Engineering Osteochondral Constructs through Spatial Regulation of Endochondral Ossification

Sheehy, E J; Vinardell, T; Buckley, C T; Kelly, D J
Trinity Centre for Bioengineering, School of Engineering, Trinity College Dublin, Ireland
kellyd9@tcd.ie

INTRODUCTION
Articular cartilage has a limited capacity for self-renewal and repair. Cartilage tissue engineering applications involve combining cells, 3D scaffolds and signalling molecules for the regeneration of chondral or osteochondral defects. Osteochondral defects are often associated with mechanical instability, which can induce osteoarthritic degenerative changes. Even in the case of chondral defects implanting an osteochondral construct may facilitate integration of the graft. Auto-graft procedures such as mosaicplasty are not ideal for the repair of such defects due to issues associated with donor-site morbidity and limited quantity of harvestable tissue.

Chondrogenically primed bone marrow derived mesenchymal stem cells have been shown to produce bone via endochondral ossification when implanted subcutaneously in nude mice. This raises the possibility of engineering osteochondral constructs by promoting endochondral ossification in one region of a bi-phase construct, and stable cartilage in another. To test the feasibility of this approach the top-half of bi-phasic agarose hydrogels were seeded with chondrocytes (chondral-phase), and the bottom half with bone marrow derived mesenchymal stem cells (osteophase). Constructs were maintained in a chemically defined chondrogenic medium and then implanted subcutaneously in nude mice in an attempt to spatially induce endochondral ossification in only the osteo-phase of the engineered tissue.

METHODS
Porcine chondrocytes (CC) and bone marrow (BM) derived mesenchymal stem cells were separately suspended in 2% agarose (type VII, Sigma) hydrogels at a density of 20 million cells/ml to produce single phase cylindrical constructs (Ø5 x 3mm). A bi-phasic cylindrical construct was created by seeding the top half of an agarose hydrogel with chondrocytes and the bottom half with BM-MSCs. Constructs were maintained in a chemically defined chondrogenic medium (CM) supplemented with TGF-β3 (10ng/ml) at 37°C and 5%CO2 for 3 weeks. Thereafter constructs were either maintained in CM, or implanted subcutaneously into the back of nude mice for an additional 4 weeks. Constructs were assessed in terms of functional properties and biochemical (DNA, sulphated glycosaminoglycan (sGAG), collagen) content. Micro computed tomography (μCT) was used to quantify mineral content. Histological stains for Alcian Blue and Alizarin Red were used to assess the spatial distributions of sGAG and calcium respectively. Statistical analyses were carried out using a general linear model for analysis of variance with a p value < 0.05 considered significant. Numerical and graphical results are reported in the form of mean ± standard deviation.

RESULTS
Culturing chondrocytes with BM-MSCs in separate layers of a bi-phasic construct significantly increased the DNA content (111.53 ± 21.17 vs. 65.08 ± 15.67 w/w ng/mg), sGAG accumulation (1.62 ± 0.27 vs. 0.9 ± 0.02 %ww), collagen accumulation (1.46 ± 0.18 vs. 0.45 ± 0.02 %ww) and equilibrium modulus (34.93 ± 7.61 vs. 22.11 ± 3.46 kPa) in the chondral-phase compared to single phase chondrocyte-seeded constructs, Fig. 1 (A). Culturing BM-MSCs with chondrocytes in a chondrogenic medium suppressed mineralisation compared to single phase BM-MSC-seeded constructs as evidenced by histology, Fig 1 (B).

Prior to subcutaneous implantation in nude mice no mineralisation of bi-phasic constructs was observed. After 4 weeks in vivo endochondral ossification of the chondrogenically primed BM-MSCs had begun to occur with hypertrophy seemingly suppressed at the osteochondral interface. The chondral-phase of bi-phasic constructs showed a native cartilage-like gradient in sGAG distribution, increasing with depth, with more intense staining at the interface of the mineralizing region, Fig. 2.

Single phase BM-MSC seeded constructs continued to mineralise in vivo with significantly increased mineral volume (0.09 ± 0.59 vs. 1.36 ± 0.42 mm³) compared to bi-phasic constructs, Fig. 3.

DISCUSSION
Bone marrow derived mesenchymal stem cells show great promise for use in tissue engineering applications. However their tendency to undergo endochondral ossification makes them a less attractive cell source for cartilage tissue engineering. This study utilises the hypertrophic phenotype of BM-MSCs along with the stable chondrogenic phenotype of chondrocytes to engineer an osteochondral construct in vivo through spatial regulation of endochondral ossification. Furthermore this study found that the in vitro culture of chondrocytes and BM-MSCs in a bi-phasic construct increased the biochemical content and functional properties of chondrocytes, and suppressed the mineralisation of BM-MSCs.

SIGNIFICANCE
Novel approaches are required for the treatment of osteochondral defects. Here we demonstrate that it is possible to engineer osteochondral tissues by implanting chondrogenically primed bi-phasic constructs containing both chondrocytes and BM-MSCs and spatially regulating endochondral ossification.

ACKNOWLEDGEMENTS
Supported by Science Foundation Ireland (08/Y15/B1336)