In Vitro Growth Trajectory and In Vivo Implantation of a Cell-Based Disc-like Angle Ply Structure for Total Disc Replacement

Introduction

Surgical strategies for treating intervertebral disc degeneration are designed primarily to alleviate pain but do not restore disc structure or function. To treat end stage disc disease, we have developed an engineered disc for total disc replacement that replicates the hierarchical structure of the native tissue. This engineered disc consists of an aligned electrospun nanofibrous scaffold annulus fibrosus (AF) and a hydrogel-based nucleus pulposus (NP); combined they form a disc-like angle ply structure (DAPS). When seeded with cells, these composites increase in compositional and functional properties with time in in vitro culture. Based on this progress, the objectives of this study were two-fold; first, to evaluate the in vitro maturation of DAPS seeded with either native AF and NP cells or with mesenchymal stem cells (MSCs) to establish a growth trajectory and, second, use our validated rat tail disc replacement model to determine if a cell-seeded DAPS can integrate into the rat caudal disc space.

Methods

AF Fabrication: Poly(e-caprolactone) (PCL) and poly(ethylene oxide) (PEO) nanofibers were electrospun onto a rotating mandrel as aligned fibrous sheets. Strips were cut 30° to the fiber direction and two strips with alternating ± 30° alignment were wrapped concentrically to form the AF region of the DAPS, sized to fit the rat caudal disc space. One layer (th = 125µm) of PEO was included for every two layers of PCL (th = 125 µm) to provide routes for cell infiltration. AF constructs were seeded with either bovine AF cells or bovine MSCs (2M cells/construct).

NP Fabrication: Methacrylated hyaluronic acid (MeHA) was produced by reacting HA with methacrylic anhydride. A 1% w/v solution was formed by dissolving MeHA and 0.05% Irgacure 2959 in phosphate-buffered saline. Bovine NP cells or MSCs were suspended in the MeHA solution (20M cells/mL), followed by photo-polymerization with UV light in a mold to form the NP region. Growth Trajectory: AF and NP regions were cultured separately in serum-free media containing TGF-β3 for 2 weeks, and then combined to form the DAPS construct.

At 2.5, 5, 7.5, and 10 weeks, mechanical properties in unconfined compression (20 cycles, 0N to −3N, 0.5 Hz, data analyzed at 20th cycle), glycosaminoglycan (GAG) content, and picrosirius red (collagen) and alcian blue (GAG) stained histological sections were evaluated.

In vivo DAPS Implantation: Athymic rats were prepared for DAPS implantation by installing an external fixator designed to stabilize two adjacent rat caudal vertebrae. AF/NP cell-seeded DAPS precultured for 10 weeks were implanted into the native disc space and rats were euthanized 5 weeks post-operatively. Vertebra-DAPS-vertebra segments were excised and scanned by micro-CT and then sectioned and stained with alcian blue/picrosirius red.

Results

The AF and NP regions of both MSC- and disc cell-seeded DAPS increased in biochemical content with time in culture (Figure 1). Collagen and GAG staining increased with time, starting at the periphery of the AF at 2.5 weeks and gradually reaching deeper portions of the AF by 10 weeks. Collagen and GAG staining in the NP increased over time, with collagen staining strongest at the center of the NP while GAG staining was evenly distributed throughout. Quantification of GAG content in each region showed a steady increase in deposition in both AF and NP regions for both cell types, with GAG production in MSC-seeded DAPS outpacing that of disc-cell seeded DAPS (Figure 2a). Mechanical properties of the DAPS decreased slightly over
of immunocompromised rats, constructs remained in the disc space, retained their morphological features, and showed signs of integration with surrounding native tissue structures. However, loss of proteoglycan in the NP region was evident, suggesting that it may be necessary to deliver factors in vivo to sustain the phenotypic production of ECM in this region.

In this study we implanted DAPS at the point of highest maturation (i.e., at 10 weeks). Implantation at earlier times, when DAPS constructs are at their maximum growth rate (as opposed to maximum growth state) may be necessary to improve integration. This strategy has proven successful for the integration of engineered cartilage into native cartilage, and a study by Bowles and co-workers reported strong vertebral integration of cell-seeded engineered discs after a shorter 2 week preculture period. Taken together, our data support the continued translation of a cell-based disc-like angle-ply structure for the replacement of severely degenerated intervertebral discs.

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References