Introduction

Intervertebral disc degeneration involves a progressive cascade of cellular, compositional and structural changes.\(^1\) Surgical treatment of disc degeneration is most commonly achieved via fusion of the degenerated motion segment, which does not restore native disc structure or function, and may exacerbate degeneration of adjacent discs.\(^2\) For the treatment of advanced degeneration, total disc arthroplasty with a cellular, engineered replacement is a promising alternative to fusion; a viable, functional substitute may restore normal mechanics to the degenerated spine. To that end, our lab has created disc-like angle ply structures (DAPS) that mimic the structure and function of the native disc by combining an electrospun nanofibrous annulus fibrosus (AF) with a hydrogel nucleus pulposus (NP).\(^3\) We have previously shown that while the DAPS are mechanically functional following in vivo implantation in the rat caudal disc space, the constructs do not integrate with the adjacent vertebral bodies and exhibit progressive reductions in MRI T2 signal and NP proteoglycan content.\(^4\) Here, we report on the in vivo performance of an endplate DAPS (eDAPS) implant that was designed to improve construct integration and promote retention of implant composition via the addition of acellular porous polymer endplates.

Methods

eDAPS Fabrication and Culture

DAPS sized for the rat caudal disc space were fabricated by concentrically wrapping aligned, angled strips of electrospun poly(ε-caprolactone) (PCL) nanofibers to form the AF region, and filling the center with a hyaluronic acid hydrogel to form the NP region. Both regions were seeded with bovine disc cells (2x10\(^6\) cells/AF and 6x10\(^5\) cells/NP) and cultured separately for two weeks in chemically defined media containing TGF-β3. After two weeks of culture, the AF and NP regions were combined, and acellular porous PCL endplates, (4 mm diameter, 1.5 mm high) fabricated via salt leaching, were apposed to each side of the DAPS to form the eDAPS construct (acellular construct viewed by µCT, Figure 1A). The eDAPS were cultured for an additional three weeks for a total of 5 weeks preculture.

Implantation Surgery

Athymic male retired breeder rats were anesthetized, and kirschner wires were passed through the C8 and C9 caudal vertebral bodies allowing the placement of a ring-type external fixator.\(^5\) eDAPS were implanted following removal of the C8-C9 disc and a partial corpectomy of the adjacent vertebral bodies. Rats were euthanized at 7 (n = 3), 17 (n = 3) and 35 (n = 7) days.

Magnetic Resonance Imaging

T2 mapping of the eDAPS was performed at 4.7T (16 echoes, TE/TR = 7.84 ms/2,000 ms, FOV = 15×15 mm\(^2\)). Average T2 maps were generated for each time point using a custom MATLAB code.\(^5\) Significant differences in eDAPS T2 values at each time point compared to native discs were assessed via a one-way ANOVA, with Tukey’s posthoc test.

Mechanical Testing and Biochemistry

Four vertebra-eDAPS-vertebra motion segments 35 days post-implantation, and four native rat tail motion segments, were subjected to mechanical testing (20 cycles, 0 to −3N/0.3 MPa, 0.05 Hz). Displacement was tracked optically using a high resolution digital camera and a custom texture tracking MATLAB code. The 20\(^{th}\) cycle of the force-displacement curve was used to calculate the toe and linear region compressive moduli, and transition and maximum strains via a bilinear fit. Significant differences in biomechanical parameters between native and eDAPS implanted motion segments were assessed via a Student’s t-test. Following mechanical testing, the eDAPS were dissected from the motion segment, separated into NP, AF and EP regions, and digested with proteinase-K. Glycosaminoglycan (GAG) content was quantified via the DMMB assay.

Histology

Vertebra-eDAPS-vertebra motion segments were fixed, decalcified and processed through paraffin. Sections were stained with Alcian blue
due in part to the PCL endplates serving as a barrier to the harsh native environment. eDAPS toe and linear region moduli were similar to that of native tissue, indicating the potential of this engineered implant for functional restoration of motion segment mechanics. Ongoing work will investigate longer durations of in vivo implantation, as well as remobilization strategies to further enhance integration and in vivo maintenance.

Conclusions
Current surgical strategies for disc degeneration do not restore native structure and function to the spine. A biologic total disc replacement that better integrates with surrounding tissue (while maintaining composition and mechanical function in the native environment) will significantly improve the standard of care for patients with low back pain.

Acknowledgments
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References
1. CDC. MMWR, 2009

Results
The NP and AF T2 relaxation times of the eDAPS were superphysiologic 7 days after implantation into the rat caudal disc space; the T2 values decreased from 7 to 17 days post-implantation. AF T2 values remained superphysiologic up to 35 days in vivo (data not shown), while NP T2 values at 17 and 35 days in vivo were not different from the NP T2 of native rat tail discs (Figure 1B). The maintenance of NP T2 signal corresponded with robust Alcian blue staining in the NP region of the eDAPS at 35 days post-implantation. DAPI staining illustrated infiltration of the acellular PCL foam endplate from the AF and NP regions of the eDAPS, in addition to infiltration of native cells from the adjacent vertebral body (Figure 1D). DAPI staining also indicated sustained cellularity of the AF and NP regions of the eDAPS from 7 days to 35 days in vivo. After 35 days in vivo, GAG content was highest in the NP region of the eDAPS (0.27% ± 0.14%ww), followed by the AF region (0.11% ± 0.01%ww) and EP region (0.03% ± 0.006%ww). The toe and linear region moduli of the eDAPS implanted motion segments were not significantly different from native discs. However, the transition and maximal strains were significantly higher in the eDAPS implanted motion segments compared to native (Figure 1C).

Discussion
Overall, the addition of engineered endplates improved integration and maintenance of DAPS matrix composition in vivo. This is in contrast with our previous findings, in which DAPS implants without endplates were characterized by a lack of integration with adjacent vertebral bodies and progressive loss of NP T2 signal and proteoglycan content. The improved in vivo performance of the eDAPS may be due in part to the PCL endplates serving as a barrier to the harsh native environment. eDAPS toe and linear region moduli were similar to that of native tissue, indicating the potential of this engineered implant for functional restoration of motion segment mechanics. Ongoing work will investigate longer durations of in vivo implantation, as well as remobilization strategies to further enhance integration and in vivo maintenance.

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Figure 1. (A) 3D µCT reconstruction of an acellular eDAPS, with cut-away illustrating the lamellar AF structure and porous EP. (B) NP T2 values for native rat tail discs and eDAPS from 7 to 35 days in vivo, compared to the NP T2 of DAPS alone (dashed line) at 5 weeks. (* = significantly different from all groups, p < 0.05). (C) Stress-strain behavior of native rat tail and eDAPS implanted motion segments. (D) Representative Alcian blue and picrosirius red stained histology of eDAPS implanted for 35 days; DAPI staining illustrates the cellularity of the vertebral body (VB) and EP interface and the AF and EP interface. Inset illustrates the appearance of the DAPS alone at 5 weeks in vivo.