Journal of Orthopaedic Surgery 2008;16(2):230-6

Autologous chondrocyte transplantation in the repair of full-thickness focal cartilage damage in rabbits

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ABSTRACT

Purpose. To compare the efficacy of autologous chondrocyte transplantation (ACT) versus non-operative measures for cartilage repair in rabbits.
Methods. Nine New Zealand white rabbits were used. Identical focal defects were created in the articular cartilage of both knees. One month later, the right knee was repaired via ACT, while the left knee was left untreated (control group). The quality of cartilage tissues in both knees was compared 3 months later, according to the quantitative analysis of glycosaminoglycan (GAG) in the cartilage and macroscopic examination of histology using the Brittberg/International Cartilage Research Society (ICRS) score.
Results. Microscopic examination showed enhanced regeneration following ACT repair. Quantification analysis revealed significantly higher cellular expression of GAG in the ACT-treated knees (1.12 vs 0.81 μg GAGs/mg protein, p=0.008). The mean Brittberg/ICRS score was significantly higher in the treated knees (6.00 vs 1.89, p=0.007).
Conclusion. ACT is superior to non-operative measures for repairing focal cartilage defects, as determined by favourable histological and immunohistological outcomes at the cellular level.

Key words: *cartilage; chondrocytes; tissue engineering; transplantation, autologous*

INTRODUCTION

Regeneration of cartilage is poor because of the absence of neurovascular supply,[1–8] and hence surgical repair following injury is difficult, and outcomes vary.[2–5,9] Tissue-engineering modalities offer biological therapies for cartilage regeneration.[6–10] Autologous chondrocyte transplantation (ACT) is a 3-stage procedure with good long-term durability and outcomes.[7] Only a few hospitals offer this modality as a standard practice,[11–13] because it is expensive (in terms of cell preparation), technically demanding, and yields similar results to other techniques requiring less expertise.[7–14]

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The efficacy of ACT is controversial because most assessments are based on patient outcome scores and investigators' own visual assessment and hence are prone to bias.[12–20] Our study was more objective because regeneration of cartilage tissues was analysed both quantitatively and qualitatively.

MATERIALS AND METHODS

Nine male New Zealand white rabbits aged 6 to 7 months weighing 2.5 kg were used. All were reared at the animal research facility of our university. The protocol was approved by the animal ethics committee. All surgical procedures were performed under general and local anaesthesia using aseptic techniques. No preoperative antibiotics were administered.

Both knee joints were opened via a medial para-patellar approach along the patellar tendon (Fig. 1a). A piece of cartilage (5 mm in diameter and 2 mm in depth) was biopsied from the medial femoral condyle using a custom-made cylindrical chondrotome (Fig. 1b). A defect was created using a scalpel until subchondral bone was reached (Fig. 1c), mimicking a clinical situation of delayed presentation and a full-thickness tear. The wound was closed and postoperative analgesia was administered for 2 days.

The harvested cartilages were sent for cell culture. They were finely chopped and digested in an incubator for 24 hours. The chondrocytes attained were passaged and propagated using Ham's F-12 and DMEM media. No scaffolding or enhancers were used.

One month later, the right knee was re-opened via the previous incision scar, and cultured chondrocytes were implanted. Tight opposition at the edges of the periosteal flap was achieved using microsutures and fibrin glue. Three dosages of antibiotics were administered following surgery.

There was no wound infection or contamination of cell cultures. The rabbits were allowed to move freely within their cages and were killed 3 months later. The knees were evaluated macroscopically based on the Brittberg/International Cartilage Research Society (ICRS) score[1,2,9] for cartilage repair by an independent observer. The distal femurs were harvested, weighed, and measured.

Half of the specimens were used for histological examination and immunohistochemical staining. They were fixed in 10% phosphate-buffered formalin (4% formaldehyde) for at least 24 hours, then decalcified and embedded in paraffin, and eventually sectioned into 5 μm thick slices on slides, deparafinised in xylene, and transferred in aqua dest with decreasing concentrations of ethanol. Slides were stained with haematoxylin and eosin (H&E) and Safranin-O.

