription of a systemic antibiotic at replantation reduced the occurrence of root resorption, 5) ankylosis was not related to splinting time, 6) pulp extirpation within 21 days did not reduce the occurrence of root resorption in teeth with open apices, but did reduce this in teeth with closed apices. Conclusion: A) Results 1), 3), and 4) are not new findings and confirm reports found in the current literature. B) Results 2), 5), and 6) are new findings not previously reported in the literature. Result 2) shows statistical significance however further data collection is warranted to reveal whether the other trends found will also demonstrate statistical significance.

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A J PAYNE* P HART, M S DUGGAL, J M LITTLEWOOD¹ & N E J CURZON. Leeds Dental Institute, Cystic Fibrosis Unit², St James Hospital, Leeds, Yeast Carriage in Healthy & Cystic Fibrosis Children.

Patients with cystic fibrosis (CF) suffer recurrent respiratory infections usually caused by *P. aeruginosa*, *S. influenzae* and *S. aureus*. Prolonged antibiotic therapy is indicated and these patients are at risk from colonization by opportunistic infections such as yeasts.

The incidence of oral yeasts in a group of 86 children attending the Regional Cystic Fibrosis Unit, St James Hospital, Yorkshire, was studied. A healthy group of children, equated for age, race, sex and social class, derived from local schools, was used as a control group. Unstimulated saliva samples were collected for yeast identification and quantification. Pathological changes of the oral mucosa were noted during a dental examination.

Results showed yeasts were isolated from 66 of the CF children compared with 26 of controls ($p < 0.001$). Mean (\pm SE) saliva candidal counts in the CF group was 2344 (± 246) cfu/ml compared with 236 (± 86) in the control group. The commonest yeast identified was *C. albicans*. Oral pathology was noted in 39 CF and 4 control children. Angular cheilitis (12) and atrophic tongue (13) were the commonest lesions noted in the CF group. In the control group the lesions were all simple oral ulceration. There was no statistical correlation between the development of pathology and the occurrence of yeast infection.

It was concluded that the colonization of the mouth by yeasts in children with CF presents a potential clinical problem. Children with CF should be followed and closely monitored by their dental practitioner for early signs of candidiasis.

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P AGARWAL*, R BARNFATHER, PJM CRAWFORD, T WRIGHT* (University of Bristol UK and *University of North Carolina, USA) Dental 'First Aid' Knowledge of Sports Trainers in North Carolina, U.S.A.

Previous work with school sports staff (Newman L, Crawford, PJM, Endod Dent Traumatol 1991; 7: 225-8) highlighted lack of knowledge of the management of dental sports injuries. An education programme has previously been established amongst sports coaches and trainers in North Carolina to cover these issues. The present study was intended to assess the dental "first aid" knowledge of this latter group.

A postal questionnaire was sent to sport coaches and trainers in schools and colleges in three areas of North Carolina (NC). The questionnaire was designed to obtain general information on their first aid training, experience of dental injuries, and knowledge of the NC programme. 2 case studies were used to assess the way in which they would manage specific types of dental injury.

Of the 70 respondents, 89% had had general first aid training and 37% had further training in dental first aid. 44% had dealt with some form of sports related dental trauma. The two case studies involved a fractured tooth and an avulsed tooth, the correct response rates were 61% and 14% respectively.

Simple dental injuries may be managed using a high degree of common sense. However, more complicated injuries require more precise knowledge. Despite a Statewide education programme, sports coaches continue to have inadequate knowledge for appropriately managing complex dental trauma.

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V E HARRISON*, S GELBIER (Department of Paediatric Dentistry and Department of Dental Public Health and Community Dental Education, KCSMD, London UK): Dentists' attitudes to and practices of reporting child physical abuse.

The aim of the study was to investigate the reporting by dentists of cases of suspected child physical abuse and factors that may deter them from reporting. A postal questionnaire was sent to all the General Dental Practitioners (GDPs) and Community Dental Officers (CDOs) in three areas of London ($N = 814$). The areas were selected because they had high rates of registration of children on the Child Protection Registers. The questionnaire was based on previous studies conducted in America (Becker D B et al. *JADA* 97: 24-28, 1978, Malecz R E. *J Dent Child* 46: 193-194, 1979, and Saxe M R and McCourt J W. *J Dent Child* 58: 361-366, 1991) and included questions on suspicion, guidelines, reporting and possible deterrents to reporting. 44.3% of the dentists replied to the questionnaire, and of those who responded, 32.3% had seen a child patient where they were suspicious that abuse may have occurred. However, only 7.3% had ever reported a case. Significantly more CDOs had both seen cases where suspicion was aroused, ($p < 0.01$) and had reported cases ($p < 0.001$) than had GDPs. The most common factors identified that may deter a dentist from reporting were related to a lack of knowledge of child physical abuse, both in the ability to diagnose a case of abuse and in the management of such a case.

It was concluded that dentists see cases of suspected child abuse, but that few are reported. Further training of all dentists, but particularly GDPs, is necessary. As an interim measure there should be local guidelines devised for the use of GDPs.

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MT ROSEY*, L SHAW, G GORDON, KELLY DA (Dep't. of Paediatric Dentistry, University of Birmingham and The Liver Unit, Childrens Hospital, Birmingham, UK): Does Cytomegalovirus influence the effect of Cyclosporin A on the gingival tissues?

The effect of Cyclosporin A on the gingivae is well known but research into the nature of the association has had conflicting results. It has been suggested that co-factors may be involved, one of these is cytomegalovirus (CMV). The aim of this study was to determine if cytomegalovirus (CMV) influenced the effect of Cyclosporin A on the gingivae in children who have received liver transplants. Sixty children taking Cyclosporin A post liver transplantation were examined. An Index of Severity of Gingival Hyperplasia was used and the circulating Cyclosporin A level and the CMV status of the patient recorded. The association between the presence and the level of gingival hyperplasia and CMV infection was examined by the chi-square test and the contingency co-efficient respectively. ANOVA was used to examine the association between the circulating Cyclosporin A and the level of gingival hyperplasia. There were 29 males and 31 females, mean age 3yrs 9months (range from 9 months to 12 yrs), mean time post transplant was 1 year 5 months (range 1 month to 4yrs 7months). The mean circulating Cyclosporin A was 162.51 ng/ml (range 0 to 413ng/ml). No significant association was found between CMV infection and neither the presence nor the level of severity of gingival hyperplasia [Chi-square .79 $p = .37$; contingency co-efficient .16]. There was no significant association between the level of circulating Cyclosporin A and neither the presence nor severity of gingival hyperplasia. The results of this study have shown no association between CMV and Cyclosporin A medication on the development of gingival hyperplasia.

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N C JONES¹*, J V SUTCLIFFE¹, R P SHELLIS¹, A C SMILLIE² and S JOHNSTON² (1: Bristol Dental School, UK; 2: Otago Dental School, Dunedin, New Zealand): Preliminary study of enamel cracks associated with restorations.

During cavity preparation and restoration, cracks may be induced by mechanical stress in the adjacent enamel. The aim of this study was to determine whether such cracks can be visualised by a resin-infiltration technique. Mesial-occlusal-distal cavities were cut in 12 surgically extracted unerupted human third molars with diamond burs. 4 cavities were restored with amalgam¹, 4 with composite resin² and 4 left unfilled. All teeth were extracted with methanol/chloroform and infiltrated with methyl methacrylate (Martin D M et al., *J Microsc* 112: 345-352, 1977). Resin overlying the crowns was ground off, the specimens were etched, washed, freeze-dried and examined by scanning electron microscopy. Despite artefacts attributable to such factors as lack of post-eruptive maturation or the extraction procedure were observed, replicas of 3 types of void specifically associated with restorations were distinguished: (1) a marginal space between amalgam and enamel; (2) cracks radiating from the restoration; (3) cracks close to the restoration and parallel to the cavity margin. All cracks followed prism junctions.

We conclude that cracks form in enamel during cavity preparation/restoration and can be visualised by replication. These cracks may influence long-term retention of restorations and initiation of recurrent caries.

¹Dispersalloy, Tytan. ²Prisma TPH, Dentsply.

400 D RUSSELL*, P A BIAGIONI AND P-J LAMBY (School of Clinical Dentistry, The Queen's University of Belfast, UK): Heat generation during pin channel preparation.

When, as a result of caries or trauma, extensive tooth destruction has occurred, auxiliary retention of plastic restorative materials is often considered. This usually takes the form of self-threading dentine pins which have to be carefully placed to avoid damage to the dental pulp on the one hand and the periodontal membrane on the other. One possible hazard to the pulp is the heat generated during the preparation of the pin channel.

The object of this pilot study was to quantify the heat generated during preparation of pin channels using 0.676 mm twist drill (Wholesome regular size link pins) in a KaVo Intra 68°C head on a KaVo 20A shank run at 4000 rpm by a KaVo Intrasept 905 motor with digital display of the speed. Twenty decoronated extracted premolar teeth were held in a bench vice, 0.05 metres from the lens of a thermal imaging camera during preparation of pin channels approximately 1 mm from the amelo dentin junction on the part of the tooth closest to the imager.

Sequential thermal images were recorded at a frame rate of 3 per second for each stage in the channel preparation technique, ie placement drill, channel drill and pin placement. The mean maximum change in temperatures for each stage were round but 35.8°C, channel drill 76.6°C and pin placement 41.6°C.

These results suggest that a significant temperature rise may occur within the pulpal chamber during pin channel preparation.

* Agema Thermovision 900 system (Danderyd, Sweden)

401 H L DOBSON*, C J WHITTERS, J MCGADEY, R H FOYE, A PAYNE, S L CREANOR, R STRANG (University of Glasgow, UK): The effect of a Nd:YAG laser on artificial white spot enamel lesions.

The aim of this study was to assess, by means of SEM and microradiography, the effects of an Nd:YAG laser on artificially created white spot lesions in enamel. Ten lower molars extracted for orthodontic purposes had two horizontal artificial white spot lesions created on the buccal surfaces, using an acidified undersaturated demineralisation solution. Next, impressions of the teeth were taken using Xantopren & Optosil impression material and resin casts created. The 320 µm optical fibre used to deliver the Nd:YAG laser beam (1.06 µm wavelength, 150 µs pulse; American Dental Laser Inc.) was held, using a mechanical jig, so that the fibre tip was in contact with the tooth surface. Teeth were laser on two spots per lesion and once on normal enamel. The laser parameters used were 50 or 100 mJ (10 pps) for 2 or 5 sec. A second impression and resin cast was then made for each tooth. The pre- and post-laser casts were examined on a Jeol T300 SEM at magnifications up to x2000. Teeth were subsequently sectioned and microradiography carried out on both the laser and unlasered areas. SEM examination of the specimens showed abundant evidence of melting of the enamel in the area of the lesion. The microradiographic analysis indicated that there was a correlation between the power of the laser and the depth of the laser area ($p < 0.05$), irrespective of the degree of demineralisation or lesion depth. With regard to normal enamel, the parameters employed had only negligible effects. In conclusion, the damage to the white spot lesions was correlated with the power settings of the Nd:YAG laser.

This study was supported by a grant from the SOHHD - K/CSO/56/3/3 and the MRC.

402 G ANSARI*, J A BEELEY*, J S REID* AND D A WEETMAN* (Depts of Child Dental Care and Oral Sciences, University of Glasgow Dental School): Chemomechanical caries removal - alternative application systems.

Chemomechanical removal of dental caries with N-mono-chloroamino butyric acid (NMCAB) and N-mono-chloroglycine (NMG) is an alternative to conventional drilling procedures. Because it selectively removes carious dentine leaving sound tooth intact, the need for local anaesthesia is minimised (Ansari *et al* *J Dent Res* 73: 795, 1994). The procedure is better suited to deciduous teeth than permanent ones (Yip H K *et al* *J Dent* in press, 1995) and the dentine remaining is bound and properly mineralised (Yip H K *et al* *Caries Res* in press, 1994). However, the applicator system normally used (Caridex Unit) is no longer commercially available and the procedure is time consuming in clinical use and requires large volumes of solution. The suitability of two other widely available instruments for this technique was therefore studied.

Carious dentine was removed chemomechanically *in vitro* from three groups of 10 freshly extracted carious deciduous teeth using a Caridex Unit*, a Kitty Water Jet† fitted with a Caridex applicator tip or a Piezon ultrasonic‡ Master 400*.

The mean time taken and volume of solution used for complete caries removal were Caridex Unit = 6.1 (± 2.2) mins, 97.5 (± 29.8) ml; Kitty Water Jet 4.6 (± 2.3) mins, 102.5 (± 26.2) ml and Piezon ultrasonic 3.1 (± 1.6) mins, 15.0 (± 5.9) ml.

The Kitty Water Jet is similar in efficiency to the Caridex Unit but the Piezon system is significantly more effective in chemomechanical caries removal ($P < 0.005$; ANOVA) in terms of time taken and volume of solution used.

* National Patient Dental Products (USA); † Mirage Dental Supplies (UK); ‡ FMS SA (Switzerland).

403 D SAMARAWICKRAMA*, M C GROOTVELD*, A SHEERIN* AND E LYNCH* (Cons. Dentistry & Inflammation Res. Group, LHM, UK): Multicomponent evaluations of the oxidising actions of a tooth-whitening dentifrice.

The nature, rate and extent of salivary reductant consumption (e.g. that of pyruvate, urate, thiocyanate, etc.) by oxidants present in tooth-whitening preparations reflect their oxidising capacity, a parameter of much relevance to their therapeutic and aesthetic actions. Therefore, high resolution proton (¹H) NMR analysis was used to investigate chemical modifications arising from equilibration of human saliva with a newly-developed dentifrice* containing carbamide peroxide [CP, 0.30% (W/W)] and peroxodisulphate [S₂O₈²⁻, 0.50% (W/W)]. Unstimulated human saliva samples obtained from volunteers (n=10) were centrifuged, the supernatant removed, and an aqueous supernatant prepared from the dentifrice product added. The mixtures were incubated for 6 hours prior to ¹H NMR analysis. The results obtained demonstrated (1) complete consumption of salivary pyruvate (from a mean value of 1.30×10^{-4} mol/dm³ in the untreated samples) by dentifrice-derived oxidants (e.g. H₂O₂), an oxidative decarboxylation reaction liberating acetate and CO₂, and (2) binding of dentifrice components (e.g. trisoxan and methyl paraben) by salivary macromolecules.

In conclusion, high resolution ¹H NMR analysis of human saliva provides much useful information regarding the molecular mechanisms associated with the therapeutic actions of active agents present in a commercially-available tooth whitening formulation.

* Ultrawhite Opal, Jarima International, UK.

404 P. ASHLEY*, R.M. DAVIES AND A.S. BLINKHORN (Dental Health Unit, University of Manchester, UK): The repeatability of Electronic Caries Monitor readings *in vivo* and *in vitro*.

The Electronic Caries Monitor (ECM) can be used to detect the presence or absence of fissure caries over the whole fissure system by first covering the surface with a conducting medium. The present study is the first in a series aimed at validating this method. 25 premolars and one permanent molar were tested *in vivo* and then retested *in vitro*. The two groups of readings were compared giving a correlation coefficient of $r = 0.75$. The *in vitro* readings were then repeated giving an *in vitro* correlation coefficient of $r = 0.90$. In a separate study 100 teeth were tested *in vivo* over a 12 month period at 4 monthly intervals. At each 3 month interval a random sample of 30 teeth were retested. The intra-examiner correlation coefficient at each retest ranged from 0.62 to 0.74. The greatest variation in readings occurred with higher ECM readings denoting sound enamel, lower readings which are thought to represent demineralisation were very repeatable.

*In conclusion, the ECM can be used to obtain repeatable readings from teeth both *in vivo* and *in vitro*. There is a strong association between the *in vivo* and *in vitro* readings, hence data from *in vitro* histological validation could be applied to a clinical situation.*

405 S E P DOWKER*, P ANDERSON AND J C ELLIOTT (Lond Hosp Med Coll, Turner St, London E1 2AD, UK): Simultaneous Sr and mineral quantification by X-ray absorption in carious enamel.

The aim was to demonstrate the possibility of using the differential absorption across the absorption edge of Sr to determine simultaneously the projected mass of enamel mineral and of Sr²⁺ diffused into a section of carious enamel. Sections ~300 µm thick, cut from human molars, were immersed in Sr(NO₃)₂ solution for 5 days. Scanning microfluorimetry, with an energy dispersive detector coupled to a 2048 channel multichannel analyser, was used to determine the X-ray attenuation at 15.27 and 16.17 keV over areas of 1 x 1 mm² in 10 µm steps. As Sr has an abrupt increase in absorption at 16.106 keV (the K absorption edge), the difference in absorption across the edge could be used to calculate the projected mass of Sr per unit area of section. The contribution of Sr was then subtracted from the observed absorption to determine the contribution due to enamel mineral alone, and hence the projected mass of enamel mineral per unit area of section. As expected, the Sr level was elevated within the region where the mineral content was reduced.

In sections of carious enamel, simultaneous measurements can be made of the projected mass of tooth mineral and of an element with a suitable absorption edge. This leads to the possibility of determining diffusion coefficients of suitably labelled chemical species for different parts of a lesion with different pore characteristics.

406 L M C CARRUTHERS*, S L CREANOR, W P SAUNDERS, R H FOYE, R STRANG (University of Glasgow): Effect of Extrinsic Fluoride Concentration on the Uptake and Release of Fluoride from Two Glass Ionomer Cements.

The aim of this study was to investigate fluoride uptake and release of two glass ionomers subjected to solutions containing three different fluoride concentrations. The two materials were Vitrebond* and Chemfil Superior*. Ten discs, 6 mm diameter with a thickness of 1.5 mm were made for each material. Test samples were exposed daily to either a 250-ppm, a 1000-ppm, or a 2500-ppm F solution for 2 min. Control samples were exposed to deionised water only. Samples were dabbed dry and then immersed in 2 ml of fresh de-ionized water for 24 hr at 37°C. All solutions were changed daily. Fluoride release was assessed over a 20 day period. Immediately after changing solutions, 1 ml of the immersant was removed and frozen at -20°C until fluoride analysis was carried out using a fluoride ion-selective electrode (Creanor *et al* *Caries Res* 1994;28:322-328). The test samples, exposed to the 1000-ppm F and 2500-ppm F solutions, consistently released more ionic fluoride than the controls and the experimental groups exposed to the 250-ppm F solution, at all time points from day 1 to day 20. The values for the 1000-ppm F samples ranged, at day 1, from 4.10 (±0.16) ppmF for Chemfil Superior and 5.33 (±0.77) for Vitrebond to 12.87 (±1.26) for Chemfil Superior and 10.34 (±1.61) for Vitrebond, at Day 20. After immersion in 2500-ppm F solution, Chemfil Superior specimens released 15.21 (±1.63) at day 1 with the comparable Vitrebond specimens releasing 17.10 (±3.76). After 20 days, Chemfil Superior released 16.43 (±2.98) and Vitrebond 14.8 (±1.58). For the total amount of fluoride released over the 20 day period, analysis of variance showed that there was a significant fluoride dose response ($p < 0.001$) and that there were significant differences between the materials ($p < 0.01$).

Vitrebond - 3M Dental Products Division Chemfil Superior - De Trey Division, Dentsply
This study was supported by a grant from the SOHHD - K/MRS/50/C1815

407 J R RADFORD*, H M BALLANTYNE, C LONGBOTTOM, N B PITTS, M ROBERTSON AND D BEIGHTON (Dept of Dental Health, Univ of Dundee, UK and Kings Coll Sch of Med & Dent, UK): Prevalence of caries-associated micro-organisms in 1 year old infants from Dundee.

Five studies have examined the prevalence of mutans streptococci and lactobacilli in infants. The aim of this study therefore was to determine the prevalence of these caries-associated micro-organisms in the saliva of 1 year old infants. Such information will form the baseline data for a longitudinal study examining the role of the microbiological factors, amongst others, as markers of future caries-risk. Using Health Visitors, a tongue-loop sample was collected from 76 one year old infants resident throughout Dundee. Samples were placed in 1 ml of transport medium dispersed by vortexing and plated on BMSA (for mutans streptococci) and Rogosa agar (for lactobacilli). After incubation, colonies were counted, then characterized using the Gram stain and catalase test. Representatives were stored for confirmatory and later detailed taxonomic identification. The lowest detection level for each of the bacterial groups was 10^3 colony forming units/ml of saliva. Mutans streptococci were recovered from 17% (when detected, range 1.00×10^3 - 8.20×10^4 , median 2.00×10^3) and lactobacilli from 9% (range 1.00×10^3 - 2.40×10^4 , median 3.00×10^3) of the infants. Therefore, even at 1 year of age these infants are harbouring cariogenic micro-organisms. Subsequent monitoring will determine if these markers can be used as predictors of caries activity.

It is concluded that mutans streptococci and lactobacilli were isolated from the saliva of 17% and 9% respectively, of 1 year old infants. The presence of these micro-organisms may indicate that these children are at risk of developing dental caries.

Supported by the Chief Scientist Office, Scottish Office Home and Health Department.

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T H GRENBYS* and M MISTRY (Oral Medicine & Pathology, UMDS Guy's Hospital, London SE1 9RT): Demineralisation of dental enamel by fruit acids and fruit juices in vitro.

Fruit juices are perceived as health-giving, but many of them are acidic, and they can contribute to dental erosion. Very little is known of the effects of the individual fruit acids and various kinds of fruit juices on dental enamel. The acidity, demineralising action and microbial inhibition by two of the commonest fruit acids and six different fruit juices were measured, and the data were analysed by ANOVA, Duncan's multiple-range probability test and Pearson's correlation test.

Malic acid dissolved more calcium from enamel mineral than citric acid did. Ascorbic acid, as used as a vitamin C supplement, dissolved very little calcium and phosphorus. Of the six juices, blackcurrant emerged as potentially the most erosive, with apple juice the least acidic. The two juices most inhibitory to the growth of oral micro-organisms were orange and apple, although these were not the most acidic ones. Titration of the acid the juices contained gave a better guide to their potential erosiveness than their pH values.

409

S L CREANOR*, J F FERGUSON, R H FOYE (Department of Oral Sciences, University of Glasgow): Comparison of the Cariogenic potential of Caloric and Non-caloric carbonated drinks.

Carbonated drinks are known to be detrimental to dental hard tissues. Diet carbonated drinks, where sucrose has been replaced by Nutrasweet, are a means by which the cariogenicity of soft drinks is being addressed. This study assessed the change in both plaque and salivary pH after rinsing with either a regular or diet carbonated drink. The drinks tested were the diet and regular brands of: Coca Cola, Pepsi Cola and Im Bru. The controls were deionised water (pH 5.32) and 5% Nutrasweet solution (pH 5.4). First, 10 samples of each drink were left on the bench for 3 hours, which allowed all the CO₂ to be blown off. The pH of all drinks did not change significantly. The lowest baseline pH was Pepsi-Cola (2.48±0.03); the highest baseline pH was Diet Coca Cola (3.18±0.03). Six subjects, who had grown overnight fasted plaque, rinsed with 10 ml of each drink on each of 4 occasions. For the two controls, the Nutrasweet produced little change in both plaque and salivary pH on any occasion. Rinsing with the deionised water, however, produced a slight decrease in pH during the initial 5 min, but had recovered its initial value by 15 min. For the test drinks, results showed that the sucrose-containing drinks always caused a drop in plaque and salivary pH, whereas the diet equivalent drinks had no net pH effect. This study indicated that diet soft drinks may be considerably less detrimental to the dental hard tissues than their regular equivalent. Frequent consumption, however, may still cause dental erosion due to their high intrinsic acidity.

410

B SPENCER*, N WEST, M ADDY. (Department of Restorative Dentistry, Bristol Dental School, Bristol): The effects of baby fruit drinks on tooth tissue in vitro.

The erosive effect of food and drink on teeth has been documented over many years. Recent evidence shows that the prevalence of erosion is increasing, particularly in children's teeth (Levin et al. *Oral Surgery Oral Medicine Oral Pathology* 1973; 35:741-746). Indeed, Smith and Shaw (*British Dental Journal* 1987; 162:65-7) drew attention to the erosive effects of baby fruit juices, frequently given as comforters in bottles and reservoir feeders. Both enamel and dentine are known to be etched by acids to some degree (Davis and Winter *British Dental Journal* 1980; 148:253-256). The aim of this investigation was to examine the effect of commercially available baby fruit drink products on tooth tissue, particularly those containing citric acid. Enamel and dentine samples were exposed to eight fruit drinks at 35°C, for ten minutes on five occasions. Tissue loss was measured by surfimetry. Results showed statistically significant differences in the degree of erosion by all products tested, on both tissue types. A redberry flavoured drink caused the greatest tissue loss on dentine and enamel of 4.35µm and 3.12µm respectively. In comparison a blackcurrant flavoured drink showed the lowest tissue loss for both tissue types. The dentine surface showed a loss of 2.04µm and the enamel 0.95µm.

In conclusion the erosion potential of baby fruit drink is extremely variable. The erosion of dentine was always greater than that of enamel with these products. This may be related to structural differences in enamel.

411

J HUGHES*, N WEST and M ADDY. (Restorative Dentistry, Dental School, Bristol): The protective effect of pellicle against citric acid erosion on tooth tissue.

It has been postulated by Darling (*Proc Roy Soc Med.* 1943; 36:499), and Mannenberg (*Arch Oral Biol* 1961; 4:59-62), that the pellicle protects the tooth against acid attack. Schulte (*Archives of Oral Biology* 1961; 4:40), added that the pellicle was freely permeable to acid, and further to calcium and phosphate ions. The aim of this *in vitro* investigation was to assess the effect of citric acid on enamel and dentine surfaces covered with a pellicle, which had been allowed to form for various lengths of time (0 to 15 hours). Five human dentine and enamel samples were soaked in saliva for 0, 1, 3, 5 or 15 hours at 35°C. They were then agitated in 0.3% citric acid adjusted to pH 3.2 with sodium hydroxide, (three successive exposures of 10 mins at 35°C), after which surface loss was measured by surfimetry. Results showed pellicle had a significant effect ($P < 0.05$) on reducing dentine and enamel loss through erosion, being more pronounced on the latter. Significant reduction in tissue loss was evident on dentine after 3 hours soaking in saliva, and on enamel after 1 hour soaking in saliva. The longer the pellicle was allowed to form, up to 15 hours, the greater was the protective effect.

In conclusion, the presence of a pellicle on enamel and dentine surfaces provides significant protection against erosive attack by citric acid.

412

D J BURDEN*, J W GARVIN and C C PATTERSON (Orthodontic Division, The Queen's University of Belfast, UK): Pilot study of an orthodontic treatment need learning package for General Dental Practitioners.

This pilot study evaluated an Index of Orthodontic Treatment Need Learning Package which was developed for use by General Dental Practitioners. Fifty-seven dentists participated in a randomised controlled trial which involved assessing the need for orthodontic treatment among two representative samples of 16 study casts. All the dentists assessed the first set of study casts without any aids or assistance. During the assessment of the second set of study casts one group of dentists used an IOTN Learning Package in the form of a poster, another group used an IOTN Learning Package in the form of a booklet, and the remaining group acted as a control and did not use any Learning Package. The results revealed that compared with the control dentists, the dentists using the IOTN poster and the IOTN booklet had, respectively, 1.8 and 2.6 fewer errors when assessing aesthetic need and 2.0 and 2.1 fewer errors when assessing dental health need.

It is concluded that in an experimental situation the IOTN Learning Package improved the GDC's assessment of orthodontic treatment need.

413

E S DAVENPORT, J E C DAVIS*, A M CUSHING, G HOLSGROVE (The London Hospital Medical College, London E1 2AD, UK): An evaluation of a DOSCE in undergraduate assessment.

This study aimed to evaluate the Dental Objective Structured Clinical Examination (DOSCE) which has been held at the London Hospital Medical College, Dental School annually for two years. Self administered questionnaires incorporating the Likert scale were used to determine staff and students attitudes towards the DOSCE. The content and face validity of the examination and reliability using Alpha Cronbach (α) were calculated. Group 1 student cohort taking the exam in 1993 (3rd yr) and 1994 (4th yr) felt that the examination was not easy, and it had been too long in the second year ($p < 0.0001$), although they considered it a good test of clinical skills. It was seen to be relevant to their professional development. The students indicated they were better motivated for the assessment in the second year. Group 2 cohort who took the exam in 1994 (3rd yr) considered the exam to be a better test of clinical skills ($p = 0.01$) and more relevant ($p = 0.02$) than Group 1. The views of the staff were very similar to those of the students and it was clear that the staff were considerably more confident about this form of assessment in its second year. α values for Group 1 and 2 in 1994 were close to the gold standard of 0.8 where $\alpha = 0.67, 0.68$ respectively.

It is concluded that the DOSCE is seen to be not only a good form of assessment by students and staff, but a valid and reliable method of assessment.

414

C E MARSHALL*, S I MORGANSTEIN, W M TAY and E LYNCH (Department of Conservative Dentistry, LHMC, UK): Long Term Use of a Dental Student Computer Network.

The BBC Torch computer system introduced in 1982 to allow clinical students to book dental chairs in which to treat their patients was upgraded to a Novell Network running a DBaseIV system in 1991 and converted to FoxPro in 1994. The change to FoxPro produced an average user time saving of around 30% when compared with DBase IV despite the fact that the key strokes required are virtually identical. The system also allows students to book their instruments from the Central Sterile Stores Department. Instrument loss can then be minimised by weighing the instruments on issue and comparing the weight on return. The fact that instruments are missing can therefore be quickly established and the students asked to find them. By storing records of the appointments made by students for patient treatment it is possible to analyse each student's practice. The frequency of appointments made for individual patients thus leads to more efficient monitoring of patient care.

A database management system of this type has many applications within a dental teaching hospital.

415

S M HOOPER*, R W VOWLES and K J MARSHALL (Department of Oral and Dental Science, University of Bristol, U.K.): Monitoring of student records for audit in restorative dentistry.

This study set out to investigate the longevity of courses of treatment being undertaken by undergraduates in an adult restorative clinic at Bristol Dental School. During a two month period every patient (592) entering the clinic was surveyed; students reported the number of appointments attended during the current course of treatment and estimated the additional number required to complete treatment. Six months later, by which time most treatment should have been completed, a random selection of the surveyed patient's records (212) was examined as part of an internal audit. Each set of notes was examined once by a member of staff from the audit panel. A report was compiled of the number of appointments attended during the course of treatment before and after the initial survey date, and whether or not the treatment was completed. Large discrepancies were found between the audit panel and student data and this led to a further examination of 109 records by two staff members. These staff independently examined all 109 records and compared their data. Where differences were found the records were re-examined jointly and discussed until agreement was reached. Of the 109 dental records examined 46 showed treatment completed; these 46 required a total of 111 visits. The students predicted a total of 83 visits (28% underestimation) whereas the panel reported 143 visits (25% overcount) to complete treatment.

This study highlights the potential unreliability of data produced by individual examination of patient records for audit purposes.

416 P B ROBINSON*, B J MILLAR, J TAYLOR (King's College Dental School, London): Assessment of UCAS forms as a predictor of dental student performance.

The aim of this study was to assess whether certain criteria on prospective dental students' UCAS application forms could help predict their undergraduate performance. Details provided by applicants and their referees from 50 UCAS forms were analyzed by an Audit Officer. Precalculated examination results in anatomy, biochemistry, physiology, oral biology and pharmacology were used as a measure of academic achievement. The continuous assessment data in conservative dentistry included the student's patient treatment quality, attendance, work volume, completed treatments and variety of procedures. Using this data the students were divided into best (A) and worst (B) achievers. Results showed that there was no difference between groups A and B in terms of their statements on the UCAS application, except that group A students had better scores for knowledge and perception about dentistry. These students also had better scores from their confidential referees' statements for conscientiousness, motivation, participation and academic and organisational skills. There was no difference between the groups' GCSE and 'A' level examinations results. In conclusion, data from UCAS forms are limited as predictors of students' performance.

418 N TOBIN*, R M SUMMERS, M J PRENDERGAST and L GUTTERIDGE (Leeds Dental Institute, Leeds, UK): Oral health of adults in Bihar State, Northern India.

There is concern that caries levels may be increasing in parts of the world where it was previously thought to be low. The aim of this study was to assess oral health among a convenience sample of adults resident on the Indo-Nepalese border. The sample consisted of 152 male and female subjects. These were examined in Champak at the village health clinic and at the Mission's hospital in Raxaul according to criteria laid out by the WHO Oral Health Surveys, Basic Methods, 1987.

The mean age of the sample was 31 years (range 18 to 54) and none were edentulous. There was an average of one tooth missing and 1.4 decayed teeth per person. In total, there were 12 fillings present. Half (48% = 73) had no evidence of active caries and about a third (34% = 51) had only between 1 and 2 decayed and untreated teeth. The periodontal assessment according to CPITN scores was found to increase with age: more than half (63%) of the sample had a CPITN score of 3 or 4 in at least one sextant, and no healthy sextants were found in the older age groups (35-44 and 45-54 years). There were very low levels of caries experience, and of dental treatment provided. Periodontal treatment need was high, although there was little evidence of associated tooth loss in this sample.

Caries levels are low but periodontal care is a more important issue for the maintenance of the dentition among the adults of the region.

420 H J WILLIAMS & S A WILLIAMS* (Dept. Psychology, University of Surrey & Leeds Dental Institute, University of Leeds, UK.): The use of 'pan' among young Bangladeshis & UK-born Bangladeshi people.

Since a high prevalence of betel quid (pan) chewing occurs among older first generation Bangladeshi (B) women in the UK (Summers et al. Community Dent Health 11: 12-16, 1994), this study aimed to investigate a younger English-speaking B population by country of birth.

Semi-structured interviews were held in community settings in West Yorkshire in English for 67 people (36 males, 31 females), mean age 17.3 years (range 12-24 years). The 37 B-born had spent, on average, 7.9 years in the UK compared with 13.9 years for the 30 UK-born, since all but 2 of the latter had spent time back in B; 74% of their time was estimated as currently spent with others in the B community; of family members-60 mothers, 41 fathers chewed pan, usually with lime & tobacco. Pan had been tried by 65 of the sample (20% by 6 years of age), for most (48/74%) initially while in UK. Currently, 51 chewed it, of whom 11 used lime and/or tobacco (traditional chewers: TCs). When chewers and non-chewers were compared, there were no differences by gender, age, place of birth, age English learnt, whether other family members chewed pan, but chewers held more positive attitudes about enjoyment and qualities of pan. TCs differed only in later age English was learnt, preference for Asian (v. English) food & positive views about arranged marriages. TCs claimed the chew made them feel good, imparted pleasant breath & attractive lips. Nut-leaf only chewers stated digestive benefits.

Pan chewing is practised by young Bangladeshi people, although to a lesser extent than among adults. In this sample, place of birth was no indicator of use. Such practices are continuing in the UK & thus future oral cancer risk may exist for successive generations.

422 S BOSOMWORTH*, S KWAN & S A WILLIAMS (Northallerton Health Services NHS Trust & Leeds Dental Institute, University of Leeds, UK.): An inquiry into social class differences in dental experiences of dental students.

Social background is strongly associated with oral health and health-related behaviours. This study aimed to explore past dental experiences of first year dental students at Leeds by social class and by family association with doctors and dentists. During the years 1989-1993, 255 students (aged 17-21 years) completed a questionnaire and were dentally examined. Of these, 186 students came from Social Class I&II backgrounds (Group A), 69 from groups III, IV & V (Group B), 38 were sons and daughters of doctors and dentists (Group C).

Results for Groups A,B&C respectively were:- mean age 19.1 years (all groups); reported 6 monthly dental checks (79%,68%,68%); distressing experiences (38%,30%,37%) or fear of dental visits (40%,39%,50%); experience of toothache (37%,38%,29%), sensitivity (53%,52%,50%), and ulcers (51%,49%,53%); or treatment received, general anaesthesia (50%,41%,42%), orthodontic treatment (51%,46%,53%) and oral hygiene advice (67%,58%,66%). Mean DMFT values were 3.5,4.6,3.0 & mean DF scores 3.4,4.5,2.8. Mean number of fissure sealants were 1.4,0.5,1.6 respectively.

It is concluded that, among this self-selected group, there are notable similarities in past dental experiences reported. However there is some variation in caries experience and treatment received.

417 R DUGUID (Department of Conservative Dentistry, Dental School, University of Dundee, Dundee, DD1 4HN): The effect of UCAS on Applications and Admissions to Dental Schools.

From 1961 to 1993 applicants for Dental Schools applied via UCCA (Universities Central Council for Admissions). Later applications were restricted to 5 Dental Schools. For the 1994 entry UCCA merged with PCAS (Polytechnics Central Admissions System) to form UCAS (Universities and Colleges Admissions Service) which allowed 8 course choices, though applicants were advised to apply to only 5 Medical or Dental Schools. Initial analysis of statistics from UCAS show that total applications for Dentistry rose from 8,293 in 1993 to 12,078 in 1994 (a 42% increase) - a much greater increase than seen for Medicine (+14%). This increase continues a trend started in 1991 (Duguid, R. Brit.Dent.J. 177: 213-216, 1994) and includes a number of applications for Dentistry from applicants placing 5 Medical Schools on the UCAS form. Previously many of these applicants did not apply for Dentistry. Only if rejected for Medicine did they then approach Dental Schools through clearing procedures.

It is concluded that the large increase in applications for Dental Schools seen in 1994 is a result of renewed interest in Dentistry as a career, but also to changes in University admission procedures.

419 SA WILLIAMS, RM SUMMERS*, A MOHAGH & I AHMED (Dept of Child Dental Health, University of Leeds, UK.): The use of tobacco and 'pan' among Bangladeshi men in West Yorkshire.

In the Indian sub-continent, oral cancer is the commonest form of malignancy, 90% of which is caused by local forms of tobacco use and/or 'pan' (betel leaf + nut + lime). A high prevalence of betel quid chewing occurs among first generation Bangladeshi women aged over 25 years, now resident in the UK (Summers et al. Community Dent Health 11: 12-16, 1994). This study aimed to investigate a corresponding male population using the same methodology.

Of the 221 men interviewed, median age 51-55 years, 67% were unemployed, 55% spoke 'little' & 28% 'no' English, & 30% had never been to a dentist although they had been resident in the UK for an average of 24.5 yrs. The betel leaf, nut, lime & tobacco were used by 79%, 79%, 57% & 31% of the men respectively. The number of quids chewed per day ranged from 0-30 (median=4). Often taken after a meal, each quid was kept in the mouth for up to 30 mins. Quid chewing was perceived as a 'good' practice by 23%, seen as an oral deodorant (25%), a digestion aid (12%) & 'good for teeth' (11%), but 2% thought it a 'bad' custom. After chewing, 22% spat it out, 3% stored it in their mouth, 55% swallowed it: 49% would be prepared to stop if advised. Tobacco powder was used as an oral hygiene aid by 14%; 66% smoked cigarettes (maximum 50/day; mode 10/day).

Compared with the previous study among Bangladeshi women, the men here were older, chewed quid less frequently, but smoked more heavily. Despite differences in tobacco & quid use, these men must also be considered at-risk group for oral malignancy. Funded by the Yorkshire Regional Health Authority.

421 S KWAN*, SA WILLIAMS & M PRENDERGAST (Leeds Dental Institute, University of Leeds, UK.): Gender differences in oral health experiences and status among first year dental students.

A previous study among first year medical & dental student found few differences in background, attitude & caries experience (Gyi et al., J Dent Res 70(4)689, 1991). This study aimed to explore gender differences in past dental experience and oral health status of first year dental students at Leeds from 1989 to 1993, using the same methodology; 131 males (M) and 136 females (F) aged 17-22 years were included in the study.

There were no gender differences in mean ages (both 19.1 years) & social class (66% M & 77% F were from Social Classes I & II); reported 6-monthly dental checks (77% M v 74% F); distressing experiences (36% M v 38% F) or fear of dental visits (36% M v 43% F); experience of toothache (55% M v 49% F), sensitivity (53% M v 56% F) and ulcers (52% M v 51% F); or treatment received, general anaesthesia (48% M v 50% F), orthodontic treatment (50% M v 52% F) and preventive advice (e.g. oral hygiene advice by 67% M v 65% F).

However, there were gender differences in the clinical findings. Mean DMFT values were 4.3(M) & 3.2(F) ($P < 0.05$) & mean DF of 4.2 (M) & 3.1 (F) ($P < 0.01$). Gingivitis scores were also higher in M (2.65) than F (1.78) ($P < 0.05$), but little difference in oral hygiene status at the time of the examination (%good: 73% M v 80% F).

It is concluded that these female dental students have better oral health status than their male peers despite similarities in past dental experiences.

423 J A SPERCHLEY*, P A HANCOCK (Dental Department, Warrington Community Health Care (NHS) Trust): A routine blood pressure screening service of adults attending the Community Dental Service.

Adult patients attending a minor oral surgery clinic and parents accompanying children were asked if they would like their blood pressure checked, as part of screening to achieve a healthier lifestyle. This was uniformly supported. The study is ongoing and has been extended to include all staff in the community service building and will be offered to the dentists in the Warrington area in the future. All readings were taken on an electronic sphygmomanometer - Dinamap[®], which minimised operator error, provided cuff placement was correct, and cuff size accurately selected. Measurements were only taken after patients had been seated for at least ten minutes. The results to date cover an age range of 21-57 years, and to some extent mirror the findings of Kilcoyne (Kilcoyne A - The Probe May 1994), in that blood pressure rises with age. However, mild hypertension has been recorded in 13% of those sampled, i.e. 7% patients, 18.8% staff. Of these, 12% are on six monthly monitoring, and 6% on weekly monitoring by their medical practitioner before drug therapy is instituted.

It is concluded that routine blood pressure monitoring will pick up abnormalities in a significant percentage of the population before symptoms supervene and preventive action can be taken.

424

J SINCLAIR* and M WILSON (Restorative Dentistry, University of Manchester, UK): Aspects of the provision of an emergency dental service to university students.

