Differentiated MSCs Seeded in a Highly Dense Collagenous Matrix Produce Novel Biphasic Osteochondral Constructs

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INTRODUCTION: Structural damage to both the articular cartilage and subchondral bone results in pain and disability for millions of people worldwide, representing a major clinical challenge. Being a hybrid of both bone and cartilage, requirements for osteochondral tissue engineering are more complex than previously investigated single tissue types. Novel methods of integrating bone and cartilage tissue constructs need to be explored. In this study, two hyper-hydrated collagen gels, containing osteogenic and chondrogenic cells, were integrated to create a biphasic osteochondral construct.

METHODS: Primary human MSCs, harvested from bone marrow of patients undergoing reconstructive surgery, were preconditioned in osteogenic (Ascorbic Acid, β-glycerophosphate, dexamethazone) and chondrogenic (TGF- β, ITS) media. These cells were then suspended in two separate collagen gels and integrated by partial setting. Upon complete setting, the resulting single gel was plastic compressed to produce a highly dense collagen sheet and spiralled (Brown *et al*., 2005). The biphasic construct was then cultured for 7 days under static conditions. Cell labelling was used to track the osteogenic and chondrogenic cells. Von Kossa and Alcian Blue stains were performed to assess bone and cartilage matrix deposition. RT-PCR was used to assess gene expression of specific markers of bone and cartilage.

RESULTS: After 7 days in culture, cell tracking (osteogenic cells: green; chondrogenic cells: red) revealed no cell migration across the osteochondral boundary. Von kossa Staining revealed matrix mineralization by the osteogenic cells. Furthermore, GAG deposition by the chondrogenic cells was indicated by alcian blue staining. RT-PCR results showed that markers of markers of bone (ALP, BSP, RUNX2) and cartilage (Aggrecan and SOX9) were expressed within the relative sections of the biphasic constructs.

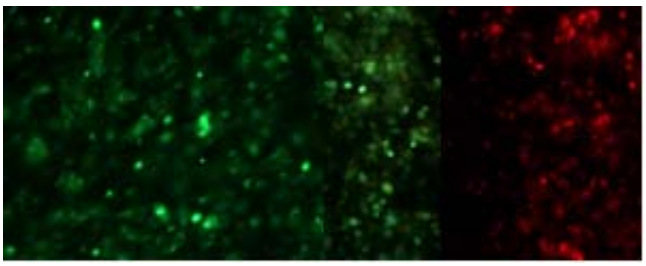

Fig. 1: Osteogenic cells (green) and chondrogenic cells (red) were tracked over 7 days and no migration over the osteochondral boundary was evident. Verifying two distinct 'bone' and 'cartilage' zones within the single biphasic construct.

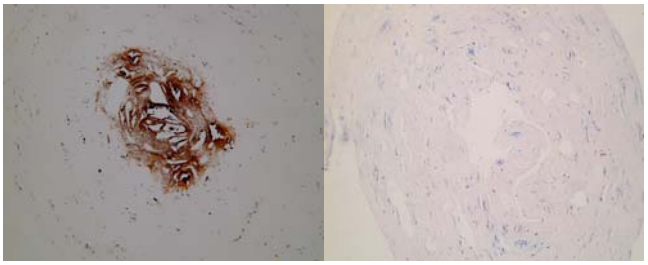

Fig.2: Von Kossa stain (left) showed evidence of matrix mineralization by the osteogenic cells and Alcian Blue stain indicated GAG deposition by the differentiated chondrocytes (right).

DISCUSSION & CONCLUSIONS: The aim of this study was to tissue engineer a biphasic osteochondral plug by integrating two cell phenotypes within a highly dense collagen matrix. Using bone (Von Kossa, ALP, BSP, RUNX2) and cartilage (Alcian blue, aggrecan and SOX9) markers, we have successfully demonstrated the formation of two distinct 'bone-like' and 'cartilage-like' zones to create a novel integrated osteochondral plug for clinical application.

REFERENCES: [1] Brown RA, Wiseman M, Chuo CB, Cheema U and Nazhat SN. Ultrarapid Engineering of Biomimetic Materials and Tissues. Adv. Funct. Mater., 2005, 15, 1762.

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