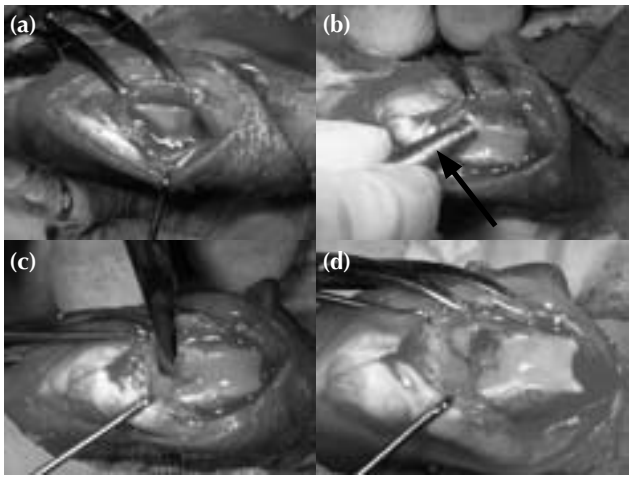

Figure 1 Creation of a defect in both knees using a customised chondrotome (arrow).

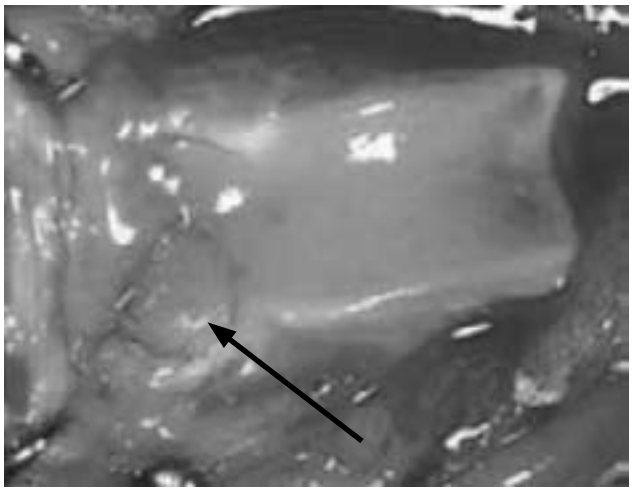

Figure 2 One month later, the defect in the right knee was covered with a periosteal flap (arrow).

Synthesis of type-II collagen was verified using immunohistochemical staining. Sections were treated with protease enzyme for about 10 minutes and washed with Tris-buffered saline prior to incubation with type-II primary antibody or phosphate-buffered saline alone (as the negative control) for 1 hour. Specimens were then incubated with horseradish peroxidase enzyme conjugated

goat-anti-mouse secondary antibody for 30 minutes prior to visualisation using the chromogen substrate diaminobenzidine. Stained slides were then mounted in dibutyl polystyrene xylene.

The other half of the specimens were used for biochemical assay of glycosaminoglycan (GAG) using the Blyscan Glycosaminoglycan Assay Kit (Biocolor, UK). GAG is a major component of the articular cartilage. Specimens were finely dissected and then digested using radioimmunoprecipitation buffer supplemented with protease inhibitor for 1 hour. Aliquots of each sample were mixed with dimethylmethylene blue dye and reagents from the GAG assay kit. The absorbance at 656 nm was measured on the spectrophotometer and compared to a plot of standards made from shark chondroitin sulphate to determine GAG content. GAG quantification indicates cellular expression of the chondrocytes within the matrix and is an indicator of tissue repair.

The GAG values complement the Brittberg/ICRS scores. The Brittberg/ICRS scores and GAG contents between the treated and control groups were compared using the Wilcoxon signed-rank test. A p value of <0.01 was considered statistically significant.

RESULTS

No obvious differences in the limp between the 2 sides was noted after the first procedure. The limp was more notable in the left knee after the second procedure, but the gait became normal at the end of the second week. On gross examination, the defect in the treated knees showed good filling with a smooth surface (Fig. 3a), with no obvious periosteal thickening. Repairs were mainly of hyaline cartilage with good ingrowth. Only one specimen showed scraping on the medial tibial articular surface. In the control knees, partial filling with an irregular surface was seen (Fig. 3b), with scraping on the articular surface of all tibial plateaus. Repairs were mainly of fibrous or fibrocartilaginous tissue.

