Cartilage Repair: A Systems Approach To Tissue Engineering

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Introduction: Cartilage is a complex multi-layered, zonally organized, aneural, avascular and poroelastic composite structure comprised of chondrocytes embedded in a water-based glycosaminoglycan (GAG) matrix reinforced with collagen fibres. Once damaged by osteoarthritis or trauma, self-repair/regeneration is limited. Current medical treatments involve microfracture techniques, autologous chondrocyte implantation or matrix-assisted chondrocyte implantation, which stimulate fibrous cartilage formation. Although in the shorter term, these techniques improve mobility and alleviate pain, fibrous cartilage does not possess the optimal biological and mechanical properties to provide a long-term solution (1). The creation of a tissue engineering solution for such a multifactorial problem requires selecting a material and design that delicately balances cell response, mechanical stability, degradation inflammation, integration and neo-tissue formation. Therefore, our hypothesis was that employing a systems approach to a tissue-engineering problem could achieve enhanced mesenchymal stem cell (MSC) engraftment, implant integration and hyaline cartilage formation. Using native hyaline cartilage as a template, a composite construct was developed to test this hypothesis, comprising a 3D support structure with functionally graded mechanical properties governed by a hierarchical pore size gradient in combination with a chondromimetic MSC milieu. This construct was characterized in vitro for chondrogenicity and using histological and biochemical techniques post-implantation in a rabbit model.

Methods: Initially, a polyactic acid poly-ε-caprolactone (PLCL) template based on the cell orientation and distribution of cells in native hyaline cartilage was produced by laser ablation in combination with thermal crimping to create a biomimetic 3D microtunnel-based architecture with pore sizes increasing from 180μm in diameter on the top to 180 μm X 600μm on the bottom and a compressive modulus of 10MPa (Fig. 1a). Upon confirmation of the cytocompatibility of the PLCL support, MSC were encapsulated in a hyaluronic acid (HA) gel using a cell seeding density of 60 X 106 cells/ml2 as previously described3, loaded on the PLCL support structure and cultured in complete chondrogenic medium (+TGFβ3) (CCM) in hypoxia for 21 days. As a method of control, cell-seeded composite structures were cultured in incomplete chondrogenic medium (-TGFβ3) (ICM) (n=3 biological replicates). Thereafter, chondrogenicity was confirmed using a dimethyl methylene blue biochemical assay for GAG accumulation and statistically analysed using a student t-test with p<0.05 regarded as significant. In parallel, the biocompatibility and tissue repair was assessed in vivo in a subchondral defect (1mm deep and 3mm in diameter) in the femoral condyle in male White New Zealand rabbits in accordance with local ethical guidelines and approval, with 3 groups, empty defect (n=3), empty scaffold (n=6) and MSC-seeded scaffold (n=6). After 4 weeks, the tissue was harvested, fixed in formalin, decalcified in Surgipath II, embedded in paraffin and sectioned at 5μm intervals through the centre of the defect. Haematoxylin and eosin (H&E) staining was employed to evaluate inflammation and cellularity, toluidine blue (TB) to examine GAG formation, while collagen type II was examined to assess hyaline cartilage development.

Results: Chondrogenesis was assessed in vitro using a DMMB assay, where a statistically significant difference in the GAG:DNA ratio was measured for the cell-seeded constructs cultured in chondrogenic environment for 21 days in hypoxia compared to the cell-seeded constructs cultured in serum free medium without chondrogenic supplements (Fig. 1b). The in vivo evaluation revealed that the PLCL biomaterial was biocompatible as evidenced by the absence of any signs of inflammation or giant cells (Fig. 1c). After 4 weeks, there was evidence of integration and neo-tissue formation with chondrocytes evident in the toluidine blue staining (Fig. 1d) and collagen type II shown by the positive brown staining (Fig. 1e) visible on and through the PLCL struts for both cell-free and cell-seeded constructs.

Discussion: Over the past 20 years, many research efforts have focused on creating tissue-engineered scaffolds with mechanical properties in the MPa range, that address the functional mechanical property requirements of articular cartilage at a macroscale and hydrogels with mechanical properties in the kPa region, which address the cell property requirements at a micro-level. However, there are no optimal biomaterials or designs that adequately address both of these materials property requirements. The results of the present study demonstrate that by employing a systems approach to cartilage tissue engineering, repair was promoted most probably by providing a biomimetic pore architecture for cell homing and ingrowth, biomechanical properties that provide functional support and biological cues that promote chondrogenesis in contrast to other studies where cartilage property requirements were investigated in isolation (2-4).

Significance: In clinical practice, hyaline cartilage repair still remains a challenge. Albeit, that the research was a short-term study, the results show that this chondromimetic composite construct holds great promise and may be a catalyst to springboard stem cell-based therapies forward toward human clinical trials.
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