Little is known of the incidence and nature of dental emergencies experienced in young adult populations. This paper reports 4-year data collected in the running of a student emergency dental service (SEDS) at the University of Manchester. Students presenting at the unit complete a questionnaire and following assessment and treatment, the diagnosis and details of treatment provided are recorded. Since the inception of the unit, data has been collected in respect of 3600 patients - data handling and analysis being computerised. The results indicate the annual demand for emergency dental care amongst the population studied is at least 39 episodes per 1000 students. No significant differences have been identified between male and female and home and overseas students in terms of DMFT and CPITN scores except in relation to the periodontal treatment needs of mature overseas students and irregular attenders. The commonest presenting emergencies relate to failed restorations and pulpal symptoms (46%), followed by pericoronitis (19%) and periodontal conditions (11%). Treatment which is generally palliative rather than definitive involves temporary dressing (28%), local measures and prescriptions (65%) or in many cases advice only (7%). It is concluded that the incidence of dental emergencies experienced amongst the student population served by the SEDS unit is relatively high, but that most of the presenting problems are of a simple nature requiring routine treatment. A SEDS unit may be found to form an important element of a University's medical and welfare services for students.

425

M LEVINKIND, C HEPWORTH*, P ANDERSON, M CALVERT (Department of Child Dental Health, London Hospital Medical College, London, E1 2AD): A relational database for clinical audit of children with acute dental trauma.

The objective of this study was to develop a database that can be implemented on a personal computer to facilitate clinical audit of the multi-disciplinary management of children who have sustained acute trauma to their teeth. It was decided to employ relational database software (Paradox for Windows) as it can be used on a personal computer and provides a standard format. Forms were designed so that data entry could be made by optical scanning of the patient records which were completed by the clinician at the time of each visit. It was possible to integrate information about each patient's dental injuries, malocclusion, caries status, periodontal health, dental treatment provided and the clinical outcome. Manipulation of the database to query the information and produce reports was provided by additional software written in ObjectPAL, which is a high-level, event driven, object based, visual programming language.

We have developed a relational database system using standard software that permits information from clinical records to be entered into the database via an optical scanner. Manipulation of the database is straight-forward and clinical outcomes can be assessed easily.

Paradox for Windows (Version 5), Borland International (UK) Ltd.
ObjectPAL (Version 5), Borland International (UK) Ltd.

426

J E CLARKSON*, H V WORTHINGTON, P J HOLLOWAY and R M DAVIES (Dental Health Unit, University of Manchester, UK): Development and evaluation of an index of dental treatment experience

The aims of this study were to develop and evaluate an Index of Dental Treatment Experience (DTE). The DTE index was developed by a group of dental experts with the aid of the Nominal Group Technique. The index scored a sound tooth 0, a tooth with a single surface filling 1, a tooth with multiple surfaces filled 3, a crowned tooth 5, a tooth replaced by a bridge 8, a tooth replaced by a denture 10 and a tooth not replaced 5. This weighting accounted for the impact of treatment on oral health and was compared with the DMF and Tissue Health Index (THI).

Clinical prevalence and questionnaire data were available for 3020 adults. These were patients of 24 dentists in general dental practice in Greater Manchester and over 25 years of age. The ability of the indices to discriminate between groups was compared. Of the 20 independent variables examined the DTE index discriminated for 18, the DMF 9 and the THI 15 ($p < 0.05$). Multiple regression analysis was used to compare the ability of the indices to explain variations in treatment experience. The THI explained most variation (34%) with p-values similar to the DTE index (31%), the DMF index explained least (23%).

An Index of Dental Treatment Experience has been developed which is more discriminatory and sensitive than the DMF index and similar to the THI.

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H V WORTHINGTON*, P S HULL, V CLEREHUGH, J E CLARKSON, R TSIRBA and R M DAVIES (Dental Health Unit, University of Manchester, UK): Validation of dentists' reasons for extracting teeth.

The aim of this study was to validate general dental practitioners' reasons for extracting teeth against independent clinical measurements. Twenty-four dentists in the Greater Manchester area provided extracted teeth from adult patients. For each extracted tooth the patient's age, gender and dental attendance were recorded, along with the reason(s) for extraction. 389 teeth were examined independently for caries, fillings and calculus. Evidence of caries or its sequelae were found on all the 110 teeth where caries was given as the primary reason for extraction.

80 teeth with a single reason given for extraction (either periodontal or caries) were randomly selected, stained, and the maximum loss of attachment measured. There was significantly more loss of attachment on teeth extracted for periodontal reasons (mean = 11.8mm) than for caries (mean = 6.5mm), ($p < 0.001$). Loss of attachment was also found to be positively associated with the age of the patient, but not with gender or dental attendance.

The general dental practitioners' reasons for tooth extraction are valid.

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DW THOMAS, J SATTERTHWAITE, EG ABSI, R WALKER* & JP SHEPHERD (Dept. of Oral Surgery, Medicine & Pathology, UWCM, Cardiff, UK): A longitudinal study of the demand for emergency dental care at a dental teaching hospital.

To investigate changes in the numbers, sources, and pattern of emergency treatment for patients which have occurred following the introduction of the new dental contract and the publication of the Poewillo report; a longitudinal study was undertaken to investigate new patient presentation at the primary care unit of the Cardiff Dental Hospital in 1989, 1993 and 1994. 500 consecutive new patients attending the primary care unit were interviewed in May/June in each of the years 1989, 1993 and 1994; data were entered and processed using the SPSS statistical analysis package. Over the study period patient throughput increased by 35%. Trends were evident in the three years studied with a decrease in the number of patients seen not having a GDP (-39%), increases in self-referred patients (+54%) and decreases in the referrals from general and medical practitioners (-41%, GDP -70%, GMP). Whilst the disease patterns showed little change, the treatment provided did, with substantial increases in the number of extractions performed (74%).

These data demonstrate increases in demand for primary care in a teaching hospital and trends in the referral pattern and treatment of these patients since 1989. This study confirms that large numbers of the patients are unregistered and would be lost to screening procedures instituted in the GDS.

429

JJ MARLEY1*, CG COWAN1, P-J LAMEY2, G LINDEN1, NW JOHNSON2, K WARNAKULASURIYA2* (1 School of Clinical Dentistry, Queen's University Belfast; 2 RCS Dept. Dental Sciences Kings College London): Management of potentially malignant oral lesions by consultant oral and maxillofacial surgeons

The aim was to investigate currently used management options for potentially malignant oral lesions in the United Kingdom. All consultants who were members of the British Association of Oral and Maxillofacial Surgeons were circulated with a questionnaire requesting information regarding their current approach to the management of such lesions. The majority 141 (61%) of those circulated responded to the questionnaire. Only 6% of responders treated more than 100 such patients in the previous year whereas 63% had treated under 50. Nearly all of the responders (96%) asked patients questions regarding smoking, the nature (91%) and amount (85%) of alcohol intake. Almost all (95%) used a written description of the oral lesions as their routine means of documentation. A small proportion of responders never photographed lesions (4%), never used a diagram (4%) or never measured the dimensions (16%). The majority of responders undertook biopsies routinely (67%) with the remainder using this investigation selectively (33%). Full blood count was used routinely by only 38% of responders. Candida isolation was conducted by the majority (77%) only selectively with 17% never attempting this. There was considerable variation in treatment patterns: (64%) undertook no active treatment for lesions without evidence of dysplasia; (16%) no active treatment for lesions with mild/moderate dysplasia. For severe dysplasia/carcinoma in-situ the commonest methods of treatment were excision (96%), followed by elimination of trauma (75%), the use of anti-fungals (64%) and laser ablation (47%).

It is concluded that there is considerable variation among consultants in oral surgery in relation to their management of potentially malignant oral lesions.

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H J WILLIAMS & S A WILLIAMS* (Dept. Psychology, University of Surrey & Leeds Dental Institute, University of Leeds, UK.): The use of 'pan' among young Bangladeshi & UK-born Bangladeshi people.

Since a high prevalence of betel quid (pan) chewing occurs among older first generation Bangladeshi (B) women in the UK (Summers et al. Community Dent Health 11: 12-16, 1994), this study aimed to investigate a younger English-speaking B population by country of birth.

Semi-structured interviews were held in community settings in West Yorkshire in English for 67 people (36 males, 31 females), mean age 17.3 years (range 12-24 years). The 37 B-born had spent, on average, 7.9 years in the UK compared with 13.9 years for the 30 UK-born, since all but 2 of the latter had spent time back in B; 74% of their time was estimated as currently spent with others in the B community; of family members-60 mothers, 41 fathers chewed pan, usually with lime & tobacco. Pan had been tried by 65 of the sample (20% by 6 years of age), for most (48-74%) initially while in UK. Currently, 51 chewed it, of whom 11 used lime and/or tobacco (traditional chewers-TCs). When chewers and non-chewers were compared, there were no differences by gender, age, place of birth, age English learnt, whether other family members chewed pan, but chewers held more positive attitudes about enjoyment and qualities of pan. TCs differed only in later age English was learnt, preference for Asian (v. English) food & positive views about arranged marriages. TCs claimed the chew made them feel good, imparted pleasant breath & attractive lips. Nut+leaf only chewers stated digestive benefits.

Pan chewing is practised by young Bangladeshi people, although to a lesser extent than among adults. In this sample, place of birth was no indicator of use. Such practices are continuing in the UK & thus future oral cancer risk may exist for successive generations.

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L GLOVER*, A MILES, S HARRISON, S PEARCE, C FEINMANN, and M HARRIS. (Dept. Maxillofacial Surgery, Eastman Dental Hospital, London): Factors affecting treatment uptake in chronic idiopathic facial pain.

The aim of this study is to assess which factors may influence patient acceptance of medical treatment for chronic idiopathic facial pain.

Methods: Patients attending a facial pain clinic were asked to complete questionnaires measuring pain and mood prior to clinical examination. Kendall rank correlation coefficients were calculated to establish which factors related to acceptance of treatment.

Results: Pain severity, depression and anxiety were positively correlated with treatment uptake ($p < 0.01$, $p < 0.005$ & $p < 0.05$ respectively). Of physical factors measured only concurrent neck pain was significantly associated with treatment uptake ($r = 0.20$; $p < 0.05$) this may merely relate to severity of the condition.

It is concluded that severity and mood influence treatment uptake rather than physical factors associated with chronic idiopathic facial pain.

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S HARRISON*, C HOPPER AND M HARRIS. (Depts. Maxillofacial Surgery, Eastman Dental Hospital and University College London Hospitals): TMJ Arthroscopy: outcome at 3 months.

The aim of this study is to assess the outcome in patients following TMJ (temporomandibular joint) arthroscopy.

All patients undergoing TMJ arthroscopy are assessed clinically and then complete a set of standardised questionnaires to assess pain and mood prior to surgery these are repeated at one and three months postoperatively.

The results at 3 months show that of those patients reporting pain prior to surgery 70% still had pain at three months although 59% report improvement, but of these half had other treatments to obtain relief. 82% of patients who reported locking of the TMJ prior to arthroscopy had complete relief of this symptom. Limitation of mouth opening was successfully treated in 68% of patients.

It is concluded that TMJ arthroscopy is most successful in treating limited mouth opening and locking of the TMJ.

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COWPE JG, ARMSTRONG RA, EVANS DJ*, BRICKLEY MR, SHEPHERD JP. (Oral Surgery, Medicine and Pathology, UWCM, Heath Park, Cardiff CF4 4XY UK): Patient perceptions regarding the costs and benefits of lower third molar removal.

The risks associated with third molar surgery are well established. Current philosophy stresses co-development of treatment plans with patients who need to understand benefits and risks of treatment options. This understanding was therefore investigated at an initial outpatient appointment (n = 91) after a verbal explanation and immediately prior to surgery (n = 73), by means of visual analogue scales to assess likelihood of each outcome. Most patients (98%) responding at an initial appointment (time = t1) felt that they had been given enough information but the proportion was lower (88%) for patients responding at the time of surgery (time = t2) ($\chi^2 = 5.4, P < 0.02$). This reduction was also found for information regarding post operative pain; (t1 = 93%, t2 = 74%, $\chi^2 = 11.84, P < 0.01$), swelling; (t1 = 95%, t2 = 77%, $\chi^2 = 11.04, P < 0.01$), trismus; (t1 = 88%, t2 = 59%, $\chi^2 = 5, P < 0.01$) and nerve damage; (t1 = 96%, t2 = 74%, $\chi^2 = 15.71, P < 0.01$). Patients estimated the likelihood of pain as (t1 = 70%, t2 = 80%), swelling (t1 = 76%, t2 = 74%) and trismus (t1 = 74%, t2 = 73%). Both groups overestimated the incidence of temporary, (transient labial t1 = 46%, t2 = 33%; transient lingual t1 = 51%, t2 = 41%) and permanent nerve damage; (labial t1 = 13%, t2 = 7%; lingual t1 = 14%, t2 = 9%). It was concluded that patients' understanding of outcomes was poor and varied with time. This might be improved by verbal explanation supplemented by provision of written information.

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D RUSSELL*, P A BIAGIONI AND P J LAMBY (School of Clinical Dentistry, The Queen's University of Belfast, UK): Heat generation during pin channel preparation.

When, as a result of caries or trauma, extensive tooth destruction has occurred, auxiliary retention of plastic restorative materials is often considered. This usually takes the form of self-dressing dentine pins which have to be carefully placed to avoid damage to the dental pulp on the one hand and the periodontal membrane on the other. One possible hazard to the pulp is the heat generated during the preparation of the pin channel.

The object of this pilot study was to quantify the heat generated during preparation of pin channels using 0.676 mm twist drill (Wholesend regular size link pins) in a KaVo Intra 68°C hand on a KaVo 20A shank run at 4000 rpm by a KaVo Intrasept 905 motor with digital display of the speed. Twenty decoronated extracted premolar teeth were held in a bench vice, 0.05 metres from the lens of a thermal imaging camera¹ during preparation of pin channels approximately 1 mm from the amelo dentinal junction on the part of the tooth closest to the imager.

Sequential thermal images were recorded at a frame rate of 3 per second for each stage in the channel proportion technique, ie placement drill, channel drill and pin placement. The mean maximum change in temperatures for each stage were round bur 35.8°C, channel drill 76.6°C and pin placement 41.6°C.

These results suggest that a significant temperature rise may occur within the pulpal chamber during pin channel preparation.

¹ Agema Thermovision 900 system (Danderyd, Sweden)

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S LANGTON* and I KARAGIOZIDIS (Department of Dental Medicine and Surgery, University of Manchester UK): Factors affecting patient satisfaction after orthognathic surgery.

This paper evaluates some of the factors that may be involved in patient satisfaction following orthognathic surgery. 52 patients who had undergone jaw osteotomies were sent postal questionnaires designed to obtain patient ratings, on a numerical scale, of factors possibly related to patient satisfaction after surgery. A response of 80.8% was obtained. Patients were asked to rate improvement in facial appearance, tooth aesthetics, dental occlusion, speech, self-confidence and eating ability. Age, degree of discomfort, duration of trismus and persistence of lip anaesthesia were also assessed. Two further ratings, an 'overall score' for the result of surgery on a 1 to 10 scale and an assessment on whether the patient would have the same surgery again, were noted. To determine which factors were considered of greatest importance, a multiple regression analysis was performed, using firstly 'overall score' and secondly rating for 'would have same operation again' as dependent variables. All other factors were considered as independent variables in the analysis.

The three most important factors in the variance of the overall score were facial appearance, trismus and improvement in occlusion. Speech, accounting for 6% of variance in 'overall rating'. These were highly significant ($p < 0.001, 0.003$ and 0.032 respectively). When considering 'would have the same operation again' lip numbness was most important, accounting for 19% of variance ($p < 0.001$).

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A JOSHI*, P J THOMSON AND J P ROOD (Department of Dental Medicine and Surgery, University of Manchester UK): Pre-emptive analgesia.

Surgical trauma may lead to hyperexcitability of dorsal horn sensory neurones resulting in amplification and prolongation of post-operative pain, which may be eliminated or reduced if the afferent stimuli are prevented from reaching the CNS by pre-injury local anaesthetic block. A pilot study of 30 age and sex-matched adult patients undergoing surgical removal of impacted mandibular third molar teeth under general anaesthesia were entered into a blind, randomised, controlled study and instructed on the use of Visual Analogue Scale (VAS) for pain assessment. Patients were randomly allocated into three groups: no local anaesthetic block; pre-operative local anaesthesia (4 ml of 4% procaine) once GA was established and post-operative local anaesthetic (as above) upon conclusion of surgery. A standard anaesthetic regime (without the use of intra-operative analgesic) was utilised throughout and demand for 'rescue' analgesia post-surgery was recorded. VAS pain scores and extent of lip numbness were measured at 30, 60, 90, 120 and 240 minutes post-operatively and the morning after surgery. Direct comparison of local analgesic intervention before and after surgery revealed lower pain scores and reduced analgesic demand during the initial post-operative period in patients who received pre-operative LA. This effect was less striking for patients who received LA post-operatively and also the next morning when pain scores and analgesic requirements had become similar within all three groups, suggesting that pre-emptive effects may only last for a limited duration.

In conclusion, pre-operative administration of local anaesthetic provided enhanced pain relief following third molar surgery compared with control patients.

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J SATTERTHWAITE, D W THOMAS, E G ABSI* & M A O LEWIS. (Dept. of Oral Surgery, Medicine and Pathology, Dental School, UWCM, Heath Park, Cardiff, UK): Antibiotic therapy in patients attending a dental hospital emergency department.

Patients presenting with acute complaints at a dental hospital emergency unit may have already been provided with antibiotics. However, little information is available in these circumstances concerning the nature of acute condition for which antibiotics have been prescribed, the type of antibiotic given and the source of initial antimicrobial therapy. A prospective study was undertaken on consecutive patients attending a dental examination and emergency clinic during May 1994. A total of 500 patients (260 male; 240 female) with a mean age 39 years (range 15-85 years) were included in the study. Complaints consisted predominantly of pulpitis (49%), dental/occlusal infection (15%), failed restorations (13%) and periodontal disease (10%). Sixty (12%) of the patients were taking antibiotics at the time of presentation and comprised of 34 (14% of those with pulpitis), 18 (25% of those with dental/occlusal infection, one (2% of those with failed restorations), four (8% of those with periodontal disease, two (10% of those with oral ulceration and one (10% of those with dental/occlusal trauma). The patients had been provided with a total of 14 different antibiotics, including penicillin (n=20), amoxycillin (n=12), co-amoxiclav (n=5) and metronidazole (n=5). Approximately half (55%) of these patients been prescribed therapy by a GDP and a third (33%) by a GMP.

It is concluded that a minority of patients presenting as an emergency have been provided with antimicrobial therapy. If antibiotics have been provided this involves a small spectrum of agents prescribed by either GDP or GMP and usually for dental/occlusal infection.

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DW THOMAS, S HODDER AND CM HILL* (Department of Oral Surgery, Medicine and Pathology, Dental School, UWCM, Cardiff, UK): Rationalisation of antimicrobial therapy in third molar surgery.

The prescription and rationalisation of antibiotic prophylaxis, for high-volume elective procedures is important. To investigate antimicrobial therapy in in-patient third molar surgery, a prospective study of in-patient and out-patient data of individuals who underwent removal of impacted third molar teeth under general anaesthesia was undertaken in March 1992. Following rationalisation of antibiotic prescribing the exercise was repeated in March 1993 (n=132 patients). Data relating to antimicrobial prescription was collected prospectively in 1992 and 1993. Outcome was measured by recording the presence of infection at 1 week, unplanned attendances at GDPs & GMPs post-operatively and the total number of post-operative visits. Following rationalisation, the number of patients who received intra-venous antibiotics decreased (19% in 1992; 2% in 1993) and the use of Augmentin¹, either I.V. or orally, ceased. The presence of post-operative wound infection (10 cases in 1992; 8 cases in 1993) and the number of post-operative hospital visits (1.37 visits in 1992; 1.54 visits in 1993) were similar, whilst the number of patients visiting practitioners outside the hospital decreased (6 in 1992; 0 in 1993).

Rationalisation of treatment suggested that the prescription of parenteral, and second generation antibiotics produces no clinically significant benefits for medically fit patients undergoing dental/occlusal surgery.

¹ Augmentin, SmithKline Beecham

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P LAU*, D THOMAS, M A O LEWIS AND P A BISHOP (Department of Oral Surgery, Medicine and Pathology, UWCM, Cardiff, UK): Bacteriology of facial lacerations.

Although the majority of facial lacerations heal uneventfully secondary infection may occasionally result in long-term aesthetic problems. Little information is presently available concerning the initial microflora of wound infection at this site. Swabs and excised tissue from patients presenting with facial lacerations at a general accident and emergency unit were investigated. Swabs, to act as wound controls, were also obtained from matched facial sites in individuals with clinically healthy skin. Tissue specimens were homogenised in 1ml of Fastidious Anaerobe Broth using a sterile glass tissue grinder. Swabs and a 50µl volume of the tissue homogenate were inoculated onto duplicate plates of Blood Agar, MacConkey Agar and Sabouraud's agar and incubated aerobically at 37°C for 48 hours. All specimens were also inoculated onto Fastidious Anaerobe Agar and incubated at 37°C for 7 days. Plates were examined daily and morphological colony types removed for identification using standard laboratory techniques. Twenty patients, comprising of 14 males and 6 females, with a mean age of 26 years (range 3-86 years), presenting with facial lacerations (eyebrow/temple, n = 14; chin, n = 2; ear, n = 1; nose, n = 1) were included in the study. The control group consisted of 20 sex and age-matched individuals. Four samples (1 swab and 2 tissue specimens from lacerations and 1 control swab) failed to yield growth. A total of 115 isolates were obtained from the remaining specimens (46 strains from test swabs, 40 strains from the test tissue and 49 strains from control swabs). The spectrum of isolates was similar from each source and comprised predominantly of coagulase-negative *Staphylococcus* spp., *Propionibacterium* spp. and micrococci.

It is concluded that the members of the commensal skin microflora are frequently present in tissues involved in facial lacerations. These bacterial species could potentially be the source of wound infection.

- 440** KJ DAVIES*, DW THOMAS, P STEPHENS and JP SHEPHERD (Department of Oral Surgery, Medicine & Pathology, Dental School, UWCM, Cardiff, UK): The *in vitro* inhibition of TGF- β induced collagen synthesis by alcohol.

Acute alcohol exposure is a common finding in trauma patients. This study investigated the potential interaction of transforming growth factor-beta (TGF- β) and alcohol in the regulation of collagen synthesis and the dermal wound healing process. The effect of alcohol on basal and TGF- β induced fibroblast collagen synthesis was investigated *in vitro*. Cultures of dermal fibroblasts were established, and maintained in DMEM at 5% CO₂ in an humidified atmosphere. Collagen synthesis was assayed in confluent cultures in serum-free medium for 48 hours. Total collagen synthesis was assayed using the commercial SIRCOT¹ collagen testing kit. At sub-clinical concentrations of alcohol (10 μ g mL⁻¹) there was no significant reduction in cell viability/proliferation ($p > 0.05$). At this concentration alcohol failed to inhibit basal collagen synthesis ($p > 0.1$) but significantly decreased TGF- β (10 ng mL⁻¹) induced collagen synthesis ($p < 0.03$).

These data demonstrate that impaired healing following the administration of alcohol may be related to inhibitions in the TGF- β induced collagen synthesis of dermal fibroblasts.

¹Sircot, Biocolor Ltd.

- 442** U BONGENHIELM* and P P ROBINSON (Department of Oral and Maxillofacial Surgery, University of Sheffield, UK): Spontaneous activity and mechanical sensitivity of inferior alveolar nerve neuromas in ferrets.

The dysaesthesia reported by a small proportion of patients who sustain inferior alveolar nerve (IAN) injuries may result from abnormal neural activity at the injury site. Using electrophysiological techniques we have, therefore, determined the level of spontaneous activity and mechanical sensitivity of IAN fibres ending in a neuroma, after different post-injury periods. In 13 anaesthetised adult male ferrets the left IAN was ligated in the region of the third premolar tooth, cut distally, and recovery permitted for periods of 3 days, 1, 2, 3, 5, 8, 12 or 16 weeks. At a terminal experiment, under sodium pentobarbitone anaesthesia (induction 42 mg/kg i.p., maintenance 3 mg/kg i.v.), recordings were made from axons in fine filaments dissected from the nerve 18-24 mm proximal to the ligature (mean 78 units examined per animal). Spontaneous activity was recorded in 0-26% of units with discharge rates of 0.1-12.9 impulses s⁻¹. The proportion of spontaneously active units appeared higher at shorter recovery periods (up to 2 weeks) but this difference was not significant (Mann-Whitney U-test, $P = 0.07$). Discharge in response to probing the neuroma was found in 0-36% of the units and was significantly higher after short recovery periods ($P < 0.05$). The majority of the spontaneously active units (92%) was also mechanically sensitive. There was no significant difference between the conduction velocity of units with (median 31 m s⁻¹) or without (30 m s⁻¹) spontaneous activity ($P > 0.7$) or with (31 m s⁻¹) or without (29 m s⁻¹) mechanical sensitivity ($P > 0.2$).

These data reveal that some axons in inferior alveolar nerve neuromas are spontaneously active and mechanically sensitive and the incidence of such units appears highest in the early stages after injury.

Supported by the Wellcome Trust.

- 444** R STRANG*, W M GRAY, D STILL (Dent Physic, Glasgow Dent Hosp & Sch; Clin Physic & Bioeng, Western Inf Glasgow; Oral Surgery, Univ of Glasgow): The Reduction of Nitrous Oxide Levels during Dental General Anaesthesia.

The aim was to measure the exposure to nitrous oxide of staff during dental general anaesthetic procedures and to investigate the ability of a scavenging system to reduce these levels. Nitrous oxide levels were measured over a one week period using diffusive samplers (Perkin-Elmer) worn by members of staff (anaesthetist, dental surgeon, nurse and nursing sister). Separate measurements were made in the morning and afternoon sessions giving 10 measurements for each staff group. Details of calibration, sample preparation and analysis have been published (Gray et al., *Diffusive sampling: An alternative approach to workplace air monitoring*, Royal Society of Chemistry 89-92, 1987). For each session, eight hour Time Weighted Averages (TWA) were calculated for each staff group, the assumption being that each member of staff only worked in Theatre one session per day. Measurements were performed on two occasions, pre and post installation of a scavenging system (PURAIR 2000, McCrae Engineering Co Ltd). The mean (SD) 8 hr TWA's for the anaesthetist were: pre-scavenging: 133 \pm 123 ppm, post scavenging: 60.8 \pm 35.4 ppm. The corresponding values for other staff were: surgeon - pre: 61 \pm 23 ppm, post: 39 \pm 14 ppm; sister - pre: 38 \pm 18 ppm, post: 11 \pm 5 ppm; nurse - pre: 38 \pm 18 ppm, post: 11 \pm 6 ppm. Apart from the values for the anaesthetist, all differences were statistically significant.

The scavenging system was effective in reducing nitrous oxide levels.

- 446** P P ROBINSON* and K G SMITH (Department of Oral and Maxillofacial Surgery, University of Sheffield, UK): An evaluation of lingual flap retraction during third molar surgery.

Lingual nerve damage during third molar surgery may sometimes be attributed to the elevation of a lingual flap and insertion of a Howarth's periosteal elevator. This technique is intended to protect the nerve from more serious damage but its efficacy has not previously been evaluated. This study has compared the incidence of temporary and permanent lingual nerve injury following 771 operations randomly allocated to be carried out with or without lingual flap retraction. The surgery was carried out under local or general anaesthesia by hospital staff of all grades and included third molars of all levels of difficulty. Details of each procedure were recorded and the outcome determined at review appointments approximately one week post-op and subsequently if sensory disturbance was present. The time taken for third molar removal without lingual flap elevation (mean 11.5 mins) was significantly less than with lingual flap elevation (mean 13.4 mins, $P < 0.0001$, Student's *t*-test). Surgery with lingual flap retraction (378 operations) resulted in lingual sensory disturbance following 26 operations (6.9%) and this persisted following 3 operations (0.8%), requiring lingual nerve repair. Surgery without lingual flap retraction (393 operations) resulted in lingual sensory disturbance following 3 operations (0.8%, $P < 0.0001$, χ^2 test), and this persisted following 1 operation (0.3%), requiring lingual nerve repair.

We conclude that avoidance of lingual flap retraction reduces the incidence of temporary lingual nerve injury and does not increase the incidence of permanent injury. These data suggest that, where possible, lingual retraction should be avoided.

- 441** W MCLEAN¹, CR BRANN¹, JP SHEPHERD¹, D WESTMORELAND² (Dept. of Oral Surgery, Medicine and Pathology¹ & Public Health Laboratory², UWCM, Cardiff, UK): Risk of hepatitis B infection through occupational glass injury in bar staff.

It has been reported that bar staff are at high risk from sharps injuries, the cause of such injuries being the glasses with which they work. Therefore, bar staff may be at risk from cross-infection, particularly from contaminated glassware. In the present study the sero-prevalence of hepatitis B in bar staff was examined. Ninety-one bar staff (mean length of service 1.8 years; 40 males, 51 females; mean age 27 years) were recruited by advertisement, with ethical committee approval, from bars in the South Glamorgan area. They were asked about injury experience and life-style risks associated with the transmission of hepatitis B. A sample of blood was also taken to screen for hepatitis B surface antigen and core antibody. Control data were obtained from the Blood Transfusion Service (BTS). When questioned about injury experience, 74 per cent reported previous lacerations from broken glassware at work. Hepatitis B core-antibody prevalence for the study group was 1.1 per cent. When compared to the prevalence in BTS donors of approximately 1 per cent, this finding suggests that bar staff are not at an increased risk of hepatitis B infection.

*The results of this study suggest that these bar staff were not at increased risk from hepatitis B infection. However, three-quarters had sustained injury from glassware whilst at work. This emphasises the need that has been expressed in a previous report (Shepherd JP et al., *Injury* 25(4): 219-220, 1994) for the introduction of a code of practice regarding bar glassware.*

- 443** K G SMITH* and P P ROBINSON (Department of Oral and Maxillofacial Surgery, University of Sheffield, UK): Recovery of mechanosensitive afferents after lingual nerve grafting or repair under tension.

Repair of a defect in the lingual nerve after removal of a damaged segment may be achieved either by insertion of a nerve graft (which creates two repair sites) or by stretching the ends and repairing under tension (which may lead to intraneural fibrosis). Previous data on the recovery of fibres in the chorda tympani revealed that a sural nerve graft did not result in better recovery than repair under tension (Smith & Robinson, *J. Dent. Res.* 1992;72:730). This study has compared functional recovery of the fibres in the lingual branch of the trigeminal nerve after each method. In anaesthetised adult cats the left lingual nerve was sectioned, a 3-4mm segment of nerve excised and the gap closed either by insertion of a 4mm sural nerve graft (3 animals) or by stretching the ends and suturing under tension (3 animals). After recovery for 6 months, under sodium pentobarbitone anaesthesia (induction 42 mg/kg i.p., maintenance 3 mg/kg i.v.), electrophysiological recordings were made from single fibres dissected from the lingual branch of the trigeminal nerve, central to the nerve injury. Each fibre was characterised by determining the mechanoreceptive field size, force threshold, adaptation time, maximum discharge frequency, conduction velocity (cv) and thermal sensitivity. Fifty nine units were examined after grafting and sixty after stretch repair. After stretch repair the purely mechanosensitive units ($n=53$) had significantly faster conduction velocities (19.5 m s⁻¹, range 15.2-24.5; grafting 15.1 m s⁻¹, range 10.3-20.4, $P < 0.02$, Mann-Whitney U test) and lower force thresholds (1mN, range 1-6; grafting 3mN, range 1-8, $P < 0.02$) than after grafting ($n=45$). There were no other significant differences between the groups.

We conclude that repairing a short lingual nerve defect under tension results in better recovery than repair with a sural nerve graft.

Supported by the Wellcome Trust

- 445** H SLABBERT*, M QUINTA, M MCGURK AND A CASH (Dept of Oral & Maxillofacial Surgery, Guy's Hospital, SE1 9RT): Full thickness skin grafts in radial forearm flap donor sites.

The radial forearm flap (RFF) can be used for the reconstruction of large intra-oral and mandibular defects. The donor site is associated with healing problems when covered with split thickness skin grafts (Elliot D et al. *Brit J Plast Surg* 41: 358-360, 1988). The aim of the present study is to report the complications and morbidity associated with full thickness grafts (FTSG) used to repair radial forearm donor defects.

Twenty-one consecutive patients whose donor site was repaired by full thickness skin grafts were selected from the archives of the Department of Oral and Maxillofacial Surgery, Guy's Hospital, London. Pertinent clinical details (tobacco and cardiovascular disease) were recorded and photographs of the donor site (at operation, 10 days and 6 months post-surgery) were assessed. Other measurements recorded were: grip strength, movement, graft area and skin loss, sensory changes, scarring and tendon exposure. Of the 21 patients whose defects were repaired with a FTSG 13 were available for review. The results indicate that in 25% of cases some mobility was reduced, partial skin loss was encountered in 14% of cases, tendon contracture in 8% and graft infection in a further 8%. In no incidence were the tendons of the forearm exposed.

The present study indicates that full thickness skin grafts used to repair donor sites in the forearm are associated with complications but these are temporary in nature and the eventual function of the arm at risk is unaffected.

- 447** SA BURNS*, KG ISAACSON, WJ HARDCASTLE and F MCDONALD (Departments of Orthodontics, Reading and UMDS, London): Relapse of mandibular osteotomies and tongue function and behaviour.

Orthognathic surgery for the treatment of severe skeletal discrepancy is a routine procedure. Instability following mandibular osteotomies is a frequent occurrence (Behrman SJ, *J Oral Surg* 30: 554-561, 1972). It is the aim of this report to examine the behaviour of the tongue using electropalatography in an attempt to rationalise our understanding of relapse. The behaviour of the tongue during speech was examined by means of the pronunciation of a series of three words and a standardised sentence. The actual movements of the tongue were inferred by the electrical contacts recorded against an acrylic appliance containing sixty two silver conducting electrodes. The case load was divided into seven cases, (age range 19-32 years), requiring advancements of between 4.5 and 9 mm for the correction of retrognathia and one case with prognathia, (age 18 years), requiring a mandibular setback of 9.5 mm. All patients underwent Straightwire appliance orthodontic treatment prior to, and following the osteotomy. The surgery was performed using a Dal Pont modified Obwegeser sagittal split osteotomy, without any form of myotomy. EPG recordings and lateral skull radiographs were taken four weeks and one week pre-operatively and six, twelve and fifty two weeks post-operatively. The results of this study showed that the tongue assumed a more anterior position following mandibular osteotomy. This change was only seen in patients undergoing surgical change in the sagittal plane of more than 5 mm. *It is concluded that this modification of tongue position and the consequent alteration in muscle balance, contributes to the relapse of mandibular osteotomies.*

448 DW THOMAS, M SAGEMAN, S HODDER* and JP SHEPHERD. (Department of Oral Surgery, Medicine and Pathology, Dental School, UWCM, Cardiff, UK): Trends in the management of fractured mandibles 1983-1993.

A number of changes have occurred in the aetiology and treatment of fractured mandibles in the UK in the past 10 years. This study characterised these changes between 1983 and 1993 and evaluated the potential in-patient resource benefits of treatment with rigid fixation versus intermaxillary fixation.

A cross-sectional study was undertaken of the aetiology, presentation and management of fractured mandibles over 10 years (1983-1993) in Cardiff. Demography of patients in both years was similar; the majority were in males (8:1, M:F), injured in assaults (>75%) and 50% of the patients presented for treatment between the hours of 10PM and 5AM. There were marked decreases in the numbers of patients waiting more than 24 hours for an operation (60%, 1983; 34%, 1993) and out-of-hours operating (60% of operations in 1983; 41% in 1993). Changes in the methods of treatment included a decrease in the use of post-operative inter-maxillary fixation (IMF) (98% of cases in 1983; 56% in 1993) and an increase in internal fixation using bone plates (2% of cases in 1983; 53% in 1993). Total in-patient stay did not change in the 2 years (mean = 3 days) and there was no difference in duration of stay between treatment modalities (internal versus intermaxillary fixation).

These data show how the management of trauma patients has been made more efficient and effective by the rationalisation of maxillofacial trauma services and by the introduction of new methods of treatment.

449 A B YOUNG*, A J DEVLIN and I M BROOK (Department of Oral and Maxillofacial Surgery, University of Sheffield, UK): The use of histomorphometric image analysis in dental research.

In many areas of dental research it is often desirable not only to gain qualitative information from objects, such as histological sections, radiographs or photographs, but also to obtain more accurate quantitative data. This, in the past, has been problematical especially when trying to measure histological sections microscopically: an eye piece graticule is inaccurate and difficult to use, especially when measuring areas or complex shapes. Where the number of certain cell types in a section are to be counted this has to be done by random sampling which may not be representative of the whole section and, therefore, not accurate. With advances in computer imaging technology image analysis programs are now available which can be applied to dental research. In Sheffield we have been using image analysis software since 1992. This software enables the acquisition of an appropriate image onto a screen using a camera. The image can then be enhanced, calibrated and a wide variety of measurements made which can be exported to data files. We have applied this technology to several research projects to produce more accurate results: the quantitative assessment of CO₂ laser wounds; the enhanced assessment of the number of immunocytochemically labelled nuclei in ferret brain stems; the quantification of new bone growth in the development of a novel membrane for guided tissue regeneration and also the assessment of the extent of osseointegration in removed implants. It has also been used in restorative dentistry to assess the extent of marginal leakage around restorations and also to evaluate plaque vitality in periodontal disease. At present it is being used to measure the fluorescence of nerve fibres (positive to various neuropeptides).

Histomorphometric image analysis is a powerful tool which can be applied to many aspects of dental research to enable accurate quantification of results.
Optimas 4.1 Biosoft ©, USA

450 A. TRIANTAFYLLOU and D. FLETCHER* (Academic Unit of Oral Diseases, University of Liverpool, UK): Histochemical observations on mast cells in salivary glands and tongue of ferret.

Previous investigators failed to demonstrate mast cells in the alimentary canal and associated glands of ferret. We therefore decided to test this and examine factors that may be of influence. Formalin-fixed major salivary glands and tongue of adult ferrets were used. A battery of histochemical procedures employing basic dyes and esterase activity were carried out for conventional times with and without previous oxidation, hot acid hydrolysis, and proteinase and mucopolysaccharase digestion. Aldehyde fuchsin (AF) and high iron diamine (HID) of all histochemical procedures revealed the presence of a few and moderately stained mast cells in the connective tissue of salivary glands and lingual musculature, and the corium of lingual mucous membrane. Pretreatment did not enhance the numbers and staining reactions of mast cells in these sites.

The results suggest that the ferret is a species of low mast cell prevalence which can be conveniently assessed by AF and HID. There is no suggestion of different distinctive mast cell phenotypes or a masking effect, but these are issues for further exploration.

451 H.E. REED* and P.M. SMITH (Oral Biology Unit, School of Dentistry, The University of Liverpool, UK): Mobilization of [Ca²⁺]_i by the microsomal Ca²⁺ ATPase inhibitor thapsigargin in acutely isolated mouse submandibular cells

The increase in [Ca²⁺]_i which links stimulus to secretion in salivary acinar cells is a complex product of Ca²⁺ release from and reuptake into intracellular stores and Ca²⁺ influx and extrusion across the plasma membrane. Previous studies in other exocrine glands have shown that the microsomal Ca²⁺ ATPase inhibitor thapsigargin gives rise to mobilization of Ca²⁺ from intracellular stores (Takemura et al J Biol Chem 264 12266-12271 1989). We have measured [Ca²⁺]_i using microfluorimetric techniques in order to investigate the extent to which thapsigargin is able to mobilize Ca²⁺ in acutely isolated mouse submandibular acinar cells.

In the absence of extracellular Ca²⁺, 2 μM thapsigargin caused a transient increase in [Ca²⁺]_i which peaked at 190 ± 44 nM (n=8). Following exposure to thapsigargin, stimulation by acetylcholine (500 nM-5 μM) did not increase [Ca²⁺]_i. Furthermore, subsequent to thapsigargin treatment, exposure to 500 nM ionomycin, which is able to release all Ca²⁺ contained in intracellular stores, produced only a small increase in [Ca²⁺]_i (<10 nM, n=4). Together these data demonstrate that thapsigargin is able to mobilize almost all the releasable Ca²⁺ sequestered within the cells including all that which is normally releasable by agonist.

We conclude that thapsigargin is an effective tool for emptying intracellular Ca²⁺ stores in submandibular acinar cells which may be used to elucidate the mechanisms which underlie the changes in [Ca²⁺]_i involved in stimulus secretion coupling.

452 P.M. SMITH* and H.E. REED (Oral Biology Unit, School of Dentistry, The University of Liverpool, UK): Amplification of the thapsigargin-evoked increase in [Ca²⁺]_i by acetylcholine in acutely isolated mouse submandibular acinar cells.

In mouse submandibular acinar cells, thapsigargin may be shown to mobilize practically all the Ca²⁺ contained in intracellular pools. Furthermore, it may be shown that slowly unloading the intracellular Ca²⁺ pools using thapsigargin does not normally cause a massive, cytotoxic, increase in cytoplasmic Ca²⁺ concentration because Ca²⁺ is rapidly extruded from the cell across the plasma membrane. Using microfluorimetric techniques to measure [Ca²⁺]_i in single cells we have observed that, in the absence of extracellular Ca²⁺, application of a sub-maximal dose of acetylcholine (500 nM) during the rising phase of the response to thapsigargin caused a 3-4 fold increase in the amplitude of the rise in [Ca²⁺]_i without any significant alteration of the time course of the response. As thapsigargin alone is capable of mobilizing all releasable Ca²⁺, this increase in amplitude is most likely the result of an inhibition of the Ca²⁺ extrusion process by acetylcholine.

We conclude that acetylcholine may be able to inhibit Ca²⁺ extrusion and thus amplify the increase in [Ca²⁺]_i following release of Ca²⁺ from intracellular stores.

453 W.M. EDGAR*, P.M. SMITH and H.E. REED (Oral Biology Unit, School of Dentistry, The University of Liverpool, UK): Calcium extrusion in acutely isolated mouse submandibular acinar cells.

