Quantitative Real-Time PCR Analysis for Chondrogenic Differentiation of Human Mesenchymal Stem Cell in Alginate Scaffolds

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INTRODUCTION: Despite the many advances seen in cartilage repair techniques, cartilage damage remains a difficult condition to treat [1]. Mesenchymal stem cells (MSCs) being capable of self-renewal and undergo multilineage differentiation was thought to be the answer to this problem [2]. It has been proposed that in order to achieve this, MSC extracted from tissues undergo cell proliferation *in vitro* prior to reimplantation to the defect site. However, the question remains as to whether MSCs are able retain their multilineage and phenotypic potential in prolonged *in vitro* culture environment. A study was therefore conducted to assess the ability of MSCs to maintain its differentiated phenotype by chemically inducing chondrogenic differentiation and using quantitative real-time polymerase chain reaction (qRT-PCR) to determine their gene expression over a period of time.

METHODS: Human bone marrow samples (2 ml) were collected from patients undergoing arthroplasty surgery whom were otherwise healthy. The mononuclear cells extracted were separated using Ficoll–Paque PLUS via centrifugation. Subsequently, suspended cells were removed after 5 days of culture, and adherent cells left to grow. Cells were detached upon reaching 80-90% confluence and sub-cultured up to 3 passages prior to further experiments. MSC antigens were recognized using monoclonal antibodies CD29, CD105 and CD166. To distinguish MSCs from resident hematopoietic stem cells, CD34 surface markers were used as negative controls. The characterised MSCs were then cultured in alginate scaffolds using chondrogenic medium. To assess chondrogenesis, gene expression analyses using RT-PCR were conducted on chondrogenic-MSCs using cartilage-specific phenotypic markers at pre-determined time points.

RESULTS: Flow cytometry analyses confirm the presence of MSCs and absence of haematopetic stem cells. The gene expression level of COMP for chondrogenic-MSCs was significantly higher than that of control (MSC cultured in basal growth medium) and up-regulated in long-term alginate 3-dimensional scaffolds culture environment. However, the gene expression levels of aggrecan appear to decrease in long-term culture conditions.

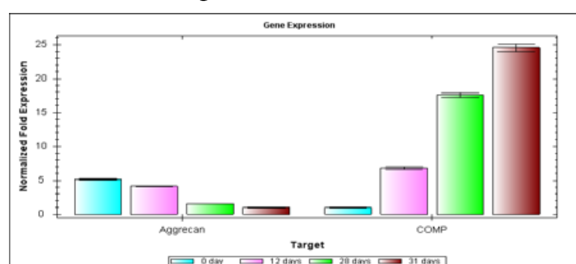

Fig. 1:The relative gene expression of aggrecan and COMP by chondrogenic-MSCs seeded in alginate scaffolds at different time point

DISCUSSION & CONCLUSIONS: Flow cytometry and RT-PCR analysis revealed that MSCs and chondrogenic-MSCs in culture retain the appropriate phenotypes comparable to that of *in vivo* MSCs. However, certain genes appear to be down regulated when maintained in prolonged cell culture conditions and may affect therapeutic outcomes when introduced in *in vivo* conditions.

REFERENCES: [1] Hirsch, MS, et al., 1997, Dev. Dyn. 210, 249–63. [2] Heng, BC, et al., 2004, Stem cells 22, 1152–1167.

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