The Effect of Remobilization on the In Vivo Function of an Endplate-Modified Engineered Disc

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Introduction

A promising alternative to fusion surgery for intervertebral disc pathology is total disc replacement with a cellular, engineered whole disc construct that restores normal structure and mechanical function to the spine. To this end, our group developed endplate-modified disc-like angle-ply structures (eDAPS) that mimic the structure and function of the native disc. Our previous work showed that, over a 5 week period of implantation in the rat tail disc space, eDAPS outperformed DAPS implanted without endplates with respect to construct composition and integration.[1] However, this previous work utilized an external fixator to immobilize the segment post-implantation. Chronic immobilization is known to be detrimental to disc health,[2] and thus the eventual restoration of physiologic loading to the implanted eDAPS will be essential for integration and long term viability.The purpose of this study was therefore to elucidate the impact of remobilization (via fixator removal) on eDAPS structure, composition and mechanics.

Methods

eDAPS Fabrication and Culture

eDAPS sized for the rat caudal disc space were fabricated by concentrically wrapping aligned, angled strips of electrospun PCL nanofibers to form the AF region, and filling the center with a hyaluronic acid hydrogel to form the NP region [3]. Both regions were seeded with bovine disc cells (2×10^6cells/AF and 6×10^5 cells/NP), and combined after two weeks culture with acellular porous PCL endplates to form the eDAPS. eDAPS were pre-cultured for 5 weeks in chemically defined media with TGF-β3.

Implantation

32 athymic, male, retired breeder rats were anesthetized, and Kirschner wires were passed through the C8 and C9 caudal vertebral bodies, allowing for external fixator placement.[4] eDAPS were implanted following removal of the C8-C9 disc and a partial corpectomy of the adjacent vertebral bodies. The effects of remobilization (R) via external fixator removal were investigated after 5 weeks or 10 weeks of implantation, with endpoints of 10 weeks (10W R, n = 6) and 20 weeks (20W R, n = 10), respectively. Control groups included animals with external fixators left in place for 10 (10W F, n = 5) and 20 weeks (20W F, n = 11). *MRI:* T2 mapping of the eDAPS was performed at 4.7T (16 echoes,TE/TR = 7.84 ms/2,000 ms, FOV = 15x15 mm^2). Average T2 maps were generated for each time point using a custom MATLAB code.

μCT Imaging and Radiographs

In the 20W R and 20W F groups, the PCL endplates were rendered radiopaque via the inclusion of zirconia nanoparticles.[5] Implanted motion segments were subjected to μCT scanning at 3μm resolution before and after application of 3N compressive loading (Scanco μCT50 Compression Device) to identify functional boney integration of the constructs. Lateral tail radiographs were taken immediately post-operative (PO), and at 10, 15, and 20 weeks PO. The angulation of the vertebral bodies (VB) of the implanted motion segment was quantified in MATLAB.

Mechanical Testing

3-4 vertebra-eDAPS-vertebra segments in each experimental group, and 4 native rat tail vertebra-disc-vertebra segments were subjected to compression testing (20 cycles, 0 to 3N, 0.05 Hx), followed by tension to failure for eDAPS samples. Mechanical properties were quantified via a bilinear fit to the 20^{th} cycle. Sugnificant differences in quantitative outcomes were assessed via a one-way ANOVA with Tukey's post-hoc test.

Results

NP T2 values (Figure 1) were maintained at native levels for up to 20 weeks *in vivo*; there were no significant differences in NP T2 across experimental groups. The toe and linear region moduli (Figure 2A) of eDAPS implanted motion segments were not significantly different from native rat tail motion segments, and there were no significant differences between experimental groups. Maximum strain was significantly greater in the 10W R group compared to the native rat tail disc; no other significant differences between groups or compared to native discs were found for transition and maximum strains (not shown). Tensile load to failure after 10 weeks implantation ranged from 4-8 N. Radiographic analysis