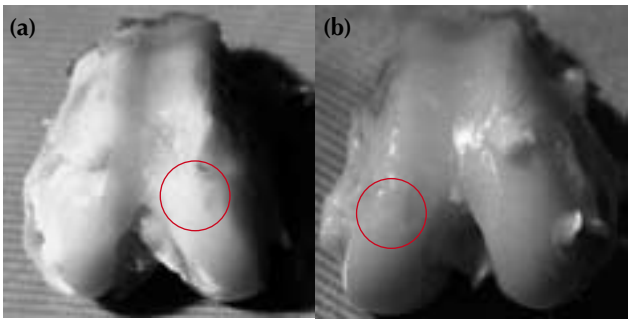

Figure 3 Gross appearance of the **(a)** right knee with a completely filled defect and **(b)** left knee with an irregular surface.

Using Safranin-O staining, tissues in the treated knees were heavily stained indicating good proteoglycan expressions within the matrix, but the stain was poor and uneven in the control knees (Fig. 4). Using H&E staining, chondrocytes in the treated knees were abundant with good-quality surrounding tissue similar to normal cartilage. In the control knees, no obvious repairs or cells were noticeable (Fig. 5).

Using immunohistochemical staining, the treated knees showed homogeneous distribution of type-II collagen similar to the surrounding normal cartilage. In the control knees, no cartilage filling was noted; only some type-II collagen was seen at the base and border of the defects (Fig. 6).

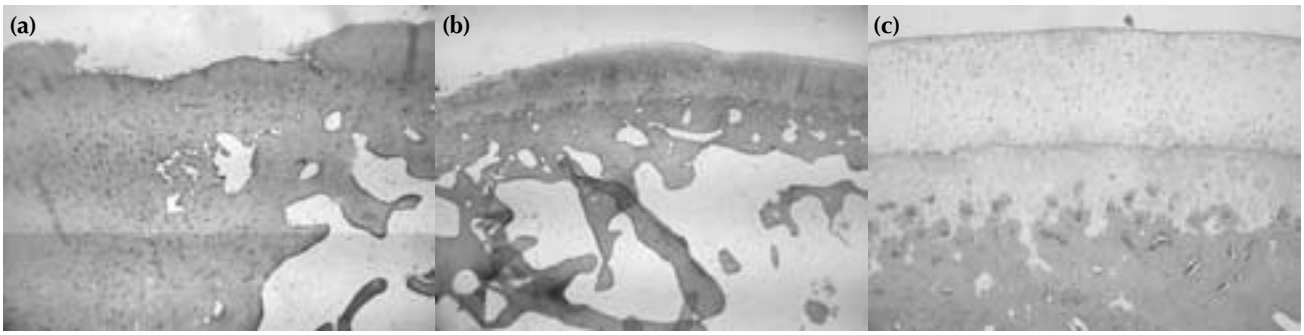

Figure 4 **(a)** Moderate-to-good cartilage growth with adequate smooth surface is seen in the right knees after 2 months; **(b)** no repair and only fibrous and fibrocartilaginous tissues are seen in the left knees. **(c)** A healthy cartilage is thick and even, with a smooth surface and well-distributed chondrocytes (Safranin-O, x40).