Mobilization of Ca²⁺ from intracellular stores and increased [Ca²⁺]_i is a critical step in stimulus secretion coupling in salivary acinar cells. However, as sustained elevation of [Ca²⁺]_i is cytotoxic, cells must also be able to rapidly remove Ca²⁺ from the cytoplasm. We have measured [Ca²⁺]_i in single isolated acinar cells using microfluorimetric techniques in order to investigate one important component of the Ca²⁺ homeostasis mechanism: Ca²⁺ extrusion across the plasma membrane. The microsomal Ca²⁺ ATPase inhibitor thapsigargin was employed to release Ca²⁺ from intracellular stores and to simultaneously prevent Ca²⁺ reuptake back into the stores. In the absence of extracellular Ca²⁺, thapsigargin stimulated an increase in [Ca²⁺]_i <200 nM which peaked 4 minutes after stimulation. Within 10 minutes [Ca²⁺]_i had returned to prestimulus levels. When Ca²⁺ extrusion was prevented using 2 mM lanthanum (Kwan et al Am J Physiol 258(6) 1006-15 1990), thapsigargin stimulated an increase in [Ca²⁺]_i >2000 nM and no recovery of [Ca²⁺]_i was observed.

We conclude that extrusion of Ca²⁺ across the plasma membrane plays a crucial role in determining [Ca²⁺]_i following Ca²⁺ release from intracellular stores.

454 S M HIGHAM*, L P LONGMAN, E A AGALAMANYI, E A FIELD and W M EDGAR. (School of Dentistry, University of Liverpool): Salivary fluoride determination in dentate patients with xerostomia: A preliminary study.

The benefit of fluoride for oral health is widely accepted and may have greater importance in patients who have low salivary flow rates. The aim of this study was to determine whether patients with a low salivary flow rate also have a low salivary fluoride concentration. 70 dentate patients attending a xerostomia clinic were chosen for this study. 35 had an unstimulated flow rate <0.2 ml/min. Some patients initially selected were rejected since insufficient saliva was obtained for F to be estimated. The patients were asked not to eat or drink for at least 4 hours prior to their appointment. Unstimulated saliva samples were collected by asking the patients to drool into preweighed sterile universal tubes for 5 minutes. Flow rates were estimated by weight and the fluoride concentration of the saliva was determined by ion selective electrode within 1 h of collection. Mean flow rates (ml/min) and F (ppm) were low flow group: 0.134 ± 0.045, 0.036 ± 0.03. Higher flow group: 0.399 ± 0.21, 0.04 ± 0.03. The differences in flow rates between the two groups was significant at the 5% level although no significant difference in F was observed. There was a poor correlation between flow rate and F for both the low flow and higher flow group (Correlation coefficient = 0.011 and 0.176).

The determination of salivary F levels in xerostomia patients may be useful because the results suggest that it is difficult to predict which patients may benefit from F supplements based on salivary flow data alone.

455 L J DAWSON (The University of Liverpool, Department of Clinical Dental Sciences, School of Dentistry, Liverpool, UK): Salivary film velocity at different sites around a single tooth.

Recent work has shown that the salivary film velocity varies at different sites in the mouth (Dawes et al, 1989; J Dent Res 68: 1479-1482). The aim now was to validate the original results, using a modified appliance, and also determine the film velocities at occlusal and interproximal sites around a single tooth, while the salivary flow rate was unstimulated. Two subjects with a missing lower molar were studied. In a single-tooth partial denture, wells of differing diameter but 1.5 mm deep were milled in the denture tooth at buccal, lingual, mesial, distal, and occlusal sites. On separate occasions the wells were filled with 1 mol/L KCl in 1% agarose and shaped with a mould to follow the tooth contour. The half-time for clearance was determined from the best-fitting least-squares line of the potassium concentration remaining in the gel plotted against the square root of time in the mouth. From a knowledge of the gel radius, the clearance half-time into a large stirred volume, and the clearance half-time in the mouth, the film velocity could be estimated. The results showed that there was no significant difference in film velocities determined by this and the original method. Significant differences were found in both subjects in the film velocities at each site around the tooth. In both subjects there was a very fast film velocity in the interproximal areas, indicating that interproximal sites may perhaps no longer necessarily be regarded as stagnation areas.

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W S LIM, N MORDAN*, J J SCIOTE, P M BARBER and N P HUNT (Department of Orthodontics and EM Unit, Eastman Dental Institute, London): A quantitative and qualitative ultrastructural study on rat skeletal muscle.

Several systems exist for the classification of skeletal muscle fibre types, including those based upon physiological properties, enzyme activities and the expression of myosin heavy chain proteins. It has been suggested that fibres can be classified on the basis of their ultrastructural characteristics, especially the Z- and M-line widths and the mitochondrial density. The aim of this study was to assess whether these ultrastructural features could provide reliable criteria for muscle fibre classification. The extensor digitorum longus (EDL), soleus and superficial masseter muscles were removed at their physiological resting length from 6 female, 84 day old Wistar rats. Following fixation in glutaraldehyde and osmium tetroxide, sections were cut, stained and viewed to a Jeol 100CX TEM. Micrographs were taken at $\times 14,000$ magnification and quantitative analysis of the Z- and M-line widths, and the mitochondrial count undertaken using computerised image analysis. Fibre typing of the muscles was also undertaken using histochemical techniques. The results indicated type I fibres had consistently wider Z-line widths compared to type II fibres, whereas M-line widths were more variable. The mitochondria numbers were greater in type I and IIA fibres as compared to type IIB fibres.

It is concluded that on the basis of the combined assessment of Z-line width and mitochondrial density, three major fibre types (type I, IIA and IIB) could be identified from the muscles studied.

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F.M. BOISSONADE*, (Dept. of Oral and Maxillofacial Surgery, Univ of Sheffield, U.K.): Fos expression in the ferret trigeminal model following tooth pulp stimulation

Expression of the nuclear phosphoprotein Fos is very low in the brain stem and spinal cord of normal animals. After noxious stimulation of the periphery, it is rapidly expressed in the appropriate postsynaptic neurones and can be detected using immunocytochemical techniques. The aim of this study was to map the regions of the trigeminal sensory complex involved in nociceptive processing, by examining the distribution of Fos in the trigeminal nuclei following tooth pulp stimulation. Ten adult female ferrets were prepared under anaesthesia (ketamine (25mg/kg) / xylazine (2mg/kg)), so that in a subsequent experiment stimuli could be applied to the upper and lower left canine teeth, recordings made from the left distal muscle and intravenous injections given, without the need for further surgery. Animals were allowed to recover and one week later were anaesthetised via an indwelling cannula with alphaxalone/alphadolone (6 mg/kg), light anaesthesia was maintained with a continuous infusion of the same anaesthetic (6-8 mg/kg/h). In 7 animals 1Hz Stimuli (train of 3, 0.5ms duration at 250Hz) were applied to the upper and lower canine teeth, at 4x threshold (4T) of the jaw opening reflex (4T=20-40 μ A) for a period of 90 minutes and then left for a further 30 minutes under light anaesthesia. In 3 control animals no stimuli were applied. All animals were then deeply anaesthetised (sodium pentobarbitone 65mg/kg, i.p.) and fixed by perfusion with 4% paraformaldehyde. Frozen sections (30 μ m) were cut from 5 mm caudal to 10 mm rostral to obex and incubated with antibodies to Fos (1: 5000, Cambridge Research Biochemicals) diluted in phosphate buffered saline containing 0.1% Triton. Sections were processed using Vectastain Elite ABC kits (Vector Labs). In stimulated animals, Fos positive nuclei were present in the spinal nucleus within subnuclei caudalis and oralis but not subnuclei interpolaris or the main sensory nucleus. In non-stimulated controls only occasional Fos containing nuclei were present.

This supports the view that rostral parts of the spinal nucleus are involved in nociceptive processing.

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Y ALAM*, B ANNAZ, A WASEEM, (Department of Craniofacial Development, UMDS (Guy's), London Bridge, UK): Isolation and characterisation of the Human Oral Cytokeratin 13 gene.

Cytokeratins are a large multigene family of proteins which constitute the major cytoskeletal component in epithelial cells. In man there are about 40 genes which express more than 20 different polypeptides grouped into two subfamilies of type I and type II cytokeratins which are differentially expressed as specific pairs of a type I and type II polypeptide. In the mitotically active basal layer of stratified epithelium, the major cytokeratins are K5/K14 and in the suprabasal layer there is expression of either K1/K10 in cornified epithelia (skin, gingiva, hard palate) or K4/K13 in non-cornified epithelia of oral and lingual mucosa. In several oral disorders such as oral hairy leukoplakia, lichen planus and squamous cell carcinoma, the pattern of K13 expression is altered. An understanding of the mechanism of cytokeratin 13 expression will provide important information about the molecular defects associated with these oral disorders. In an attempt to elucidate the mechanism underlying cytokeratin expression in oral mucosa we have cloned K13 gene by screening a human chromosome 17 specific library. Eleven cosmids reactive with both 5' and 3' ends of K13 cDNA, and therefore containing both ends of the K13 gene, were identified. A comparative analysis of the Southern hybridisation using riboprobes from 3' and 5' ends of the full-length cDNA revealed a single 10kb fragment, containing the entire sequence of K13 gene, could be obtained by digestion with *Bam*HI. Preliminary data on the identification of the cis-regulatory elements responsible for the tissue-specific expression of this gene will be presented.

These results indicate that we have successfully identified and cloned the gene encoding cytokeratin K13 from a human chromosome 17 specific cosmid library, which appears to contain all the regulatory elements necessary for its expression.

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N D JOHNSON*, E LYNCH, A SHEERIN*, M ATHERTON*, D NAUGHTON* and M GROOTVELD* (Conservative Dentistry & Inflammation Research Group*, LHMIC: Multicomponent investigations of the oxidising actions of a chlorite-containing oral rinse.

A multicomponent evaluation of the oxidative consumption of salivary biomolecules by a commercially-available oral rinse preparation containing chlorite anion [ClO_2^- , 2% (W/V)] has been investigated using high resolution ^1H NMR spectroscopy. Unstimulated human saliva samples were obtained from twenty patients. Immediately after collection all samples were centrifuged, supernatant removed, and an equivalent volume of oral rinse added. The mixture was incubated at a temperature of 37°C for a 30 minute period prior to ^1H NMR analysis. Aqueous solutions containing 1.00×10^{-2} mol. dm^{-3} sodium pyruvate, L-cysteine or L-methionine were prepared in 4.00×10^{-2} mol. dm^{-3} phosphate buffer (pH 7.00). Aliquots of these solutions were then treated with an equivalent volume of oral rinse. The results obtained demonstrated that ClO_2^- present in this preparation effected the oxidative decarboxylation of salivary pyruvate (to acetate and CO_2). Experiments conducted on chemicals model systems confirmed the oxidative decarboxylation of pyruvate by this oral rinse, and also demonstrated that the amino acids cysteine and methionine (precursors to volatile sulphur compounds responsible for oral malodour) were oxidatively consumed.

High resolution ^1H NMR spectroscopy is a technique of much utility concerning multicomponent evaluations of the oxidising actions of therapeutically-active agents present in oral rinse preparations towards salivary biomolecules.

* Janina International, UK

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P MAILLOU*, J P NEWTON and S W CADDEN (Department of Dental Prosthetics & Gerontology, University of Dundee, UK): Selective depression of part of an inhibitory jaw reflex in man.

Studies in this laboratory have shown that inhibitory jaw reflexes can be depressed by remote noxious cutaneous stimuli (Cadden S W & Newton J P, *Archs oral Biol*: 30, 177-180, 1994). More recently we have found that noxious stimulation of remote deep somatic structures also depresses this reflex. We now report that the effects of the deep somatic stimuli are more selective and apply to just one part of the reflex response. EMG recordings were made from a masseter muscle in 10 volunteer subjects while they clenched at a constant level with the aid of visual feedback. A reflex inhibition was evoked by applying electrical stimuli to the skin of the upper lip. The recordings were full-wave rectified, averaged and smoothed, and down-going waves representing the inhibitory reflexes were identified. In control sequences, the reflex had a latency of 40 ± 0.8 ms and a duration of 57 ± 3.0 ms (means \pm SEMs). Five minutes later, immediately after a 1 min conditioning period during which the subjects had undertaken vigorous exercise of a lower arm while its blood supply was blocked, the reflex duration was reduced to 43.7 ± 4.3 ms. This was due almost entirely to an effect at the beginning of the reflex as evidenced by a significant increase in its latency (by 13 ms; $P < 0.005$, Student's paired *t*-test). By contrast, in similar experiments when noxious cutaneous conditioning stimuli (submerging a hand in 48°C water) were used, significant effects ($P < 0.05$; $n = 10$) occurred at both ends of the reflex with it starting 10 ± 3.2 ms later and finishing 14 ± 5.1 ms earlier than in controls. *Thus it appears that noxious stimulation of remote deep somatic, but not cutaneous, structures can selectively depress just the early part of an inhibitory jaw reflex.*

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SI CAIN*, RE EVANS and F McDONALD (Department of Orthodontics, UMDS, UK): Supplementary assessment of skeletal pattern using Natural Head Posture.

There has been increasing recognition that facial aesthetics should be assessed in relation to the natural head position (NHP) because it reflects the true appearance. This investigation involved 20 children (16 girls, 4 boys) requiring a lateral skull radiograph as part of their orthodontic diagnosis. They were of Caucasian descent, with a mean age of 12.4 years (range 10-16 years). A series of profile photographs were taken of each subject, registered in NHP. The photographs were digitised using a digital camera and software system. This allowed the indirect transfer of an extracranial reference line to the digitised radiograph. A cephalometric analysis was undertaken with reference to the transferred true horizontal. The NHP was shown to have an acceptable reproducibility which was 2.5 times less variable than conventional intracranial reference lines. Conventional analyses of the anteroposterior skeletal pattern were compared with a supplementary analysis based on the true horizontal (Hor). A skeletal Class I normal range for AB-Hor of 6° - 11° , originally derived from pre-existing data, was supported. The AB-Hor angle (6° - 11°) showed the highest agreement (74%) with the clinical impression and was the most sensitive test for a Class II skeletal pattern but its specificity index was low (37%). No analyses recorded both high sensitivity and specificity indices for the sagittal skeletal pattern.

As yet NHP is not sufficiently accurate and reproducible to influence clinical registration.

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C IRWIN*, C RICE*, M SMYTH* and S SCHOR* (Queen's University, Belfast, Dundee Dental Hospital): The effects of IL-18 on fibroblast-induced collagen gel contraction are dependent on the presence of serum.

Cell-induced collagen gel contraction is considered to closely reflect wound contraction *in vivo* and as such provides a simple model to study the contraction process. Human gingival fibroblasts were incorporated into collagen gels to investigate the effects of the inflammatory cytokine Interleukin-18 (IL-18) on gel contraction. Over the concentration range 10-1000 U/ml IL-18 significantly inhibited ($p < 0.01$) gel contraction in serum-containing cultures (2.5% FCS), but stimulated contraction ($p < 0.01$) to a striking degree under serum-free conditions. There were no significant differences in the effects of the various concentrations of IL-18 studied. Addition of indomethacin (2×10^{-4} M), an inhibitor of prostaglandin synthesis, partially reversed the inhibitory effects of IL-18 in the presence of serum, but had no effect on the IL-18-induced stimulation of contraction in the absence of serum.

Thus the effects of IL-18 on collagen gel contraction, and the mechanisms through which these effects are mediated, are dependent on the presence or absence of serum components in the culture medium.

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S SPRAGUE*, S MILSOM, D DYMOCK, W.G. WADE and A.J. WRIGHTMAN (Dental School, Bristol, UK and Pure and Applied Biology, UWCC, Cardiff, UK): PCR-RFLP and protein profiles for differentiation of *P. intermedia* and *P. nigrescens*

The differentiation of *P. nigrescens* and *P. intermedia* is not easy by conventional means. Protein profiles and ribotyping have been proposed to be reliable methods but these techniques are relatively time-consuming. The aim of this study was to evaluate restriction enzyme digestion of amplified 16S rRNA genes (PCR-RFLP) as a method for differentiating *P. intermedia* and *P. nigrescens*. 16S rRNA sequences were obtained from GenBank via SEQNET (Daresbury, UK) and the GCG MAPSORT programme used to screen enzymes for their ability to discriminate between the two species. On this basis restriction enzymes *Cfo*I, *Hinf*I and *Taq*I were chosen for use. *P. nigrescens* strains ATCC 33563⁺ and ATCC 25261, *P. intermedia* ATCC 25611⁺ and 31 fresh isolates were included in the study. Genomic DNA was extracted, 16S rRNA genes amplified by PCR with primers 27f and 1492r and the PCR products cut with the restriction enzymes. Whole cell protein profiles were generated by SDS-PAGE using the PhastSystem. Protein profiles identified 13 of the fresh isolates to be *P. intermedia* and the remainder as *P. nigrescens*. The PCR-RFLP patterns were in agreement and were consistent with the patterns predicted by MAPSORT.

*In conclusion, PCR-RFLP provides a rapid and reliable method for the differentiation of *P. nigrescens* and *P. intermedia*.*

- 464** A.J. MIGHELL*, P.A. ROBINSON, W.J. HUME. (Division of Dental Surgery, Leeds Dental Institute, Leeds, UK): PCNA immunoreactivity in multinucleated cells of giant cell fibroma and peripheral giant cell granuloma.

Multinucleated cells (MnC) in giant cell fibroma (GCF) are thought to originate from local fibroblasts (Odell *et al.*, *J Oral Pathol Med* 23: 284-7, 1994), whereas MnC in peripheral giant cell granuloma (PGCG) probably form by fusion of differentiated mononuclear cells derived from bone marrow (Bonetti *et al.*, *Oral Surg* 70: 471-5, 1990). We hypothesised that the two MnC populations may have different patterns of nuclear immunoreactivity to proliferating cell nuclear antigen (PCNA), a marker of deoxyribonucleic acid (DNA) synthesis and repair. To test this hypothesis, we investigated archival, formalin-fixed and paraffin-embedded specimens of GCF (n=6) and PGCG (n=6) for PCNA immunoreactivity. Consideration was given to the complexities of PCNA immunohistochemistry (McCormick *et al.*, *Histopathology* 22: 543-7, 1993) with evaluation of different anti-PCNA antibody dilutions, and comparison of microwave antigen retrieval (AR), autoclave AR and no AR. Optimum immunoreactivity was observed after autoclave AR, and the results described were consistent for anti-PCNA antibody dilutions between 1 in 10 and 1 in 600 (v/v). PCNA immunoreactivity was observed in nearly all nuclei of each GCF MnC. However, within a single MnC, nuclei could be either PCNA+, or PCNA- with marked variation in staining intensity. By contrast, PGCG MnC nuclei were PCNA-, although intensely PCNA+ nuclei were observed around MnC. The patterns of PCNA immunoreactivity described support: 1) GCF MnC formation by either fusion of recently cycling mononuclear cells, or intracellular cell cycling without cytoplasmic division. 2) PGCG MnC formation by fusion of differentiated mononuclear cells. Supported by the MRC.

- 466** A.WIGHT¹, V.SIVARAJASINGAM¹, P.RICE², J.COWPE¹ & G.OGDEN¹ (Dept of Dental Surg & Perio, Univ of Dundee¹, Tayside Alcohol Problems Service², UWCM, Cardiff UK): Quantitative cytology of buccal mucosal cells: effect of alcohol

The aim of this study was to measure the effect of alcohol consumption and smoking on nuclear and cytoplasmic areas of oral buccal mucosal cells. 25 individuals ranging from 22 to 61 years attending a centre for alcohol related problems and 11 controls ranging from 28 to 76 years were included in the study. Detailed alcohol and smoking habits were recorded for each subject. A full blood count and liver function tests were also carried out. Ethical approval was obtained from the Tayside Medical Ethics Committee. For the purpose of analysis, subjects from the alcohol centre were placed into three distinct groups (regular drinkers and smokers, regular drinkers and nonsmokers and binge drinkers and smokers). Smears from clinically normal right buccal mucosa were taken in a reproducible manner from all subjects, transferred to a clean, dry slide, fixed and stained with Papanicolaou. Nuclear (NA) and cytoplasmic (CA) areas of 50 randomly selected cells from each slide were measured using the VIDS V image analysis system¹. Two sample t-test showed a significant reduction in the buccal mucosal cytoplasmic areas ($p < 0.05$) and a significant increase in the nuclear/cytoplasmic ratio ($p < 0.05$) of all three alcohol groups compared to the controls. Nuclear area, cytoplasmic area and nuclear/cytoplasmic ratio within the alcohol groups showed no significant difference ($p > 0.05$). In conclusion, alcohol either alone or in combination with smoking significantly reduced the CA of buccal mucosal cells. A significant increase in the NA was only apparent in the regular alcohol and smoking group.

Acknowledgement: VS was in receipt of a Vacation Research Grant, Scottish Home and Health Department. ¹ Analytical measuring system, Cambridge.

- 468** A.J. WIGHT¹, J. DEWAR¹, D.P. LANE¹, G.R. OGDEN¹ (Depts of Dental Surgery & Perio¹, Radiotherapy & Oncology², C.R.C. Labs³, Univ of Dundee, UK): Can oral cytology detect p53 in response to DNA damaging agents?

Wild type p53 is thought to accumulate in response to DNA damage. This study aimed to investigate if DNA-damaging agents (radiotherapy and cytotoxic chemotherapy), used in the treatment of malignant disease, induced increased p53 expression which could be detected by oral cytology. Local Ethics Committee approval was granted for taking smears from the oral mucosa of such patients, before and at various time intervals during and after treatment. Smears were obtained from normal buccal mucosa of: a) 9 patients whose oral mucosa was irradiated during the treatment of a tumour in the maxillofacial region and b) 15 patients receiving cytotoxic chemotherapy for a tumour distant from the oral cavity. The smears were fixed and stored at -70°C until required. An avidin-biotin method⁴ was employed for the immunocytochemical detection of p53, using the antibodies DO1 and CMI1, which recognise both wild and mutant p53. p53 was not detected in any of the smears, but was identified in the positive control (PANC-1⁵). In conclusion, oral exfoliative cytology was unable to detect increased expression of p53 in these patients, either because p53 was not induced or was a transient phenomenon and therefore not detectable in exfoliated cells.

¹Vale Smear Fix, Vale Labs, UK ²Vectastain ABC Kit, Vector Labs, UK ³ECACC 87092802

- 470** M. AWAD¹, JR HEATH and DC WATTS, (University of Manchester, UK): Polymerisation shrinkage kinetics in Dual-Cure Resin-Composites.

Dual-Cure resin-composites are often formulated with increased monomer proportions relative to restorative composites. The aim of this study was to evaluate polymerisation shrinkage-kinetics over a range of temperatures for eight representative materials. Pastes were mixed rapidly and fabricated, within 45-60 s, as disks (7 mm diam. x 1.5 mm) within an upgraded shrinkage-measurement instrument (Watts & Cash, *Dent Mater* 7, 281, 1991). The temperature of the specimen-mounting platform was fixed ($\pm 0.3^\circ\text{C}$) at 23, 30, 37, 44 & 70 °C during separate measurement runs. Cure was initiated by visible-light application (Luxor, ICI plc) for 60 s. Volumetric anisotropic shrinkage values (%) were recorded digitally from uniaxial LVDT measurements for 1 hour. For each temperature, the time-dependence of shrinkage was initially linear for up to 2 min followed by a more gradual approach to a final value S_p . A linear regression ($p < 0.01$) of S_t with temperature was obtained, with one exception: for which $p < 0.05$. $\{S_t\}_{77}$ ranged from 2.21 - 5.72 %. $\{S_p\}_{77}/\{S_t\}_{77}$ ranged from 0.64 - 0.88. Relative to restorative composites, Dual-Cure resin-composites may typically exhibit slower and sub-optimal final-cure characteristics at oral temperatures combined with greater magnitudes of final shrinkage.

- 465** G.BOWES¹, V.SIVARAJASINGAM¹, J.CHISHOLM¹, F.RICE² & G.OGDEN¹ (Dept of Dent Surg & Perio¹, Univ of Dundee, Tayside Alcohol Problems Service², UK): Cell sampling from whole saliva: reproducibility and influence of alcohol abuse.

The aim of this study was to evaluate the reproducibility of Cytospin (Shandon, Runcorn, UK) preparations from whole saliva, with respect to cytomorphology. Saliva samples were collected from 13 patients attending an alcohol problem service and 9 controls. In addition smears were taken from normal buccal mucosa in the alcohol group, for comparison with the Cytospin preparations. Saliva samples were treated with Sputasol (Oxoid, London), then centrifuged using Cytospin (1000 rpm for 5 minutes). The preparations and smears were immediately fixed and stored at -70°C until required. The Cytospin preparations (two from each patient) and smears were stained with Papanicolaou and mean nuclear (NA) and cytoplasmic (CA) values calculated for 50 cells using the VIDS V image analysis system. Cytochrome expression was also assessed. When the two Cytospin preparations from the same saliva were compared, using a two sample t-test, no significant difference in either mean NA or mean CA value was found for both alcohol and control patients. For alcohol patients comparing Cytospin preps. with smears, a significant increase in mean NA, but not mean CA was found for the smears. When the Cytospin preparations for alcohol and control patients were compared a highly significant reduction in mean CA ($p < 0.001$) was found for the alcohol group. In conclusion this study has shown that for Cytospin preparations (i) whole saliva produces adequate cell harvest (ii) mean NA and CA values are reproducible and (iii) mean CA reduces in those who abuse alcohol. G.B. was in receipt of a Vacation Research Grant from the Scottish Office Home & Health Department.

- 467** J.COWPE¹, E.CONNOR², M.GREEN³, & G.OGDEN¹. Dept. Oral Surg & Pathol¹, UWCM Cardiff², Dept. of Dental Surg & Perio³, Univ of Dundee UK): Quantitative cytology of oral smears: effect of long term storage.

The aims of this study were to see whether a significant variation occurred in quantitative cytomorphological analysis of oral smears, with respect to duration and temperature of storage. Smears were taken from normal buccal mucosa (BM) and normal dorsal tongue (DT) of 5 healthy patients (age 20-30 yr) and stored at either 4°C or -70°C for up to one year. After the following time intervals a smear was taken from each site for each temperature: 1 day, 1 week, 1 month, 3 months, 6 months, 1 year. The nuclear (NA) and cytoplasmic (CA) area values of 50 randomly selected cells were then calculated using the VIDS V image analysis system. All smears had previously been processed for cytokeratin expression using a standard protocol (Vectastain, Vector Labs., England) and the pan-epithelial marker LP34. Storage at -70°C showed little variation of mean NA or CA with time for both buccal mucosa and dorsal tongue. At 4°C, there was evidence for an increase in mean NA and mean CA values for both sites. Confidence intervals were calculated using the Wilcoxon score. In conclusion, archival oral smears stored at -70°C can be utilised for quantitative cytomorphological analysis of nuclear and cytoplasmic area for up to one year.

- 469** T-Y YANG*, P B ROBINSON and R D RAWLINGS (King's College and Imperial College, London): Assessment of PVD TiN coatings for dental instruments.

The aim of this study was to compare the cutting efficiency of stainless steel (SS) curettes and instruments with those coated with titanium nitride (TiN) by physical vapour deposition (PVD). A jig, which was fitted on to a horizontal milling machine, was constructed to provide controlled cutting of a rotating (40rpm) ceramic cylinder (macro glass-ceramic). A constant cutting force of 2.3N was applied to the instruments through the balance system of the jig. The advance of the instruments into the ceramic was sensed by a transducer and plotted on a chart recorder. During a complete test each instrument made a cut of approximately 20m through the ceramic. The roughness of the cut ceramic surface was measured with a Taly-Surf. The surface roughness of extracted human teeth prepared with coated and uncoated curettes was also measured. The results showed that the cutting efficiency of PVD TiN coated instruments was better than with SS. PVD TiN instruments also produced a smoother ceramic surface which was consistent with the findings that root surfaces prepared with SS instruments had an average roughness of 2.65µm compared with 0.20µm for TiN. In conclusion, PVD coated dental instruments improve the cutting performance by remaining sharp for longer than SS. They create smoother root surfaces which may delay the nucleation of calculus.

- 471** J VERRAN* and C MARYAN (Dept Biological Sciences*, and Dept Polymer Technology, Manchester Metropolitan University, UK): Adhesion of Candida to acrylic and silicone of different surface topography.

Candida albicans adheres in higher numbers to roughened denture material (acrylic) than to smooth (Verrian *et al.*, Biofouling, 3:183-19, 1991). Silicones used for facial prostheses are also susceptible to microbial colonisation. This investigation compares C.albicans adhesion to acrylic and silicone of different surface topography. Silicone¹ was processed against a sheet of acrylic². 'Rough' silicone was processed against a vacuum mixed dental stone. Acrylic was roughened manually using medium grade emery paper. R_a values of 0.02, 1.26 and 1.93 µm were obtained for smooth acrylic, roughened acrylic and dental stone respectively (silicone samples were too thin to be used). Washed, standardised yeast cell suspensions were incubated with surfaces for 1h at 37°C, after which time adherent cells were stained with acridine orange, and counted, using incident beam fluorescent microscopy. There was no significant difference in Candida adhesion to smooth (acrylic and silicone) surfaces (although the silicone was slightly more hydrophilic). Significantly higher ($p < 0.005$) numbers of adherent cells were observed on roughened surfaces (Silicone/acrylic) than on smooth surfaces. Silicones used in facial prostheses are normally processed against stone. The resultant surface roughness may facilitate microbial colonisation of the silicone, and should therefore be minimised.

¹Silekin 2, Chas. F. Thackray, Leeds ²Perspex (polymethylmethacrylate), ICI Darwen

472 BJ MILLAR, DJ WOOD*, NL BUBB, I GABRIELSON (King's College Dental School & Brunel University, London): Tear strength of hydrophilic and hydrophobic polyvinylsiloxane materials

Addition cured silicone impression materials have poor surface wetting and manufacturers have added intrinsic surfactants in an attempt to overcome this. This study aims to compare the tear strengths of hydrophilic and hydrophobic polyvinylsiloxane impression materials. Samples of 12 commercially available polyvinylsiloxane materials were tested for tear strength according to ISO 4648(C) specification. Materials were tested for surface wetting using a Dynamic Contact Angle apparatus based on the Wilhelmy technique. Tear strengths ranged from 5.2-9.2 N/m and the advancing contact angle ranged from 87.4°-111.3°. There was a direct relation between the median tear strength values and the advancing contact angles which was significant (Spearman's Ranking Correlation Coefficient $R=0.794$, $P<0.01$). It therefore appears that the inclusion of an intrinsic surfactant adversely affects the physical properties of these materials. In conclusion, addition cured silicone impression materials which showed lower advancing contact angles, and are therefore more hydrophilic, have lower tear strengths.

474 J T LILICO* and J F McCABE (Department of Restorative Dentistry, Newcastle upon Tyne Dental School, UK): Effects of thermocycling and water storage on resin to alloy bonds

Current trends in Restorative Dentistry highlight the importance of bonding resin to alloy. This study determined which combination of alloy/adhesive/resin produced the highest value of bond strength and is able to resist water storage and thermocycling. Samples of a gold alloy A. Matcraft 45¹ and a Ni Cr alloy B. Unitech Forte² were air abraded and steam cleaned, composite was then bonded to each with 1. Art Bond³, 2. Metabond⁴, and 3. All Bond², and tested in shear (1mm/min) at either 24h or after 5 days and subjected to 250 thermocycles (5°C, 23°C and 60°C) with a dwell time of 1 minute. Test groups of 30 or 12 specimens were used. Results showing mean bond strengths and standard deviations for combinations before and after thermocycling are as follows (all values in MPa): 24h test: A1 19.6 ± 4.1 , A2 22.4 ± 4.5 , A3 17.9 ± 3.3 , B1 19.5 ± 4.0 , B2 17.5 ± 8.6 , B3 23.8 ± 3.9 . Thermocycle test: A1 19.9 ± 6.6 , A2 25.1 ± 9.1 , A3 23.1 ± 5.8 , B1 28.0 ± 5.9 , B2 22.5 ± 12.3 , B3 32.2 ± 6.6 . Statistical analysis (ANOVA) revealed that thermocycling increased the bond strengths in all cases and that this was significant in all cases except A1 ($\alpha<0.05$). Highest bond strengths were produced using combination B3.

¹ Johnson Matthey, ² Orthomax UK, ³ Coltene AG, ⁴ Parkell Bio Materials, ⁵ Bisco Inc.

475 NJ WOODLEY*, SM GRIFFITHS, EH DAVIES, GJ PEARSON, HN NEWMAN (Eastman Dental Institute, London): A preliminary evaluation of in vivo fluoride uptake by overlay denture abutments from two topical agents.

6 patients (3 male, 3 female, age range 58 to 74 years, mean 67.1 years) with advanced tooth wear restored with provisional overlay dentures entered the study. Each patient required re-contouring of one or more of the abutments before the construction of definitive dentures. A dentine sample approximately 1 x 1 x 0.5mm was dissected from each abutment using a fine diamond bur under water spray. The patients were provided, at random, with either:

1. Amine fluoride/stannous fluoride (0.14 F) toothpaste and amine fluoride/stannous fluoride (0.025% F) mouth rinse¹
- or 2. Sodium fluoride (0.14 F) toothpaste and sodium fluoride (0.025% F) mouth rinse.

Both operator and patient were blind as to the fluoride regime. After one month a second sample was dissected from the abutments from an area adjacent to the first biopsy.

The dimensions of each sample were measured and then dissolved in 1M hydrochloric acid. Fluoride determination was carried out using the differential electrode cell method (Tyler J and Poole D, *Arch Oral Biol* 34: 985-988, 1989). Fluoride uptake per surface area of the dentine was calculated.

The fluoride uptake by the dentine in patients using the amine fluoride/stannous fluoride regime ranged from 0 to 60 µg/cm² and in patients using the sodium fluoride regime ranged from 4 to 18 µg/cm². This research was sponsored by GABA International Ltd., Switzerland.

¹ Meridol, GABA International Ltd.

477 H TORABZADEH* and Y E Y ABOUSH (Department of Oral and Dental Science, University of Bristol, UK): Translucency of light-activated glass-ionomer restoratives and a compomer

This study assessed the changes in the translucency of the light activated glass-ionomers Fuji II LC¹ and Photac-Fil² and the compomer Dyract³ over a period of 1 year. For comparison, a conventional glass-ionomer (Fuji Cap II)⁴ and a composite (Tetric)⁵ were used. Discs (5 per group), 5mm in diameter and 1.5mm thick, were made and their translucency assessed immediately after preparation, and at 1 week, 6 months and 1 year thereafter. The translucency (expressed in terms of optical density/mm) used a photometric technique which employed a light dependent resistor to measure the light intensity. The data were subjected to ANOVA and the Scheffe test at the 0.05 level. The light activated glass-ionomers behaved in a similar manner to the composite where there was no change at 1 week, but the translucency value improved significantly at 6 months and remained at this level at 1 year. The translucency of the compomer did not change significantly over the test period. The translucency of the conventional glass-ionomer continually improved over the 1 year period, albeit that the results at 1 week and 6 months were not significantly different. At 1 year, Photac-Fil was significantly more translucent than the other materials, Fuji Cap II was the least translucent material, and the translucency values for Fuji II LC, Dyract and Tetric were not significantly different. It is concluded that the translucency of the glass-ionomers and composite improves over time whereas the compomer acquires optimum translucency immediately after preparation.

¹ GC-International, ² ESPE, ³ Dentsply, ⁴ Vivadent.

473 R G JAGGER* and M G J WATERS* (Cardiff Dental School, UK): A modified RTV silicone soft lining material with low water absorption.

Room-temperature vulcanising (RTV) denture soft lining materials have been associated with high water absorption. A previous investigation demonstrated that the high absorption of an experimental RTV silicone lining material was due to the filler (Waters M G J, and Jagger R G, *J Dent Res* 73: 807). The aim of present study was to modify the filler content of the experimental material in order to produce a material with low water absorption. Three new formulations were prepared, each containing different hydrophobic silane treated silica fillers. The materials were cured for 24 hours at room-temperature. Water absorption, solubility and volume change of the formulations were determined using standard experimental techniques. All formulations demonstrated greatly reduced water uptake at 3 months. One formulation was favoured because of its better handling characteristics and water absorption of 1.82%, solubility of 0.75%, and volume change of 1.13% at equilibrium. Modification of the filler content of an experimental RTV silicone soft lining material has produced a material with low water sorption characteristics.

474A MJ WHALLEY MJ, (Dept. of Clinical Dental Sciences, The University of Liverpool)

The determination of elastic modulus for 17 composite restoratives

9 standardised specimens of each of 17 composite restorative materials and one amalgam alloy were prepared for mechanical testing according to ISO4049. A 3 point bending test was performed on 3 samples of each material at each of 1 hour, 1 day and 1 week post-curing. A Hounsfield testing machine was used to compress the samples with a crosshead speed of 0.1mm/min; the samples being supported by metal rods positioned exactly 20mm apart. An extensometer was positioned beneath the centre of the sample and the force applied directly above this. The extensometer was set to zero with a 2N preload on the sample for all tests. The elastic modulus for each test was determined by the standard formula. The mean values (GPa) recorded for the all the composite resin materials were 4.0 (1.2-12.5) at 1h; 7.2 (3.1-18.5) at 1 day and 10.4 (4.2-28) at 1 week. The mean values recorded for the amalgam alloy tested were 17.4 (15.5-20.5) at 1h; 51.6 (33.9-63.6) at 1 day and 59.3 (56.4-64.4) at 1 week.

Currently available composite restorative materials have a wide range of elastic moduli and there are significant changes in the values even after 24 hours setting.

476 E MATHER*, A W G WALLS, J F McCABE (Dental School, Newcastle upon Tyne, UK): The physical properties of a glass ionomer cement used for ART.

Automatic Restorative Treatment is a method for management of caries in less well developed countries of the world where sophisticated dental equipment is not available. Caries is simply excavated from teeth and the resultant defect restored using a glass ionomer cement. One manufacturer has produced a commercial glass ionomer cement which, it is claimed, has enhanced physical properties relative to a normal material. The aim of this study was to measure some of the physical properties of this new material and compare them to a conventional glass ionomer from the same manufacturer, and to a composite resin. The materials used were Fuji IX¹ (FIX) and Fuji II¹ (FII) glass ionomer cements and Tetric² (T) a composite resin. The properties that were measured were: setting kinetics (differential thermal analysis method), flexural strength (24h and 30d Instron machine) and fluoride release (specific ion electrode method). The setting reaction of FIX was slightly, though significantly slower than FII (time to T_{max} 3.96 ± 0.06 mins of 3.12 ± 0.1, time to 5% T_{max} 8.66 ± 0.24 mins of 7.11 ± 0.51). The flexural strength of FIX after 30d (24.4 ± 8.5 MPa) was significantly greater than that for FII (7.6 ± 1.7 MPa) $p<0.001$ Student t-test, although it was much lower than that for Tetric (117.1 ± 15.9 MPa). The pattern of fluoride release for the glass ionomers was similar over a period of 30d, both products releasing ~ 20µg (cumulative).

Fuji IX has significantly greater flexural strength than Fuji II, and a similar pattern of fluoride release. The setting of Fuji II is slightly slower.

¹ GC Japan, ² Vivadent, Liechtenstein

478 Y E Y ABOUSH*, H TORABZADEH and A R LEE (Department of Oral and Dental Science, University of Bristol and MRC Dental Group, UK): One-year fluoride release from fluoride-containing restorative materials.

This study measured the amount of fluoride released from three light-curing glass-ionomers (FLC¹, P², V³) a chemically-setting glass-ionomer (F⁴), a compomer (D⁵) and a composite (T⁶) over a period of 1 year. Five discs (7 mm x 2 mm) were made from each material. These were sequentially immersed in 4 mL portions of deionised water at 37°C. Before each measurement, the sample was rinsed with 1 mL of deionised water. The amount of fluoride released was measured using an Orion Model 901 microprocessor digital analyzer and converted into µg/cm². The fluoride release was measured 86 times during the test period. The cumulative fluoride release after 360 days was (mean ± SD): P (1154 ± 86); V (593 ± 41); FLC (580 ± 17); F (480 ± 42); D (87 ± 17); T (22 ± 2). The amount of fluoride released over the last 24 hour period was: P (5.0 ± 0.8); V (2.5 ± 0.3); FLC (2.3 ± 0.2); F (1.3 ± 0.2); D (0.21 ± 0.04); T (0.23 ± 0.02). The data were subjected to ANOVA and the Scheffe test at 0.05 level. For the two sets of data, the values for V and FLC, D and T were not significantly different. All other comparisons were significantly different. It is concluded that the light-curing glass-ionomers tested release more fluoride than a chemically-setting glass-ionomer, a compomer or a composite.

¹ Fuji II LC, ² Fuji-Cap II (GC-International); ³ Photac-Fil Aplicap (ESPE); ⁴ Vitremer (3M); ⁵ Dyract (Dentsply); ⁶ Tetric (Vivadent).

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X ZHANG*, MA WILSON and MW WILSON (Restorative Dentistry, University of Manchester, UK): The effect of mouthwashes on the surface hardness of resin composite and glass ionomer cement.

This *in vitro* study investigates the effect of mouthwashes on the surface hardness of specimens of a resin composite (RC) and glass ionomer cement (GIC). 30 specimens (10x5x3mm) of a RC¹ and GIC² were prepared and divided at random into 5 groups. Each group of RC and GIC specimens was allocated at random to one of 4 mouthwashes^{3,4} or water (W). Specimens were tested for surface hardness using the Wallace indentation test - 10 measurements being taken from each specimen. Specimens were immersed in the allocated mouthwash or water 2 min/day for 3 months and the surface hardness remeasured. Results (mean [s.d.]) for the RC: baseline - Cora, 1.0675 (0.0090); Plax, 0.9602 (0.1276); Lis, 1.0375 (0.1697); Mac, 0.9815 (0.1000); W, 0.8652 (0.0477); 3mth - Cora, 1.1581 (0.1381); Plax, 1.1417 (0.0730); Lis, 1.2262 (0.1070); Mac, 1.2508 (0.0454); W, 0.8884 (0.0413). Results (mean [s.d.]) for the GIC: baseline - Cora, 0.8157 (0.078); Plax, 1.0387 (0.1052); Lis, 0.9378 (0.1174); Mac, 0.8880 (0.0627); W, 0.824 (0.0714); 3mth - Cora, 0.8929 (0.067); Plax, 1.0413 (0.12); Lis, 1.0517 (0.0589); Mac, 1.0830 (0.1513); W, 0.8240 (0.0713). Using Scheffé statistical procedure the results indicate that the surface hardness of the RC was significantly reduced ($P < 0.05$) by Cora, Plax, Lis and Mac. The surface hardness of the GIC was significantly reduced by Lis and Mac. It is concluded that the use of certain mouthwashes may be found to reduce the surface hardness of resin composite and glass ionomer cement.

