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

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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.[1] 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[2] to determine if a cell-seeded DAPS can integrate into the rat caudal disc space.

Methods

AF Fabrication: Poly(ε-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.[2] 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.[3] 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.[2] 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

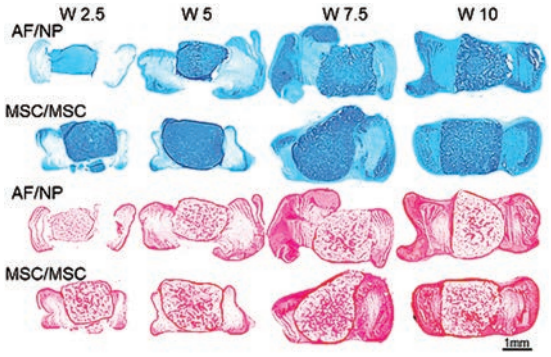

Figure 1. Alcian blue (top rows) and picrosirius red (bottom rows) of AF/NP and MSC/MSC DAPS with time in *in vitro* culture.

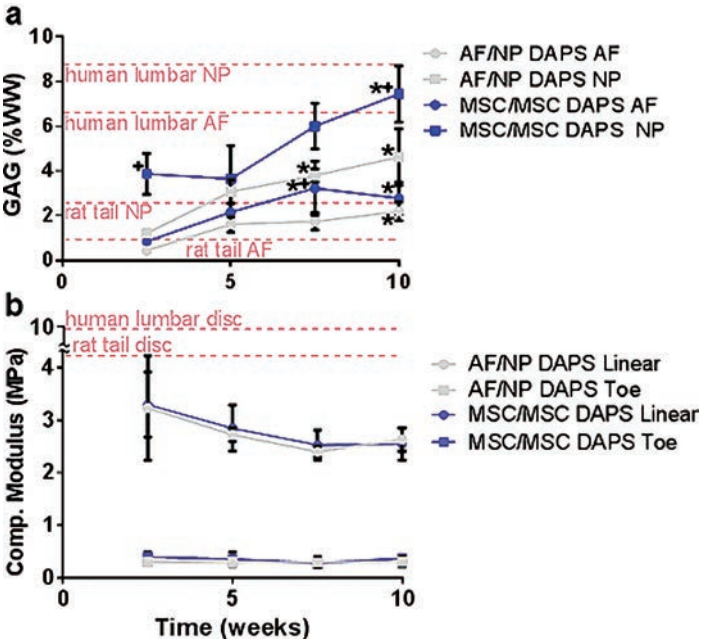

Figure 2. (A) GAG contents of the AF and NP regions of AF/NP and MSC/MSC DAPS with time in culture. (B) Linear and toe region compressive moduli of AF/NP and MSC/MSC DAPS. Native rat and human values are from[6]. *, $p < 0.05$ vs. 2.5 weeks; +, $p < 0.05$ vs. AF/NP at specific timepoint.

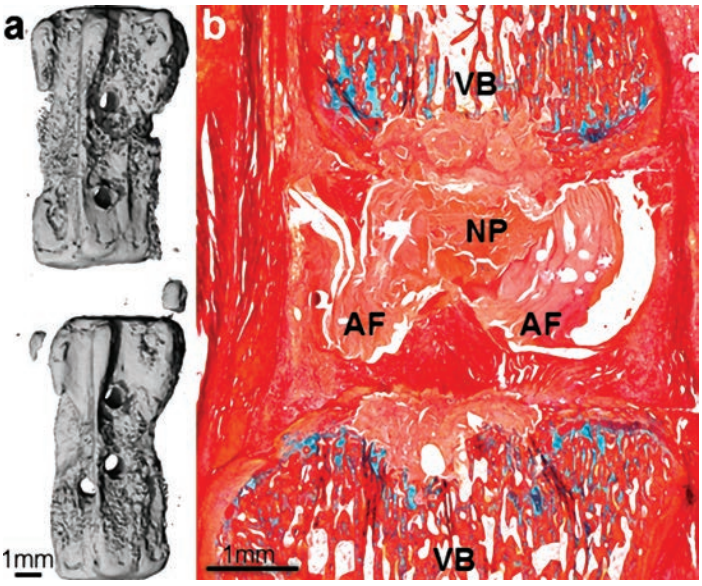

Figure 3. (A) Micro-CT reconstruction and (B) alcian blue/picrosirius red stained section of an AF/NP DAPS precultured 10 weeks and implanted for 5 weeks in the rat caudal spine.

the first 7.5 weeks of *in vitro* culture, and remained constant thereafter, with no differences between MSC and disc cell-seeded DAPS (Figure 2b). After 10 weeks of pre-culture, AF/NP cell seeded DAPS were implanted into the caudal spine of athymic rats. Five weeks after implantation, micro-CT scans revealed normal vertebral morphology, with some new bone deposition around the k-wire holes, but no signs of intervertebral fusion (Figure 3a). Alcian blue/picrosirius red stained vertebra-DAPS-vertebra sections showed that DAPS maintained their structure and integrated with the adjacent soft tissue (Figure 3b). Interestingly, the NP region of the implanted DAPS stained strongly for collagen, but weakly for proteoglycans, suggesting a possible shift in cell phenotype after implantation.

Discussion

This study demonstrates that an engineered disc composed of an electrospun AF and a hydrogel NP, seeded with either native disc cells or MSCs, matures compositionally with time in *in vitro* culture. The combination of bovine disc cells or MSCs with media containing TGF-β3 proved to be effective for *in vitro* maturation, as construct approached rat caudal and human lumbar disc compositional and functional benchmarks. Here, DAPS achieved GAG content and compressive moduli higher than those reported previously for other engineered discs[1,4] and also engineered cartilage. Interestingly, the mechanical properties of the engineered disc were high from the outset, and relatively stable over the long-term. This likely reflects the mechanical contributions of the electrospun AF region, whose primary constituent (PCL) degrades slowly over time in *in vitro* culture. When pre-matured DAPS, seeded with native disc cells, were implanted into the caudal spine 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[5], and a study by Bowles and co-workers reported strong vertebral integration of cell-seeded engineered discs after a shorter 2 week preculture period.[4] 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.

Acknowledgement

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