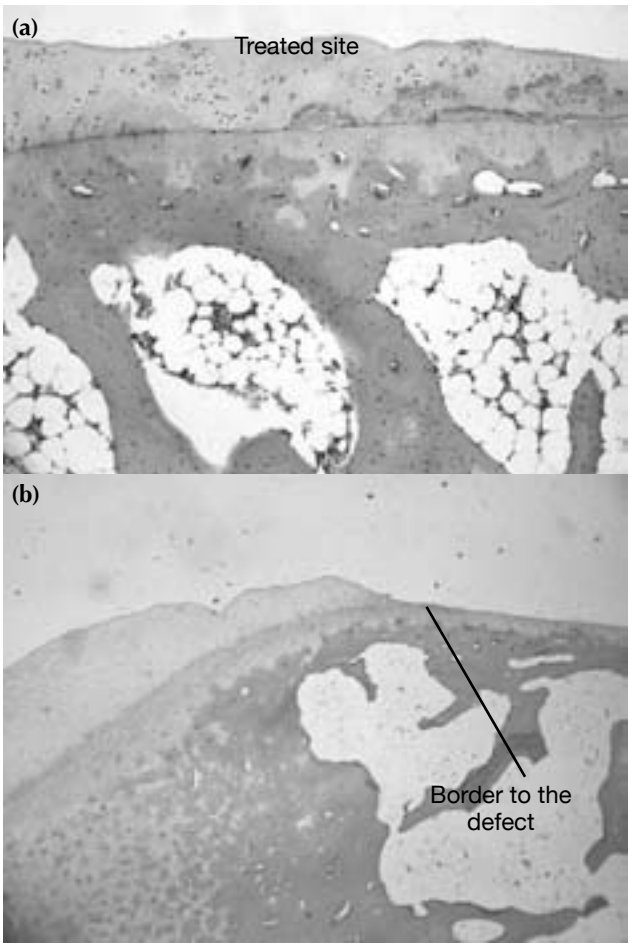

Figure 5 **(a)** Good filling with viable cells in the matrix of the repair site (H&E, x40). **(b)** The defect site remains unfilled. The subchondral bone is exposed (H&E, x40).

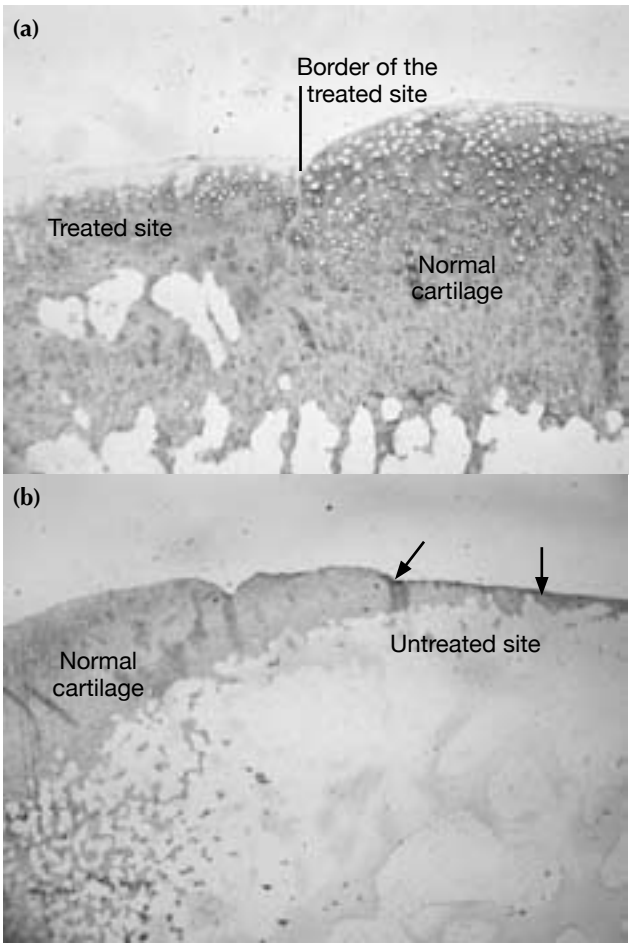

Figure 6 **(a)** Abundant type-II collagen is seen in the treated knees (immunohistochemical staining, x100). **(b)** Type-II collagen is only seen at the bottom and border of the defect (arrows) [immunohistochemical staining, x40].

In the right and left knees respectively, the mean GAGs were 1.12 and 0.81 μg GAGs/mg protein (p=0.008), whereas the mean Brittberg/ICRS scores were 6.00 and 1.89 (p=0.007) [Table]. ACT is superior to non-operative measures in repairing focal cartilage defects, with favourable histological and immunohistological outcomes at the cellular levels.

DISCUSSION

Articular cartilage consists of an extracellular matrix (mainly type-II collagen) and a cellular component (chondrocytes). The collagen is embedded in a firm, hydrated gel of proteoglycans. GAG is a component of proteoglycans and hyaluronic acid and helps

Table
Mean glycosaminoglycan (GAG) and Brittberg/International Cartilage Research Society (ICRS) scores for the right and left knees

Parameter	Knee		p Value (Wilcoxon signed-rank test)
	Left	Right	
Mean (SD) GAG (μg GAG/g protein)	0.81 (0.17)	1.12 (0.48)	0.008
Mean (SD) Brittberg/ICRS score	1.89 (1.54)	6.00 (1.23)	0.007

maintain tissue homoeostasis.[12,13] It repels water and prevents disintegration of matrix materials during injury,[21,22] absorbs shock, and lubricates the synovial joint.