1, TPH, De Trey Dentistry, 2, Chemfil, De Trey, 3, Conodyst (Cora), Smith, Kline & Beecham; 4, Plax, Colgate; 5, Listerine (Lis), Warner-Lambert; 6, Macleams (Mac), MacLeams.

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P W-Y CHEUNG*, D N FEMMER and P B ROBINSON (King's College London): Thermal shock in a restored premolar: a three-dimensional finite element analysis.

A three-dimensional finite element (FE) model was developed to predict the evolution of the temperature distribution in a sound and restored premolar subject to thermal shock. A finite element model incorporating elements representing enamel, dentine and a Class I amalgam restoration was constructed. The FE method program ABAQUS was used to predict isotherms in a sound and restored tooth during transient heat conduction corresponding to high (65°C) and low (0°C) temperature thermal cycles each of 15s duration. The results showed that a maximum temperature of 56.2°C was reached at the buccal tip of the crown after 3.6s in both the sound and restored tooth. Isotherms showed that after 2s in either cycle the temperature gradient in the restoration was much lower than in the corresponding region of the sound tooth. After 12s the effect of the restoration on the isotherms was less apparent. The model will be used to investigate thermally-induced stresses at the interface between the restoration and tooth.

481

NM NUTTALL* (Dental Health Services Research Unit, University of Dundee, UK): Previous tooth loss as an indicator of future dental extractions.

There are several plausible models for the pattern of tooth loss in adult dental patients. The aim of this study was to explore the relationship between the number of existing teeth and the subsequent experience of tooth extractions in adults to determine whether previous tooth loss is a reliable indicator of future experience of extractions.

The subjects were 720 Scottish adults who were dentate in 1978. All were dentally examined as part of the Adult Dental Health survey in 1978 and gave permission for their NHS dental treatment records to be monitored longitudinally. 20 patients are known to have been lost to follow up. Subjects who had fewer than 20 teeth in 1978 ($n=201$) experienced an average of 2.7 extractions in the next 10 years compared with an average of 1.1 extractions among those with 20 or more teeth ($n=499$) (Mann-Whitney U Test, $p > 0.001$).

The findings indicate that previous tooth loss appears to be an indicator of future dental extractions.

Supported by the Scottish Office Home and Health Department.

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G LINDEN*, C IRWIN and B MULLALLY (School of Clinical Dentistry, Queen's University of Belfast): Clinical characteristics of sites losing periodontal attachment in regular dental attenders.

The aim was to investigate factors associated with the progression of periodontitis. Twenty three regular dental attenders (13 men, 10 women) enrolled in a longitudinal study of periodontal disease were examined at an interval of 5.5 (SD 0.6) years and the mean age at the second examination was 41.1 (SD 7.3) years. Bleeding on probing, subgingival calculus, probing depth and clinical attachment level were recorded at four proximal sites per tooth. There were 2089 sites which had complete data and 444 (21%) of these had periodontal breakdown (periodontal attachment reduced by ≥ 2 mm between assessments). Odds ratios (OR) were calculated for the association between clinical characteristics at the initial assessment and subsequent loss of attachment. There was an increased risk of periodontal breakdown in sites which bled at the first assessment (OR 1.49; c.i. 1.2-1.85). A higher proportion of sites with profuse bleeding (90.5%) subsequently lost attachment than those with pin point (24.8%) or no bleeding (19.1%), Chi square 15.1; $P=0.005$. The presence of calculus was associated with subsequent breakdown (OR 2.2; c.i. 1.66-2.88) while periodontal pocketing (≥ 4 mm) was not (OR 0.92; c.i. 0.67-1.26). Shallow non-bleeding sites were as likely as deeper (≥ 3 mm) bleeding sites to lose attachment (OR 1.12; c.i. 0.74-1.69).

It is concluded that the clinical characteristics of sites were only moderate predictors of subsequent periodontal attachment loss.

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B MULLALLY* and G LINDEN (School of Clinical Dentistry, Queen's University of Belfast): Furcation involvement in subjects with periodontitis who smoke.

The aim was to investigate the relationship between cigarette smoking and furcation involvement of molar teeth. A consecutive group of 50 smokers (>10 cigarettes/day for >10 years), aged 35 or over, were recruited from referrals to a periodontal department and age and gender matched with never smokers. The smokers consumed an average of 18.0 (SD 6.7) cigarettes per day and had smoked for 20.7 (SD 6.5) years. Radiographs of all molar teeth were examined twice by an investigator who was blind to the smoking status. Differences between the radiographic assessment of furcation involvement at the two examinations were subsequently resolved by discussion between two investigators before the code was broken. The smokers had almost the same number of molar teeth 6.7 (SD 2.6) as the never smokers, 7.3 (SD 2.3), ($t=1.2$, $P=0.22$). A higher proportion of the smokers (72%) had evidence of furcation involvement than the never smokers (36%), Chi square 13.0, $P=0.0003$. The odds ratio for a smoker having one molar with radiographic evidence of furcation involvement was 4.6 (c.i. 2.0-10.6). Smokers had more molars with furcation involvement 1.94 (SD 1.7) compared with never smokers, 0.94 (SD 1.4), ($t=3.1$, $P=0.003$).

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OS' AI-HOKAIL*, TF WALSH and LG DAVIS (Department of Restorative Dentistry, University of Sheffield, UK): The relationship between dental panoramic tomography and basic periodontal examination.

This investigation was undertaken to determine the relationship between bone loss observed on dental panoramic tomographs (DPT) and the basic periodontal examination (BPE). To improve accuracy and reliability, computer enhancement and digital analysis were used. A total of 199 posterior sextants on DPT radiographs from 50 patients (29 female and 21 male) with a mean age of $42 \pm SD$ were examined.

The results showed a statistically significant relationship between BPE and measurement from the enamel-cement junction to the bone margin (A), which correlated closely with the BPE. The relationship between A and BPE was found to fit the following equation:

$\sqrt{A} = B_i + F$ where, F was a constant and B_i the coefficient corresponding to the i th codes of BPE.

It was concluded that there was a close correlation between the BPE and DPT radiographs of the subjects. The bone loss corresponded well to the main BPE codes. This data would support the use of dental panoramic radiographs as a periodontal screening tool, which is contrary to current advice (Hirschmann et al. *Br Dent J* 176: 324, 1994).

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M B CHABANSKI, D G GILLAM, J S BULMAN and H N NEWMAN* (Eastman Dental Institute University of London, UK): Clinical evaluation of patients self-reporting tooth sensitivity.

The prevalence of tooth sensitivity (Cervical Dentine Sensitivity (CDS)) in adult populations indicates that 8-35% of subjects reported CDS depending on the population studied and the methodology used. Few studies, however, have reported on the prevalence of CDS in periodontal patients. The aim of the study was to determine the prevalence, severity and distribution of CDS in patients referred for specialist periodontal diagnosis. 51 patients (27M/24F, Mean Age 48.5 years [SD 11.63]) who gave their informed written consent were clinically evaluated for CDS using recognised methods of assessment, namely Yeaple probe, cold air blast and subjective evaluation. Other clinical variables (e.g., plaque and recession scores) were also recorded at this visit. Regression analysis and correlation coefficients were used to determine the relationship between the clinical variables. The results demonstrated a prevalence of CDS ranging between 72.5% to 98% of patients, with no significant gender difference. Results for the distribution of tooth types showed that molar teeth were mainly affected, followed by left canines and premolars. No correlation was noted between plaque, recession, response to tactile or thermal stimulation. Pain response from tactile and thermal stimulation showed no significant difference between tooth surfaces. Cold stimulation was perceived to be the dominant pain-producing stimulus as had been previously reported. The results of this investigation support earlier findings of Collett & Speelman *Rev Behav Med Dent* 46: 63-73, 1991, suggesting that prevalence of CDS is higher in periodontal patients than has been reported elsewhere. This finding would suggest that previous periodontal treatment and/or periodontal disease may play a role in the aetiology of CDS.

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P J MOYNIHAN* R A SEYMOUR and S NERY (The Dental School, University of Newcastle upon Tyne, UK): The relationship between antioxidant vitamin intake and the severity of experimental gingivitis.

Antioxidant vitamin status may influence the response of the periodontal tissues to plaque and its products and, through their role as free radical scavengers, may have a part to play in the treatment and prevention of periodontal disease. This study aimed to ascertain whether a relationship existed between dietary intake of antioxidant vitamins (B-carotene, vitamin C and vitamin E) and the severity of inflammation of experimentally induced gingivitis. Male volunteers ($n=28$) abstained from oral hygiene in the upper right quadrant of their mouths for 21 days. Gingival health (Plaque score, modified gingival index (MGI) (Lobene et al., *Clin Prev Dent* 8 3-6, 1986) and gingival crevicular fluid (GCF) flow) were assessed at baseline and at 7 day intervals thereafter. Dietary intake of antioxidant vitamins was quantified using a food frequency and amount questionnaire (O'Brian and Nelson, *Proc Nutr Soc* 52 83s, 1992). Relationships were investigated using Pearson's correlation analysis. No significant correlations were found between intake of any of the vitamins and GCF or MGI scores. These data do not show a relationship between dietary intake of antioxidant vitamins and gingival inflammation. The relationship between blood and GCF antioxidant concentrations and development of experimental gingivitis needs to be examined.

- 487** GM MASON*, ILC CHAPPLE and JB MATTHEWS (School of Dentistry, University of Birmingham, UK): Local and systemic antioxidant capacity in chronic adult periodontal disease.

The potential importance of free radical damage in the pathogenesis of periodontal disease has led to anecdotal reports of the use of antioxidant treatment regimens (eg. vitamin Q). The aim of this study was to determine whether total antioxidant status (saliva and serum) is altered in chronic adult periodontal disease.

Antioxidant capacity was determined using an enhanced chemiluminescent method (Whitehead TP, Thorpe G & Maxwell S, *Anal Chimica Acta* 266: 265-277, 1992). The method was calibrated with a water soluble tocopherol analogue (Trolox) and results expressed as $\mu\text{mole/ml}$ trolox equivalents. Paired serum and mechanically stimulated (5 min.) saliva samples were collected from healthy controls ($n=8$) and patients with chronic adult periodontal disease ($n=8$). All samples were stored in liquid nitrogen prior to analysis and were assayed in triplicate.

Total serum antioxidant capacity was similar in healthy controls ($0.53 \pm 0.08 \mu\text{mole/ml}$) and periodontitis patients ($0.48 \pm 0.1 \mu\text{mole/ml}$). By contrast, salivary antioxidant levels were significantly lower in periodontitis ($0.17 \pm 0.05 \mu\text{mole/ml}$) compared with controls ($0.31 \pm 0.12 \mu\text{mole/ml}$). These results indicate that chronic periodontitis may be associated with reduced salivary antioxidant capacity, possibly due to increased free radical release from disease sites. Further studies are in progress to investigate whether similar changes can be detected in gingival crevicular fluid.

- 489** E VARGA* and L H MAIR (Department of Clinical Dental Sciences, The University of Liverpool, UK): Periodontal factors influencing the development of gingival hyperplasia in renal transplant patients.

The periodontal status of 35 patients was monitored on two occasions prior to renal transplant and then at 4, 6, 10, 16 and 20 weeks postoperatively. The following statistics were recorded: the Muhlemann bleeding index, the plaque index, the presence of calculus, the pocket probing depth and a simple colour index. The presence of defective restorations was noted together with habits such as mouthbreathing. At each visit alginate impressions were taken and stone models fabricated as a record of the hyperplasia. The post transplant serum cyclosporin levels were recorded.

From the models it was possible to divide the sample into three groups having severe ($n=13$), mild ($n=16$) and no hyperplasia ($n=6$). Although the severe group had higher pre-transplant plaque levels and bleeding scores with evidence of an associated pre-transplant hyperplastic response: these differences were not significant (Wilcoxon Rank Sum $p > 0.09$). Also there were no statistically significant differences in the cyclosporin levels between the three groups.

It is concluded that only a minority of renal transplant patients develop severe hyperplasia. The pre-transplant periodontal status does not predict the severity of post-transplant hyperplasia.

- 491** C MCGREEVY* and C IRWIN (Department of Restorative Dentistry, Queen's University of Belfast): The effects of 5 α -dihydrotestosterone on human gingival fibroblasts.

This study was designed to determine the effects of the 5 α -reduced metabolite of testosterone, 5 α -dihydrotestosterone (5 α -DHT), on human gingival fibroblasts and its possible role in cyclosporin-induced gingival overgrowth. Gingival fibroblasts were derived from the cyclosporin-induced overgrown gingiva of three individuals on long-term cyclosporin therapy and from the normal healthy gingiva of age- and sex-matched controls who had never received cyclosporin. Monolayer cultures of each of the cell lines were incubated in DMEM plus 2.5% FCS and exposed to concentrations of 0.1, 1, 10 and 100 ng/ml of 5 α -DHT. Negative controls were set up in each case. Parameters studied included (a) cellular proliferation, measured by ^3H -thymidine incorporation, (b) protein synthesis, estimated by the incorporation of ^3H -proline into non-dialysable macromolecules and (c) collagen production, assayed by a specific enzyme degradation assay. Results indicated that 5 α -DHT had no significant effect on the proliferative capacity or biosynthetic activity of either population of gingival fibroblasts.

This study suggests that, if 5 α -DHT is of aetiological importance in the pathogenesis of cyclosporin-induced gingival overgrowth, then its effects appear to be mediated through functional parameters other than cellular proliferation or matrix biosynthesis.

- 493** R McANDREW* and T LOYN (Department of Periodontology, Dental School, U.W.C.M., Cardiff): Effect of drug impregnated collagen sponges on human gingival fibroblasts (HGF) *in vitro*.

Human Type I collagen sponges were manufactured following the method outlined by Chung and Miller (Science 183: 1200-1211, 1974) and impregnated with Tetracycline, Clindamycin, Metronidazole, Ibuprofen 25 and Ibuprofen 50. Extraction of soluble materials by immersion and incubation in MEM at 37°C for 24 hours was carried out. Extract solutions were added in triplicate at different concentrations (neat, $1/2$, $1/4$, $1/8$) to 96 well microtitre plates containing HGF (1×10^4 , 5×10^4 , 1×10^5) and maintained at 37°C for 72 hrs. Proliferation and/or cytotoxicity was assessed through spectro-photometric assay by calculating the percentage of the alterations in the mean optical density values in the HGF cultures. Results revealed that both concentration of extract and number of cells were influential in assessing cytotoxicity and proliferation. When used "neat", at the cell concentration of 5×10^4 , all extracted solutions were stimulatory to HGF except Ibuprofen 50, rank order being: Tetracycline (+791%) > Plain (+322%) > Clindamycin (+205%) > Metronidazole (+205%) > Ibuprofen 25 (+87%) > Ibuprofen 50 (-23%).

It would appear from this study that incorporation of drugs into manufactured collagen sponges can influence the proliferative capability of human derived gingival fibroblasts, this is dependent on drug type, drug concentration and cell concentration.

- 488** FBURKE*, ELYNOCH*, AKALLI*, DM WILLIAMS*, MGROOTVELD* and D BEHNTON* (Depts of Cons Dent*, Oral Pathology*, Infl Res Grp*, UJMC, Oral Microbiology, KCSMU*, UK): Human saliva metabolic status assessed by high field proton NMR spectroscopy.

High field proton (^1H) nuclear magnetic resonance (NMR) spectroscopy was used to detect organic acid anions associated with microbial metabolism in saliva samples from subjects with periodontal disease before and after treatment. Unstimulated saliva samples were collected from subjects with gingivitis ($n=10$), exhibiting bleeding on probing and pocketing ≤ 4 mm. The saliva was collected before and twenty-four hours after periodontal treatment which involved scaling, root planning and rinsing with a mouthwash of 0.2 % w/v chlorhexidine gluconate. Immediately after collection, all samples were centrifuged at 16,000g for 30 min. ^1H NMR analysis was conducted immediately after centrifugation, or after storage at -20°C for up to 18 hours. A matched paired *t*-test was used to determine the significance of the results acquired. Mean metabolite concentrations detected before treatment were ($10^{-3} \text{ mol dm}^{-3}$): propionate 0.29, acetate 2.24, formate 0.32, lactate 0.18, pyruvate 0.09, succinate 0.07 and glycine 0.17. Twenty-four hours after treatment the mean metabolite concentrations detected were ($10^{-3} \text{ mol dm}^{-3}$): propionate trace ($p < 0.01$), acetate 0.35 ($p < 0.01$) formate 0.49 (n.s.), lactate 0.39 ($p < 0.05$), pyruvate trace ($p < 0.05$), succinate 0.04 (n.s.) and glycine 0.04 ($p < 0.01$).

Since the organic acid anions detectable predominantly arise from microbial metabolism high resolution ^1H NMR analysis may have a useful role in evaluating the pathogenesis and treatment of periodontal diseases at the molecular level.

- 490** J A JAMES*, C R IRWIN and G LINDEN (School of Clinical Dentistry, Queen's University Belfast): Enhanced cellular activity by gingival fibroblasts in response to cyclosporin A and Transforming Growth Factor β_1 *in vitro*.

Gingival overgrowth is frequently associated with cyclosporin A (CsA) therapy. This study aimed to investigate the effects of CsA and the inflammatory mediator TGF β_1 on growth of and protein production by gingival fibroblasts derived from 3 patients with enlarged gingiva (OG) induced solely by cyclosporin A, and fibroblasts derived from normal gingiva (N) in aged and sex matched controls. Cultures were maintained in control medium under standard conditions (5% CO $_2$, 37°C, Dulbecco's Modified Eagle's Medium supplemented with 2.5% fetal calf serum) either as monolayers on plastic or with cells embedded in a type I collagen gel. Growth and protein were measured in control medium and medium containing: a) 500 ng/ml CsA; b) 1 ng/ml TGF β_1 ; or c) 500 ng/ml CsA and 1 ng/ml TGF β_1 . In both culture models the addition of CsA alone had no effect on the growth characteristics of any fibroblast cell line. TGF β_1 and the combination of CsA and TGF β_1 significantly increased the growth of 5 of the cell lines in monolayer culture (3OG, 2N) but had no effect on cells grown in collagen gels. In all cell lines protein production in collagen was elevated compared with on plastic. CsA had little effect on protein production in either culture model. Monolayer cultures, but not cultures in collagen, were significantly stimulated by the combination of CsA and TGF β_1 , but to a markedly lesser degree than TGF β_1 alone, which caused approximately two fold increase in protein production.

It is concluded that the soluble mediator TGF β_1 may have a role as a co-factor in the pathogenesis of gingival overgrowth.

This work was supported by the Sir Jules Thorn Trust

- 492** R.A. WALKER*, B.J. MOXHAM, C.W.A. ARCHER, (Anatomy Unit School of Molecular and Medical Biosciences, UWCC, Cardiff): The expression of laminin epitopes by cultured ovine periodontal ligament fibroblasts

Laminin is a large multidomain glycoprotein of the extracellular matrix and basement membrane, in addition to having multiple functions in cell differentiation, adhesion, migration and proliferation. Periodontal tissues were excised from both the mid-root and apical surfaces of central incisors extracted from 9 month-old animals and collagenase pre-treated (22.5 units/ml) in the presence of 10% fetal calf serum (FCS) for 24h before being transferred to 100mm petri dishes. Explants were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS for 6 days after which cells with a fibroblast-like morphology were observed. The cells were treated with the ionophore monensin (5 μM for 5 hours) which blocks Golgi translocation and facilitates intracellular localisation of matrix components. The cells were fixed in 95% ethanol, air dried and rehydrated in phosphate buffered saline (PBS). Laminin epitopes were localised using a rabbit anti-laminin polyclonal antibody, L9393 (Sigma) in conjunction with a standard immunofluorescence procedure. Results revealed the expression of the laminin epitope by nearly all of the cultured periodontal ligament fibroblasts. A previous immunohistochemical localisation of laminin *in situ* using L9393 confirmed the general location of laminin to the basement membranes of vessels and epithelium in the periodontium. Laminin was detected under junctional, sulcular, and oral epithelium of the marginal gingiva and around epithelial cell rests of Malassez but not within the mesenchymal periodontal fibroblasts. The observation that periodontal ligament fibroblasts synthesise laminin *in vitro* was not expected. We have yet to establish whether this result is a culture artefact or if indeed laminin is a constitutive component of the ligament. Further work is underway to ascertain in more detail the phenotype of these cells and the laminin isoform(s) expressed.

- 494** DM WILLIAMS, SJ CANNON* and MA SCRAGG (Dept. Oral Path., Lond. Hosp. Med. Coll. UK): Exposure of human gingival fibroblasts to *Porphyromonas gingivalis* secreted products damages cell-substrate interactions.

The aim of this study was to assess the effects of the culture supernatant of *Porphyromonas gingivalis* (PGSN) strain W83 on the cytoskeletal and cell-substrate interactions of human gingival fibroblasts (HGF). HGF were exposed to PGSN for up to 18 hours. Integrin and cytoskeletal components were identified using fluorescence labelled antibodies. Within 6 hours of exposure to PGSN HGF showed morphological changes leading to detachment from the surface of the culture vessel. Loss of attachment was due primarily to heat-labile component(s) of the supernatant, but continued MTT reducing activity indicated that it was not as a result of cell death. Dose dependent losses of β_1 and α_5 integrin subunit expression and fibronectin occurred within 30 minutes, became more marked on prolonged incubation and were abolished by heating the supernatant. Reductions in both α_1 and β_3 integrin subunit expression were also apparent but less marked and less rapid than those of β_1 and α_5 . Although F-actin stress fibres were abolished in cells exposed for 18 hours to the higher concentrations of the supernatant the actin-binding protein vinculin was unaffected.

These selective effects of PGSN on membrane-associated integrins and the cytoskeleton of HGF may contribute to the pathogenic mechanisms involved in the loss of connective tissue which characterises destructive periodontitis.

495 JU WADDINGTON¹, MS LANGLEY¹, G EMBERY¹, L GUIDA² and G RUORIO² (¹Basic Dent. Sci., Cardiff Dental School, UK; ²Naples Dental School, Italy): Longitudinal study of sulphated glycosaminoglycans in crevicular fluid as markers of active periodontal disease.

The presence of sulphated glycosaminoglycans (GAG) in human gingival crevicular fluid (GCF) has been implicated as an indicator of active destruction of the periodontal tissues, particularly bone. Previous biochemical studies have identified this GAG to be a chondroitin sulphate proteoglycan, similar to those found in human alveolar bone (Waddington *et al.*, 1994 Arch. Oral Biol. 39, 361-368). A clinical longitudinal study was carried out to analyse the potential of sulphated GAG in human GCF as a biomarker for predicting active periodontal tissue destruction. The relationship between the observed increase in attachment loss and sulphated GAG was investigated at three sites in 10 subjects with adult chronic periodontitis. GCF was collected into capillary tubes placed at the gingival margin for 10 min and attachment loss recorded at each site at 3 month intervals, for 21 months. Sulphated GAG content was quantified by cellulose acetate electrophoresis and densitometric scans of the electrophoretic sheets. Active tissue destruction was presumed to be present at those sites presenting an increase in attachment loss of 1.5 mm or more over 3 months. Inactive control sites were regarded as those with unchanged attachment loss for 6 or more months. A significantly higher level of sulphated GAG was detected in the GCF at sites presumed disease active compared to the inactive control group ($p=0.0018$). Regression analysis also suggested a limited relationship between recorded attachment loss and increasing sulphated GAG present in GCF. The study represents the first longitudinal assessment of the detection of sulphated GAG in GCF and demonstrates its potential use as a biomarker for monitoring active periods of destruction of the periodontal tissues.

497 M D PATEL, J ASHMORE*, P GALOUT, M WILSON*, B HENDERSON*, I OLSEN (Depts of Periodontology, Microbiology and Maxillofacial Surgery, Eastman Dental Institute): Gapstatin - an *Actinomyces* protein which inhibits the cell cycle of oral fibroblasts.

The surface-associated material (SAM) of the oral pathogen *A. actinomycetemcomitans* contains a protein, which we have termed gapstatin, that prevents the proliferation of human fibroblasts *in vitro*. This is not due to direct inhibition of DNA synthesis, as occurs within 1 h of the addition of drugs such as cytosine arabinoside, since the inhibitory effect of SAM was found to be exerted only 12 h after the initial exposure of the cells. Moreover, SAM-treated cells accumulated a 4n level of DNA, the same as cultures incubated with the anti-mitotic drug colchicine, which blocks the cell cycle in metaphase. To investigate the mechanism of action of SAM, we established synchronized cultures of gingival fibroblasts with hydroxyurea, a reversible inhibitor of S phase. When hydroxyurea was removed, the cells in control cultures proceeded through S, then G2 and into mitosis (M). These phases were measured by the incorporation of ³H-thymidine. FACS analysis of propidium iodide-stained cells and the presence of cells containing condensed chromosomes, respectively. However, when hydroxyurea was removed and SAM was added for only a brief period early in the S phase, it caused the subsequent arrest of the cells in the G2 phase and prevented their progression into M.

These findings suggest that Gapstatin may be a specific inhibitor of the expression and/or function of cyclins A and B1, proteins which have a fundamental role in regulating the mammalian cell cycle and thereby in periodontal repair processes.

499 T G HEANEY* and G HILLS (Department of Clinical Dental Sciences, University of Liverpool, England.): Comparison of healing of inverse bevel and intrasulcular periodontal flaps.

The aim of this study was to determine whether a flap procedure using an intrasulcular (IS) incision was as effective as a conventional inverse bevel (IB) flap procedure for eliminating periodontal pockets. A total of 8 IB and 7 IS full thickness flaps were used in anterior and posterior segments in 8 patients with informed consent. There were no significant differences ($P=0.05$) between the sites chosen for the two procedures in Gingival, Plaque and Bleeding Indices (GI, PI, BI), gingival sulcus fluid flow rates (GSF), pocket probing depths (PPD) and gingival recession (GR) immediately before surgery, and they were monitored post-operatively at intervals up to 3 months. Both techniques resulted in similar ($P>0.05$) but significant reductions in PPD and increases in GR by 3 months ($P=0.0001$). However, restoration of PI to pre-operative levels took appreciably longer with the IB procedure (3-4 wks IB, 4-5 wks IS, $P=0.003$), BI returned to pre-operative levels between 5-6 wks (IB) and 3-4 wks (IS), ($P=0.0001$) and abolition of GSF occurred more rapidly after IS flap surgery ($P=0.0001$). IS flaps were of equal efficacy to IB flaps under the conditions of study and healed more rapidly initially than the latter, judging by clinical criteria. This may result from faster reestablishment of a functioning dentoalveolar seal because of better initial root adaptation and retention of pocket epithelium achieved with IS flaps.

501 R O'LEARY*, AM SVED, EH DAVIES, TG LEIGHTON, M WILSON and JB KIESER (Department of Periodontology, Eastman Dental Institute, UK): The bactericidal effects of dental ultrasound *in vitro*.

The aim of this study was to investigate the acoustic effects of the dental ultrasonic scaler. Firstly 0.2 ml suspensions of pure cultures of *Actinobacillus actinomycetemcomitans* (Aa) and *Porphyromonas gingivalis* (Pg) were subjected to the ultrasonic vibrations of the Cavitron P1 insert, for 2.5 and 5.0 minutes, in an acoustically simulated pocket model and survivors determined by viable counts. Five test and five non-activated control samples of each bacterial species were used in each experiment, and then repeated. The presence of cavitation, to which any bactericidal activity observed might be attributed, was also investigated by sonoluminescence production (Walton AJ and Reynolds GT, *Advances in Physics* 33: 595-660, 1984). The necessary deflection of the water coolant from the insert tip operating within the simulated pocket, resulted in temperatures of 47.6°C and 52.3°C after 2.5 and 5.0 minutes respectively. Finally, the effects of such temperatures on the viability of the target bacteria were determined.

After 5 minutes sonication, the viable counts of both Aa and Pg were significantly reduced by 62.6×10^4 and 200×10^6 cfu respectively. Sonication for 2.5 minutes resulted in significant reductions in the viability of Pg only (1.9×10^6 cfu). However, no sonoluminescence was detected and equivalent temperature rises resulted in bactericidal activity comparable to that due to sonication.

It was therefore concluded that no additional bactericidal effect from acoustic phenomena exists under these experimental conditions. The relevance of this finding to subgingival instrumentation under normal clinical conditions remains unclear.

496 TF WALSH, A RAWLINSON* and RB WINSTANLEY (Department of Restorative Dentistry, University of Sheffield, UK): The effect of topical subgingival 25% metronidazole on subgingival neutral proteolytic enzyme activity.

The aim of the present study was to compare the effects of 25% metronidazole dental gel and mechanical treatment on the subgingival neutral proteolytic enzyme activity of subjects with adult periodontitis. A split mouth study was utilised with two pairs of sites identified on contralateral teeth in each subject. Treatment either by mechanical therapy as described by Rawlinson & Walsh (*J Dent J*, 173, 161-166, 1993) or by two subgingival applications of 25% metronidazole 7 days apart, was randomly applied to the contralateral sites. Subgingival neutral proteolytic enzyme activity was monitored at baseline, one week, one month and three months using the remazolbrilliant blue collagen-powder method (Walsh TF *et al.*, *J Dent Res* 72, 719, 1993). The results showed that there was no differences between the two treatments with regard to changes in the neutral proteolytic enzyme activity when assessed using the Chi-squared test.

It was concluded that the subgingival application of 25% metronidazole gel was equally effective as mechanical treatment at reducing the level of pocket inflammation as assessed by the level of neutral proteolytic enzyme in the crevicular fluid.

498 TF WALSH, G RICCARDI* and PI VARELA-CENTELES (Department of Restorative Dentistry, University of Sheffield, UK): The effect of non-surgical therapy on subgingival pocket temperature.

The aim of the present study was to compare the effects of non-surgical periodontal therapy on subgingival temperature in patients suffering from adult periodontitis. 52 sites in 15 patients, diagnosed by clinical and radiographic criteria as suffering from adult periodontitis, were examined at baseline, one week, one month and three months. Subgingival temperature was measured using a specially constructed sterilisable thermocouple connected to a digital thermometer with an accuracy of 0.1°C. Non-surgical periodontal therapy using hand and ultrasonic instruments as described by Rawlinson & Walsh (*J Dent J*, 173, 161-166, 1993), was undertaken and the subgingival temperature subsequently monitored. The mean temperature at baseline was 33.2°C \pm 2.96. One week after non-surgical treatment it had fallen to 32.9°C \pm 2.96 but had risen again to baseline levels by 4 weeks after baseline. None of these differences were significant when subjected to analysis of variance ($p=0.883$, $DF=207/3$, $F=0.22$).

It was concluded that, in this group of selected patients, mean subgingival temperature did not significantly change following non-surgical therapy.

500 R P ALLAKER*, C R JARROLD, K A YOUNG, J M HARDIE and M R HEATH* (Depts of Oral Microbiology & *Prosthetic Dentistry, London Hospital Medical College): Evaluation of topical minocycline in the treatment of elderly patients with periodontitis.

The efficacy of a 2% w/w minocycline gel (Dentomycin™) was evaluated in a split-mouth study of 22 older adults (mean age 60 years; age range 53-73 years) with chronic periodontitis. At baseline, patients received conventional scaling and root planing. Test and placebo gels were administered at contralateral disease sites at baseline, and at weeks 2 and 4. Clinical measurements and microbiological sampling were carried out at baseline, and at weeks 2, 4, 6 and 16. Reductions in gingival bleeding and plaque scores occurred with both test and placebo gels. Reduction in pocket depth was more pronounced at test sites in comparison to control sites. Levels of *Porphyromonas gingivalis* and *Prevotella intermedia* were reduced until week 6 at test sites only. *Actinobacillus actinomycetemcomitans* was not isolated from any sites sampled.

This study has shown that administration of a 2% minocycline gel, in addition to scaling and root planing, may be a useful adjunct in the treatment of older adults with periodontitis.

502 S HADDAD*, T LOYN, R McANDREW and P DOWELL (Department of Periodontology, Dental School, Cardiff): The cytotoxic effect of mouthwashes on fibroblasts *in vitro*.

This project investigated the cytotoxic effect of seven commercially available mouthwashes (Corsodyl, Corsodyl mint, Periogard, Macleans, Rembrandt, Listerine and Plax) on human gingival fibroblasts (HGF) *in vitro*. Eight concentrations of each mouthwash (100%, 75%, 50%, 25%, 10%, 4%, 2% and 1%) diluted with distilled H₂O were added in triplicate to 96 well microtitre plates containing HGF (1×10^5 / μ l) at day 2 of growth for 30 seconds and then washed (control wells were exposed to water). Results obtained were analysed by spectrophotometry after staining by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Mosmann T, *J Immunol Meths* 65: 55-63, 1983). The mean, standard error and percentage of viable cells for each concentration was determined. In addition, the cytotoxicity for each mouthwash was qualitatively assessed using transmission electron microscopy. Results showed that all mouthwashes were cytotoxic, to a certain level, for HGF in vitro. When the full concentration of the mouthwash was applied, percentage of viable cells were: 23.7% for Listerine, 36% for Periogard, 45.9% for Plax, 0% for Rembrandt, 1.5% for Corsodyl, 9.8% for Corsodyl mint and 18.8% for Macleans.

This *in vitro* study concluded that all tested mouthwashes had a cytotoxic effect on HGF. It is possible that mouthwashes may interfere and delay the initial healing process after periodontal surgery.

503 A.L. COOKSON*, A. WRAY, A.E. JACOB, P.S. HANDLEY, (School of Biological Sciences, University of Manchester, U.K.): Characterisation and Speciation of *Prevotella intermedia* and *Prevotella nigrescens*.

The black pigmented obligate anaerobes *Prevotella intermedia* and *Prevotella nigrescens* have been implicated in periodontal disease and endodontic infections respectively. Despite being genetically heterogeneous, both species share many phenotypes that makes speciation difficult to perform without DNA probes. Two methods not requiring DNA probes to speciate *P. intermedia* and *P. nigrescens* have been published (Multilocus enzyme electrophoresis, Shah and Gharbia, *Int. J. Syst. Bacteriol.* 42 p.542-546, 1992 and serotype-specific monoclonal antibodies Devine *et al.*, *FEMS Microbiol. Lett.* 120 p.99-104, 1994), but these methods cannot be easily used in all laboratories. The aim of this study was to find an alternative method to speciate both organisms. Potential properties investigated were the presence of surface structures, the production of proteases and surface protein profiles. Fibrillar surface structures were observed on all strains examined and strains of both species produced proteases. However, clear species discrimination of 14 *P. intermedia* strains and 19 *P. nigrescens* strains was possible by SDS-PAGE analysis of SDS cell surface extracts. *P. intermedia* was characterised by the presence of a 31kDa protein, whilst *P. nigrescens* was characterised by a doublet of proteins of approximately 28 and 35kDa. Protein profiles from other black pigmented anaerobes (*Porphyromonas gingivalis*, *Prevotella denticola*, *Prevotella loeschii* and *Prevotella melaninogenica*) were clearly different from the *P. intermedia* and *P. nigrescens* profiles. The use of protein profiles from *P. intermedia* and *P. nigrescens* therefore offers a rapid and convenient way of unambiguously distinguishing them from each other and from other black pigmented oral anaerobes.

505 H WARDLE¹, K BENNETT¹, P HULL¹, L JOSEPH¹, D DENNING^{1,2} and D DRUCKER^{1,2} (Hope Hospital, Salford¹, University of Sheffield², University of Manchester³): GLC characterisation of spirochaetes

Isolation and identification of oral spirochaetes is of great importance for determining the putative pathogenic role of these organisms. The aim of this study was to isolate spirochaetes from subgingival plaque and to compare them with known spirochaete strains using GLC. A collection of spirochaetes was obtained from the culture of subgingival plaque samples using modified New Oral Spirochaete medium and nitrocellulose membrane filters. Five ATCC spirochaete strains, *T. pretiosum* ATCC 35405 and ATCC 33520, *T. vincentii* ATCC 35580, *T. pectinovorum* ATCC 33768 and *T. solarskii* ATCC 35534, were cultivated in the appropriate media. All the isolates were grown in broth. Fermentation end-products were extracted from the broth using ether and chloroform extractions for volatile and non-volatile fatty acids respectively. Peaks thus obtained were compared to those produced by sterile broth. Known standards were used to identify the peaks produced. Clinical isolates tested so far appear to have similar fatty acid profiles to those of *T. denticola*. Prominent peaks of volatile fatty acids corresponded to acetic and propionic acids and of non-volatile acids to lactic acid.

These preliminary results suggest that GLC is a very useful aid in the characterisation of clinical isolates of oral spirochaetes.

507 R HARPER-OWEN*, D DYMOCK, WG WADE and AJ WRIGHTMAN (Dental School, Bristol, UK and PABIO, UWCC, Cardiff, UK): Differentiation of *Fusobacterium nucleatum* sub-species by 16S rRNA gene restriction analysis.

Four sub-species of *F. nucleatum* have been proposed but there are no known phenotypic tests which discriminate between them. The aim of this study was to evaluate the use of restriction analysis of amplified 16S rRNA genes for this purpose. *F. nucleatum* ss. *nucleatum* ATCC 25586, ss. *fastiforme* NCTC 11326, ss. *polymorphum* ATCC 10562, ss. *vincentii* ATCC 49256 and 30 fresh isolates identified as *F. nucleatum* were included in the study. 16S rRNA genes were amplified by means of the polymerase chain reaction (PCR) and the PCR products digested with restriction endonucleases *CfoI*, *HinII*, *RsaI* and *SplI*. The four type strains could be distinguished by the patterns generated by the four enzymes taken together. However, the fresh isolates gave a multiplicity of patterns; only two strains could be positively identified and were both ss. *polymorphum*. Compared to other genera examined by this technique, strains of *F. nucleatum* exhibited considerable diversity. In addition, a number of strains exhibited allelic variation in 16S rRNA sequence.

In conclusion 16S rRNA PCR-RFLP is not suitable for the differentiation of *F. nucleatum* sub-species.

509 D LAW*, W WADE and D B DRUCKER (Hope Hospital, Salford; University of Bristol and University of Manchester): *Carnocytophaga* phospholipids analyzed by fast atom bombardment mass spectrometry.

Almost nothing is known of the phospholipid composition of *Carnocytophaga*. The aim of this study was to obtain detailed information on the phospholipid composition of *Carnocytophaga* by use of fast atom bombardment mass spectrometry (FAB MS). Strains representing all seven clusters of Khwaja *et al.* (1990) were chosen for study. Lipid extracts were suspended in m-nitrobenzyl alcohol matrix fluid and bombarded with xenon to produce anions in a Kratos Concept II mass spectrometer. Large numbers of anions were observed. Major peaks included those which had mass/charge (m/z) values consistent with the presence of anions of the individual phosphatidylethanolamines, PE(24:2), PE(25:2), PE(27:1) and PE(30:0).

We conclude that *Carnocytophaga* has phospholipid analogues which are unique to *Carnocytophaga* and thus have possible value for chemosystematic purposes.

504 SE GHARIBIA, D RAJENDRAM* & HN SHAH (Dept. of Microbiology, Eastman Dental Institute): Implications of 16S-rRNA sequence homology and secondary structure with reference to *Prevotella intermedia* and *Prevotella nigrescens*.

Prevotella intermedia and *P. nigrescens* are phenotypically so closely related that present methods of detection are based largely on nucleic acid probes. In the present study, 8 oligonucleotides based upon regions of the small subunit 16S ribosomal RNA gene sequences were analysed against a background of their position within the molecule and their two dimensional structure to rationalise their use in recognising both species. 41 clinical isolates from both oral and respiratory sites and 2 reference strains were subjected to DNA-DNA hybridisation and multilocus enzyme electrophoresis to confirm their identity. Restriction patterns generated from digestion of PCR amplified rRNA genes yielded no species specific bands. Alignment of oligonucleotide probes designated 1B1-2 to 1B1-6 (for *P. intermedia*) and 2B1-2 (for *P. nigrescens*) with the 16S rRNA suggested that these probes lacked specificity or were constructed from hypervariable regions. A 52 mer oligonucleotide (designated B1) reliably detected both species. Because of the high degree of concordance between the 16S rRNAs of both species, it was necessary to vary the stringency of hybridisation conditions for detection of both species. Thus probe 1B1-1 recognised *P. intermedia* while 2B1-1 detected both *P. intermedia* and *P. nigrescens* at low stringency. However, under conditions of high stringency only *P. nigrescens* was recognised by probe 2B1-1. These probes were highly specific and did not hybridise with DNA from the closely related *P. corporis*, nor other periodontal pathogens such as *F. nucleatum*, *A. actinomycetemcomitans*, *T. denticola* and several pigmented species such as *P. melaninogenica*, *P. denticola*, *P. loeschii*, *P. asaccharolytica*, *P. endodontalis*, *P. gingivalis*, *P. levi*, and *P. macacae*. The results of this study indicated that of the many potential rRNA probes, only two were found to be highly specific in recognising *P. intermedia* and *P. nigrescens*.

506 J M SLANEY*, J ADUSE-OPOKU, P SHEPHERD¹, J CRIDLAND¹ and M A CURTIS (MRC Mol. Path. Group, LHMC, London & Dept. Immunol., UMDS): Analysis of the adhesion domain of the ArgI protease of *P. gingivalis* W50 using monoclonal antibodies.

The major arginine-specific protease of *P. gingivalis* W50 is synthesised as a precursor, PrpRI, with large N- and C-terminal extensions. Processing of PrpRI, *in vitro*, generates monomeric species (ArgIA & ArgIB) and a dimeric form (ArgJ) in which the protease is associated with a second polypeptide derived from the C-terminus of the precursor (β region). Analysis of the sequence suggests that the β region of PrpRI shares significant similarity to adhesin/antigen proteins of other micro-organisms. In the present study the role of the β region in the haemagglutination reaction of *P. gingivalis* cells and culture supernatants was examined. An internal fragment of prpRI, corresponding to residues 784-1132 within the β region was cloned in pUC18 in *E. coli* (JM11) and the recombinant product examined via western blotting using a panel of monoclonal antibodies raised against *P. gingivalis* whole cells (Cridland *et al.* 1994 J. Perio. Res. 29: 339-347). Two antibodies, mAbs 1A1 and 2B/H9, recognised the JM11 recombinant protein and the heterodimeric form of ArgI from *P. gingivalis* whilst ArgIA and ArgIB were not immunoreactive. Following affinity purification of the antibodies, mAb 1A1 inhibited the haemagglutination of *P. gingivalis* cell sonicates and culture supernatants. 2B/H9 and a control mAb to *P. gingivalis* LPS were ineffective in the same dose range.

