Can the application of hydrostatic pressure to 3D ESC-models enhance the maturation of engineered cartilage tissue?

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INTRODUCTION: Degeneration of cartilage resulting from injury or disease, combined with its notoriously poor capacity for repair, has created a high clinical demand for regenerative medicine constructs. Human embryonic stem cells (hESC) are pluripotent, thus offering an allogeneic cell source for this application. Recent advances in culture techniques have allowed for the production of xeno-free chondroprogenitors from hESC [1]. Previous work demonstrated that the application of these hESC-derived chondroprogenitors, when encapsulated in fibrin gels, enhanced cartilage repair in osteochondral defects of rat models [2]. Mechanical cues contribute to early cartilage development *in vivo*. The application of hydrostatic pressure an important physical stimulus has a positive effect *in vitro* [3]. This study aims to investigate the effects of hydrostatic pressure on *in vitro* 3D models using hESC-derived chondroprogenitors and human bone marrow derived mesenchymal stem cells (hMSC).

METHODS: hESC were differentiated into chondroprogenitors following a chemically defined protocol [1-2]. Chondroprogenitors and human bone marrow derived MSC were then either embedded into hydrogels or allowed to form cell spheroids. Hydrogels and cell spheroids underwent mechanical stimulation with intermittent hydrostatic pressure for 1 hour daily at 270 kPa and 1 Hz over 8 days. To assess the effect of hydrostatic pressure samples were compared with statically cultured controls. Biochemical assays, qPCR, histology and immunocytochemistry were utilised to investigate matrix production, expression levels of chondrogenic markers, cell viability and proliferation. Furthermore, chondroprogenitor-seeded fibrin gels were implanted into a cartilage defect at the epiphyses of *ex vivo* cultured chick femurs and stimulated for 5 days at 1Hz and 270 kPa for 1hour per day and then cultured statically for another 5 days.

RESULTS: Results suggest that mechanical stimulation of hMSC and chondroprogenitor hydrogels and spheroids using intermittent hydrostatic pressure resulted in increased production total protein and matrix proteins such as collagen and GAG. In addition, upregulation of chondrogenic markers was observed.

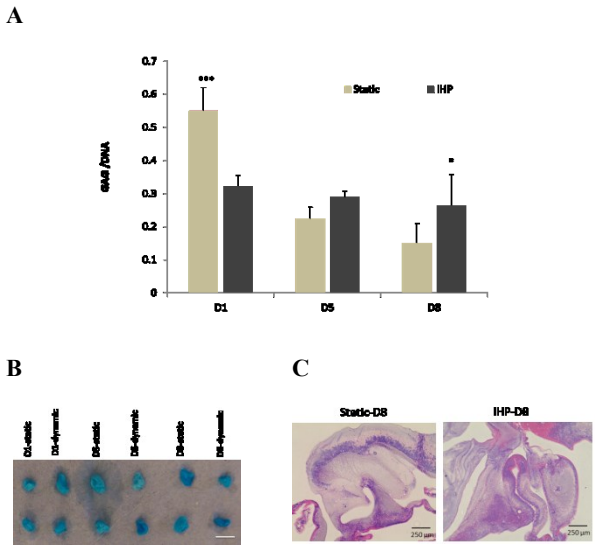

Fig. 1: Chondroprogenitor cells embedded in hydrogels and cultured for 8 days statically and dynamically. DMMB assay (A), Alcian Blue stain (B) and H&E stain (C) indicate increased matrix production. Scale bar: B= 2mm, C=250µm. Colour intensity in B represents amount of GAG produced.

DISCUSSION & CONCLUSIONS: hMSC and chondroprogenitor cells remain viable in static and dynamic culture. Upon mechanical stimulation in the hydrostatic force bioreactor, increased protein content and protein release was observed for cell spheroids and hydrogels. Future work will include the optimisation of stimulation regime for the ex vivo culture chick femurs and the investigation of alternative hydrogel materials.

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