GAG and type-II collagen were heavily expressed in the treated but not the control knees (except at the base and border of the defects).[7,16,21,22] A 'bioactive chamber theory'[2,17–19] postulates that new cartilage may originate from normal cartilage surrounding the defect, calcified zones, chondrocytes in the cryptae of the irregular subchondral bone, bone marrow of the osteochondral defect, or synovial fluid. Cells in the adjacent cartilage show mitotic activity after injury, but not sufficient for any noticeable repair. Therefore, patients may attain partial symptomatic relief despite no treatment. This may lead to delay in treatment and further cartilage damage (even in the opposite articular surface).

The transplanted chondrocytes can remain viable for a long time.[23–26] They can be traced using radio-isotope cell labelling or fluorescence cell labelling and visualised using fluorescent or confocal microscopy or polymerase chain reaction. A mean of 87% of cell viability is maintained for up to 4 weeks; only 14% of the one million chondrocytes transplanted remained, but the total numbers of cells increase with time. This suggests that ACT keeps the transplanted chondrocytes viable and promotes cell migration from the surrounding tissues.[23–25] Nonetheless, the source and fate of these cells were not determined. They may migrate into the surrounding tissues, participate in cell-mediated immune death or even in apoptosis. Long-term survival of transplanted chondrocytes is difficult to follow because of (1) degradation of fluorescent dyes and (2) chondrocytes losing tagged dyes in their progeny when undergoing *in vivo* proliferation/differentiation.[27]

ACT is preferred over subchondral drilling and mosaicplasty as a treatment of choice,[13] but the high cost incurred does not justify its use in standard practice. Subchondral drilling creates channels connecting marrow into the defects and enables mesenchymal-cell migration and differentiation into mature chondrocytes to promote cartilage repair. However, it has not been useful or beneficial,[15,22,28] because (1) the defects are repaired by callus formation resulting in stiffer cartilage and trauma to the opposite articular surface[2]; (2) breaching the subchondral bone may disturb the elastic resilience equilibrium of cartilage and subchondral bone functioning as a shock absorber; when under high loads, both the cartilage and subchondral bone may deform resulting in a global deformity of the articular architecture[29,30]; (3) type-I collagen and fibrous (and fibrocartilage) materials filling in the defect may damage the surrounding cartilage structure.

Mosaicplasty uses osteochondral plugs obtained from the non–weight bearing portion of the articular surface to replace the lost cartilage at the defective sites.[15,22,28,31] Its rationale is based on the notion that autologous tissues rather than cells promote faster healing. However, multiple cylindrical grafts are required to fill the defect[13]; spaces between these grafts are filled with fibrocartilage and even new bone, possibly damaging both the opposite articular and treated surfaces. Large defects cannot be repaired because of limited donor osteochondral grafts. Donor-site morbidity may be an issue because of the large amount of autologous tissue required.[32]

Randomised controlled studies comparing ACT with other treatment modalities have shown mixed outcomes. ACT appears to produce better mid-term results.[13,22,28,31–36] Despite its success in treating cartilage defects, its use in developing countries is limited because tissue culture/transplant facilities are very costly.[13,37–39] Cell-culture techniques with appropriate standards are difficult to master, and the transport and processing costs are unaffordable for the general public.[11–13,32–6]

In our study, the biomechanical properties pertaining to the Brittberg/ICRS score and GAG quantification were not assessed. The biomechanical property (tensile strength and deformity resistance) of the repaired cartilage is best assessed using indentometry analysis. It must be comparable to normal cartilage so as not to disintegrate upon normal use or damage the opposite and adjacent articular surfaces. The ICRS histological scoring system[10] was not used, as it is qualitative in nature and not subject to statistical analysis. The amount of type-II collagen was not quantified because specimens available for testing were limited. Larger animals should be used in future experiments to enable more diverse tests on repair outcomes and compare ACT to mosaicplasty, because only larger animals (e.g. goats or dogs) have sufficient amount of cartilage for mosaicplasty.[34]

ACKNOWLEDGEMENTS

We thank Nor Azeera Bakar, Sukanya Subramaniam, Rasanayagam, and Lim Saw Kim for the animal handling and experiments and tissue processing. We also thank the National Bio Directorate, Ministry of Science and Technology of Malaysia for funding this research (grant No.: PR-06-03-01).

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