These results indicate that the β region of PrpRI contains an epitope which can mediate attachment to red blood cells and help clarify the relationship between haemagglutination reactions and arg-x protease activity in this organism.

508 S FATHI¹, M ABDI¹, DRUCKER^{1,2}, H WARDLE² and L JOSEPH² (Turner Dental School, Manchester¹ and Hope Hospital, Salford²): *Fusobacterium* phospholipid profiles analyzed by FAB-MS.

The major phospholipid of *Fusobacterium* is known to be phosphatidylethanolamine with smaller amounts of other polar lipids such as phosphatidylglycerol. However, few strains have been examined and only lipid families have been sought. The aim of this study was to analyze individual polar lipid analogues, within each family present, using fast atom bombardment mass spectrometry (FAB MS). Polar lipid extracts were prepared, washed and dried. Sample dissolved in a matrix of m-nitrobenzyl alcohol was analyzed by negative-ion FAB MS using Xenon. Major anion peaks observed in the low mass region of mass/charge, m/z, 211, 225, 227, 241, 253, 255, 279 and 281 were consistent with presence of C_{15:1}, C_{16:1}, C_{15:0}, C_{16:0}, C_{18:1} and C_{18:0}. In the high mass region, major anion peaks observed of m/z 618, 634, 644, 660, 662, 672, 688, 693 and 719 which were consistent with the presence of PE(27:1), PE(28:0), PE(29:2), PE(29:1), PE(30:1), PE(30:0), PE(31:2) and PE(32:1) phosphatidylethanolamines and of PG(30:0) and PG(32:1) phosphatidylglycerols.

We conclude that FAB MS can provide data on individual analogues of PE and PG from *Fusobacterium* not readily obtained by other means. Furthermore the phospholipid profile is diagnostic for *Fusobacterium*.

510 S ALI¹, D DRUCKER^{1,2}, H WARDLE², M ABDI¹ and L JOSEPH² (Turner Dental School, Manchester¹ and Hope Hospital, Salford²): *Treponema* phospholipid profiles analyzed by FAB-MS in negative-ion mode.

Very little is known regarding polar lipids of *Treponema*. The limited information so far published contains conflicting reports. No data are available with respect to which specific analogues are present of particular phospholipid families. The aim of this study was to determine detailed information on individual phospholipid analogues of *Treponema*. Lipid extracts of *Treponema denticola* were analysed by FAB MS in negative-ion mode. Major anions in the low mass region were of mass/charge, m/z, 211, 225, 227, 241, 255, 279, 281 and 283 which are consistent with the presence of C_{15:1}, C_{16:1}, C_{16:0}, C_{15:0}, C_{18:1}, C_{18:0} and C_{18:0} carboxylate anions. In the high mass region, the major anions observed were of m/z 618, 632, 646, 660, 663, 677, 691 and 705 which may be putatively identified as PE(27:1), PE(28:1), PE(29:1), PE(30:1), PG(28:1), PG(29:1), PG(30:1) and PG(31:1). These putative identifications are supported by the carboxylate profiles.

It is concluded that *Treponema* phospholipid profiles are amenable to analysis by FAB MS and that absolutely novel information can thereby be gathered.

511 M ANDERSON¹, H WARDLE², R GRADY¹, D DRUCKER^{1,2} AND D DENNING^{1,2} (University of Manchester¹, Hope Hospital, Salford²): Characterisation of oral treponemes of known and unknown species by RAPD

The Random Amplification of Polymorphic DNA (RAPD) technique has been used to differentiate closely related species of many different bacterial genera and to type different strains of the same species. The potential for using RAPD to differentiate and characterise *Treponema* species was investigated. Genomic DNA was extracted from four different *Treponema* species (ATCC strains) including *T. pectinovorum*, *T. sokratiki*, *T. vincentii* and two different serovars strains of *T. denticola*. Five different primers (9-, 17-, 19- and 20-mers) previously used with other spirochetes and oral bacteria, were used to amplify polymorphic regions of the genomes. The four different species gave obviously different patterns of amplified fragments after agarose gel electrophoresis with only a few bands in common. Several *treponema* isolates of unknown species were cultured and purified from dental plaque samples (*vide Wardle et al. poster*). These isolates were also characterised using the five primers and were scored for number of bands in common with known species. The matrix was used to show how similar the unknowns were to the known species.

Preliminary data shows that four of the unknown isolates from three different patients all produce very similar RAPD patterns to each other.

512 MJ NEAL¹, R GOODACRE, S HIOM, AJ WEIGHTMAN AND WG WADE (Biological Sciences, Aberystwyth, Dental School, Bristol and PARO, UWCC, Cardiff): Identification of oral *Eubacterium* spp. by pyrolysis mass spectrometry

Pyrolysis mass spectrometry (PyMS) has been shown to be a valuable technique for use in bacterial systematics and, specifically, identification. The aim of this study was to evaluate PyMS with neural network analysis for the identification of oral anaerobic *Eubacterium* spp. Curie-point pyrolysis mass spectra were obtained from 28 *Eubacterium* strains representative of named species and unnamed taxa, together with 6 abcess isolates provisionally identified as *P. heliotrinreducens*. Artificial neural networks (ANNs) were then trained by supervised learning (with the back-propagation algorithm) to identify isolates from their pyrolysis mass spectra. Analysis of the spectra generated clustering of strains consistent with that obtained from previous phenotypic and genotypic studies. The neural network correctly identified the known *Eubacterium* strains. The abcess isolates were identified as unnamed *Eubacterium* taxon C₂.

This study has demonstrated that the combination of pyrolysis mass spectrometry and ANNs provides a rapid and accurate identification technique for oral Eubacterium spp.

513 MJ WILSON¹, WG WADE AND AJ WEIGHTMAN (Dental School, Cardiff, UK; Dental School, Bristol, UK and Pure and Applied Biology, UWCC, Cardiff, UK): The design and validation of DNA probes against un-culturable bacteria

Culture-independent analysis of the subgingival microflora by 16S ribosomal RNA sequencing has revealed the presence of three previously uncharacterised bacteria. The aim of this study was to design specific oligonucleotide probes against these bacteria and use the probes to screen samples of subgingival plaque. Areas of greatest sequence variability were chosen as targets for probe construction and the suitability of these sequences for use as DNA probes was assessed using the OLIGO package. Total nucleic acid was extracted from 46 samples of plaque from both healthy and periodontally diseased sites and hybridised to each of the three probes under stringent hybridisation conditions. Three probes (B4, A18 and A19), each exhibiting low self-complementarity and little tendency towards dimer formation, were used. No cross-reactivity was detected against 20 other oral bacteria but a strongly positive signal resulted with the recombinant plasmid DNA used as positive controls. The target bacterial sequences were not detected in any of the 46 subgingival plaque samples.

In conclusion, DNA probes to unculturable bacteria were successfully constructed. However, the presence of the target bacteria in clinical specimens was not detected.

514 J PARRY^{1,2*}, R HOLT, H N SHAH¹, M WILSON¹ (Dept. of Microbiology and Children's Dentistry, Eastman Dental Institute, London): Use of impedance measurements for the detection of mycoplasmas in saliva samples

Bacterial growth can result in changes in the electrical conductivity (impedance) of the medium and measurement of such changes can be used to detect the presence of bacteria, and their growth rate. The aim of this study was to determine whether mycoplasmas could be detected in saliva by measuring changes in impedance (using the Rapid Bacterial Impedance Technique - RABIT) and to determine which of three media would be the most suitable for use with this system. Saliva samples were taken from 10 adult volunteers and a portion of each was plated onto a medium selective for mycoplasmas (Mycoplasma Experience Medium - MEM³). These were incubated for 10 days and examined for characteristic mycoplasma colonies. Further portions of each sample were added to 5 ml of the following selective mycoplasma media: Hayflicks (H), Oxoid (O)² and MEM. The electrical impedance of the cultures was monitored every 9 mins over a 72 h period. Mycoplasmas were cultured on agar plates from 7 of the 10 samples. Growth was detected by impedance measurements in 5 of the samples in ME medium, 3 of the samples in H medium and 2 of the samples in O medium.

In conclusion, impedance measurements were less sensitive at detecting mycoplasmas than traditional culturing methods. However, the convenience of the impedance technique and its usefulness in physiological studies, justify further work on developing a medium suitable for use with the technique.

¹Don Whitley Scientific Ltd, Shipley. ²Mycoplasma Experience Ltd, Reigate. ³Oxoid Ltd, Basingstoke.

515 HN SHAH¹, N ADAMS², DMA ANDREWS¹, K GULABIVALA¹ & SE GHARIB¹ (Dept. Microbiology & Conservation, Eastman Dental Institute, UK); Energy assimilation by *Porphyromonas endodontalis* and *Peptostreptococcus micros* as a model for studies of microbial activity in the root canal system

The root canal system harbours a range of microbial species that are well adapted for growth in such an exacting ecosystem. Growth and survival of bacteria at these sites must ultimately depend on nutrient availability and is likely to vary with the clinical condition. In the present study we utilised measurements of conductive changes to study energy assimilation by *Peptostreptococcus micros* and *Porphyromonas endodontalis*, two species that are frequently associated with these sites. Previously we constructed a multiple electrode cell and used it to optimise conditions for physiological studies of periodontal pathogens. Here, we used the Don Whitley Scientific Ltd, (Shipley, Yorks, BD17 7SB) 'Rapid Automated Bacterial Impedance Technique' equipment to measure bio-electrical impedance changes in these species in response to amino acid utilisation by *P. endodontalis* and *P. micros*. In the former, glutamate, lysine and histidine were utilised after the first 5 hours of anaerobic incubation while asparagine uptake was initiated after 13 hours. This was reflected in a decrease in bio-impedance for 20 hours, after which a two fold increase was apparent. By contrast, the uptake of amino acids was considerably faster in *P. micros* and followed a linear response except for asparagine which was initiated within the first hour and resulted in the reduction in the conductivity of the culture medium for 14 hours followed by a rapid and linear increase in conductivity.

The results of this study suggest that amino acids represent an important source of nutrients for *P. endodontalis* and *P. micros* and, by inference, may also be an important substrate for other species infecting the root canal. These results further indicate that *P. micros* is able to process these amino acids more efficiently than the former species. The slower utilisation of asparagine by both species suggests a deamidation reaction followed by uptake of the corresponding amino acid.

516 K A YOUNG¹, R P ALLAKER, J M HARDIE AND R A WHILEY (Department of Oral Microbiology, London Hospital Medical College): Pathogenic mechanisms in mixed infections with *Escherichia coli* and *Streptococcus milleri*-group organisms

Streptococcus milleri-group organisms (*S. intermedius*, *S. constellatus* and *S. anginosus*) and *E. coli* together produce localised suppurative infections at oral and other body sites. Coaggregation, enhancement of hydrolytic enzyme activities and growth stimulation were investigated with combinations of these organisms. Coaggregation reactions occurred frequently between *S. anginosus* (83% of strain combinations) or *S. constellatus* (87%) and *E. coli* isolates, but were infrequent between *S. intermedius* and *E. coli* (28%). No enhancement of enzyme activities against lipid, phosphate, peptide and saccharide substrates were detected with combinations of species in comparison to the species alone. Exponential growth of *S. constellatus* and *S. intermedius*, in mixed culture with *E. coli*, occurred within 6h post inoculation, in comparison to 25h without *E. coli*. No growth stimulation of *S. anginosus* was observed.

It is concluded that both coaggregation and growth stimulation occur between *E. coli* and *S. milleri*-group organisms, and may be important mechanisms in the establishment of mixed infections involving these bacteria.

517 V.S. LUCAS¹, D BEIGHTON², G.J. ROBERTS¹ (Great Ormond Street Hosp., Dept. of Dental Sciences, King College Hosp.): Oral Streptococcal Changes following Total Body Irradiation / Chemotherapy for Bone Marrow Transplantation

Objectives: Evidence implicating oral streptococci as opportunistic organisms in systemic infections in bone marrow transplant (BMT) patients has been accumulating for several years (Heimbold et al. *Oral Surg Med Path* 68: 711-716). In this study the changes in the oral flora of children following total body irradiation (TBI) and chemotherapy have been investigated.

Patients and methods: Eleven children with relapsed leukaemia were matched with normal children. Pre-transplantation conditioning comprised fractionated TBI and chemotherapy. Saliva was collected on 4 occasions: (1) Before conditioning (baseline), (2) 7 days post-transplantation (neutrophils = 0), (3) Neutrophils $\geq 0.5 \times 10^9/L$ and (4) 120 days post-transplantation. Saliva was processed for speciation of viridans streptococci (Beighton et al. *J. Med. Micro* 35: 367-372).

Results: (1) *S. mitis* and *S. oralis* were the predominant species isolated when the neutrophil count was 0. The mean counts of *S. mitis* and *S. oralis*, as a percentage of the total colony count, increased from 8.2% at baseline to 47.2% at 7 days post transplantation ($p < 0.01$). (2) There was a decrease in the total anaerobic count from baseline to zero neutrophil count, [$p < 0.03$]. (3) The mean number of streptococcal species isolated per patient also decreased from baseline, 2.82 ± 1.1 to 1.27 ± 0.05 . (4) There was no significant difference in the microbial counts at the baseline or at 120 days post-transplantation between the BMT and normal children.

Conclusions: Oral flora changes following TBI and chemotherapy may increase the risk of systemic infection from *S. mitis* and *S. oralis*.

(This work was supported by the Sir Jules Thorne Charitable Trust)

518 P TOWNES¹, M A O LEWIS AND A J C POTTS (Department of Oral Surgery, Medicine and Pathology UWCC, Cardiff, UK): Routine diagnostic service provided by an oral microbiology unit in a dental teaching hospital

The aim of this study was to provide information on the source, specimen type, disorder and culture results for material submitted to an oral microbiology unit that provides a bacteriology and mycology service. A total of 1,433 consecutive request forms received in the oral microbiology unit between January 1993 and November 1994 were examined. Specimens were received predominantly from the oral medicine clinic (69%), examination and emergency unit (18%) and oral surgery unit (7%). The conditions investigated most frequently were suspected candidosis (25%) white lesions (14%), burning mouth syndrome (10%) and dental abscesses (13%). The majority (80%) of requests originated from mucosal conditions with 20% coming from dentofacial lesions. Samples from suspected candidosis comprised of 44% oral rinses (OR), 41% of which yielded candida, and 42% imprint cultures (IC), 58% of which were positive. White lesions were sampled predominantly (53% cases) by OR, 24% of which were positive for candida. The majority of samples from patients with burning mouth were OR (60%) of which 15% were positive whilst 24% of imprint cultures in these patients yielded candida. Samples from dental abscesses included 69% aspirates and 24% swabs. Culture of these aspirates revealed strict anaerobes alone in 30%, facultative anaerobes alone 2% and a mixture in 49%. Scanty or no growth occurred in 18% of aspirates. In contrast, swabs from abscesses yielded only strict anaerobes in 11% of cases and facultative anaerobes alone in 13%. The present study has confirmed that microbiological investigations play a role in the clinical management of a variety of oral conditions. However, in some disorders ensuring the most appropriate specimen may increase the value of the subsequent microbiological report.

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B MILLNS¹, M V MARTIN¹, R H K VAN SAENE² (¹Departments of Clinical Dental Sciences and ²Medical Microbiology, The University of Liverpool, UK): Selective decontamination of oral flora during anticancer therapy in children.

Selective decontamination is the elimination of micro-organisms known to be opportunistic pathogens in medically-compromised individuals. The aim of this study was to determine the efficacy of selective decontamination (SD) of oral Gram-negative bacteria (GNB), known to cause serious systemic infections in children suffering from leukaemia and Non-Hodgkins lymphoma. Weekly oral washings were taken by rinsing 10 ml sterile pyrogen free water for 30 sec in 12 leukaemic children (11 male, 1 female, mean age 10 years, range 4-16 years) during antineoplastic therapy. The washings were inoculated onto blood agar and McConkey agar and the frequency of GNB carriage was calculated and this was compared to the febrile episodes. The frequency of GNB carriage was compared to a group of age and sex matched healthy children. The SD regimen was 2% w/v Tobramycin, 2% w/v amphotericin and 2% w/v Colistin given as an oral gel. The children were also given Tobramycin (20 - 80 mg / ml per day) and Polymyxin (0.6 - 12 million units per day) systemically during intensification periods. Only one febrile attack was suffered by the 12 leukaemic / lymphomic children, associated with oral GNB carriage; oral frequency of GNB carriage during SD was 2%: in the control group, oral frequency of GNB carriage was 7%.

SD appears to prevent oral GNB carriage associated with febrile attacks in leukaemic and lymphomic children.

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P BARTON^{*} and M V MARTIN (Department of Clinical Dental Sciences, School of Dentistry, The University of Liverpool, UK): Incidence of occult blood in hospital orthodontic practice.

The aim of this study was to evaluate the incidence of occult blood in a hospital orthodontic practice. The presence of blood in the surgery following complete arch debonding / debanding and multiple molar banding procedures was assessed. The detection of blood utilised the Kastle Meyer Test (McColl *et al.*, *Brit Dent J* 1994, 176: 65-67). Cotton wool swabs were taken from the bracket tray, bracket table, operating lamp, air / water syringe, handpiece motor and band trays after the completion of each procedure and again following standard disinfection regime. The swabs were placed in 9.95 ml PBS and vortexed for 60 s and manually agitated for 10 s and then assessed for the presence of blood using the Kastle Meyer Test. Blood was found on all the surfaces tested, prior to disinfection. After disinfection the bracket table, lamp, handpiece motor and band-containing tray all had occult blood present.

Orthodontic procedures such as banding and debonding are associated with persistent blood contamination and could therefore be a source of cross infection. Simple disinfection procedures are not sufficient to remove all of this occult blood.

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J O McLAUGHLIN, W A COULTER, A COFFEY^{*} and D J BURDEN (Division of Orthodontics and Department of Microbiology, Queen's University UK): The risk of bacteraemia following orthodontic banding.

The aim of this study was to assess the incidence of bacteraemia following orthodontic banding. Guidelines on the prevention of endocarditis published in the United Kingdom do not consider the adjustment of orthodontic appliances to be a significant risk. There is considerable uncertainty among orthodontists about the need for antibiotic prophylaxis when fitting and removing orthodontic bands.

Thirty adult volunteers with good oral health were included in this study. An orthodontic band was placed on a first molar tooth of each subject. Venous blood samples were taken before, and after the molar band was fitted. Microbiological tests carried out on the blood samples revealed a comparatively low incidence of bacteraemia (10%) following orthodontic banding.

It is concluded that the risk of bacteraemia following orthodontic banding in patients with good oral health is low.

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T M HUNTER, E N HORROCKS^{*} and M WILSON (Departments of Orthodontics and Microbiology, Eastman Dental Hospital and Institute, London): Cross-infection control in the orthodontic environment.

The aim of the study was to assess the effectiveness of sterilization methods and instrument and accessory storage in preventing contamination by oral bacteria. Twenty-five orthodontic pliers were autoclaved¹ at 134°C for 3 mins. The effectiveness of the sterilization cycle was monitored using *Bacillus stearothermophilus* spores. The instruments were then stored in drawers or on an instrument rack whilst the surgery was used for routine treatment of patients, although the test pliers were not disturbed. The pliers were tested for contamination, after storage intervals ranging from 1 hour to 1 week, by immersion in nutrient broth and then plating aliquots of the broth onto blood agar and mini-salivarius (m-s) agar. The plates were examined for bacterial growth after 72 hrs. Appropriate controls were included. Elastomeric modules and elastic chain² were removed from 17 surgeries at the end of a treatment session and placed directly onto m-s agar. Previously unopened samples were taken as controls. The results showed the sterilization technique to be effective and that instrument contamination was negligible following storage up to 1 week. Both elastic accessories, however, showed a highly significant level of streptococcal contamination. **It is concluded that improvements need to be made in the presentation, storage and handling of orthodontic accessories in order to avoid cross-infection.**

¹Little Sister 2, SES. ²Durachain, Orthocare (UK) Ltd

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P.HART^{*}, S.WITT and K.LAIDLER (Divisions of Dental Surgery & Restorative Dentistry, Leeds Dental Institute, Leeds): The Dental Articulator - A Cross-infection Hazard?

This study investigated the potential for cross-infection associated with the travels of a cohort of 15 dental articulators from the dental laboratory to the clinic and back to the laboratory. The articulators were freshly drawn from stores, numbered, bagged and sterilised in the autoclave. Swabs were taken to confirm sterility at the time of issue to the technician. Further swabs were taken on receipt of the try-in dentures in the clinic and again in the laboratory following the clinical try-in. Swabs were cultured aerobically in brain heart infusion broth prior to subculture on blood agar.

All of the articulators were sterile at the time of issue to the technicians. All subsequently gave growth of potentially pathogenic micro-organisms following both laboratory and clinical use. *Staphylococcus aureus* was the commonest isolate present on 12 of the articulators following the laboratory set-up and on 10 following the clinical try-in stage. *Acinetobacter calcoaceticus* was present on four occasions pre-clinically and twice later. *Vibrio fluvialis* was present on one articulator at both stages. The majority of the articulators were contaminated by the same micro-organisms on both occasions.

It is concluded that the common dental articulator presents a serious cross-infection hazard, especially when immediate denture construction is involved. The sources of contamination appear to be the hands of the various operators and the tap water used in the laboratory.

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C I PANKHURST^{*}, D CLARK, D A LEWIS, G F TWSLEY & D BEIGHTON (Community Dentistry & Oral Microbiology, KCSMD): The recovery of oral yeasts using three different selective media.

The isolation of non-*Candida* species, resistant strains and multiple isolates from oral infections is increasingly reported. We therefore compared the recovery from two differential chromogenic media, modified Pagano-Lavin (PL, Orica) and CHROMagar Candida (CHROMagar Co., Paris) with Sabouraud dextrose agar (SDA). A total of 41 patients attending either an HIV clinic (N=18) or an oral medicine clinic, with clinical evidence of oral candidiasis, was recruited. Samples were taken using an oral rinse technique (10 ml of PBS for 60s). 100 µl of neat and serially diluted rinses were plated onto the media, incubated aerobically at 37°C for 72 h. Morphology and colony colour, with reference to the manufacturer's guide, were recorded and checked determined. Ten isolates per plate and those with colour or morphology variation were identified using the UAG test (Perry J L, *J Clin Micro* 25:2424-2425, 1987) or API 20C AUX. 95% of patients harboured yeasts with multiple isolates being recovered from 7. There was no difference in the counts from the SDA or CHROMagar ($p > 0.05$) but a lower count was found with the PL medium, $p < 0.01$. Non-*Candida* species were detected with an equal frequency on both differential media. The correlation between presumptive identification by colony colour of *Candida* on the CHROMagar and the formal identification was excellent. In parallel cultures from clinical specimens there was no difference in the total counts obtained on the SAB and CHROMagar. CHROMagar facilitated the reliable presumptive identification of *Candida* and aided the recognition of mixed cultures.

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M ABDI^{*} and D B DRUCKER (Turner Dental School, University of Manchester): Phospholipids of *Candida* analyzed by FAB-MS.

Individual analogues and homologues of phospholipids can now be readily detected using fast atom bombardment mass spectrometry (FAB MS). The aim of this study was to obtain detailed information on *Candida* phospholipids (PL) using FAB-MS. Lipids were extracted from 43 *Candida* isolates and prepared for FAB-MS analysis in negative-ion mode. Each isolate yielded several hundred anion peaks.

The major peaks consistent with presence of PL included those of m/z 710, 716, 740 and 742 tentatively identified as PE(34:4), PE(34:1), PE(36:3) and PE(36:2).

Major peaks consistent with the presence of carboxylate were of m/z 241, 253, 255, 277, 279, 281, 283 and 307 putatively identified as C_{15:0}, C_{16:1}, C_{16:0}, C_{18:1}, C_{18:2}, C_{18:1}, C_{18:0} and C_{20:1} which also support the putative PL identifications. Such PL profiles differ from those published for other microorganisms.

Quantitative differences were observed between different species.

It is concluded that *Candida* has a unique combination of PL analogues which thus have potential chemosystematic significance.

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J M SHARGANI^{*}, D B DRUCKER and A J DUXBURY (Turner Dental school, University of Manchester UK): Effect of negative air ion (NAI) streams on *Candida albicans*.

The aim of this study was to investigate the effect of humidity, probe distance, and exposure time (at 37°C) on the action of a NAI stream directed against *Candida albicans*. Ten isolates of *Candida albicans* were identified, cultured (to stationary phase), spread (0.4ml) on the surface of electrically-earthed Sabouraud dextrose agar allowed to dry and contained in a closed chamber of relative humidity set at either 0%, 55% or 100%. Plates were exposed to the NAI streams on three different quarters while the fourth acted as a control. Plates were incubated overnight and zones of growth inhibition were photographed and measured with a planimeter. Results showed significant ($p < 0.05$) differences in area of inhibition for humidity, probe distance (inversely proportional) and exposure time (directly related) for all strains.

It is concluded that an increase in the probe distance or relative humidity or a decrease in exposure time decreases the efficiency of killing of *Candida albicans* by a NAI stream.

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H PATEL^{1,2*}, M WILSON¹ and J NOAR² (Depts. of ¹Microbiology and ²Orthodontics, Eastman Dental Institute, London): Effect of sucrose on corrosion of oral magnets by multi-species biofilms.

The purpose of this study was to determine the effect of sucrose on the corrosion of intra-oral magnets by multi-species biofilms. Using pooled human saliva as an inoculum, biofilms were grown on the surfaces of 90 neodymium/iron/boron (Nd-Fe-B) magnets in a constant depth film fermenter under aerobic conditions at 37°C. The fluid phase was a mucin-containing artificial saliva (1.2 litres/day) and, after 15 days, 100 g of sucrose was added (in three 30-minute pulses of 33.3 g) for a further 15 days. 6 magnets with attached biofilms were removed periodically. On each sampling occasion the numbers of aerobes, anaerobes, streptococci, veillonellae and actinomyces in each biofilm, the pH of the fermenter effluent and the dry weight of the magnets were determined. There was a 0.02% weight loss in the magnets during the first 15 days whereas during the remaining 15 days (when sucrose was added) the weight loss was 28-fold greater, amounting to 0.56%. The decrease in weight of the magnets corresponded to a fall in pH (from a mean of 6.94 to a mean of 4.96), an increase in the proportion of streptococci and a decrease in the proportion of veillonellae comprising the biofilms.

The results of this study have shown that the presence of sucrose affects the microbial composition of multi-species biofilms growing on Nd-Fe-B magnets and results in a marked increase in corrosion of the magnets.

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JR NAGLIK*, SP SWEET and SJ CHALLACOMBE. (Department of Oral Medicine & Pathology, UMDS Guy's Hospital, UK): Immune responses to *Candida* cell wall antigens in serum and saliva.

Currently there are gaps in the understanding of the relationship between host immunity, the virulence of *Candida* and the onset of *Candida* infection. The aims of the study were to investigate mucosal and serum responses to blastospore and hyphal cell wall antigens of different *Candida* species. Separated antigens were probed with pooled human whole saliva (IgA), pooled human serum (IgG) and hyperimmune mouse serum (IgG) raised against blastospore and hyphal forms of *C. albicans*. Western blotting experiments revealed that up to 25 blastospore and hyphal antigens were detected by human saliva and serum but fewer antigens were recognised by the mouse sera. A 49kD hyphal specific antigen expressed by both *C. albicans* and *C. tropicalis* was detected by whole saliva and weakly by human serum but was not detected by the mouse sera. Yeast specific antigens were only detected with the mouse sera. Human salivary and serum recognition patterns were similar. Different *Candida* species possessed unique immunogenic antigens but other antigens were shared between species.

This study has revealed species, blastospore and hyphal specific antigens which may prove useful in analysing protective human responses.

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D RAHMAN*, S J CHALLACOMBE* & D T O'HAGAN*. (Oral Med & Path UMDS, Guy's Hospital & *United Biomed Inc. Hauppauge, NY). Oral immunisation with microparticles gives rise to a common mucosal response.

Oral immunisation with polylactide co-glycolide microparticles containing antigen can efficiently induce salivary and systemic immune responses. The aims of this study were to determine whether oral immunisation with bioresorbable microparticles might lead to a common mucosal response including vaginal secretions. Female Balb/c mice were immunised orally with bioresorbable microparticles containing ovalbumin at 0 and 4 weeks. Antibody responses were assayed by ELISA in saliva, gut washings, vaginal washings and serum, and antibody producing cells were assayed by ELISPOT in salivary glands and nasal cavity. After primary immunisation, IgA antibodies were detected in vaginal washings, saliva and in gut washings which were significantly greater than those detected with soluble antigen alone ($p < 0.05$). Secondary immunisation greatly enhanced antibody titres. Oral immunisation produced antibody forming cells in salivary glands and in nasal cavity and responses were seen primarily after the second immunisation.

The results suggest that immunisation with antigen in bioresorbable microparticles induces common mucosal immune and systemic responses efficiently and may be effective against infections affecting a number of different mucosal surfaces.

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N BHADIA*, S MEGHJI, M WILSON, B HENDERSON, S HAIR (Maxillofacial Surgery, Eastman Dental Institute, UK): Bacterial surface-associated material stimulates nitric oxide production.

Nitric oxide (NO) is a potential pro-inflammatory mediator produced by various cell populations (e.g. macrophages and endothelial cells) when exposed to endotoxin or pro-inflammatory cytokines. The possibility that the easily solubilized surface-associated material (SAM) from periodontopathic bacteria can induce NO synthesis has now been examined. The murine macrophage cell line J774 was cultured in 96 well plates in the presence of various concentrations of SAM or SAM plus γ -interferon from *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Elkanella corrodens*, *Prevotella intermedia*, *Campylobacter rectus* or *Staphylococcus aureus*. The effect of endotoxin and lipopolysaccharide from enteric bacteria was also tested. The production of NO was measured by assay of nitrite using the Griess reagent with colorimetric determination at 540nm. The SAM's from *E. corrodens* and *A. actinomycetemcomitans* at 100ng/ml and 10µg/ml respectively were potent stimulators of nitric oxide synthesis macrophages while the SAM's from *P. gingivalis*, *C. rectus* and *S. aureus* only stimulated nitric oxide production in combination with γ -interferon. The SAM from *Prevotella intermedia* was not able to induce macrophage nitric oxide production at the highest concentration tested (200ng/ml). In the presence of γ -interferon SAM from *E. corrodens* had an ED₅₀ of 10ng/ml. NO production was blocked by addition of the competitive inhibitor L-monomethylarginine (L-NMMA). Bacterial surface-associated material stimulates nitric oxide production by macrophages.

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SJ RAVENALL*, SR STONE, MA CURTIS & GL HOWELLS (Oral Pathology & MRC Group, LHM&C & Haematology, Camb.): Thrombin, but not a proteolytically inactive mutant, stimulates IL-6 production by human gingival fibroblasts.

Fibroblasts are an important source of IL-6 in many chronic inflammatory conditions. Thrombin has recently been shown to stimulate fibroblast proliferation and chemotaxis *in vitro*, but its effect on IL-6 production is unknown. The aim of this study therefore was to determine whether thrombin is able to stimulate IL-6 production by human gingival fibroblasts (HGF) and also whether this effect is dependent upon proteolysis. HGF were grown to confluence in 24 well plates in RPMI and 10% fetal calf serum, starved of serum for 24 hours and stimulated with IL-1 (20 U/ml), thrombin (10^{-12} M - 10^{-6} M) or a proteolytically inactive thrombin mutant (S195A) (10^{-12} M - 10^{-6} M) for 72 hrs and the supernatants harvested. Using ELISA (R&D), a dose dependent stimulation of IL-6 production by thrombin was observed between 10^{-12} M (323 \pm 16 pg/ml) 10^{-6} M (1598 \pm 75 pg/ml) and compared with unstimulated levels (27.6 \pm 14 pg/ml). IL-1 stimulated concentrations were 84153 \pm 4179 pg/ml. By contrast, S195A had no effect at any concentration up to 10^{-6} M. Northern analysis of HGF using a cDNA probe for human IL-6 (a gift of Dr. L. McKay) after 16 hrs stimulation showed a significant increase in levels of IL-6 mRNA.

We conclude that thrombin stimulates de novo IL-6 production by HGF *in vitro* which is dependent upon proteolytic activity and probably mediated by the platelet type thrombin receptor.

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GL HOWELLS*, MA CURTIS, E SCOTT & SR STONE (Oral Pathology & MRC Group, LHM&C & Haematology, Cambridge): Polymorphonuclear cell chemotaxis in response to thrombin and a proteolytically inactive mutant thrombin *in vitro*.

Thrombin is a chemotactant for a number of inflammatory cells including neutrophils. However it is unclear whether neutrophil chemotaxis is triggered by the proteolytic cleavage and activation of the recently cloned platelet thrombin receptor. Recently we found, using flow cytometry, that antibodies against this receptor bound to platelets but not neutrophils (Howells G L, B J Haem 84: 156-160, 1993). The aim of this project was to investigate the mechanism of thrombin-induced neutrophil chemotaxis *in vitro*. Using a 48 well Boyden chamber and freshly isolated neutrophils, we compared human thrombin, a recombinant proteolytically inactive form of thrombin (S-195A) and the thrombin receptor agonist peptide (TRAP). Neutrophils were counted in 2 fields from each of 4 replicate wells using computer imaging (Seescan, Cambridge UK). Thrombin triggered directional movement of neutrophils (10^{-11} M - 10^{-6} M) which could be completely blocked by the thrombin inhibitor hirudin. However, TRAP (0.5 - 500 µM) had no significant effect. A dose dependent chemotactic response was also produced by the enzymatically inactive S-195A thrombin (maximal at 10^{-8} M).

We conclude that the chemotactic effect of thrombin for neutrophils is not dependent upon activation of the cloned platelet thrombin receptor and may be mediated by an alternative receptor.

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D SHAKIR, DR GEATCH* and JJ TAYLOR (Departments of Oral Biology and Restorative Dentistry, The Dental School, University of Newcastle upon Tyne, UK): Detection of IgH gene rearrangements in DNA extracted from GCF.

Immunoglobulins are produced in B-lymphocytes as a consequence of clone specific rearrangements in the immunoglobulin heavy chain (IgH) gene locus; therefore the detection and analysis of these gene structures are of interest in defining the role of B-lymphocytes in inflammatory disorders such as periodontal disease. The presence of DNA containing rearranged IgH genes in extracts of gingival crevicular fluid (GCF) was investigated using PCR. Due to the complexity of the IgH gene locus 5 separate primer pairs were required for comprehensive analysis of gene rearrangements within the locus. GCF samples were obtained from 2 periodontally healthy individuals and from 3 patients with chronic periodontal disease using filter paper strips and DNA was extracted by heating in the presence of NaOH. Analysis of the patterns of PCR products obtained from neutralised GCF extracts showed different size and intensity of amplified IgH rearrangements with different variable region primers. The overall pattern of gene rearrangements was almost identical in GCF from different individuals irrespective of their periodontal status. Interestingly comparison of the pattern obtained for the GCF samples and the peripheral blood lymphocyte DNA indicated some differences.

It is concluded that this approach is useful in the analysis of IgH genes in GCF samples and therefore in the study of the role of the B-lymphocytes in the immune response at this site.

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J COWPE, G OGDEN, N MOLLAOGLU* and M RAWASDESH (Departments of Oral Surgery, Universities of Cardiff & Dundee) Comparing morphometry of oral smears using two image analysis systems.

A Seescan image analysis system has recently replaced the M85 microdensitometer and Vids V analyser which were previously used for the assessment of nuclear DNA content of Feulgen stained smears and measuring nuclear (NA) and cytoplasmic (CA) area of Papanicolaou stained smears, respectively. The Seescan system performs both DNA and NA and CA measurements. We have previously reported the use of a "discriminant line" superimposed on an NA/CA scatterplot to identify suspicious oral lesions and promote biopsy (Cowpe et al, J Roy Soc Med 81: 509-513, 1988). The aim of this study was to detect any variation in the mean NA and CA values for a selection of oral smears measured using the Vids V and the Seescan systems. Twenty clinically normal and abnormal Papanicolaou stained smears were measured with both systems. Fifty randomly selected cells and their nuclei were measured in each specimen using both systems. Analysis of method comparison was performed (Brown RA & Beck JS, J Clin Path 42: 4-12, 1989). The mean NA value recorded with Seescan (93.36µm²) was significantly elevated by a "constant amount" compared with values recorded with the Vids (81.36µm²). There was no significant variation between mean CA values of 2035µm² and 1905µm² for the Vids and Seescan system respectively.

In conclusion, because NA values appear to be larger when using the Seescan smears measured using Seescan cannot be directly compared with those previously measured using Vids. A new "discriminant line" will need to be constructed.

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C FROST*, G F COPE and I L C CHAPPLE (School of Dentistry & Wolfson Applied Technology Laboratory, The University of Birmingham, UK): A new salivary assay for nicotine metabolites for analysis of smoking habit.

Studies have demonstrated that self report of smoking habit is inaccurate and that objective tests for nicotine and its metabolites are more useful in assessing active and passive smoke exposure. We report a new chair-side assay (CSA) for salivary cotinine, modified from the method of Cope et al (*J Smoking-Rel Dis* 5:163-167, 1994) which expresses cotinine concentration as $\mu\text{g/ml}$ nicotine equivalents ($\mu\text{g/ml}$ n.eq). This study aimed to calibrate the CSA against a standard radioimmuno assay (RIA), to measure within and between assay coefficients of variation (CV) and to relate salivary cotinine levels to nicotine intake in smokers and non-smokers. A saliva sample from a smoker was aliquoted and assayed 8 times under uniform conditions. A second saliva sample from a non-smoker was loaded with $15\mu\text{g/ml}$ cotinine and diluted with phosphate buffered saline to form 1.875 & $0.9375\mu\text{g/ml}$ cotinine standards, which were assayed 9 times on separate days. Stimulated saliva was collected (5 mins) from age-matched smokers ($n=24$) and non-smokers ($n=18$) by chewing a rubber band and related to smoking habit. The within batch CV was 15% and between batch CV's were 15.7% ($1.875\mu\text{g/ml}$) and 13.8% ($0.9375\mu\text{g/ml}$). The correlation (r) between the CSA and RIA was 0.6 ($p < 0.0001$). There was a significant relationship between salivary cotinine concentration and number of cigarettes smoked daily (RIA, $r = 0.5$ $p < 0.01$; CSA, $r = 0.43$ $p < 0.04$) and number smoked on the day (RIA, $r = 0.5$ $p < 0.009$; CSA, $r = 0.54$ $p < 0.007$). Using a threshold of $0.25\mu\text{g/ml}$ n.eq, the CSA correctly diagnosed smoking habit with 93% sensitivity & 94% specificity. *We conclude that the salivary cotinine assay has potential value as a chairside test of smoking habit.*

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NM HOWIE*, AT CRUCHLEY and DM WILLIAMS (The London Hospital Medical College, U.K.): The Effect of Ethanol on the Permeability of Human Oral Mucosa to Albumin and Sucrose.

Ethanol has been implicated in the aetiology of oral cancer, through its local action as a solvent on the lipid permeability barrier of oral mucosa, enhancing penetration of carcinogens into the epithelium. Previous studies have exposed mucosa to ethanol for prolonged periods, but recently we have shown that short-term exposure can increase the permeability to tritiated water (Triglas *Tet al. J Dent Res* 72: 694, 1993). We now report the effect of 1 hour and 15 minute exposure of oral mucosa to ethanol on its permeability to FITC-albumin and ^{14}C -sucrose. Specimens of human ventral tongue mucosa were obtained at autopsy from 16 individuals, aged 31-67 years, snap frozen and stored at -70°C until use. The tissue was pre-treated with PBS (control) for 1 hour or with 15% ethanol for 1 hour or 15 minutes, then exposed to FITC-albumin for 18 hours, using a continuous flow perfusion chamber system. Tissue morphology was examined before and after the permeability experiments, and the depth of albumin penetration measured using computer-assisted image analysis. The albumin penetrated significantly further ($p < 0.05$) through the mucosa after pre-treatment with ethanol, whether for 15 minutes ($65.3 \pm 63.1 \mu\text{m}$) or for 1 hour ($74.5 \pm 49.5 \mu\text{m}$), compared with the PBS pre-treated tissue ($11.7 \pm 11.6 \mu\text{m}$). Morphologically, the surface layers of the epithelium were seen to lift off after treatment with ethanol, and the extent of this effect was time-dependent. Further tissue samples were exposed to ^{14}C -sucrose after pre-treatment for 1 hour with PBS or 15% ethanol. Permeability co-efficients (K_p) were determined, but no significant difference was found (7.81 ± 2.60 and $7.11 \pm 3.41 \times 10^{-5} \text{ cm/min}$, respectively). Morphological examination showed disintegration of the epithelium after both forms of pre-treatment, probably due to osmotic shock. *These results suggest that ethanol is capable of enhancing the penetration of large molecular weight molecules into the oral mucosa, which may be important in the pathogenesis of mucosal disease.*

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J G MCGIMPSBY*, P A BLAGIONI and P J LAMBY (Dental School, Queen's University, Belfast UK): Thermographic Imaging subclinical inflammatory changes following surgical removal of impacted lower wisdom tooth.

Surgical removal of an impacted lower wisdom tooth will inevitably produce an inflammatory response causing unpleasant symptoms for the patient. To date clinical parameters have usually been employed to quantify this inflammatory response but such analysis is relatively crude and subjective. Thermographic imaging offers a non-invasive means of quantifying such an inflammatory response accurately and objectively.

The purpose of this prospective study was to measure thermographically the inflammatory response on the operated and unoperated sides prior to surgery, 20 minutes after surgery and on the third and fifth post-operative days.

In this pilot study 10 patients were recruited between 20-50 years, who required the removal of an impacted lower wisdom tooth under local anaesthesia. Pre-operative assessment of the tooth in all cases indicated the necessity for removal of bone using a dental drill. Patients recruited were only permitted to use paracetamol for post-operative analgesia. The results at each of the measurement times indicated temperature changes from normal of 2.2°C immediately postoperatively, 2.0°C on the third day post operatively and 1.3°C and the fifth day after the operation.