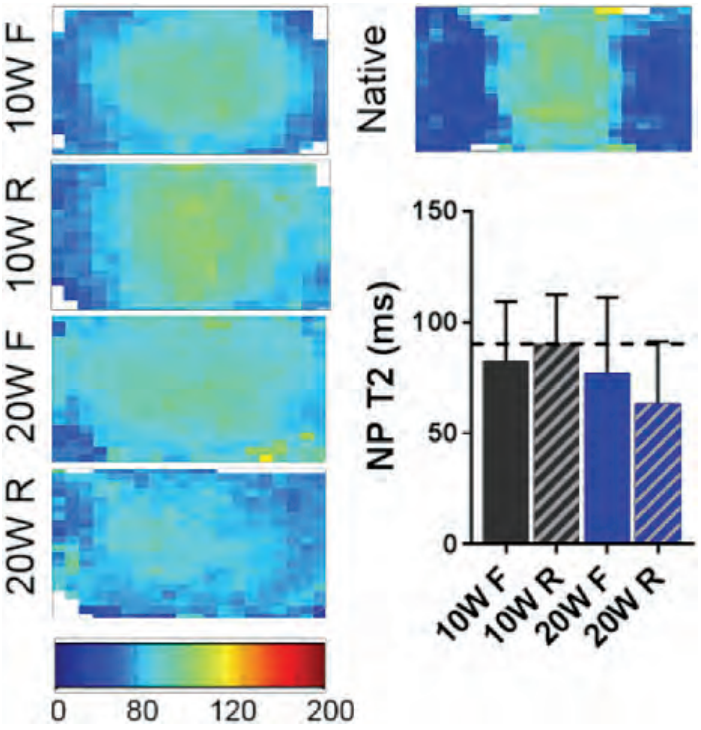

Figure 1. Average T2 maps for each experimental group, and quantification of NP T2. Dashed line = native rat tail NP.

revealed a progressive increase in vertebral body angle in eDAPS implanted motion Segments following remobilization. Vertebral body angle (Figure 2B) also increased from PO to 10 weeks in the fixed group, but remained stable from 10 to 20 weeks. Vertebral body angle was significantly higher in the remobilized group (9.6º) compared to the fixed group (5.0º) at 20 weeks. Vertebral body angulation in the remobilized group was further evident on μCT, and led to shearing of the implanted eDAPS under physiologic compressive loading. In contrast, the eDAPS remained well aligned in the fixed group, resulting in uniform axial compression of the implanted construct similar to the native disc (Figure 3).

Discussion

These data suggest that long-term *in vivo* implantation of the eDAPS results in maintenance of construct composition and functional integration. While mechanical properties and MRI T2 values were not different between fixed and remobilized groups, remobilization had adverse effects on motion segment morphology, regardless of whether the external fixator was removed at 5 or 10 weeks post-implantation. It is likely that even after 10 weeks *in vivo*, eDAPS integration is not sufficient to fully support restoration of native loading, particularly in the hypermobile rat caudal spine, which lacks stabilizing posterior elements. Ongoing work is further investigating the

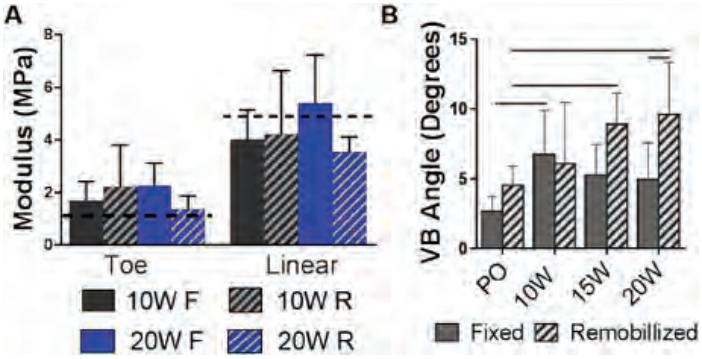

Figure 2. (A) Moduli for each experimental group. Dashed lines = native rat tail properties. **(B)** Vertebral body angle over time for fixed and remobilized groups. Bars denote significance.

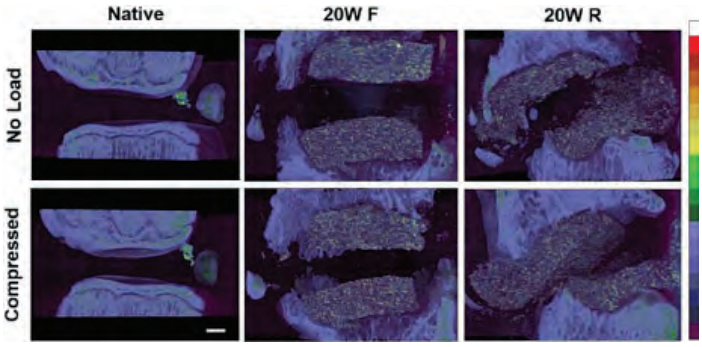

Figure 3. 3D reconstructions of μCT scans of motion segments from the fixed and remobilized groups, compared to the native rat tail disc, before and after application of 3N compression using the Scanco *in situ* compression device. Color scale represents bone mineral density.

biochemistry, histology and tensile properties of the 20 week implantation groups. Future work will evaluate the eDAPS in larger pre- clinical animal models, which have a more human-like morphology and motion.

Significance

Current surgical strategies for treating disc pathology do not restore native disc structure or function. A tissue-engineered disc replacement capable of integrating with the native environment, while maintaining composition and mechanical function, will significantly advance treatment options for patients with degenerative spinal pathology.

References

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