The Effect of Hypoxic Pre-Culture on Chondrogenic Differentiation of Human Amniotic Fluid-Derived Stem Cells in a Collagen-Hyaluronic Acid Scaffold

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Introduction: Articular cartilage is a resilient tissue with a poor regenerative capacity due to its avascular nature and the poor migratory capacity of chondrocytes. As a result, articular cartilage is unable to heal even the most minor injuries [1]. The poor long-term outcome of conventional treatment methods used clinically shows that there is an inherent need for alternative approaches in cartilage defect repair. Tissue engineering strategies have shown potential as one of these alternative approaches. Human amniotic fluid-derived stem cells (AFSCs) have recently been explored as a unique source of cells for tissue engineering due to their pluripotency and present a potentially novel stem cell for chondrogenic differentiation. Also, taking into account that articular cartilage resides in a hypoxic environment, the use of such a stimulus to enhance in vitro chondrogenesis bears significant implications in advanced cartilage tissue engineering strategies. Within our laboratory, collagen-hyaluronic acid (HyA) scaffolds were recently developed and optimised to support mesenchymal stem cell chondrogenesis [2]. In this context, the aims of this study were two-fold: (i) to investigate the ability of ASFCs to differentiate down a characteristic chondrogenic lineage and (ii) investigate the effect of hypoxic (2% O2) pre-culture period on the enhancement of the chondrogenic differentiation of AFSCs within these highly porous collagen-HyA scaffolds.

Methods: Collagen-HyA scaffolds composed of micro-fibrillar type I bovine tendon collagen and HyA sodium salt were fabricated using a previously described freeze-drying process [3]. AFSCs were seeded onto these scaffolds and cultured in chondrogenic media containing TGF-B3 and pre-cultured under either normoxic (21% O2) or hypoxic conditions (2% O2) for 7 days subsequently followed by a culture period of up to 35 days in normoxia. The chondrogenic differentiation of AFSCs in each pre-culture group was analysed at day 7, 14, 21, 28 and 35 of culture. Following culture, the scaffolds were assessed for gene expression using qRT-PCR, sulphated GAG (sGAG) matrix production using a di-methyl-methylene blue assay and safranin-O histological staining, as well as Collagen type II and Aggrecan deposition using immunohistochemistry. Statistical analysis was carried out using two-way ANOVA.

Results: The results of this study demonstrated that AFSCs were capable of chondrogenic differentiation while seeded on collagen-HyA scaffolds as evidenced by robust deposition of both Collagen II and Aggrecan at all time points (Figure 1). Deposition of sGAG was also observed around the periphery of the scaffold, becoming more prominent throughout the construct at later time points. qRT-PCR also revealed that chondrogenic gene markers were up-regulated in both hypoxic and normoxic pre-culture groups in comparison to an undifferentiated AFSC control, indicating differentiation was taking place (Figure 2). Furthermore, it was found that hypoxic pre-culture may have a beneficial role in the enhancement of the chondrogenic differentiation of AFSCs as evidenced by increases seen in cell density and the expression of the early stage chondrogenic gene SOX9 in comparison to scaffolds pre-cultured in normoxia at early time points.

Discussion: The results of this study indicate that AFSCs can be successfully differentiated down a chondrogenic lineage while seeded on collagen-HyA scaffolds whether pre-cultured in either normoxia or hypoxia. This novel combination of cells and biomaterial potentially offers a new type of construct for cartilage repair, as evidenced by the robust cartilaginous matrix deposition and the synthesis of sGAG seen throughout the scaffold. This study also demonstrates the potential of utilising low-oxygen environments for the enhancement of therapeutic constructs for use in cartilage repair.

Significance: AFSCs represent an important new stem cell source in tissue engineering. This study is the first to demonstrate the potential to of these cells to differentiate down a chondrogenic lineage on a porous biomaterial in vitro and thus may have significant implications in development of advanced tissue engineering strategies for articular cartilage defect repair.

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References:
Figure 1: Immunohistochemical staining of collagen-HyA scaffolds for Collagen II and Aggrecan at day 21 of culture. Scale bar represents 100μm.
Figure 2: qRT-PCR analysis showing the up-regulated expression of early stage chondrogenic marker Sox9 at day 21 by differentiating AFSCs pre-cultured in normoxia and hypoxia. *** denotes p<0.0001 statistical significance.