The baseline results will be used to study the inflammatory responses to different and-inflammatory regimes.

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S A HIBBERT*, J K FIELD*, B B J LOVUIS and J WHITTAKER* (Departments of Clinical Dental Sciences and Child Health² The University of Liverpool UK: Genetic linkage analysis of apparently dominant inheritance of cleft and palate.

Cleft lip with or without cleft palate (CLP) is one of the most common congenital malformations, with a mean incidence in the Caucasian population of 1:1000 live births. Familial recurrences have suggested a heritable aetiology and a genetic component is now universally accepted. However, attempts to identify the genetic locus have so far been unsuccessful. Analysis of records in the Mersey Regional Cleft Lip and Palate Unit has identified 38 families (101 affected individuals) with apparent dominant inheritance of CLP. Blood is currently being collected from these families for DNA extraction from lymphocytes. We are using PCR amplification of microsatellite loci to test linkage to candidate chromosome bands. This research group has successfully used this technique to locate the tylosis, oesophageal cancer gene, to chromosome 17q (Risk *J et al., Nature Genet.* In press).

Studies have suggested the role of a major gene in CLP families. Current molecular techniques are now available to investigate the location of the gene(s) in these families.

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L ROZ*, C L WU*, P SPEIGHT, S PORTER, C SCULLY, N S THAKKER* (*University of Manchester, Eastman Dental Institute, London): Loss of heterozygosity on chromosome 3p in oral premalignant lesion and squamous cell carcinoma.

Loss of heterozygosity (LOH) indicative of presence of tumour suppressor genes on chromosome 3p is commonly observed in carcinomas of various tissues. We have examined LOH on chromosome 3p in 27 oral squamous cell carcinomas (SCCs) and 28 precancerous lesions (PCLs) with severe dysplasia, using 15 highly informative microsatellite polymorphisms and constructed a detailed deletion map of chromosome 3p. Overall, LOH was observed in ~52% of SCCs and 50% of PCLs. A majority (~80%) of samples with LOH showed a loss in more than one area. Interstitial losses in both SCCs and PCLs defined three major noncontiguous regions of loss at 3p13-21.3 (centred on marker D3S1228), 3p21.3-p22 (centred on marker D3S1007) and 3p25 (centred on markers D3S1110 and D3S656).

These data suggest a role for at least three tumour suppressor genes on chromosome 3p in oral SCCs. Furthermore the high frequency of LOH in oral PCLs suggest that genetic alterations of chromosome 3p are an early event in oral carcinogenesis.

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K T ALLISON* and TEKESA BEST (Department of Oral Surgery, Medicine & Pathology, UWCM, & Cardiff Institute of Higher Education UK): p53, PCNA & Ki67 expression in oral SCC and the effects of some preparatory procedures.

This investigation builds on previous studies of proliferation markers in a defined group of oral SCCs. Specifically, the effects of microwaves on p53, PCNA & Ki67 were examined in alcohol and formalin fixed tissues and the distribution of antigens compared. 100% of tumour cells were positive for p53 using a standard immunostaining method following microwaving for 20 minutes in citric acid buffer. There were many 'false' positive cells, especially inflammatory cells. That the primary antibody dilution had to be optimised casts doubt on indiscriminate use of antigen retrieval methods. Microwaving prior to demonstration of PCNA is contra indicated. Like p53, both PCNA and Ki67 staining was enhanced following alcohol fixation. More PCNA positive cells were revealed following alcohol fixation. p53 showed nuclear, cytoplasmic, perinuclear and a distinctive inter epithelial cell distribution in different tumours. There was no correlation between tumour differentiation and expression of these antigens. There was no commonality between Ki67 expression and either p53 or PCNA expression.

It is concluded that ethanol is the fixative of choice for proliferation markers and that antigen retrieval methods require careful interpretation.

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J PENHOLLOW*, R MAHER, S WARKAKULASURIYA, N W JOHNSON, (King's College School of Medicine & Dentistry, London, IPMC, Karachi): Relative infrequency of H-ras codon 12 mutations in carcinomas arising in oral submucous fibrosis.

We earlier reported that point mutations in the H-ras oncogene of oral squamous cell carcinomas are infrequent in British cases (Warkakulasuriya et al. *J Oral Pathol Med* 1992; 21: 223-9) but are common in chewing tobacco-related oral carcinomas in India (Sarnath et al., *Br J Cancer* 1991; 63:573-8). We now report on the prevalence of H-ras mutations (codon 12) in a mixed sample of oral submucous fibrosis (OSF) ($n=16$), oral leukoplakia ($n=2$) and an additional 16 cases with concurrent carcinomas. DNA was extracted from formalin-fixed, paraffin-embedded tissues following proteinase digestion by the isopropanol method. In vitro amplification of a 111 base pair fragment flanking H-ras codons 12/13 was performed by polymerase chain reaction (PCR). Amplification of DNA was evaluated by gel electrophoresis. 31 samples were successfully amplified. The PCR product was then digested with a restriction enzyme (MSP1) for codon 12 mutant specific Restriction Fragment Length Polymorphism (RFLPs) on gel electrophoresis (Campbell et al. *Br J Derm* 1993; 128: 111-4). An experimental tumour derived from a rat fibroblast line expressing human mutated H-ras served as a positive control. Only 1/16 squamous cell carcinomas (transformed from OSF) was found to have a H-ras codon 12 mutation. Tissues from OSF and leukoplakia cases (with no concurrent carcinomas) showed no evidence of H-ras mutations at this site. *ras Oncogene activation in oral carcinomas arising from OSF appears to be infrequent compared to a previously reported Indian sample (with no OSF).* This suggests that the natural history and multi-stage mechanisms may be different during oral carcinogenesis following different precancerous stages.

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K RAI*, ND EDMONDSON, J HAMBURGER and JB MATTHEWS (School of Dentistry, University of Birmingham, UK): p53 expression in oral lichen planus.

Whilst over expression of p53 is often interpreted as indicating the presence of abnormal product, due to mutation of the p53 gene, overexpression of 'normal' p53 may be associated with local repair mechanisms. The aim of this study was to examine expression of p53 in oral lichen planus and simple keratoses. Paraffin sections of lichen planus ($n=13$) and keratosis ($n=14$; specimens without histological evidence of dysplasia) were stained for p53 using a sensitive Biotin-Streptavidin immunoperoxidase technique (Biogenex) and monoclonal antibody to p53 (clone BP53-12; Biogenex). Staining was performed with and without microwave pretreatment. Positive cells were counted manually and related to length of basement membrane as assessed by TV image analysis. In both groups, positive cell numbers were increased by microwave treatment ($p < 0.001$). More p53+ cells were seen in lichen planus ($74.5 \pm 5.2 \text{ cells/mm}$) than in keratoses ($27.7 \pm 2.8 \text{ cells/mm}$; $p < 0.0001$). Within the lichen planus group, there were significantly more p53+ cells associated with areas overlying dense inflammatory cell infiltrates compared with those associated with moderate or mild infiltrates ($p < 0.005$). The results suggest that over expression of p53 may play a role in the reparative process of epithelium in lichen planus and that levels of p53 reflect the extent of underlying inflammatory infiltrate.

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A KIRBY*, I OLSEN†, P FARTHING+ and SR PORTER (Depts of Oral Medicine and Periodontology, Eastman Dental Institute; +Dept of Oral Pathology, London Hospital Medical College; Lymphocyte function-associated antigen-3 (LFA-3) in oral lichen planus.

Lichen planus is a chronic inflammatory mucocutaneous disorder characterised by massive infiltration of leukocytes, particularly T-cells, into the lamina propria of lesional tissue. Since lymphocyte function-associated antigen-3 (LFA-3) plays a major role in T-lymphocyte binding, we have examined the expression of this adhesion molecule in oral lichen planus. Immunohistochemical staining of cryostat sections of normal buccal mucosa (n=6) revealed LFA-3 to be present predominantly on epithelial Langerhans cells and particularly on keratinocytes in close proximity to the basal lamina. However, in oral lichen planus (n=12), LFA-3 was expressed at higher levels, and more discontinuously, within the epithelium. Moreover, two unique LFA-3 staining patterns were consistently observed in the sub-epithelial region. Firstly, infiltrating macrophages had no detectable surface-associated LFA-3, but intense cytoplasmic staining, suggesting the presence of LFA-3 mRNA lacking the membrane insertion domain. Secondly, extensive LFA-3 staining was found to be associated with the extracellular matrix, possibly as a result of shedding from fibroblasts, which had no surface-associated staining. Molecular studies are now in progress to determine whether macrophages in lichen planus produce an alternatively spliced form of the LFA-3 mRNA transcript and if a 'soluble' LFA-3 could facilitate the retention and activation of T-cells within lesional tissue of oral lichen planus.

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J JONES*, PM SPEIGHT, M SUGIYAMA†, FM WATT† (Eastman Dental Institute and Imperial Cancer Research Fund, London, and Osaka University*, Japan): The role of the vitronectin receptor in malignant oral epithelium.

Integrins are heterodimeric cell surface adhesion molecules involved in cell-cell and cell-matrix interactions. The α_v subunit associates with various β subunits to form a vitronectin receptor. In melanoma cell lines expression of $\alpha_v\beta_3$ is associated with invasiveness and metastasis but the role of α_v in modulating the behaviour of malignant epithelial cells is not known. The purpose of this study was to investigate the role of α_v in oral squamous cell carcinomas (SCC). A cell line derived from an oral SCC, H357, that is naturally deficient in α_v was transfected with an expression construct for α_v , and positive clones were sorted by FACS. Empty vector control clones were also made. Growth studies were carried out on tissue culture plastic and in 0.3% soft agar. Differentiation was investigated by Western blotting for involucrin production. The expression of α_v and $\alpha_v\beta_3$ in tumours was investigated by immunoperoxidase staining of frozen sections of 18 oral SCC and 10 specimens of normal oral mucosa. Clones expressing α_v grew more slowly and formed colonies less efficiently than the α_v -negative parental cells. Involucrin expression was increased in the α_v clones, α_v and $\alpha_v\beta_3$ were weakly expressed in normal oral mucosa, but expression was increased in all SCCs. The results show that vitronectin receptors are upregulated in oral carcinomas but their role appears to be complex since *in vitro* expression was associated with reduced cell proliferation and the onset of terminal differentiation. Further studies are needed to elucidate the role of vitronectin receptors in oral carcinomas.

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J.WESTERMAN*, P.ROBINSON and W.HUME (Division of Dental Surgery, Leeds Dental Institute, Leeds): Development of a mini-collagen gel system for the investigation of fibroblast behaviour *in-vitro*.

Investigations into fibroblast behaviour are being carried out more frequently in collagen matrices as this allows the cells to grow in three dimensions which is more analogous to the *in-vivo* state. In our earlier investigations we employed 50mm gels for radiolabelling experiments which required large stocks of collagen, cells and radiolabel. To overcome this we developed a mini-collagen gel system in which a known number of fibroblasts was mixed in a collagen matrix and allowed to gel in the wells of a 96 well microtitre plate. This enabled large numbers of different cell samples to be investigated simultaneously and under identical conditions. It also allowed timed observations to be carried out ranging from zero hour to several days. We have used this method for radiolabelling fibroblasts with ^3H -thymidine and ^{35}S -methionine as a measure of DNA and protein synthesis respectively. We have also investigated the effect of drugs on fibroblast behaviour (e.g. cyclosporin) using this technique.

We have developed a mini-collagen gel system which enables rapid investigation of different fibroblast sample behaviour in collagen simultaneously and under similar conditions with more economical use of reagents.

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CS DOWNER* and SS PRIME (Department of Oral and Dental Sciences, University of Bristol, UK): The expression of keratinocyte growth factor receptors on human oral keratinocytes but not fibroblasts.

Previous studies have investigated the response of tumour-derived oral keratinocytes and contiguous fibroblasts to keratinocyte growth factor (KGF) (Downer C and Prime SS, *J Dent Res* 73: 833, 1994). The results indicated that keratinocytes but not fibroblasts are stimulated by this ligand in a dose-dependent manner. The purpose of the present study was to determine whether the differential response of keratinocytes and fibroblasts to KGF reflected a differential expression of cell surface KGF receptors. Subconfluent dishes of cultured cells were incubated with ^{125}I -KGF for three hours at room temperature. The receptors were cross linked with disuccinimidyl suberate (30 ng/ml) for 30 minutes at room temperature and transferred to eppendorf and lysed with 1% (v/v) Triton. Samples of the supernatant were loaded onto an 8% polyacrylamide gel and resolved for 4-12 hours at 200 v 6-30 mA. Gels were removed, dried and exposed for 2 weeks at -70°C prior to developing. In certain experiments competitive inhibition of ligand binding with excess KGF was used to determine the specificity of ^{125}I -KGF binding. The results indicated that keratinocytes but not fibroblasts expressed cell surface KGF receptors.

This study demonstrates that the distribution of KGF cell surface receptors reflects the differential response of keratinocytes and fibroblasts to this ligand.

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AJ COLLIER*, JR SANDY, and SS PRIME (Dept of Oral and Dental Sciences, University of Bristol, UK): TGF- β 1 signal transduction may be mediated through guanine nucleotide binding proteins (G proteins) in human oral keratinocytes.

The intracellular mechanisms by which transforming growth factor β 1 (TGF- β 1) mediates its response remain largely unknown. This study examined the relationship between TGF- β 1 and G proteins in a series of human oral carcinoma-derived cell lines. The expression levels of the α subunit of one form of G-protein, G_{α} , were determined in the presence or absence of TGF- β 1 (10 ng/ml for 3 hr at 37°C; 1% FCS) using western and northern blot analysis. All cell lines (n=7) expressed the G_{α} subunit and TGF- β 1 upregulated the expression of G_{α} . In order to establish whether TGF- β receptors mediate their response to exogenous ligand via a G protein, two bacterially-derived toxins from *V. cholerae* (CTX) and *B. pertussis* (PTX), which interfere with G protein action, were used in studies of ^{125}I -TGF- β 1 binding affinity and TGF- β 1-mediated transcription factor regulation. Both toxins changed the profile of TGF- β cell surface receptors from one of high affinity to that of high and low affinity. PTX interfered with TGF- β 1-induced *jun-B* upregulation but did not have an effect on TGF- β 1-induced downregulation of *c-myc*.

The results of this study show that TGF- β 1 signal transduction may be mediated through pertussis sensitive G proteins. In addition, the data indicate that TGF- β 1-induced control of cell proliferation (*c-myc*) and ECM (*jun-B*) may be regulated at the cell surface.

This work was supported by an MRC project grant G9123775.

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M FAHEY*, A STONE, AJ COLLIER, IC PATERSON and SS PRIME (Dept of Oral and Dental Sciences, University of Bristol, UK): TGF- β isoform autocrine production by cultured *ras*-transfected and tumour-derived human keratinocytes.

An ELISA to quantify TGF- β production in conditioned medium from cultured cells has been described previously (Fahey M *et al.*, *J Dent Res* 73: 833, 1994). The present study extends this preliminary work and reports the autocrine production of TGF- β 1, - β 2, - β 3 by immortal (n=1), *ras*-transfected (n=4) and tumour-derived (n=7) cultured human keratinocytes. Detection limits were 39 pg/ml for TGF- β 1, 78 pg/ml for TGF- β 2 and 1.875 ng/ml for TGF- β 3. Negative staining controls and cross-reactivity of TGF- β isoform-specific antibodies were consistently less than 3.5%. All of the cell lines produced TGF- β 1 (mean 526.5 pg/10⁶ cells/48 hr; n=12); over-expression of this ligand occurred in *ras*-transfected keratinocytes (mean 485.8 pg/10⁶ cells/48 hr; n=4) compared to their more normal immortal counter-part (mean 194.9 pg/10⁶ cells/48 hr). TGF- β 2 was produced by 8 of 12 cell lines (mean 274.4 pg/10⁶ cells/48 hr) and decreased expression was associated with the tumorigenic phenotype. The production of TGF- β 1 and - β 2 was unrelated to the degree of cellular differentiation. TGF- β 3 was undetectable in all cell lines.

The results demonstrate that the ratio of TGF- β 1: TGF- β 2 isoform production reflects tumour cell behaviour in atypical mice.

This work was supported by an MRC project grant G9123775

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J.LI, P.M.FARTHING†, G.W.IRELAND and M.H.THORNHILL* (The University of Manchester and The London Hospital Medical College, UK): Comparison of IL-6 production by oral and skin keratinocytes

Keratinocytes (KC) play an important role in immune and inflammatory processes by producing pro-inflammatory cytokines such as IL-6. IL-6 is an important mediator of the host response to injury and infection and has pleiotropic effects on wound healing as well as immune and inflammatory processes. Here we compared IL-6 production by human oral (OK) and skin keratinocytes (SK) in culture, and studied the effect of cytokine stimulation on IL-6 production, using an IL-6 specific sandwich ELISA and the IL-6 specific B9 cell line proliferation assay. The two assays produced comparable results.

There was no constitutive IL-6 production by OK or SK. However, stimulation with TNF- α or IFN- γ induced significant levels of IL-6 production by both cell types. Optimal stimulation of OK occurred after 48 hr and 48-72 hr for SK using 100-1000 U/ml TNF- α . TNF- α had a significantly greater effect on OK than SK (p<0.001). IL-4 also induced IL-6 production which was greater for OK than SK (p<0.001). The IL-4 effect peaked after 48 hr on OK but did not peak until sometime later than 72 hr on SK. In contrast, IL-1 α induced a significant increase in IL-6 production by OK (p<0.05) but not SK. TGF- β and IL-8 had no effect. When used in combination, IFN- γ and TNF- α had a large synergistic effect on IL-6 production by both cell types. IFN- γ and IL-4 had a similar but smaller synergistic effect on both cell types.

Compared to SK, OK exhibited a different pattern of responsiveness to cytokine stimulation and the resulting increase in IL-6 production was larger and more rapid. Differences in IL-6 production by OK and SK may help to explain the different pattern of skin and oral lesions in mucocutaneous diseases such as lichen planus and the different patterns of wound healing at the two sites.

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J. LI*, P. FARTHING†, and M. THORNHILL (Dept of Oral Medicine, Univ Dental Hospital of Manchester and Dept of Oral Pathology, The London Hospital Medical College, UK) Effect of serum on ICAM-1 expression by oral and skin keratinocytes.

Intercellular adhesion molecule-1 (ICAM-1; CD54) expression by keratinocytes is important in the interepithelial and interepithelial accumulation of lymphocytes in inflammatory disorders such as lichen planus which may affect both the skin and oral cavity. However, there are differences in ICAM-1 expression, and cytokine induction of ICAM-1 expression, between skin (SK) and oral keratinocytes (OK) cultured in growth medium (GM) containing 10% fetal calf serum (FCS) (J. Li, *et al.*, *J Dent Res* 72: 739, 1993). The aims of this investigation were to compare the induction of ICAM-1 expression on OK and SK grown in GM and serum free medium (SFM) using a cell-based ELISA to see if a factor in serum was responsible for these differences.

OK cultured in GM expressed high levels of ICAM-1 expression which was abolished by culture in SFM. No evidence of ICAM-1 expression on SK cultured in either GM or SFM was observed. Of the supplements present in GM, only FCS induced ICAM-1 on OK and maximal expression was reached after 8 hrs in 20% FCS. Human serum had the same effect as FCS but there was no effect of either agent on SK. IFN- γ induced a larger increase in ICAM-1 expression on OK grown in GM than on OK grown in SFM (p<0.05) but conversely the increase in ICAM-1 expression on SK grown in GM was significantly less (p<0.05) than on SK grown in SFM.

These results show that OK and SK in culture respond differently in their expression of ICAM-1 in the presence of serum, and their response to IFN- γ stimulation is also different. These observations suggest that a factor in serum promotes ICAM-1 expression on OK and inhibits it on SK. The factor responsible for this has yet to be identified.

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T-J LI*, RM BROWNE and JB MATTHEWS (Unit of Oral Pathology, University of Birmingham, UK): TGF α and EGF in odontogenic jaw cysts.

TGF α and EGF are structurally related molecules that regulate cell growth and differentiation via EGF receptor (EGFR). Our recent studies have demonstrated differential expression of EGFR by different types of odontogenic cyst lining (Li T-J, Browne RM & Matthews JB, *Virchows Archiv A* 423: 137-144, 1993). The aim of this study was to investigate the pattern of expression of TGF α and EGF in the three major types of odontogenic cyst (odontogenic keratocysts [OKC], n=27; dentigerous cysts [DC], n=10; radicular cysts [RC], n=10). Growth factors were detected in paraffin sections using a biotin-streptavidin peroxidase technique with monoclonal antibody to TGF α (clone 213-4.4, Oncogene Science) and polyclonal antibody to EGF (Z-12; Santa Cruz).

The epithelial linings of all cysts showed reactivity for TGF α which was mainly localised to basal and suprabasal layers. 89% of OKC linings exhibited a strong positive reaction. By contrast, expression in DC and RC linings was patchy and weaker, with only 50% exhibiting strong immunoreactivity. In all specimens, EGF reactivity was weaker than that for TGF α with 2 DC and 2 RC being negative. The staining pattern also differed, with EGF staining being predominantly suprabasal. TGF α and EGF were also detected in endothelial cells, fibroblasts and inflammatory cells within the cyst walls. These results, together with our previous studies of EGFR, indicate differential expression of TGF α , EGF and their common receptor between the different types of odontogenic cyst, suggesting that these growth factors (via autocrine &/or paracrine pathways) may be involved in their pathogenesis.

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RM GRAVELEY*, A HOUGHTON, RGG RUSSELL and BMJ STRINGER (Dept of Oral Pathology and HMCB, University of Sheffield, UK): Modification of differential display polymerase chain reaction for bone and cartilage studies.

The differential display polymerase chain reaction (dd-PCR) technique is an extremely powerful method which enables the rapid identification of expression differences between cell populations. In short, all mRNA species expressed by a cell or tissue sample are displayed as a series of size fractionated mRNA fragments in polyacrylamide sequencing gels, after PCR amplification with specified primers. By running in parallel eg. the same sample which has undergone a different treatment, it is possible to discern differences in mRNA expression resulting from that treatment, and isolate those expressed differences directly from the gels (Liang et al. *Nucleic Acids Research* 21: 3269-3275, 1993). We reported to the BSDR in 1994, our application of this procedure to studies of IL-1 α induced expression in human bone cells. Although this approach has provided us with interesting data, isolating differentially expressed sequences from the polyacrylamide gels without the carry over of surrounding but non-differentially expressed sequences, remains difficult. Often it is necessary to subclone after gel extraction, to isolate the single fragment(s) of interest. To avoid subcloning, and consequently make substantial resource savings, we have applied a recent modification of this technique (Sokolov and Proctor, *Nucleic Acids Research* 22: 4009-4015, 1994) to our studies. This provides for differentially displayed sequences in agarose gels, and a ten-fold decrease in band numbers per lane when compared to standard dd-PCR, making single band isolation possible. Also, differential expression is of RNA sequences upstream of the 3' untranslated region, unlike Liang et al's technique.

In conclusion, we have applied a modified differential display technique to our bone and cartilage studies and found a significant improvement in the isolation and sequencing of differentially expressed sequences identified in our bone and cartilage studies.

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X CAO*, A HOUGHTON, RGG RUSSELL & BMJ STRINGER (Depts of Oral Pathology and HMCB, University of Sheffield): Immobilisation of human foetal bone precursor cells by retroviral temperature-sensitive oncogene transduction.

In recent years, very considerable advances have been made in bone cell biology and it is now clear that bone is a very active tissue which is continually remodelled throughout life. In addition to regulating the functioning of differentiated bone cells, though, it is now understood that controlling the commitment of bone precursor cells to the mature phenotype, may also be crucial to bone homeostasis. In order to provide the human cellular tools to study this differentiation process, we have immortalised human osteoblast precursor cells (Houghton et al. 1995 BSDR meeting). To complete this picture though and to give greater opportunity to understanding the whole of the human bone cell differentiation process, we have attempted to produce large homogeneous populations of human osteoblast precursor cells from foetal tissues at a stage representing early commitment of differentiation to the osteoblast lineage. Utilising our oncogene transduction technology, we have shown that amphotropically packaged retroviral constructs are capable of transducing human foetal bone precursor cells at 6-9 weeks of gestation. Also, that transduction with a temperature sensitive form of the simian virus 40 derived large tumour antigen induces sustained cell replication when incubated at the oncogene's non-permissive temperature. Furthermore, these cells can be induced to inspect characteristics expected of differentiating human bone cells.

We conclude that these foetal clones could provide a useful model of human cells at an early stage in the commitment to an osteoblast lineage, and as such may allow the study of very early events involved in the human bone cell differentiation process.

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M.F. HAQUE*, M. HARRIS, B. HENDERSON and S. MEGHLI (Department of Oral and Maxillofacial Surgery, Eastman Dental Institute, London WC1X 8LD): An immunohistochemical study of inflammatory cells in oral submucous fibrosis.

Oral submucous fibrosis (OSF) is characterised by progressive fibrosis of the lamina propria stimulated by areca alkaloids from areca catechu nut. There is also a lichenoid infiltrate of inflammatory cells but has not been fully characterised. To study the inflammatory infiltrate sections of OSF tissue were stained with an immunoperoxidase technique using antibodies to pan T cells (CD3), T helper/inducer cell (CD4), T suppressor/cytotoxic cell (CD8), macrophage (CD68) and B and activated T cells (HLA-DR alpha chain antigen). 15 patients and 5 normal controls entered the study. 4 mm diameter punch biopsies were taken from the cheek mucosa and snap frozen in liquid nitrogen for cryostat sectioning. In normal tissues there were occasional CD3 and CD4 positive cells. In the lesion most cells were CD3 positive. CD4 cells predominated with only occasional CD8 and CD68 cells scattered in the infiltrate. In 8 cases CD3 and CD4 positive cells infiltrated the epithelium, in all cases almost all cells were HLA-DR positive.

The results show that the inflammatory infiltrate in OSF is dominated by activated T cells with a high CD4/CD8 ratio. This is similar to that seen in a number of immune-mediated mucosal diseases including lichen planus, and suggests that in OSF there may be a local immune response to altered epithelial antigens.

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M ONG'UTI*, GL HOWELLS, IM LEIGH & DM WILLIAMS (Oral Pathology & Experimental Dermatology, LHM, London): An immunohistochemical study of keratins 6 & 16 and Ki-67 expression in African ameloblastomas.

A significant proportion of ameloblastomas in Africans are aggressive and appear to grow rapidly. These may be different in aetiology or show differences in tumour cell phenotype. The aim of this study was to investigate cell proliferation in variety of ameloblastomas from Kenyan patients using monoclonal antibodies to keratins 6 and 16, which are expressed by proliferating epithelium and Ki-67 antigen, a cell proliferation marker. Representative tissue was taken from 14 cases of ameloblastomas on excision. 5 μ m cryostat sections were prepared and immunocytochemistry performed using monoclonal antibodies to keratin 6 (KA12) keratin 16 (L1025) and Ki-67 (MB-1, Dako) and the avidin biotin peroxidase system. In all cases a patchy staining for keratins 6 and 16 was seen. The intensity of staining for both keratins was similar and exclusively suprabasal. By contrast Ki-67 staining was mainly confined to the basal cells and most frequently seen in areas which also showed keratins 6 and 16 staining. Ki-67 antigen expression was variable. The percentage of epithelial cells staining for Ki-67 antigen ranged from 1.6% - 17.9% based on counts of more than 5000 cells for each case.

We conclude that keratins 6 and 16 are expressed in suprabasal epithelial cells in a range of ameloblastomas. The variation in the expression of Ki-67 antigen from case to case may prove to be a useful prognostic marker in further studies.

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J COWPE*, G OGDEN, N MOLLAOGLU and R WALKER (Departments of Oral Surgery, Universities of Cardiff & Dundee): Measuring nuclear DNA-content of oral smears using a Seescan image analysis system.

Cytophotometry has been widely used to characterise the DNA-content of cytological smears. Until recently we used a microdensitometer to measure DNA-content of Feulgen stained oral smears. Following the acquisition of image analysis equipment, with optical density software, the aim of this study was to compare the DNA content of a selection of normal and abnormal smears when measured using three different machines. Twenty five oral smears were measured using a) a Vicker's M85 microdensitometer, b) an IBAS image analysis system and c) a Seescan image analysis system. Randomly selected Feulgen stained nuclei were measured in each smear and DNA range profiles compiled for each specimen. Eleven normal, five non-dysplastic and nine histologically confirmed dysplastic or malignant smears were assessed. There was 92% and 88% agreement between the M85 and the IBAS and Seescan systems respectively, with 80% agreement between the IBAS and Seescan systems. Only IBAS displayed one false negative result. False positive results were observed; three for Seescan and one each for M85 and IBAS.

It is concluded that the seescan system provides a reliable replacement for the M85. It is more user-friendly, easier and quicker to operate, more cells and additional parameters can be measured which should improve the accuracy of quantitative cytology in the detection of malignancy in oral smears.

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J A WOOLGAR*, E D VAUGHAN*, J S BROWN*, J SCOTT* and C R WEST* (Clinical Dental Sciences, Walton Hospital, Public Health, Liverpool): Behaviour of oral cancer and an outcome - audit of its management.

The study details the behaviour and outcome of 123 newly-diagnosed cases of oral squamous cell carcinoma treated by primary local resection and simultaneous neck dissection, and, in 52% of cases, post-operative radiotherapy. Outcome was recorded as survival, cause of death and where applicable, site and timing of recurrence.

Actuarial survival analysis showed the overall one-year survival probability was 84%, falling to 69% at two years, and 65% at five years. For patients without lymph node metastasis, the corresponding survival probabilities were 95%, 86% and 86%, respectively; and for patients with metastasis, 71%, 52% and 44%, respectively. Thirty patients (24%) had died of their oral cancer: 16 (13%) of local disease, 10 (8%) of regional disease and 4 (3%) of systemic metastases. In addition to metastasis, survival was related to the clinical T stage ($P = 0.005$), the histological pattern of invasion ($P < 0.0001$) and status of the resection margins of the primary tumour ($P = 0.0001$). In cases with metastasis, outcome was related to the number ($P < 0.001$) and anatomical position of positive nodes ($P = 0.005$), and the presence and extent extracapsular spread ($P = 0.001$).

We conclude that prognosis is good in early stage oral cancer but is profoundly affected by lymphatic metastasis. Furthermore, the pathological stage maintains its prognostic significance even when adjuvant radiotherapy is given.

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K M BARNARD* & S J CHALLACOMRE (Depts of Paediatric Dentistry, KCSMD and Oral Medicine & Pathology, UMDS, Guy's, London).

Clinical characteristics of patients presenting with orofacial granulomatosis.

Orofacial granulomatosis (OFG) is a chronic non-caseating granulomatous disorder involving the tissues of the mouth and face. It may exist as a localised entity or represent the oral manifestations of Crohn's disease or sarcoidosis. The aim of this study was to characterise specific oral lesions in 29 patients (14 males and 15 females) presenting with OFG. The median age was 29 years (range 4-72) and the median age of onset 28.5 years. The prevalence of lesions was oral swelling (82%), epithelial tags (41%), erythema (72%), depigmentation (21%), fissures (21%), cobblestoning (45%) and ulceration (72%). The main sites involved were buccal mucosa (76%), gingiva (69%) and lips (69%) with tongue, palate and buccal sulcus involved in over 33%. Thirteen (45%) patients presented with symptomatic gastrointestinal involvement including abdominal pain, diarrhoea, constipation, rectal bleeding, mucus, vomiting, tenesmus and perianal signs. Four patients had a previous diagnosis of Crohn's disease and 2 (6.9%) patients had a known diagnosis of sarcoidosis.

In conclusion there was involvement of four or more oral sites in 55% of cases and this multifocal oral involvement was more commonly associated with systemic disease.

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M A O LEWIS, A J C POTTS, J G COWPE and H L LEWIS* (Department of Oral Surgery, Medicine and Pathology, UWCM, Cardiff, UK): Views of general dental practitioners on antibiotics available for the treatment of acute orofacial infection.

Twelve systemic antimicrobials are included in the Dental Practitioner's Formulary (DPF). Little information is available concerning the usage of these agents by general dental practitioners (GDPs). A postal questionnaire with pre-coded and open questions related to acute bacterial infection and frequency of prescribing of these antibiotics ("often", "sometimes" or "never") was sent to 500 randomly selected GDPs in the UK, stratified by geographical area. Replies were received from 279 practitioners (response rate 56%). Each of the antimicrobials was prescribed on occasions. Whilst four agents (metronidazole, amoxycillin, erythromycin and penicillin V) were used "often" or "sometimes" by 76-98% of GDPs, six drugs (ampicillin, cephalexin, cephadrine, doxycycline, clindamycin and oxytetracycline) were "never" prescribed by 57-93% of GDPs. A quarter of GDPs felt that the number of patients presenting with acute infections had increased and 32% felt that penicillin resistance in dental infections had become more prevalent. Two thirds (65%) of GDPs indicated that first line antibiotic therapy failed in less than 6% of their patients. When failure occurred the patient received one or more of the following: alternative antibiotic (85%), referral to Oral Surgeon (22%), referral to GMP (5%), further local measures (13%). Despite the low failure rate and the small range of drugs employed by the majority of GDPs, 27% felt that the antibiotics presently available in the DPF were insufficient for their treatment needs.

It is concluded that antimicrobial prescribing by the majority of GDPs involves a small range of the agents available in the DPF. It would appear that such restricted prescribing is associated with low prevalence of clinical failure.

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C MASON, SR PORTER, A MEE*, C CARRY, T McEWAN†. Dept of Children's Dentistry, *Dept of Oral Medicine, †Anaesthetic Dept, Eastman Dental Institute, London, UK. Significance of abnormal haematology of children requiring general anaesthesia.

Haematological investigation of Caucasians prior to general anaesthesia for dental surgery is rarely of clinical advantage, however, there is little data of the possible value of such screening in groups of children liable to haemoglobinopathies. The present investigation has examined to frequency and clinical outcomes of reduced haemoglobin in a group of children attending a dental outpatients unit in central London. The study group comprised 1000 children requiring urgent or elective dental surgery at the Eastman Dental Institute and Hospital, London, for dentistry under general anaesthesia during 1992. The ethnic background of the children comprised Caucasian (24.5%), Afro-Caribbean (28.6%), Indian/Pakistani (25.2%), Mediterranean (10.5%), Oriental (6.4%), Arabic (4.4%) and South American (0.4%). Haemoglobin levels were estimated in all children and appropriate haematological investigations undertaken in children at risk of haemoglobinopathy.

Thirty one children had haemoglobin levels of 10.0g/dl or less. In addition, seventeen had sickle cell trait, and 10 had possible thalassaemia trait but there was no correlation between haemoglobinopathy tendency and low levels of haemoglobin. The planned general anaesthesia was undertaken in 22 of the 31 children with low levels of haemoglobin. Only 6 children ultimately did not undergo general anaesthesia, all failing to return to clinic.

It is concluded that pre-anaesthetic haematological assessments of children requiring minor dental surgery is infrequently of any clinical value.

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B D THEAKER*, P SLOAN, S G LANGTON AND D H CARTER (Tuner Dental School, University of Manchester, UK): Application of cryopling and cryomicrotomy to diagnostic histopathology.

We have developed a methodology which enables the preparation of fresh-frozen sections of mineralised tissue for research (Carter D H, *Histochem J* 26: 103-109, 1994). The aim of the present study was to assess the potential of this technique in diagnostic and forensic histopathology when combined with cryopling (Rauschning W, *Spine* 8: 170-180, 1983). Jaw resections were fresh frozen and embedded in 1.6% carboxymethylcellulose cooled by liquid nitrogen. Specimens were then sequentially sectioned in a PMV 450 mp cryomicrotome. Sections were taken at intervals using polyvinylpyrrolidone support films. The histological preservation of the tissue in cryosections was comparable or superior to that of conventionally processed tissues. It was possible to examine microscopic tissue relationships and sample excision margins. Results were obtained within a few hours.

It is concluded that cryopling and cryomicrotomy can be combined to provide a rapid and accurate method for pathological examination of bony specimens.

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N AGRAWAL*, P J REDFERN, L H MAIR (Department of Clinical Dental Sciences, The University of Liverpool, UK): The effects of the use of 3,4 Methylene-dioxy-methamphetamine (Ecstasy) on occlusal toothwear.

3,4 MDMA is a Category A drug which is currently abused for recreational purposes by many young people. The drug has many reported side effects including: teeth grinding, dehydration and dry mouth (Solowij N *et al.*, *Br J Addict* 87: 1161-1172, 1992). The latter effects are countered by drinking carbonated drinks. A sample of 28 users together with a matched comparison group was established by a peer network snowball starting at a drug counselling centre. Both groups completed a questionnaire concerning their drug usage and diet. Soft wax was used as an impression to make stone models which were then scored using the criteria of Smith and Knight (*Br Dent J* 156: 435-438, 1984). The overall mean scores for the user group (0.67 ± 0.45) was significantly different from the comparison group (0.16 ± 0.30) ($t = 4.77$, $p < 0.001$). The differences were greatest in the molar region. The questionnaire indicated that 72% of the users were aware of grinding their teeth 12 hours after taking the drug and in 35% the effect was present for up to 24 hours. Users reported consuming more soft drinks, but less alcohol than the comparison group.

It is concluded that this group of Ecstasy users had more toothwear than a comparable group of non-users.

We are grateful to the staff of the Maryland Centre, Liverpool for their help and support.

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L SARNA, P-J LAMEY*, J A BEELEY AND A B LAMB (Glasgow Dental School and School of Dentistry, Queen's University, Belfast): Electrophoretic analysis of parotid salivary proline-rich proteins in Burning Mouth Syndrome.

Whilst there is a considerable amount of information available on a number of precipitating factors in Burning Mouth Syndrome (BMS), virtually nothing is known about salivary composition in these patients. A study of salivary proline-rich protein profiles was therefore undertaken on BMS patients.

Parotid saliva samples were collected from seventy patients with BMS, 12 age and sex matched controls and 29 younger control subjects. Salivary protein profiles were studied by SDS-PAGE (Beeley *et al.*, *Electrophoresis* 12: 1032-1041, 1991). In this system, proline-rich proteins stain pink-violet whereas other proteins stain blue.

Seventy four per cent of BMS patients exhibited an unusual proline-rich protein (designated band 'X') which occurred with a frequency of only 17% in age and sex matched controls and in 21% of younger control subjects ($P = < 0.05$).

Proline-rich protein 'X', the frequency of which is significantly elevated in BMS, needs to be characterised in order to understand what role, if any, it plays in this disorder.

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P-J LAMEY, A B LAMB, T D REES AND S KILLOUGH* (School of Dentistry, Queen's University, Glasgow University (UK), Baylor College of Dentistry (USA): Burning Mouth Syndrome Subtypes: A UK and USA Comparison.

Burning Mouth Syndrome (BMS) has been recognised as not being a homogeneous condition. Three subtypes are recognised: In Type 1 BMS patients have no burning on waking but it develops as the day goes on, in Type 2 BMS the condition is present on waking and continues throughout the day. In Type 1 and Type 2 BMS the burning is unremitting being present every day whereas in Type 3 BMS the burning is intermittent. The significance of subtypes is that the precipitating factors involved in BMS are different between subtypes and the prognosis is different. A comparative prospective study was undertaken of the prevalence of subtypes in a UK and USA population. The UK group comprised 150 patients and the USA group 130. All patients were directly questioned about their BMS history and then categorised as having Type 1, 2 and 3 BMS. Despite wide differences in dentate status of the UK (20%) and USA (70%) patient groups virtually identical figures for the prevalence of subtypes were recorded. These were Type 1 (34% UK vs 27% USA), Type 2 (55% UK vs 56% USA) and Type 3 (11% UK vs 17% USA).

The conclusion is that similar subtypes of BMS are present in a UK and USA population and this seems unrelated to dental status.

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L FARTASH*, T J CLIFFORD AND P-J LAMEY (School of Clinical Dentistry, The Queen's University of Belfast, UK): Migraine, temporomandibular joint disorders and mandibular tori.

Migraine is a common condition with a number of recognised precipitating factors. Recently nocturnal parafunctional activity has been added to this list and may be of particular importance since 70% of migraineurs have their attacks on waking, suggesting a nocturnal trigger. Temporomandibular dysfunction syndrome (TMJDS) is similarly multifactorial but parafunction has long been established as a significant trigger factor. Mandibular tori can be inherited and claims have been made that they are in some way related to parafunction. To test this hypothesis 86 unselected migraineurs and 24 patients with TMJDS were compared with an equal number of age and sex matched controls with no known history of parafunction, migraine or TMJDS. The prevalence of mandibular tori was 36% (migraine) 54% (TMJDS) and 33% (control subjects). There is a significant difference ($p < 0.0001$) between control subjects and migraineurs and TMJDS patients in relation to the presence of mandibular tori.

This study lends support to the hypothesis that mandibular tori are related to parafunctional activity. In addition the presence of mandibular tori in migraineurs may have important implications for management.

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M LONG*, W LLOYD, D BOWSHER AND P-J LAMEY (School of Clinical Dentistry, The Queen's University of Belfast, Pain Relief Foundation, Liverpool, UK): Relaxation tape therapy and its impact on migraine.

Stress is one of the many trigger factors reported by patients as triggering attacks of migraine. Whether stress and migraine are linked directly or stress acts via increasing nocturnal parafunctional activity is unclear. One way of potentially reducing stress is by the use of relaxation tape therapy. A tape especially designed to relax the muscles of the head and neck has been produced by ourselves recently. This open study reports on the patients documented response to using the tape prior to sleep. In total fifty six patients were studied all but two of whom had their condition diagnosed medically. Forty three per cent of patients used the tape daily, thirty per cent used the tape weekly and the remainder infrequently. Fifty per cent of patients reported the tape as being very beneficial, thirty six per cent claimed it to be beneficial and fourteen per cent found no benefit. A reduction in frequency of migraine was reported by forty six per cent of patients and no change in fifty four per cent. Severity of migraines were reduced in thirty four per cent and unchanged in sixty six per cent. Twenty seven per cent reported a reduction in duration of migraine and forty six per cent used less medication.

Relaxation tape therapy is beneficial in a significant proportion of unselected migraineurs.

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J BRIGGS*, T J CLIFFORD, L FARTASH AND P-J LAMEY (School of Clinical Dentistry, The Queen's University of Belfast, UK): Parafunctional activity, anxiety and depression in migraineurs.

