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ATDC5: an Ideal Cell Line for Development of Tissue Engineering Strategies Aimed at Cartilage Generation

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INTRODUCTION: Pivotal in the advancement of clinical approaches for cartilage regeneration will be the development of robust models of cartilage formation, which can also aid in delineating the steps of chondrogenesis. This study was therefore aimed at engineering facile, reproducible three-dimensional (3D) models of cartilage generation utilising ATDC5 cells, a murine chondrocytic cell line widely used as a monolayer culture system to study chondrogenic differentiation, in comparison to the clinically suitable options of human articular chondrocytes and adult mesenchymal stem cells from human bone marrow.

METHODS: Femoral heads and bone marrow samples from patients undergoing routine hip replacement surgeries were used to isolate articular chondrocytes and Stro-1+ mesenchymal stem cells respectively. Proliferating ATDC5 cells, confluent human articular chondrocytes and Stro-1+ cells, grown as monolayer cultures, were harvested for micromass pellet cultures under chondrogenic conditions (10 ng/ml TGF-β3, 1X ITS, 10^{-8}M dexamethasone, 100 μM ascorbate-2-phosphate) and dynamic seeding onto polyglycolic acid (PGA) fleece within a 'high aspect ratio vessel' rotating bioreactor. In addition, micromass pellet cultures of ATDC5 cells were harvested at 7, 14, 21 and 28 days to study the process of chondrogenic differentiation in response to insulin and TGF-β3. After a culture period ranging between 21 and 28 days, explants were analysed for chondrogenic differentiation by histology (Alcian blue/ Sirius red {A/S}, Safranin O and Alkaline phosphatase {ALP} staining), immunohistochemistry and RT-PCR, for protein and gene expression of typical chondrogenic markers – sox-9, aggrecan and type II collagen.

RESULTS: Chondrogenic differentiation, evident by chondrocytes expressing typical chondrogenic genes and proteins, and lodged in distinct lacunae embedded in a cartilaginous matrix of proteoglycans and type II collagen, was observed in ATDC5 and articular chondrocyte pellets at 21 days, and at 28 days in pellets of Stro-1+ cells. The ATDC5 pellet culture time-course illustrated a gradual progression from an aggregation of cells at day 7, to the initiation of matrix synthesis and development of chondrocytic phenotype at day 14, followed by differentiation as pre-hypertrophic chondrocytes synthesizing proteoglycan and type II collagen-rich matrix at day 21 and maintenance of the pre-hypertrophic phenotype in response to TGF-β3 at day 28 (Fig. 1). Explants of ATDC5 cells cultured on PGA fleece in the bioreactor for 21 days were reminiscent of cartilaginous structures composed of numerous pre-hypertrophic chondrocytes, staining for typical chondrocytic proteins, lodged in distinct lacunae embedded in proteoglycan and type II collagen matrix (Fig. 2). In comparison, 21-day articular chondrocyte and Stro-1+ explants exhibited a mesh-work of PGA fibres supporting chondrocytic cells, in combination with discrete islands of chondrocytic cells embedded in proteoglycan-rich matrix.

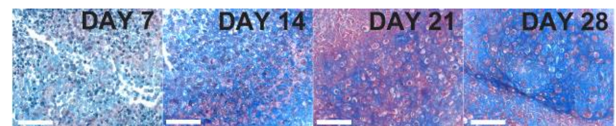

Fig. 1: Chondrogenic differentiation in A/S-stained ATDC5 pellets. Scale bar 100 μm

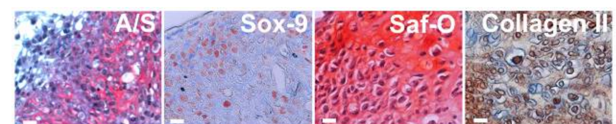

Fig. 2: Chondrogenic differentiation in tissue engineered ATDC5 constructs. Scale bar 20 μm

DISCUSSION & CONCLUSIONS: The study has highlighted suitability of ATDC5 cells in 3D environs, in comparison to human primary cells, to delineate the steps of chondrogenic differentiation and generate cartilaginous structures exhibiting morphology, gene and protein expression profiles reflective of the *in vivo* scenario. Although cell lines cannot replace human primary cell models, our study has shown that they can furnish important information concerning seeding densities, time frames, biocompatibility and judicious use of growth factors and differentiating agents, thereby significantly reducing time and cost associated with the tissue engineering of primary human cells.

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