The aim of this study was to investigate whether a relationship exists between parafunctional habits and anxiety and depression in a group of migraineurs. Seventy patients who were diagnosed as suffering from migraine in accordance with criteria defined by the International Headache Society (Cephalgia 1988; 8: 1-96) were assessed for a parafunctional activity by clinical examination and were also assessed for anxiety and depression using HAD scales. All patients were drawn from those referred to a facial pain clinic in Belfast. Fifty-nine patients were female, a female to male ratio of 6:1, ages ranged from 14-77 with a median age of 43 years. Thirty-six patients were assessed as having parafunctional activity of whom 18 had anxiety scores of 8 and over suggesting possible or frank anxiety while 11 had depression scores of 8 and over. When anxiety in migraineurs with parafunction was compared with those without parafunction a significant difference $p < .01$ (X^2 test) was observed. A similar $p < .01$ was observed when depression was considered. Paterson, et al (Journal of Dental Research 1994; 73 (4): 806) have shown a highly significant relationship between parafunction and anxiety in RMS patients and this study supports a similar relationship between anxiety and parafunction in migraineurs.

It is concluded that anxiety and parafunction may play a role in the aetiology of migraine headaches.

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T J CLIFFORD*, L FARTASH AND P-J LAMEY (School of Clinical Dentistry, The Queen's University of Belfast UK): Development of a Migraine Severity Index to predict treatment outcome.

Nocturnal parafunctional activity is an important precipitating factor in some patients with migraine. Acrylic appliance therapy is highly effective in dentate migraineurs and the worst outcome appears to be a reduction in the number of attacks to 40% of previous, although clearly many patients are rendered asymptomatic. In an attempt to predict the likelihood of a successful outcome to acrylic appliance therapy in migraineurs a migraine severity index (MSI) was developed. The MSI quantifies clinical features of the patients migraine attack including duration, severity and accompanying clinical features. One hundred dentate migraineurs were treated with nocturnal acrylic appliance therapy for one year and outcome assessed using the MSI.

While the overall success rate of treatment was high, - 70% of migraineurs were asymptomatic - no single factor or group of factors appears to enable prediction of outcome of treatment using the MSI.

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F T LUNDY* AND P-J LAMEY (School of Clinical Dentistry, The Queen's University of Belfast, UK): Salivary neurokinin A levels and migraine.

Migraine is recognised as being capable of being triggered in susceptible individuals by a number of factors such as cheese, chocolate, red wine, overtiredness and citrus fruits. Nocturnal parafunction activity can also trigger attacks in susceptible individuals and acrylic appliance therapy can be very successful in rendering some migraineurs asymptomatic. This study sought to investigate the relationship between salivary neurokinin A levels and the triggering of migraine attacks by withdrawing acrylic appliance therapy from successfully treated patients. Ten patients were studied and all provided informed consent to participate. All were migraineurs rendered asymptomatic by acrylic appliance therapy for at least three months and were receiving no medication. Daily whole saliva samples were collected on waking for two days whilst still wearing the appliance, daily until an attack of migraine and for two days following return to wearing the appliance. Neurokinin A was measured by radioimmunoassay with appropriate controls. The results demonstrate that neurokinin A levels are greatly elevated prior to an attack and return to baseline following re-institution of splint therapy.

This drug free model allows detailed salivary neuropeptide analysis before and during a migraine attack and enables comment to be made of their role in disease.

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D G MACDONALD*, A STEVENSON, C J WHITTERS (Glasgow Dental Hospital and School, UK): Effects of pulsed Nd: YAG laser on normal tooth pulp.

Laser treatment has been proposed as a means of achieving pulpal analgesia. It is not known if this causes any pathological changes to normal pulp *in vivo*. Eighteen teeth were studied from six orthodontic patients who required one or more pairs of premolar teeth extracted. Under a protocol approved by the Local Ethics Committee a pulsed Nd: YAG laser with a 320 μ m diameter optical fibre delivery system was used to irradiate the labial and palatal enamel surfaces of nine test teeth with 113 mJ pulses at 15 pps for 3 mins. Test teeth were extracted two hours after laser. All root apices were removed immediately and the teeth were fixed in formalin. Paraffin processed sections were prepared and stained H and E. Coronal pulps of test and control teeth were assessed blind, by subjective quantitative scoring on a scale of 0-4, for vacuolation of odontoblasts, vascular dilatation, margination of neutrophils, neutrophils in tissue, oedema and 'eosinophilic degeneration'. By Mann Whitney U Test the laser treated teeth showed significantly higher total scores than controls ($P < 0.05$). The only two instances of neutrophils in tissue were in laser treated teeth. Some of the changes were those of early autolysis in relation to delay in diffusion of fixative. This was found in both groups, but was more marked in laser teeth.

It is concluded that laser teeth with the protocols advised for pulpal anaesthesia produced demonstrable pathological changes when pulps are examined two hours later. The changes seen were mild and probably reversible, but confirmation of this will require further study.

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BE KELLIS*, PA BIAGIONI and JG KENNEDY. (Department of Restorative Dentistry, Queen's University, Belfast): Thermographic assessment of pulpal blood flow.

Pulpal blood flow has been estimated using various techniques including laser Doppler, oximetry, Xe 131 clearance and transillumination. None of these have gained clinical acceptance and conventional thermal and electrical neural pulp tests remain the diagnostic tools of choice in assessing pulpal vitality. The aim of this study was to assess the usefulness of the Agema thermovision 900 system¹ to detect pulpal blood flow. One subject with one root-filled upper lateral incisor tooth and the contralateral tooth sound and responding positively to thermal and electrical pulp tests was recruited for the trial. The teeth were isolated with rubber dam and imaged prior to testing. The non vital tooth was cooled with a stream of water at 20°C for 30 seconds, dried with gauze and imaged until re-warming to baseline temperature was achieved. The process was repeated for the contralateral vital tooth and the profile of re-warming compared. Measurements recorded on three occasions showed that there was no demonstrable difference in baseline resting temperature between the vital and non vital tooth. However, a more rapid rate of return to baseline temperature was consistently demonstrated in the known vital tooth than in the non vital tooth. Further trials are in progress. *The results suggest that infrared thermography may be useful as a predictor of tooth vitality based on blood supply rather than nerve supply.*

¹AGEMA Infrared systems, Danderyd, Sweden

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D.L.HUSSEY*, J.P.McCULLAGH, P.A.BIAGIONI and P-J.LAMEY (School of Clinical Dentistry, Queen's University, Belfast, Northern Ireland): Thermographic assessment of endodontic thermomechanical obturation.

The temperature changes on the root surface of human premolar teeth during thermomechanical root canal obturation were measured, *in-vitro*, using an infrared thermal imaging camera.¹ Thirty human premolar teeth were prepared to a size 50 master apical file using stepback technique. Each tooth had a gutta percha (GP) point fitted to the working length and this was verified radiographically. The sample teeth were then divided into three equal groups and each canal was obturated with GP using a thermomechanical technique.² Handpiece working rotation speeds of 8, 12 and 16 thousand rpm were used. The thermal images were recorded at 2s intervals for a period of 20s and stored for later analysis.

Results: The baseline temperature for all groups was approximately 24°C. The mean maximum temperatures recorded on the root face during the obturating sequence were 73.5(±19.0), 67.5(±15.3) and 78.8(±13.1)°C for the rotation speeds of 8, 12 and 16 thousand rpm respectively. These relate to a maximum deviation from baseline of approximately 49, 43 and 52°C. The most rapid temperature rise was recorded in the fastest rotation group.

Conclusion: *Very high temperature rises are produced at the root surface during thermomechanical obturation of root canals.*

¹ Thermovision 900, Agema, Danderyd, Sweden.

² Gutta-Condensor, Maillefer, Ballaigues, Switzerland.

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F WASTI*, A C SHEARER and N H F WILSON (University of Manchester): The use of a radiopaque contrast medium in endodontic radiography.

A series of *in vitro* studies were carried out to investigate the use and application of a radiopaque contrast medium in conventional periapical dental radiography for the diagnosis and evaluation of root canal systems. The radiographic images of 30 maxillary and 30 mandibular first permanent molar teeth with a radiopaque medium (Omnipaque¹) in the root canal systems were compared and contrasted with plain radiographic images of the same teeth. Further comparisons were made with the teeth rendered transparent. The results indicated that by standardising the diagnostic criteria the inter-examiner reliability was within good agreement: it was independent of the radiographic technique used (mean weighted Kappa 0.825-0.967). The validity of diagnostic radiographs was enhanced by the use of radiopaque contrast medium. The results confirm that radiopaque medium radiographic images of root canal systems are easier to identify, read and interpret than plain radiographic images of root canal systems.

It is concluded that the use of radiopaque contrast medium in endodontic radiography may be a valuable aid in the diagnosis and evaluation of root canal systems and that this method would complement rather than replace plain radiography.

¹ Iohexol, Nycomed, Oslo.

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P.A.BIAGIONI*, J.P.McCULLAGH, D.L.HUSSEY and P-J.LAMEY (School of Clinical Dentistry, Queen's University, Belfast, Northern Ireland): Thermographic assessment of post channel preparation.

The temperature changes on the root surface of root filled human premolar teeth during post channel preparation were measured, *in-vitro*, using an infrared thermal imaging camera.¹ Ten permanent human premolars previously root filled with GP had a post channel prepared by sequentially drilling with a number 2 Peeso² reamer, a number 3 Peeso reamer and a red Parapost³ drill at 8000 rpm to approximately two thirds of the root length. The thermal images were recorded at 1s intervals over a period of 5s for the Peeso reamers and 10 s for the Parapost drill.

Results: Mean maximum temperature rises 39.3°C, 51.5°C, 61.9°C were measured on the root surface for the number 2 Peeso reamer, number 3 Peeso reamer and a red Parapost drill respectively.

Conclusion: *High temperature rises are produced at the root surface during mechanical post channel preparation. This occurs during removal of GP at 8000 rpm and is greatest for the Parapost drill stage.*

¹ Thermovision 900, Agema, Danderyd, Sweden.

² Peeso Reamers, Products Dentaires SA, Vevey, Switzerland

³ Parapost, Whaledent International, New York, USA

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S A WHITEHEAD¹ and N H F WILSON (University of Manchester, UK) Evaluation of Vertical Barrelling Effects Using Surface Profilometry.

The true aetiology of the non-carious cervical notch shaped lesion remains obscure. However, it is postulated that "abfractions" occur at the apex of the notch possibly caused by tooth flexure and vertical barrelling (Grippio J.O., *J.Aesthet.Dent* 4:55-64.1992). The aim of this study was to validate this concept and to assess the legitimacy of the surface profile measuring system employed. Extracted human permanent upper canine teeth were embedded rigidly in epoxy resin. The labial profile was taken using a diamond stylus mounted in a Perthometer S8P Profilometer (Feinpruf-Perthen GmbH, Göttingen, Germany). The teeth were then loaded in an axial direction and the labial surface re-profiled. Comparisons of profiles taken before and after loading were undertaken and differences noted. The results indicate that changes occur in the labial profile of canine teeth that are subjected to axial loading which could be considered to represent vertical barrelling.

It is concluded that the concept of vertical barrelling could be considered valid and may influence the development of non-carious cervical notch lesions and that surface profilometry offers a potential method to quantify this effect.

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A WATTS¹ and R C PATERSON² (¹The University of Liverpool, UK, ²University of Glasgow, UK): Histological assessment of root canal preparation techniques.

This study was designed to demonstrate the responses of the periodontal tissues to three methods of root canal preparation technique, in order to select the best method of canal preparation for use during the biological testing of root canal sealing materials.

Thirty-six nine month old ferrets were anaesthetised by intramuscular injection of alfaxalone/alfadolone (dosage 12-15 mg per Kg body weight). They were intubated and maintained on halothane and oxygen. The canine teeth were isolated with rubber dam and the canals prepared by hand filing (Maillefer K-flexofiles) alone or in combination with either sonic (Micromega) or ultrasonic (Caviendo, Dentsply) cleansing. Only the coronal portions of the canals were sealed leaving the apical portions empty after instrumentation. The animals were sacrificed by intrap

eritoneal overdosage of barbiturate 1,2,4 or 12 weeks after instrumentation. The tissues were processed for light microscopy and stained with either H & E or specific bacterial stains. A number of short term specimens exhibited inflammatory infiltration of the periapical tissues with all three methods of canal preparation but long term responses appeared minimal. Varying quantities of debris were present in the root canals prepared by all three methods. Least debris was observed following ultrasonic cleansing.

It is concluded that hand instrumentation followed by ultrasonic cleansing is the most suitable method of root canal preparation during the biological testing of root canal sealing materials. Supported by an E.C. BIOMED I Grant.

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F MCDONALD¹, K BRADY, TR PITT FORD and M TORABINAJAD (Departments of Orthodontics and Conservative Dental Surgery, UMDS, London): Mineral trioxide aggregate as a substrate for osteoblasts.

In this report we have examined cultured osteoblasts in the presence of Mineral Trioxide Aggregate (MTA). Firstly we examined the material, MTA as set in moist conditions; secondly we examined the behaviour of MG-63 cells with respect to time and with respect to the MTA material. For the analysis of the material we used the KVEEX quantitative x-ray absorption analysis.

To examine the reactions of the osteoblast-like cell lines, MG-63 cells were grown to confluence in Hams F12/DMEM media which contained 10% foetal calf serum, 50 µ/ml streptomycin, 50 µ/ml penicillin and 100 µ/ml amphotericin. Prior to incorporating the cover slips within the media, MTA had been placed with sterile water 7 days previously to this and allowed to set in the moist atmosphere. The cells were returned to the incubator together with the MTA and stopped at 12 hours, 24 hours and 48 hours. Examination demonstrated that the former appeared as discrete crystals and the latter appeared as an amorphous structure with no apparent crystal growth but more a granular type appearance. The mean value of calcium in the prisms was 87% calcium and 2.47% silica, the remainder being comprised of oxygen. The cells appeared to lay down processes over the granular substrate while they were completely absent from the crystalline structure.

In conclusion therefore, it appears that mineral trioxide aggregate offers a reasonable substrate for osteoblast ingress.

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SJ HODGES¹, RE EVANS and F MCDONALD (Department of Orthodontics, UMDS, London): Assessment of magnetic alloy cytotoxicity determined by cell migration.

The inherent sensitivity of magnets to corrosion, together with their use in a hostile environment, means that some level of patient exposure to the leachable and corrosion products can be assumed. Presently there is little information on the biodegradation of magnets *in vitro*, and even less on the biologic effects of these breakdown products. The magnetic alloy cytotoxicity was investigated by the Under-Agarose cell migration technique, using human foetal fibroblast cells. Both magnetised and unmagnetised, coated and uncoated, forms of Neodymium-Iron-Boron magnets were examined. The results show that the static magnetic field and the leachable and corrosion products of Neodymium-Iron-Boron magnets do not have a statistically significant effect on the behaviour of foetal fibroblasts. Trends are shown, however, towards a temporary decrease in the migration of fibroblasts affected by the magnet breakdown products and a decrease in migration, at all times measured, by the cells exposed to the magnetic field. This report highlights the need both for care in the use of magnets until the consequences of the static magnetic field are fully elucidated.

Precautions should be taken to prevent the escape of breakdown products when using magnets in vivo.

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N BUBB¹, D WOOD, B MILLAR, L GABRIELSON (King's College Dental School & Brunel University, London): Comparison of Dynamic Contact Angle and traditional measurements of advancing contact angles.

During the use of elastomeric impression materials the Advancing Contact Angle (ACA) is important during the taking of an impression and pouring a die and affect the fit of a finished restoration. Traditionally, ACAs have been measured by recording the angle formed by a droplet of liquid, water (method 1), calcium sulphate solution or setting die stone on the substrate. Ensuring that the ACA rather than static contact angle is recorded is an inherent problem. Alternatively a Wilhelmy based procedure may be used; such as the Dynamic Contact Angle analysis system (method 2). This records the ACA over a relatively large surface area over a period of time. A pilot study into the differences in values for the ACA obtained between water and impression materials using method 1 and method 2 was carried out on a selection of 19 addition cured silicone impression materials. Values ranged from: 37-102° for method 1 and 85-114° for method 2. Close agreement (<5% difference) occurred in six cases, however, a comparison of the rank order (Spearman's Ranking Correlation Coefficient) of results from the two methods show significant (P<0.05) similarity between the rank orders of the ACA.

In conclusion, the two methods gave similar trends but little direct agreement of ACAs. Difficulties associated with the recording of ACAs would support the use of a dynamic method.

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S M DUNN¹, B J MILLAR, D WOOD, and N BUBB (King's College Dental School, London): Investigation of a novel retention system for InCeram¹ and Spinel² core dental ceramics.

This study aims to examine the surface topography of InCeram and Spinel specimens, prepared using the Bateman "Etch" Retention System (PCT/GB93/01739) and to measure any effect on flexural strength. Twenty specimens measuring 20mm by 2mm by 2mm were made in each of the two core ceramics. Ten specimens in each material remained untreated, while ten were "etched" by incorporating plastic retention chips, of diameter 50-300µm, on one surface of the specimen, which were subsequently burnt out. One specimen from each of the four groups was prepared for electron microscopy, while the remainder were subjected to a three point flexural strength test. The treated InCeram surfaces possessed many retentive pits, similar in size to the retention chips, while the treated Spinel surfaces had fewer and smaller pits. The mean flexural strength values for untreated and treated InCeram and Spinel specimens were 27.6±6.1Nmm², 16.6±1.9Nmm² and 20.5±4.6Nmm², 8.8±2.7Nmm², respectively. The flexural strength of both materials was significantly reduced by "etching" (P<0.05). However, there was no significant difference in the flexural strength between untreated Spinel and treated InCeram specimens. The system appears promising for use with InCeram core ceramics.

¹Vita, UK.

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FJT BURKE¹, JR MAIN and DC WATTS (Restorative Dentistry, University of Manchester, UK): Effect of contamination of etched porcelain surfaces by operating gloves.

Gloves are worn by a high proportion of dental health care workers while treating patients. It is the purpose of this study to investigate whether contamination of etched porcelain surfaces by operating gloves may result in a reduction of the shear bond strength of the porcelain to a resin-composite luting material. Fifty specimens (7 mm diam x 2 mm) were constructed in fieldspathic porcelain. These were placed in copper rings (15 mm diam.) using dental stone and divided at random into 5 groups of 10 specimens. The porcelain surface of each specimen was etched with hydrofluoric acid. Samples of glove material were held against the etched porcelain surface for 10 s. Four different glove types were used, 1 - Biogel D¹, 2 - Featherflex², 3 - Microtouch³, 4 - Tru-Touch⁴. A resin composite based luting material⁵ of standard shade was placed against the etched porcelain surface in a PTFE mould (3.5 mm diam. x 2 mm) and light-cured for 60 s. One (control) group was not treated with glove material. Mean shear bond strengths (Mpa) of 22.5, 21.3, 16.4, and 23.2 were recorded for Groups 1,2,3 and 4 respectively, and 25.6 for the control group. Statistical analysis of the results by one-way ANOVA showed no significant difference between the groups. While results may suggest a diminution of shear bond strength of porcelain specimens to resin-composite luting material when contaminated with some glove specimens, it is concluded that there is no significant difference in shear bond strengths between specimens the surfaces of which have been contaminated by gloves and those which were not. 1,2, Regent Hospital Products, London, UK; 3, Johnson & Johnson, Livingston, Scotland, UK; 4, Becton-Dickinson, NJ, USA; 5, 3M Indirect Porcelain Bonding Kit, 3M, MN, USA.

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GEARY JL¹, CUNNINGHAM JL, KINIRONS MJ (School of Clinical Dentistry, Queen's University of Belfast, UK.): A subjective assessment of the shade of dental laboratory-fired samples of dentine coloured ceramic.

The aim of this investigation was to compare the observed shade of laboratory-fired, dentine coloured ceramic discs, with their expected colours as defined by the manufacturers. Eight shades from each of four different manufacturers' products were selected for testing. The fired ceramic samples (n=32), which were constructed in thicknesses characteristic of ceramic veneers, were mounted on shade guide sticks. Comparisons were made using the same shade guide¹. A group of twenty 'adept' observers examined the samples in controlled lighting conditions. No single shade was determined to match twelve of the samples tested. Of the remaining twenty samples for which a single shade match was identified, only six corresponded to the manufacturers designated colours. Of the eight different shades tested for one product six were judged to match the same colour. Samples were matched predominantly to shades higher in colour 'value' than the manufacturers' designated shades.

For the materials and shades examined in this study alternative shade determination systems and/or modified coloured ceramic materials are required to improve perceived colour fidelity between laboratory-fired ceramic and the shade guide to which it is matched.

1. Vita Lumin®-System, Vita Zahnfabrik

583 F J SHAIN*, P M MARQUIS and A C C SHORTALL (School of Dentistry, University of Birmingham, UK): Clinical performance of porcelain laminate veneers fitted in Birmingham Dental Hospital (BDH).

This investigation aims at monitoring the clinical performance of porcelain veneers (PV) fitted to unprepared teeth in BDH between November 1984 and February 1992. 372 PV fitted for 104 patients were followed-up for a maximum of 95 months. The veneers were constructed using platinum foil technique, the bonding surfaces were prepared by grit blasting with 50µm Al_2O_3 , a layer of silane coupling agent (Fusion) was applied to the prepared surfaces and the PV were cemented using micro-filled composite resin. At each review time, the restorations were classified as clinically satisfactory, presenting with a repairable problem or failed. Survival analysis showed that the probability of survival for these restorations was 88% after 1 year decreasing to 76%, 68%, 62%, 58%, 48%, 47% after 2, 3, 4, 5, 6, 6.5 years respectively. The major cause of failure was the fracture of the restoration 54%, followed by debonding 21%. The highest percentage of failures was seen in the group of P.V. cemented over existing restorations. The study showed a higher failure rate than the previously published work (Dunne S M and Miller B J, *Br Dent J*, 175: 317-321, 1993).

It is concluded that P.V. fitted to unprepared teeth face a high probability of failure especially if the enamel surface area is reduced by the presence of restorations.

584 J WALSH*, P V HATTON, A JOHNSON, A CLIFFORD and R HILL (Restorative Dentistry, University of Sheffield, UK & Materials Science, University of Limerick, Ireland): Glass-ceramics for dental applications.

Glass formulations used for the manufacture of glass-ionomer cements (GICs) also have the potential for use as castable glass-ceramics for the production of crowns and inlays. These high fluoride glasses offer the advantages of low liquidus temperatures with bulk nucleation of fluoapatite and mullite through an amorphous phase separation process. They also have advantages over existing ceramic systems in that they can be cast using conventional dental technology equipment, are easily bonded using GICs and are capable of releasing fluoride ions which are known to stimulate apatite deposition as well as possessing the cariostatic properties. A range of glasses with $1.5SiO_2-Al_2O_3-0.5P_2O_5-CaO-xCaF_2$ formulation, using thermal analysis techniques (DSC) with SEM and XRD full characterisation of the glasses was undertaken. Their suitability for dental applications was assessed by producing crowns and inlays with centrifugal (Galloni Modular S3 Italy) and air pressure/vacuum (Heraeus CL-G77 Germany) casting machines. Melt temperatures were between 1200 and 1400°C, and sintering was carried out at 1000°C for up to six hours. The results showed that the current glass formulations were too viscous in the mould state for air pressure/vacuum casting. However the results of centrifugal casting were more promising, with the fabrication of model crowns and inlays with a good fit. Further adjustment of the investment composition reduced tolerances to < 100 µm, while initial problems with carbon contamination from the graphite liner were eliminated using a platinum crucible. *It was concluded that glass-ceramics based on ionomer glasses have the potential for use as crowns and inlays.*

585 M A O LEWIS, A J C POTTS, J G COWPE and H L LEWIS* (Department of Oral Surgery, Medicine and Pathology, UWCM, Cardiff, UK): Views of general dental practitioners on antibiotics available for the treatment of acute orofacial infection.

Twelve systemic antimicrobials are included in the Dental Practitioner's Formulary (DPF). Little information is available concerning the usage of these agents by general dental practitioners (GDPs). A postal questionnaire with pre-coded and open questions related to acute bacterial infection and frequency of prescribing of these antibiotics ("often", "sometimes" or "never") was sent to 500 randomly selected GDPs in the UK, stratified by geographical area. Replies were received from 279 practitioners (response rate 56%). Each of the antimicrobials was prescribed on occasions. Whilst four agents (metronidazole, amoxycillin, erythromycin and penicillin V) were used "often" or "sometimes" by 76-98% of GDPs, six drugs (azithromycin, cephalexin, cephradine, doxycycline, clindamycin and oxytetracycline) were "never" prescribed by 57-93% of GDPs. A quarter of GDPs felt that the number of patients presenting with acute infections had increased and 32% felt that penicillin resistance in dental infections had become more prevalent. Two thirds (65%) of GDPs indicated that first line antibiotic therapy failed in less than 6% of their patients. When failure occurred the patient received one or more of the following:- alternative antibiotic (85%), referral to Oral Surgeon (22%), referral to GMP (5%), further local measures (13%). Despite the low failure rate and the small range of drugs employed by the majority of GDPs, 27% felt that the antibiotics presently available in the DPF were insufficient for their treatment needs.

It is concluded that antimicrobial prescribing by the majority of GDPs involves a small range of the agents available in the DPF. It would appear that such restricted prescribing is associated with low prevalence of clinical failure.

586 M MAK*, A JE QUALTROUGH and F JT BURKE (Restorative Dentistry, University of Manchester, UK): Fracture resistance of dentine-bonded crowns; use of different ceramics

The purpose of this study is to investigate fracture resistance of dentine-bonded crowns constructed from different ceramic materials. Forty extracted, sound maxillary premolar teeth were selected and divided into 4 groups of 10 teeth of similar buccopalatal width. Standardised minimal crown preparations were carried out on all teeth. Group 1 crowns were constructed from a low-fusing porcelain¹, Group 2 from a leucite-reinforced glass-ceramic², Group 3 from a castable ceramic³ and Group 4 from aluminous porcelain⁴. Ceramic fitting surfaces were etched with hydrofluoric acid and the crowns luted with a dual-cure resin composite using a standardised technique first treating the dentine with a dentine bonding agent⁵. Compressive loads were applied at 1mm/min via a 4mm steel bar. Fracture loads and mode of fracture were recorded. Mean fracturing forces were 0.76 KN, 0.95 KN, 1.12 KN, 0.72 KN respectively for Groups 1, 2, 3, & 4. Statistical analysis by Tukey's Multiple Comparison Test showed that loads required to fracture specimens in Group 3 were significantly greater than for the other groups ($p = 0.0099$). Three Group 2 specimens suffered fracture of underlying tooth substance, compared to 1 in Groups 1 and 3 and 0 in Group 4. *It is concluded that use of a castable ceramic material³ significantly increases fracture resistance of dentine-bonded crowns without increasing risk of underlying tooth fracture.* ¹Mirage; Chameleon Dental Products, KN, USA; ²Empress; Ivoclar-Vivadent, Liechtenstein; ³Dicor; DeTrey/Dentsply, Weybridge, UK; ⁴Vitadur N; Vita Zahnfabrik, Bad Säckingen, Germany; ⁵Mirage ABC; Chameleon Dental Products, KN, USA.

587 K SEYMOUR*, D SAMARAWICKRAMA, L ZOU, E LYNCH (Dept of Conservative Dentistry, LHM, UK): Shoulder dimensions and angles of bonded crown preparations *in vivo*.

It is generally accepted that the ideal labial preparation for porcelain bonded to metal crowns consists of a shoulder 0.8-1.5mm wide with a shoulder angle of 90-110°. Such a preparation will provide for adequate material thickness, correct shape for porcelain and minimal unsupported tooth structure. Recent studies have indicated that when experienced clinicians cut preparations on extracted teeth there was a tendency to under prepare the labial shoulder and to over angle it. This may have implications for restoration longevity and periodontal health if extrapolated to the clinical situation. A logical extension of this work is to investigate teeth that have been prepared *in vivo* to receive these type of crowns. In the current study, the working dies of twenty five single rooted teeth prepared by the same group of experienced clinicians to receive porcelain bonded to metal crowns were scanned in the mid-buccal plane using a co-ordinate measuring machine (International Metrology Systems, UK) with a non-contact laser probe (Renishaw, UK) recording x, y, and z surface co-ordinates from which measurements of shoulder width and angle were obtained and analysed. The results show a shoulder width of 1.01 ± 0.35 mm and shoulder angle of $103.42 \pm 9.84^\circ$ (mean \pm SD).

*The data suggest that the short falls seen in preparations of extracted teeth do not appear to be repeated in the *in vivo* situation, as all but four preparations fell within the accepted ideal range in terms of shoulder width and angle.*

588 V JOVANOVSКИ*, L ZOU, W M TAY, E LYNCH and M G COX* (Department of Conservative Dentistry, LHM, UK and National Physical Laboratory, UK): Superposition of co-ordinate data obtained from a sequence of replicas.

Measurements from a sequence of replicas of a tooth were used to supply accurate information on the changes in its surface. The replicas were digitized by a co-ordinate measuring machine (Jovanovski, V *et al*, *J Dent Res* 72:742, Abs 446, 1993). The orientation and positioning of the replicas on the digitising platform might vary, therefore the data sets generally would not be in the same frame of reference. The superposition of two data sets consisted of a rigid transformation of one of the sets with the result that pairs of corresponding points (those which represent the same point on the surface) had essentially the same co-ordinates. Such pairs were selected from stable regions by the operator and a least-squares procedure applied to determine the parameters of the required transformation. The resulting alignment was within 10µm, which was within the accuracy of the digitized data.

Superposition is an effective method of studying sequential changes on tooth surfaces.

589 L ZOU*, V JOVANOVSКИ, W M TAY and E LYNCH (Department of Conservative Dentistry, LHM, UK): The influence of three parameters on laser probe scanning of irregular surfaces.

Quantification of the morphology of tooth surfaces to a high precision was carried out by a co-ordinate measuring machine with a semiconductor laser probe. (Bedford, J *et al*, *J Dent Res* 72:742, Abs 445, 1993). Observations were made of the influence of the scanning speed, the incident angle of the probe to the surface and the laser probe sensitivity threshold level on the accuracy of measured co-ordinates of surface points. Two reference surfaces were used - one flat, the other with a $3mm \times 3mm$ corrugation. Each of the two surfaces was scanned two times, at three different probe speeds (4, 10 and 50 points/second), at two incident angles (0° and 45° to the surface normal) and at five threshold levels. There was a marked drop in accuracy on the corrugated surface at speeds above 10 points/second but no change on the smooth surface. The sensitivity threshold did not influence the measurements significantly. The optimum value of the probe incident angle was found when the bisector of the incident and reflected beams was set parallel to the mean surface normal.

This pilot study has produced determinants of the optimal scanning speed, incident angle and sensitivity threshold necessary for accurate quantification of tooth morphology.

590 B C M PATEL, N MATOORIAN*, A BOWLER (School of Technology & Information Science, Thames Valley University): Dental Electromagnetic Tomography: Properties of tooth and plastic restoratives.

A safe and accurate means of detection/early diagnosis of caries would offer a considerable advantage in clinical dentistry. Conventional dental radiography is routinely used, but does not provide an accurate means of assessing the degree of demineralisation, and represents a major source of ionising radiation. We examine a totally safe means of imaging tooth structures using Electromagnetic Tomography (EMT). The concept of EMT is based on mutual inductance measurement (conductivity and permeability changes) resulting from induced inductance between an exciting coil and a sensing coil. A non-contact EMT system for conductive and/or ferromagnetic imaging was designed and constructed, consisting of an array of miniature induction coils (inductance of 10mH). These sensors were driven by auxiliary electronics to generate x, y, z co-ordinates (conductivity and permeability) across sensing pairs to ultimately construct a 3D image. Healthy human dental enamel, dentine, demineralised dentine, amalgam, glassionomer and composite were examined in the form of slabs (0.5-3mm thickness). The specimens were either tissue-dry or wet. In addition, complex-impedance analysis was carried out at 1MHz to establish fundamental electrical and magnetic properties. Between 3 to 10 readings were taken (up to 7 sites) on the specimens. The impedance measurements varied between specimens by 20-100kOhms and was dependent upon degree of hydration. Hydrated specimens showed a clear relative difference in impedance between enamel and dentine (1:2) and dentine to demineralised dentine was 1:20. Similarly, the impedance ratios for glassionomer, composite and amalgam were 1:50:1, respectively.

The principle of mutual inductance electromagnetic tomography can be used to clearly differentiate between various tooth tissue/plastic restoratives and may form the basis of a 3D imaging system.

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P LIKEMAN*, A JUSZCZYK and D RADFORD (Prosthetics Dept, UMDS, London SE1 8RT, UK): An examination of the surface of investment poured against different duplicating media.

Master casts for the construction of partial dentures are duplicated in investment using media commonly based on agar. Lately duplicating media based on polyvinylsiloxane (PVS) have become available for the purpose. The object of the study was to compare the surface of a phosphate bonded investment poured into moulds of an agar based and a PVS based duplicating medium. Casts were viewed by the scanning tandem microscope and Roughness (R) values of surface roughness were derived from the computer held images. Brinell hardness was measured using a 10mm ball indenter and a force of 980.7N. Specimens of investment were investigated: as set, and after dipping in beeswax and drying at 200°C for one hour. Investment poured in agar showed more surface roughness [R=2.29(29)] than investment poured in PVS moulds [R=1.93(15)]. Hardness values were significantly greater for investment poured in PVS, as set: BHN=34.8(3.6) than in agar BHN=18.4(2.7); $t=14.7$ $p<0.000$ df=27. The hardness was much reduced after dipping and drying, PVS: BHN=18.3(2.9); Agar: BHN=15.1(2.3); $t=2.9$ $p<0.01$ df=18.

The surface of investment poured in PVS duplicating media is harder and smoother than the same investment poured in agar.

*Crotom WB, Davis Schottlander, Letchworth, Herts, UK. *Ortagel, Krupp, Essen, Germany. *Elle Double, Ziemack, Badia Polesina (Rovigo), Italy.

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KANCHANAVASITA W*, PEARSON GJ and ANSTICE HM (Eastman Dental Institute, London): The temperature rise in ion-leachable cements during setting reaction.

It is a well established fact that composite resins undergo an exothermic setting reaction. The resin-modification of GICs is therefore likely to lead to an increase in temperature during the setting reaction. This study investigated the temperature rise during setting for four ion-leachable cements and a composite resin. Five specimens of each of the resin-modified cements (Fuji II LC¹ and Dyract²), the conventional GICs (Fuji II³ and Opusfil⁴) and the composite resin (Silux Plus⁵) were placed in PTFE mould with an internal diameter of 6 mm and a thickness of 3 mm. The specimens were maintained at either 25°C or 37°C, being light-cured if required for 20s. The temperature rises which occurred during setting were measured using a thermocouple connected to a digital thermometer. The mean maximum temperature rises (in °C) for Fuji II LC, Dyract, Silux Plus, Opusfil, and Fuji II at 25°C and 37°C were 17.5 (1.5) and 20.2 (0.3), 14.4 (0.3) and 9.5 (2.1), 12.0 (1.1) and 5.0 (0.1), 6.0 (1.3) and 1.3 (0.6), and 1.0 (0.6) and 2.4 (0.5), respectively.

The temperature rises for resin-modified cements were significantly higher than those for the conventional GICs at both experimental conditions.

¹GC Dental, Japan. ²Deutsply, Germany. ³GC Dental, Japan. ⁴David Schottlander & Davis Ltd, UK. ⁵3M, USA.

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T W RISELEY*, R WADGE and M LEVINKIND (London Hospital Medical College, London E1 2AD, UK): Evaluation of alterations in ion motion in dental materials following water uptake.

The aim of this investigation was to use impedance spectroscopy to study ion motion in dental materials as they hydrate and measure the conductivity of the water in which the specimens were left over a one month period in vitro. Three restorative materials were studied: a glass ionomer (Chemfil, De Trey), a composite (Herculite XRV, Kerr) and a glass ionomer-composite hybrid (Vitremere, 3M). Discs (21mm diameter and 1mm thick) were immersed in separate 10ml aliquots of distilled water which were changed at regular intervals. Electrical impedance measurements were made by sandwiching each disc between two platinum foil electrodes and applying a 200 mV alternating potential over a frequency range of 10 Hz to 100kHz. Impedance measurements were made under the same conditions with interdigitating electrodes placed on one surface of each disc. Each time the water in which the discs were immersed was changed, the conductivity of the water in which each disc had been immersed was also measured. These measurements showed that the glass ionomer exhibited the greatest release of ions, composite had the lowest and the hybrid ion release values were in between those from the other materials. Results of the impedance measurements using interdigitating electrodes were equivocal. However, measurements of electrical impedance using platinum foil electrodes were more successful. Typical DC resistance values for the materials after 1 month in distilled water were: Glass Ionomer = 0.16MΩ, Hybrid = 1.55MΩ, Composite = 6.90MΩ.

We conclude that AC electrical impedance measurements are a useful technique to study ion motion in dental materials and release of ions into the water in which the specimens are hydrating. We gratefully acknowledge financial support from 3M.

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BW BERTENSHAW* and DC WATTS (University of Manchester, UK): High Temperature degradation of restorative materials: forensic implications.

House fires have shown that the temperature on the ground floor can reach 1200°C, presenting major problems of identification of fire victims. Tooth-coloured anterior fillings are the most difficult features to establish and visual examination alone may be insufficient for this purpose. The aim of this investigation was to assess the effect of high temperatures on representative dental restorative materials under laboratory conditions. Discs of diameter 3 mm x 2 mm depth were prepared of the following materials: glass ionomer, composite, ZOE, zinc phosphate, zinc polycarboxylate, gutta percha. Each material was subjected to a programme of temperature rise. Specimens groups were held for 5 min at, successively, temperature intervals of 100°C in the range 100 - 1100°C, unless disintegration occurred beforehand. After each stage, the samples were cooled to room temperature and were, a) observed under a stereo microscope for physical changes and b) measured with a tristimulus colour analyzer for reflective surface-colour characteristics. The L*A*B* colour measurement system was used. Each material showed colour and physical changes through the temperature rise. For example, a temperature versus lightness factor plot for glass ionomer compared to composite in the region of 500 to 800°C revealed a decrease in percentage lightness from 90% to 40%, followed by an increase back to the original lightness value. Significant differences in colour parameters may be pertinent to identification of either patient restorations, or where these are known to the temperature attained by the fire in the vicinity of the victim.

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DC WATTS and AJ CASE* (University of Manchester Dental School, UK): Fracture-toughness and creep-recovery of resin/ionomer restoratives.

This study aims at *in vitro* stress-evaluation of two resin/ionomer 'hybrids' (DYR¹ & FJL²), including a Compomer system (Vitremer³), with controls of glass-ionomer (CHF⁴) and resin-composites (TPH⁵). Three products^{1,2,4} were from the same manufacturer¹. Fracture-toughness (K_{IC}) was determined using protocols (BS 5447, 1977) employing SEN specimens 34x24x6 mm. Specimens ($n=6$) were prepared incrementally in moulds, commencing from the vicinity of the central scalpel-blade as notch-former. Materials requiring light-curing^{1,2,4} were thoroughly irradiated. Any requiring initial aqueous protection were so treated. After 24 h, they were conditioned at 37°C in 1 N NaCl for 1 month. Measurement of K_{IC} was at 0.1 mm s⁻¹. Creep specimens ($n=5$) were similarly fabricated (6x4 mm) and aq. stored for 1 month at 37°C. Compressive stress (32 MPa) was then applied for 8 h in 37°C water and strain was recorded digitally. Creep-recovery was monitored for a further 8 h. K_{IC} data are as follows [X (SD)]: TPH 1.68 (0.31) > ($p<0.05$) DYR 1.32 (0.02); FJL 1.28 (0.13) >> ($p<0.01$) CHF 0.44 (0.02). Mean creep (%) increased in the sequence: DYR 0.67 < DYR 0.71 < CHF 0.72 < FJL 0.99. Recovery (% of max) decreased in the sequence: DYR 92.8 > TPH 86.5 > CHF 74.1 > FJL 57.4. Statistical CV for creep was circa 4%. Both 'hybrid' restoratives exhibited acceptable fracture-toughness in the range characteristic of resin-composites and a factor of 3 greater than a conventional glass-ionomer. The 'hybrids' however were differentiated markedly in viscoelastic-recovery with the Compomer³ exhibiting stability equivalent to resin-composite. ¹Dyract² Fuji II LC, GC Corporation, Tokyo. ²Chemfil Sup. ³TPH. ⁴Deutsply, Constance, Germany.

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BM GRIFFITHS* and TF WATSON. (Eastman Dental Hospital and UMDS, Guy's Hospital, London, UK): Confocal Microscopic investigation of the sealing ability of five dentine bonding agents.

The interdiffusion zone between bonding agent and dentine may be permeable to pulpal fluid movement. Interfacial porosity when using five dentine bonding agents (DBAs) was investigated by observing microleakage of fluorescent labelled saline from the pulp cavity. The DBAs ranged from U (control) a single stage adhesive resin, to more complex all-in-one systems: CL with an autopolymerising primer/conditioner, or OB, E1 and E2 employing H₂O₂, each. E1 and E2 were similar, but E2 was light-cured. OB and CL are used with 'stress distributing' composite liners. Wedge shaped cavities were cut in 50 teeth, treated with a DBA and restored with Herculite XRV (Kerr Mfg.). The pulp chamber was filled with rhodamine B for 3 hours and the teeth longitudinally sectioned. The samples were examined with fluorescence confocal microscopy. Interfacial micro leakage was measured as a % of pulpal wall length by image analysis. Control U gave 100% dentine smear layer leakage. 60% of OB restorations were fully sealed; the leaking OB restorations showing porosity < 50% along the pulpal wall. Results for CL were comparable to OB, but 40% showed no leakage. E1 and E2 gave variable results. E1 showed a leakage distribution from 0 to 100% whereas E2 showed a polarisation towards 0 or 100% leakage. This study indicates the importance of primer polymerisation in all-in-one systems, but also the beneficial effect of a stress distributing layer superficial to the adhesive interface.

¹Unif bond, ESPR. ²CL: Chemfil Liner 2, Kazzrey. ³OB: Optabond, Kerr. ⁴E1 E2: exp primers used with U.

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B R DAVIES*, S M DUNNE and B J MILLAR (King's College Dental School, London): A survey of the effectiveness of light-curing units and testing devices.

This study aims to: a) survey the output from 49 light-curing units in clinical use; b) measure the effect on depth of cure of composite resins caused by a range of light intensity; c) assess the relationship between radiometer readings and depth of cure in a human tooth and a Heliotest¹ model. The mean meter reading produced by the lights surveyed using a Lampchecker radiometer² was 4.4±2.4, range 0.3 to 10.0. The manufacturer consider optimal light output to be within the range 5.0 to 7.0. However, lights of very low output (0.2±0.1) were found to be capable of curing, after 20 seconds, a 1.9±0.3mm thickness of composite. The mean depths of cure of composite placed in the Heliotest were significantly ($P<0.01$) greater than those observed in the natural tooth model. Correlation coefficients of meter readings and depth of cure for the Elos Cure Ritz³, Demetron 100⁴ and Lampchecker radiometers, were 0.98, 0.94, 0.90 (20 second cure) and 0.97, 0.95, 0.90 (60 second cure), respectively. The mean output of lights surveyed was lower than recommended. However, the depth of composite cure was compatible with clinical technique. Radiometer readings correlated well with depth of composite cure in a natural tooth, while the Heliotest gave exaggerated results.

¹Ivoclar Vivadent, UK. ²Clarke Dental, UK. ³Efos Inc, Canada; ⁴Demetron Research Corp, USA

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CA MITCHELL*, E OHAGAN and JM Walker (Dep't Restorative Dentistry, The Queen's University of Belfast, UK): Probability of Failure of Orthodontic Brackets Bonded with Glass-Ionomer or Composite Cement.

The aim of this study was to compare the maximum loads at failure and the probability of failure of three glass ionomer cements and a composite cement bonding orthodontic brackets to human teeth. The cements studied included a conventional glass ionomer cement (A), two resin-modified glass ionomer cements (B, C) and a composite cement (D). The roots of two hundred human premolar teeth were embedded in acrylic resin and the buccal enamel surface of the crown prepared as required. Each cement used to bond the bracket to the enamel was weighed, and light-cured where required. The specimens were stored for 10 minutes or 24 hours at 37°C and 100% humidity. A tensile shear force was applied via a wire loop placed under the wings of the bracket. The maximum load at failure was noted and the data obtained analysed using a Kruskal-Wallis test followed by comparison of the groups using Mann-Whitney tests. Comparison of the loads at failure revealed that the composite cement (D) was significantly stronger than the glass ionomer cements (A, B, C) at 10 mins and 24 hrs ($P<0.05$). Weibull analysis of the results gave values for the Weibull moduli and probabilities of survival for an orthodontic bracket under a given load for each cement at 10 mins and 24 hrs.

Glass ionomer cements give a number of clinically significant advantages over composite cement in the retention of brackets. Resin-modified glass ionomer cements have a higher probability of survival than conventional cements at 24 hrs and further improvements in their early bond strength would be clinically beneficial.

Cement A = Chemfil II Express, Deutsply Ltd, De Trey Division, Weybridge, UK. Cement B = Vitremer, 3M Dental Products Division, St Paul, MN, USA. Cement C = Experimental cement, 3M Dental Products Division, St Paul, MN, USA. Cement D = Concise, 3M Dental Products Division, St Paul, MN, USA.

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S.M.C. GOULD*, M. SHERRIFF. (Dept. of Orthodontics, Guy's Hospital Dental School, London U.K.): Friction in a Speed bracket/archwire system - A Finite Element Investigation.

There is some confusion in the literature over the relative importance of the different variables of orthodontic sliding friction. The finite element method (F.E.M.)¹ was used to build an idealised three-dimensional model of a bracket/archwire system to study these variables. Following recommended finite element procedure, a simplified two-dimensional model was built and a friction analysis carried out using a variety of loading conditions. Results from these analyses indicated that expected areas of high frictional force. This data was then used to refine mesh design and loading conditions for the detailed three-dimensional model. Results showed that in an idealised mode system, increasing the ligation force, will increase the frictional force. More surprisingly, the distribution of this applied force was the dominant factor in determining the level of friction. Result from the two-dimensional frictional analysis indicated that a light, evenly distributed ligation force will produce the least friction.

It is concluded that finite element analysis is a viable method for studying friction in an idealised orthodontic bracket/archwire system. Indeed, if friction is ignored when using the F.E.M. then results will be unreliable. The analyses showed that ligation force and the distribution of this force are important factors in sliding friction.

¹ Using Mystro / Lusas version 11 software.

600

JEDYNAKIEWICZ NM, MARTIN N*, FLETCHER JM (Department of Clinical Dental Sciences, The University of Liverpool) A clinical evaluation of a new ionic-matrix composite

Dyract¹ direct restorative is a light-activated composite material containing carboxyl side-chains on the polymerisable molecule. The setting reaction takes place in two stages. The first stage is a light-activated polymerisation reaction. The second stage is an acid-base reaction between the carboxyl groups and metal ions released from the glass filler. This study examines the clinical performance of the material over a period of three years. The new material is evaluated against a control material (Fuji II LC²) for the restoration of cervical class V lesions. 60 matched pairs of restorations were placed in human subjects and evaluated according to modified Ryge criteria. The evaluation was performed single-blind by two examiners. The parameters under evaluation are; surface finish, surface contour, colour match, gingival index, secondary caries and marginal integrity. Baseline observations were recorded and the restorations re-evaluated at one year. There was no statistical difference between the test and control materials for any parameter except surface finish. The surface finish of Dyract was scored grade 1 in all test restorations. All control restorations were scored grade 2. At one year, all retained restorations placed with Dyract were clinically acceptable and showed a superior surface finish to the control material.

¹ De Trey Dentaply, Konstanz, Germany. ² GC Int. Tokyo, Japan

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JEDYNAKIEWICZ NM*, MARTIN N, FLETCHER JM (Department of Clinical Dental Sciences, The University of Liverpool) A clinical evaluation of a new self-priming dentine adhesive

Dyract PSA¹ is a new single component dentine adhesive which uses acetone to transport polymerisable monomers into the subsurface dentine. This study was designed to evaluate the retention rates of composite restorations placed in non-retentive cavities. The adhesive is under evaluation against a control material² in a three year clinical trial. 60 pairs of restorations were placed in non-retentive cervical cavities in human subjects. All cavities had more than 50% of their margins lying in dentine. A single application of the adhesive was painted directly onto the exposed dentine, blown to evaporate the solvent and then photopolymerised. The restorative composite (Dyract¹) was applied immediately, photopolymerised and finished. 49 patients (2x58 restorations) attended at one year and the restorations were evaluated. 57 test restorations and 58 control restorations were retained.

One year results indicate that Dyract PSA establishes an adhesive bond adequate for the retention of cervical restorations. Long term durability of the bond must be demonstrated.

¹ De Trey Dentaply, Konstanz, Germany. ² GC Int. Tokyo, Japan.

602

JEDYNAKIEWICZ NM, MARTIN N, FLETCHER JM* (Department of Clinical Dental Sciences, The University of Liverpool) A clinical evaluation of a high-fluoride self-priming adhesive

The adhesive under evaluation, K81, is a self-priming single-component dentine adhesive which has been modified to release fluoride after setting. This study is designed to evaluate the retention rates of composite restorations placed in non-retentive cavities. The adhesive is under evaluation in a three year clinical trial. 85 restorations were placed in non-retentive cervical cavities in 37 human subjects. All cavities had more than 50% of their margins lying in dentine. A single application of the adhesive was painted directly onto the exposed dentine, blown to evaporate the solvent and then photopolymerised. The restorative composite (Dyract¹) was applied immediately, photopolymerised and finished. 36 patients (83 restorations) attended for review at three months and the restorations were evaluated. All the test restorations were retained.

Initial results at 3 months indicate that the high-fluoride adhesive K81 establishes an adhesive bond adequate for the retention of cervical restorations. Long term durability of the bond must be demonstrated.

¹ De Trey Dentaply, Konstanz, Germany.

603

A. ROBINSON*, J F McCABE. (Department of Restorative Dentistry, University of Newcastle upon Tyne, Dental School.): Effect of 4 surface treatments on cement to gold bonding.

The effect of 4 surface treatments on shear bond strength of 6 cements (4 resin, 1 glass ionomer and zinc phosphate) to gold alloy¹ were evaluated. Adhesives were bonded to the alloy using the gelatin capsule method (Barkmeier WW, et al. J Esth Dent, 1991, 3(4)148-53) and debonded after 24 hours at 37°C RH 100% at a cross head speed of 1mm/minute. The treatments were tin plating², Ga/Sn amalgamation³, heat treatment 400°C for 4 minutes and sand blasting (control). Test groups of 30 were used and the results were subject to Weibull analysis from which a probability of failure at 6 Mpa was computed, as follows.

	Panavia Ex ⁴	Panavia 21 ⁵	Metabond ⁶	Comspan ⁷	Aqua Cem ⁸	Zinc Ph ⁹
Tin plate	0.775	0.536	0.073	0.385	0.896	0.963
Ga/Sn	0.171	0.141	0.122	0.363	0.923	0.974
Heat	0.012	0.020	0.059	0.363	0.921	0.923
Control	0.230	0.056	0.005	0.127	0.784	0.883

The probability of failure is significantly reduced by the use of heat treatment and Panavia (Ex or 21)

¹Degulor C, Degussa Co, Ltd. ²Kada Aoe Mini, Nippon Avionics, Co, Ltd. ³Goodfellows Ltd. ⁴Kuraray Co, Ltd. ⁵Parkell Bio Materials Co Ltd. ⁶Caulk, Dentaply. ⁷De-Tray, Dentaply.

604

SOO S*, LEUNG T (Prosthetics Dept., Eastman Dental Institute, U. of London): The Hidden-clasp System vs. Conventional Clasp - A Comparison of Retention Forces.

Partial dentures rely on clasps for direct retention, but their poor appearance has often been a barrier to patient acceptance. Many so-called aesthetic clasp systems have been proposed in an attempt to provide unobtrusive retention, but it is unknown how well they perform their primary function of retention. This study examined the Hidden-clasp design, and compared its retention to C-Clasps and I-Bars. A number of cast Co-Cr frameworks of each design were produced at a commercial dental laboratory and their retention measured on a model with natural teeth and simulated periodontal ligament. The frameworks were pulled from the test model using a Universal Testing Machine and the retention recorded on a chart recorder. The three clasp designs were tested as a whole framework and then as a single clasp, under dry and wet conditions. The mean retention measured in Newtons are as follows:

	C-clasp	I-bar	Hidden clasp
Whole framework, dry	10.7	5.4	8.6
Whole framework, wet	17.5	7.6	13.1
Single clasp, dry	11.8	5.7	7.8
Single clasp, wet	20.2	11.7	8.8

The results indicate that the Hidden-Clasp provides comparable retention to that of C-Clasps and I-Bars.

605

PRESTON G*, LEUNG T (Prosthetics Dept., Eastman Dental Institute, U. of London): Effect of heat treatment on the behaviour of cast cobalt chromium clasps.

This study investigated the effects of heat treatment on some of the properties of the cobalt chromium alloy Bayer SP Chrome Cobalt. Test specimens consisting of 15mm long half round tapering clasps were tested as cast, after heat treatment for one hour at 600°C, 800°C or 1000°C followed by quenching. The investigation involved: 1. Measuring the deflection of the clasps and load at the proportional limit, as well as the ratio of load: deflection, an indication of their stiffness. 2. Measuring permanent deformation of the clasps following deflections simulating 6 months' use. 3. Vicker's hardness testing was carried out on the test plates carrying the clasps. The mean deflection and load at proportional limit for the as cast, 600°C, 800°C and 1000°C groups were (0.34mm, 6.83N), (0.31mm, 6.07N), (0.33mm, 7.13N) and (0.30mm, 5.30N) respectively. The mean load: deflection ratios were 20.75Nmm⁻¹ [as cast], 19.74Nmm⁻¹ [600°C], 21.68Nmm⁻¹ [800°C], 17.53Nmm⁻¹ [1000°C]. Mean permanent deformation after 1460 deflections was highest for as cast specimens, and decreased with increasing heat treatment temperature. Vicker's hardness values rose as heat treatment temperature was increased. It was concluded that no direct relationship was present between heat treatment temperatures and changes in stiffness or proportional limit of the clasps relative to their 'as cast' state. Permanent deformation after repeated flexure had large within-group variability. Heat treatment at all three temperatures tested increased the hardness of the alloy.

606

N A ORCHARD*, E H DAVIES, G J PEARSON, J A HOWLETT (Departments of Prosthetic Dentistry and Biomaterials Research, Eastman Dental Institute, London, UK): Bonding of resins to cast cobalt-chromium alloy.

Adhesive resin systems are reported to improve the bond strength between resins and Co-Cr alloy. The study compared the behaviour of three resin systems, Cesead¹, Biodent K&B² and Chromasil/Spectralink³ in the presence of mechanical retention. Sixty 8mm diameter cylinders and alloy bars, 30°4'0.8mm, had 0.6mm diameter retention beads cast on to the bonding surface at regular intervals. These surfaces were then abraded with 50µm AL₂O₃ then ultrasonically cleaned in distilled water immediately prior to bonding of the resins, 4mm deep on the cylinders and 2mm deep on the bars. Polymerisation was initiated by heat and pressure or light as appropriate for the resins. Specimens were stored in water for 7 days or 91 days with 1000 thermocycles between 4°, 37° and 60°C before shear load testing in a Hounsfield H25K universal testing machine (UTM). Three point bend testing of the bar specimens was carried out in an Instron 4505 UTM. The nature of the failure of the specimens was examined in an optical microscope. Mean shear bond strengths initially were 23MPa¹, 25MPa², 9MPa³ and following thermocycling 30MPa¹, 27MPa², 10MPa³. Flexural failure occurred at 779GPa¹, 348GPa², 415GPa³ initially and fell to 575GPa¹, 291GPa², 343GPa³ following thermocycling.

The investigation showed that the light-cured resin system¹ performed significantly better than the heat and pressure-cured conventional resin² in turn, the latter performed significantly better than the heat and pressure-cured resin with adhesive properties³.

¹Cesead-Kuraray Co Ltd, ²Biodent K&B Plus- DeTrey GmbH, ³Chromasil/Spectralink Ivoclar AG

607 P AL-DEGHAISEH* & J A HOBKIRK (Eastman Dental Institute, London, UK): The effect of maxillary anterior teeth appearance on personality projection in society.

The objectives of this study were to investigate the effects of the appearance of maxillary anterior teeth on elderly patients' personality as perceived by others. Four male and 3 female subjects, dentate, partially edentulous and edentulous were chosen to provide 9 different dental appearances by giving the edentulous subjects dentures with various designs. The subjects were photographed full-face and close-up using standard techniques to produce colour prints. These were then scored in a two stage process by five groups of twenty observers consisting of child and adult patients, dental surgery assistants and two groups of dentists one of which worked in restorative dentistry. In the first phase observers were asked to score both of the views in ranking order of preference. The second phase was carried out on the top, middle, and bottom two preferred pictures and involved the observers in scoring the images for a series of personality characteristics using a standard pro-forma. Perceptions varied depending on whether a full face or close up view was shown. There was degree of consistency between the groups although the children occasionally preferred a different picture. Edentulousness or severe tooth surface loss was perceived as unattractive, and a dentate appearance most attractive. Full face views of the same subjects were associated with a wider range of scores. While lack of teeth was not a bar to humorous or homely characteristics it was associated negatively with intelligence and friendliness.

608 J.P. HINDLE* and A. HARRISON (Restorative Dentistry, Dental School, Bristol, UK): Application of 3-dimensional profile gauges in primary recording of extra-oral defects.

This investigation is designed to determine the suitability of a 3-dimensional profile gauge in the primary recording of extra-oral defects in patients who have undergone major head and neck surgery. Five gauges were constructed with pin densities of 9, 16, 25, 36 and 49 pins/cm². Each gauge was assessed for accuracy of the model obtained when compared with control models obtained from the same patients using conventional alginate impression techniques. Perceived patient comfort during the use of each gauge was also assessed. Results showed that whilst pin densities of 25+ pins/cm² gave adequate defect detail patients felt that only the gauge with a pin density of 25/cm² was comfortable in use. Patients also reported feeling much less claustrophobic when the profile gauge was applied to their defect and surrounding tissues when compared with the alginate impression technique.

It is concluded that a 3-dimensional profile gauge is suitable for use in recording surgically created extra-oral defects. A pin density of 25/cm² appears to give sufficient detail of the defect whilst maintaining patient comfort during the recording procedure.

609 M.K. HILAL*, M. Y. SHAREEF and R. VAN NOORT (Department of Restorative Dentistry, University of Sheffield, Sheffield S10 2TA) Development of a calcium phosphate/zirconia bioactive ceramic composite.

Powder pressing of HAP/partially stabilised zirconia, aimed at producing a high strength bioactive ceramic composite, requires excessively high temperatures for densification causing breakdown of the HAP (Hilal *et al.* JDR 1994, p853 abstr.537). Hot pressing is now being studied in an effort to obtain full densification at lower firing temperatures. HAP@ and ZrO₂@ powder compositions ranging from 0 to 100 vol% ZrO₂ were prepared using a wet mixing process. Ten discs were made for each composition and hot pressed at 1250°C for 1 hour. The % theoretical density and biaxial flexural strength (BFS) were measured and microstructural changes were examined using XRD and SEM. The addition of a small amount of zirconia to the HAP and vice versa resulted in problems of densification. This was especially pronounced for small additions of ZrO₂. Only for pure HAP, pure ZrO₂ and a 55/45 vol% ZrO₂/HAP composite was near theoretical density achieved. This was reflected in the biaxial flexural strengths, which for the 55/45 composite was 451±31 MPa. Although a considerable improvement on pure HAP, this was still some way short of the BFS of pure ZrO₂ at 1213±223 MPa. The XRD data confirmed that the HAP was retained. SEM examination revealed a two-phase microstructure of HAP and zirconia crystals. The rapid improvement in strength at the 55/45 composition can be ascribed to the formation of a zirconia matrix in preference to an HAP matrix. *It is concluded that a high strength HAP/zirconia ceramic composite can be produced using hot pressing without causing breakdown of the HAP.*

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610 G HOAD-REDDICK* (Department of Dental Medicine and Surgery, University of Manchester, UK): Is post-extraction bone resorption inevitable? - Results of an investigation after one year.

The aim of this study was to measure the effect on alveolar bone resorption, of the insertion of non-resorbable hydroxyapatite granules (Calcitra® 2040) into fresh extraction sockets immediately post-extraction. Two groups of subjects matched for age and sex were studied. A total of 36 subjects (20 male, 16 female, average age 54 years) were rendered edentulous by the extraction of canine teeth and were provided with immediate dentures. Particulate HA was placed into the extraction sockets of half the sample. Assessment was undertaken by means of clinical photographs, radiography and profilometry. Patient compliance with the procedure was good and healing appeared normal in all cases. Results after one year showed that the material worked effectively retaining bone not only in the immediate area of the canine socket but also in the midline and in the region of the mental foramen. Not only was statistically significantly more height of bone retained by the implant group but also more width. In the canine region, one year post-extraction, three times more bone was retained by the implant than the control group when measuring ridge height ($p=0.0006$ on the right side; $=0.022$ on the left). When measuring width reduction, it was found that at one year the implant group had retained twice as much ridge width as the control group ($P=0.059$ on the right side; $=0.005$ on the left).

Preservation of the residual alveolar ridge in both the vertical and horizontal dimensions was achieved. This could benefit many patients who are about to be rendered edentulous as, not only is the implantation procedure simple, it is also cheap.

611 D R RADFORD (Department of Prosthetic Dentistry, UMDS, London): An investigation into the surface of failed Brånemark fixtures.

Although osseointegration is now a dependable procedure it is difficult to comply with the 1988 NIH implant consensus which requires that the cause of failed fixtures be identified (Rizzo AA (ed), *J Dent Educ* 52: 678-827, 1988). The aim of this study was to assess the value of SEM and Energy-dispersive x-ray analysis (EDX) and to analyse the surfaces and attached material on 12 failed fixtures. Implants were retrieved after they had failed clinically and stored in neutral buffered formalin. Fixtures were washed in sterile water, then in 100% alcohol and air dried. They were mounted on aluminium stubs, carbon coated, and viewed in a SEM Hitachi S520 with an integral EDX Kevex Delta.

The results showed areas of integrated bone and other areas totally devoid of calcified tissue. This was confirmed with back-scatter images and EDX analysis. Colonisation by bacteria and yeasts was observed on four of the fixtures.

Failed fixtures appeared to have been only partly osseointegrated. The SEM combined with EDX analysis is ideal instrumentation for analysis of osseointegration failure as it allows confident interpretation of the electron microscope image.

612 L SARKAR*, SJ JONES and A BOYDE (Department of Anatomy, University College London, UK): Human bone associated with Ti dental implants: confocal microscopic observations.

This study demonstrates the utility of confocal microscopy in examining the microstructure of the bone:implant interface in surgical scrap associated with titanium dental implants. Specimens retrieved, after being left in situ for periods between 6 months and 3 years, included whole loaded implants and peri-implant bone fragments from implants which had not been loaded. Reflection and fluorescence confocal optical microscopy was employed to study the tissue deep to polished block faces of PMMA embedded material. The pure titanium implants had successfully stimulated osseointegration. The autofluorescence and reflection modes of the confocal laser microscope allowed the recognition of mineralised extrinsic (Sharpey's) fibres and woven bone next to the implant surface, clearly distinguishable from lamellar bone. The organisation of vascular channels and the presence of woven bone in both long term implants and non-loaded bone fragments is taken to reflect continual modelling and turnover.

It is concluded that the dental implant:bone interface is dynamic in nature, and that confocal microscopy is ideal for the 3D study of surgical scrap in the implant field.

613 S BODELL*, I M BROOK and P V RATTON (Biomaterials Group, School of Clinical Dentistry, University of Sheffield, UK): Human response to bioresorbable self reinforced polyglycolic acid used for orbital floor fracture repair.

Fractures of the orbital floor are repaired using autogenous grafts, which are associated with increased donor site morbidity, or allografts the use of which is associated with infection and extrusion. We have demonstrated the *in vitro* osteoconductive properties and clinical potential of self reinforced biodegradable polyglycolic acid (RPGA) (*J Dent Res* 73: 741 1994) for orbital floor repair. Following implantation polyglycolic acid is hydrolysed to water and carbon dioxide via the tricarboxylic acid cycle, and should not suffer the problems encountered with non resorbable allografts (*Br J Oral & Max Surg* 31 (3) 154-157 1993). Two patients who had orbital floor repair with RPGA membrane 6 and 18 months previously required re-operation for removal of miniplates. In both cases an intact bony orbital floor was found with a thin layer of non-inflamed fibrous tissue separating the orbital fat from the periosteum. A biopsy was taken, fixed in glutaraldehyde, resin embedded. Thin sections were stained with uranyl acetate in 50% ethanol and Reynold's lead citrate and viewed using a Philips CM 10 transmission electron microscope, or with Toluidine Blue for light microscopical examination. At 6 months the structure of the RPGA membrane had been lost and isolated PGA fibres were embedded in a collagenous extracellular matrix containing fibroblasts. There was no evidence of the mineralization as reported *in vitro*. At 18 months the PGA had been replaced by fibrous tissue and in places there was some calcification of the collagen fibres. There was a pattern to the deposition of collagen suggesting initial infiltration of tissue between the RPGA fibres followed by resorption and replacement of the RPGA which in places appeared to be replaced by a collagen free ground substance.

RPGA is a suitable material for orbital floor repair and resorbs between 6 and 18 months.

1. Biofix®, Bioscience Ltd, Tampere, Finland

614 R AKEEL, M R HEATH* and A M FERMAN (Prosthetic Dentistry, London Hosp, E1 2AD): A new method for measuring masticatory forces on implant stabilised mandibular overdentures

The aim of this study was to develop and test a method for measuring masticatory forces in implant stabilised overdenture wearers. The first transducer included a rosette strain gauge and was designed to attach to an Astra implant: this has been validated and reported (Akeel *et al J Dent Res* 71: 717, 1992). Since then, commercial pressure sensors have also been used to monitor mucosal loading unilaterally under the saddle mesial and distal to the implant. Intraoral calibration was achieved by loading defined parts of the occlusion using a bite gauge. The output from all transducers were analysed with multiple regression analysis so that unilateral masticatory forces can be recorded regardless of the site of loading. The implant acts as a fulcrum during loading which precludes simultaneous output from both mucosal pressure transducers. The use of the transducers cover both incision and mastication. The multiple regression model gave a tight fit (0.99) and a standard error of 3 N. The percentage of occlusal forces received by the implant ranged from 51 to 89%, depending on the site of loading.

It is concluded that the method described can be used to measure vertical masticatory forces and its distribution in subjects with this type of prosthesis without interfering with their occlusion.

615

C A BURNETT* and T J CLIFFORD (Division of Restorative Dentistry, The Queen's University of Belfast, UK): Reproducibility of closest speaking space in three phonetic tests.

The purpose of this investigation was to determine if the closest speaking spaces (CSS), measured during three separate phonetic tests, in a subject group with normal dentitions, were reproducible after a six month time period. The mandibular movements of thirty subjects were measured using an electrognathograph and correlated by computer during the recitation of three phonetic tests, one containing all the phonemes of the English language in a twelve line paragraph, one containing the six sibilant sounds in a continuous sentence and one with six single words each containing one of the sibilant sounds. This experimental procedure was repeated after a six month time interval. Statistical analysis (one-factor ANOVA test with repeated measures, significance level 98%) demonstrated no significant difference among the mean CSS determined initially or among those determined after six months. However only the CSS determined for the test containing all phonemes was not significantly different when results were compared between the two recording sessions. It was concluded that the CSS was reproducible for a phonetic test containing all phonemes and not reproducible for tests containing only the sibilant sounds.

617

A T INGLIS*, B J MILLAR, P B ROBINSON (King's College School of Medicine and Dentistry, London): Clinical evaluation of an anterior hybrid composite resin over eight years

The aim of this prospective longitudinal study was to evaluate the performance of an anterior hybrid composite resin restorative material (OPALUX, 3C) in 24 selected patients (12 male, 12 female). Scores were obtained using USPHS criteria for marginal adaptation, anatomic form, colour match, surface roughness and marginal discolouration. Baseline data was collected on 45 restorations (25 class III, 3 class IV, 16 class V) and reviewed independently by two of the three clinicians who contributed to the study who then reached a consensus score. Restorations were reviewed, and scored in the same way, at 3 months (n=33), 1 year (n=33), 2 years (n=28), 3 years (n=17) and 8 years (n=25). The total number of alpha ratings at baseline, 3 months, 1 year, 2 years, 3 years and 8 years for the following parameters were: marginal adaptation (33, 28, 29, 27, 12, 17); anatomic form (39, 30, 32, 26, 17, 15); surface roughness (34, 26, 24, 23, 11, 4); colour match (24, 25, 26, 24, 15, 12) and marginal discolouration (39, 31, 30, 25, 10, 9). The incidence of secondary caries was very low. Life table analysis suggests a 73% survival, with no C scores, at 8 years. In conclusion, the majority of these composite resin restorations continued to perform satisfactorily.

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J L CUNNINGHAM*, K E PATTISON and I C BENINGTON (Department of Restorative Dentistry, Queen's University of Belfast, N. Ireland): Shear bond strength of denture tooth adhesives.

In the United Kingdom over £7 million is spent annually on the repair of acrylic resin dentures. It is estimated that one-third of the repairs results from tooth debonding (Debar et al. Br Dent J 1994; 178: 343-345). A number of tooth surface treatments have previously been investigated with the most promising bonding results being obtained with liquid or adhesive coatings. Rubber moulds were made to accommodate each tooth in a set of six anterior teeth. A 5mm diameter channel in the mould fitted accurately on the ridge lap surface of the tooth. Molten wax was poured through the channel and allowed to cool. The tooth and wax patterns were invested in dental plaster and the wax eliminated. Before packing the acrylic resin the bonding surfaces of the teeth were treated: (a) dewaxed only, (b) the application of an adhesive agent¹ and (c) the application of an experimental cement (n = 108). Shear loading was applied at a rate of 2.5 mm min⁻¹ at the tooth/resin interface. Results showed that the adhesive agent produced a non-significant increase in strength (p < 0.05) while the experimental cement significantly improved the bond (p > 0.001). It is concluded that the experimental cement, in comparison to the commercial product, imparts a significantly higher bond strength to denture teeth.

1 Vitacoll, Vita Zahnfabrik, Germany.

We gratefully acknowledge support from the Wellcome Trust.

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B J MILLAR, M NESBIT*, D WOOD, B BUDD (King's College Dental School, London): The effect of disinfection regimes on the wettability of impression materials.

Surface disinfection of addition cured silicone impression materials is generally recommended. Cullen et al (J Prosthet Dent, 1991, 6, 261-5) showed that higher advancing contact angles between water and elastomeric impression materials resulted in a higher incidence of voids on the surface of gypsum dies. This study aims to assess the effect of 3 surface disinfectants on the advancing contact angle of water on the surface of addition cured silicone impression material. Contact angles were measured by direct measurement from photographs. Three surface disinfectants, HM520¹, Sporidicin² and Dentiro³, were applied to the surface of 17 impression materials. Contact angles of the treated surfaces were compared with a control (untreated) group. In all, 35 of the treated surfaces showed a reduced contact angle when compared with the control samples, 14 treated samples showed an increased contact angle and 2 samples remained the same. This result was significant (Chi² = 9, P < 0.05). In conclusion, all three surface disinfectant treatments appeared to increase the surface wetting of addition cured silicone materials.

¹ Durr, SS White, London, UK

² Dentsply, Weybridge, UK

³ Panadent, London, UK

616

S. DJEMAL* AND D J SETCHELL (Department of Conservation, Eastman Dental Institute, UK): An investigation of upper anterior crown preparations.

The aim of this study was to examine upper anterior crown preparations performed by general dental practitioners with regard to palatal and incisal reduction, degree of taper and the resultant gingival wall height. Working casts of crown preparations and associated preoperative study casts were obtained from 12 practitioners with 2 - 9 years experience. The mid-axial palatal surfaces of the teeth before and after preparation were profiled using the Reflex Microscope (Reflex Measurement, Somerset BA6 8SP, UK) and Comp3d software. 50 preparations were measured. The results showed that the palatal reduction at 14 predetermined sites in the mid-axial palatal surface ranged from 0 - 3.08 mm. The overall mean value was 0.9 mm. A lingual concavity consistent with preoperative form was infrequently produced. The incisal reduction varied from 0.25 - 6.17 mm with a mean of 2.26 mm. The mean degrees of taper were 24.50° labiolaterally and 17.18° mesiodistally. Only 23 tooth preparations displayed a discernable gingival wall and 6 preparations were undercut.

It is concluded that departures from optimal form during anterior crown preparations are prevalent and that desirable features are either poorly understood or difficult to achieve.

618

M. LEONG, MA WILSON and RHF WILSON (Restorative Dentistry, University of Manchester, UK): The influence of matrix technique on the marginal adaptation of composite inlays.

This study investigates the influence of three matrix techniques on the marginal adaptation of two types of composite inlay. Thirty sets of three teeth comprising one upper first premolar, one upper second premolar and one upper first permanent molar tooth were set in separate blocks in correct anatomical relationship. A standardised MOD preparation was completed in each of the second premolar teeth and lined with a glass ionomer cement. A silicone impression was then taken of each set of teeth. Inlays were fabricated using one of two systems^{1,2} and placed with the aid of one of three matrix techniques: polyester strip alone, polyester strip tightened and wedged prior to inlay insertion, polyester strip tightened and wedged following inlay placement - five preparations being allocated at random to each test group. Following finishing, the marginal adaptation of each inlay was assessed and scored at 16 defined sites under a stereomicroscope. Excellent marginal adaptation was found at 63% of the sites - more excellent scores being recorded for occlusal (82%) than proximal (51%) sites. Mann Whitney and ANOVA analyses failed to reveal any significant differences between the inlays of the two systems and in respect of the three matrix techniques in terms of percentage excellent scores. It is concluded that for inlays of the two systems tested, the three matrix techniques investigated may not be found to significantly influence the excellence of the marginal adaptation. In inlays of both systems occlusal marginal adaptation may be found to be superior to proximal marginal adaptation.

¹ Coltene ID Inlay system, Coltene AG, Altstätten, Switzerland

² SR-Inlay System, Ivoclar-Vivadent, Schaan, Liechtenstein

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COLLIS J* JONES E L and LEYSHON RJ (Department of Prosthetic Dentistry, Dental School, Cardiff, UK): A comparative study of acetyl resin with lucitone 199 and trevalon

Manufacturers claims for Dental D (acetal resin) suggest that it has physical properties that enable it to be used for the construction of skeletal components of partial dentures, as well as denture bases and other prosthetic components. To determine how this new resin compares with those already on the market, a series of tests were carried out on specimens of Dental D, Lucitone 199 and Trevalon. Modulus of elasticity was determined using the Lloyds Transverse Bend Test, surface hardness used the Minotovo Micro Hardness test, an impact test was performed using Zwick impact hardness apparatus and glass transition temperature was recorded using Dynamic Mechanical Thermal Analysis. The findings for each material were analysed using the ANOVA package. Student T-tests results with p < 0.05 were accepted as significant. Dental D had a high modulus of elasticity, low surface hardness and a low impact resistance compared with Lucitone 199. When compared with Trevalon, Dental D had a high modulus of elasticity, a similar surface hardness and similar impact resistance. Dental D displayed a higher glass transition temperature than both Lucitone 199 and Trevalon, but all were dimensionally stable within applicable limits (0-100°C).

It is concluded that the properties of Dental D (Acetal resin) differ significantly from those of Acrylic resins.

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B J MILLAR*, S M DUMME, P B ROBINSON (King's College Dental School, London): In-vivo study of a surfactant used with polyvinylsiloxane impression materials.

A topical surfactant¹ has been shown (Robinson et al, J Prosthet Dent 1994 71 390-393) to significantly reduce the number of surface voids in-vitro when used with a polyvinylsiloxane impression material. The aim of this study was to determine whether the use of this surfactant also reduced the number of air-bubbles visible on the surface of three polyvinylsiloxane materials when used in-vivo. A total of 57 impressions were taken of the maxillary arch in 57 subjects. Hydrosystem was applied, prior to the insertion of the impression, to the left or right premolar teeth allocated at random. The untreated side acted as a control. These impressions were examined for surface voids by three examiners, unaware on which side the surfactant had been applied, and a consensus view was used in the analysis. Following the revealing of the code the impressions could be graded as: unreadable (4), no difference between treated and untreated sides (4), less voids on the treated side (33) and less voids on the untreated side (16). Hydrosystem was found to significantly reduce the number of surface voids (Chi² = 5.9, P < 0.05). There was no difference with regard to the three impression materials used. In conclusion, in-vivo use of Hydrosystem reduced the number of bubbles on the surface of addition cured silicone impressions.

¹ Hydrosystem: Firmadenta, UK; Thermack, Italy

623 C A HASELDEN*, J A HOBKIRK, J R GOODMAN, S P JONES and P KING (Eastman Dental Institute, London, UK): The morbidity of retained deciduous teeth in patients with hypodontia.

One of the characteristics of hypodontia is the retention of deciduous teeth which lack permanent successors. The morbidity of these teeth is of importance in clinical dentistry. The purpose of this investigation was to study patterns of root resorption in deciduous canines, and molars with no permanent successors in patients with hypodontia. The study was carried out using OPT's of these patients which had been recorded routinely for clinical purposes. A total of 356 radiographs which clearly showed such teeth in patients with ages up to 40 years was available for the study, and these represented the reported gender distribution of hypodontia in the population (9:5=3:2). Root resorption was banded into four groups, little/none, <25%, <50% and <75% using defined criteria. In multi rooted teeth the longer root was scored. Scoring was carried out by one observer who had been shown to have a reproducibility of >0.8 using the unweighted Kappa system. Inter-observer error between four different observers was 0.6 - 0.8 using the same test. It was found that the lower C's were the teeth least likely to be resorbed, and the upper D's the most. The upper C's also had a long lifespan, although less than that of the lower C's. While the lower D's had a more rapid resorption rate this was less than that of their maxillary counterparts. The resorption pattern for all these teeth was consistent. Resorption patterns for the E's were much more variable. No gender related differences in rates of root resorption were found.

624 A AGARWAL* and J A HOWLETT (Department of Prosthetic Dentistry, Eastman Dental Institute, London, UK): Assessment of two biometric guides to the positioning of the maxillary incisors.

Recommended guides for determining the natural antero-posterior position of the maxillary incisors in edentulous patients are the nasolabial angle and incisive papilla. This study assessed the accuracy of these guides by investigating: i) the relationship of the maxillary incisor to maxillary plane angle (UI-Max) to the nasolabial angle (NLA) using 91 standardised cephalometric lateral skull radiographs of dentate adults. Angular measurements, using a protractor were made to the nearest 0.5°. Assessment of measurement error included use of a paired t-test, Dahlberg's formula and coefficient of reliability; ii) the distance between the labial surface of the maxillary central incisors to the posterior edge of the incisive papilla (IP/I), measured on 100 casts of subjects with Class I or Class II (ii) incisor relationships. Casts were oriented according to the occlusal plane on an XY table and moved horizontally beneath the vertical arm of a surveyor and distance IP/I was measured to the nearest 0.1mm on two occasions. Results were analysed by paired and unpaired t-tests.

In subjects with normal inclination of the incisors mean NLA was $111.5^\circ \pm 10^\circ$, there was a slight inverse linear relationship between NLA and UI-Max (Pearson's $r=0.349$). The suggestion of Prosthetic Texts that the NLA be restored to 90° would appear to be unsubstantiated. A significant difference ($p \leq 0.01$) was found in the mean IP/I between Class I subjects ($12.84 \pm 1.5\text{mm}$) and Class II (ii) subjects ($11 \pm 1.43\text{mm}$), however the difference of 2mm may not be of any clinical significance.

625 T FRIEL* and R D WELFARE (Department of Prosthetic Dentistry, Eastman Dental Institute, London, UK): The Hospital Anxiety and Depression (HAD) scale and complete denture satisfaction.

Researchers have sought to establish relationships between satisfaction with complete dentures and personality traits for over forty years. This study aimed to investigate the relationship between levels of anxiety and depression and satisfaction with complete dentures. Out of an initial group of 111 patients who had new complete denture constructed at the Eastman Dental Institute only 68 were included in the study. These all completed: a Hospital Anxiety and Depression (HAD) Scale (Zigmond A & Snaith R, *Acta Psychiatrica Scand* 67: 361-370, 1983) on three occasions, before, during and after denture construction; the satisfaction questionnaire for their old dentures before treatment; the satisfaction questionnaire for their new dentures after treatment. Although the study group had levels of anxiety and depression which were higher than the population as a whole no strong relationship was found between the HAD score and denture satisfaction using the Spearman rank correlation coefficient.

It is concluded the HAD scale is not a suitable predictor of satisfaction with complete dentures.

626 P. LIU*, P.M. SMITH and J. SCOTT (School of Dentistry, The University of Liverpool, UK): Functional correlates of morphology in acutely isolated rat parotid acinar cells following duct-ligation induced atrophy.

In the rat, ligation of the parotid duct gives rise to profound atrophy of the gland. Furthermore, the fraction of gland volume morphologically identifiable as acinar cells drops from 84% to <1% after 1 week ligation. We have used microfluorimetric techniques with single isolated acinar cells in order to determine whether those cells which retain the appearance of acinar cells following ligation-induced atrophy also retain their functional ability to respond to a secretory agonist with an increase in intracellular Ca^{2+} .

Acinar cells from control glands showed a dose dependent increase in $[\text{Ca}^{2+}]_i$ following agonist stimulation. Maximal response was obtained with $1\text{-}5\mu\text{M}$ acetylcholine ($178 \pm 53\text{ nM}$ $n=9$) or $10\mu\text{M}$ carbachol ($176 \pm 52\text{ nM}$ $n=4$). Acinar cells from atrophied glands following 1 week duct ligation also responded to $5\mu\text{M}$ acetylcholine with an increase in $[\text{Ca}^{2+}]_i$ of $125 \pm 9\text{ nM}$ ($n=5$).

We conclude that in the atrophied gland there is a good correlation between morphology and function in individual cells.

The consequence of this for the secretory ability of the whole gland following atrophy, the effects of more prolonged atrophy and recovery of the gland following de-ligation are all currently under investigation.

627 C G KELLY¹*, J HADDEN², P I HARIS², D CHAPMAN², S TODRYK¹ (¹Div. of Immunology, UMDS at Guy's Hospital, London SE1 9RT, ²Dept. of Protein and Mol. Biol., Royal Free Hospital School of Medicine): Structure of streptococcal antigen I/II.

Colonisation of the tooth surface with *Streptococcus* mutans is the main cause of dental caries. Initial adherence of *S. mutans* involves stereospecific recognition of salivary receptors, which are adsorbed to the tooth surface, by a cell surface protein adhesin termed streptococcal antigen I/II (SAI/II). The aim of this study was to analyse the secondary structure of SAI/II by Fourier transform infra-red (FTIR) and circular dichroism (c.d.) spectroscopy. To determine the distribution of structural elements more precisely, a series of overlapping recombinant polypeptide fragments which spanned the predicted extracellular portion of SAI/II were also analysed. Both analyses indicated that SAI/II comprises approximately 36% α -helix and 40% β -sheet. Recombinant SAI/II, expressed in *E. coli* possessed similar levels of these structural components but was less thermally stable. The recombinant polypeptide comprising the N-terminal region of SAI/II (residues 39-481) adopted predominantly an α -helical conformation (60%) and underwent reversible thermal denaturation consistent with the proposal that it adopts an α -helical coiled coil conformation. The recombinant fragment comprising residues 475-824 also possessed considerable secondary structure (45% β -sheet, 20% α -helix). The recombinant polypeptide encompassing the adhesion binding-site of SAI/II (residues 816-1213) possessed approximately 40% β -sheet. The C-terminal fragment (residues 1155-1538) was insoluble at the concentrations required for analysis and no data were collected. These results suggest that recombinant SAI/II and polypeptide fragments adopt conformations similar to those of the native molecule from *S. mutans*. The results are also consistent with the proposed model for the structure of SAI/II.

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