Engineered Total Disc Replacements in a Large Animal Model Recapitulate Native Disc Structure and Function

Sarah E. Gullbrand[1,2]
Beth G. Ashinsky[1,2,3]
Dong Hwa Kim[1,2]
Lachlan J. Smith[1,2]
Dawn M. Elliott[4]
Thomas P. Schaer[1]
Robert L. Mauck[1,2]
Harvey E. Smith[1,2]

[1]University of Pennsylvania, Philadelphia, PA

[2]Corporal Michael J. Crescenz VA Medical Center, Philadelphia, PA

[3]Drexel University, Philadelphia, PA

[4]University of Delaware, Newark, DE

Introduction

Intervertebral disc degeneration is commonly associated with back and neck pain, and current surgical treatments for end-stage degeneration, including spinal fusion, do not restore spine function. Replacement of the degenerative intervertebral disc with a living, tissue engineered construct has the potential to restore normal structure and function to the spine.[1] Towards this end, we developed endplate-modified disc-like angle ply structures (eDAPS) that recapitulate the structure and function of the native disc. These implants combine a cell-seeded hydrogel nucleus pulposus (NP) and an electrospun poly(ε-caprolactone) (PCL) annulus fibrosus (AF) with acellular PCL foam endplates.[2,3] We previously showed in a rat tail disc replacement model that eDAPS functionally mature *in vivo*, recapitulating many of the characteristics of the native disc and that eDAPS could be fabricated at human length scales for evaluation in a large animal model.[4] Here, we compare eDAPS structure and mechanical function following *in vivo* implantation to healthy and degenerative (discectomy) goat cervical discs at 8 weeks.

Methods

eDAPS sized for the goat and human cervical disc space (9 mm height, 16 mm diameter) were fabricated as previously described[4] and seeded with allogeneic goat bone-marrow derived mesenchymal stem cells. eDAPS were cultured for 13-17 weeks in a chemically defined media with TGF-β3 prior to implantation. With IACUC approval, 5 male large frame goats underwent a surgical procedure to implant the eDAPS at the C2-C3 level of the cervical spine. Implanted motion segments were immobilized with an anterior cervical plate to ensure construct retention. The C3-C4 discs adjacent to the eDAPS implants were utilized as healthy controls. 3 additional goats underwent a surgical procedure to induce degeneration of the C2-C3 disc via sub-total discectomy. Animals in both cohorts were euthanized at 8 weeks for analyses. Cervical spines were subjected to MRI at 3T to obtain both a T2-weighted image, and an image series for quantitative T2 mapping. Following MRI, cervical motion segments from each experimental group (healthy, degenerative, and eDAPS implanted) were isolated and subjected to 20 cycles of compression, where the applied compressive stress was equivalent to that of the human cervical disc, due to the weight of the head (0 to −25 N, 0.084 MPa). Mechanical properties were quantified via a bilinear fit of the toe and linear regions of the stress-strain curve. Motion segments in each group were then fixed, decalcified and processed through paraffin for histology. Sections were stained with alcian blue (proteoglycans) and picrosirius red (collagens). Significant differences ($p < 0.05$) in quantitative outcomes were assessed via a Kruskal-Wallis with Dunn's multiple comparison test.

Results

eDAPS implants generally recapitulated the structure and composition of the healthy disc after 8 weeks *in vivo* (Figure 1A), with a proteoglycan rich nucleus pulposus and a lamellar and collagen rich annulus fibrosus. Furthermore, histology demonstrated robust integration of the PCL endplates with the adjacent vertebral bodies. In contrast, discectomy resulted in substantial degeneration of the motion segment, including severe annular disorganization, boney endplate remodeling, NP fibrosis, and loss of disc height. T2 weighted MRIs (Figure 1B) demonstrated high signal intensity within the eDAPS, similar to the control disc, compared to a loss of NP signal intensity in the discectomy group. T2 relaxation time in the NP (Figure 1C) was significantly reduced in degenerative discs compared to controls. There was no detectable difference in NP T2 between eDAPS implants and healthy or degenerative discs. Degeneration induced via discectomy also compromised disc mechanical function, with significant increases in compressive strain and reductions in toe and linear moduli, compared to eDAPS implants (Figure 2). Although the moduli of eDAPS implanted motion segments trended higher than controls, transition and maximum strains were within the range of controls.

Discussion

Our results demonstrate that surgically induced disc degeneration significantly alters the structure, composition and mechanical function of the healthy disc, while eDAPS implantation improved motion segment structure and function toward healthy levels.

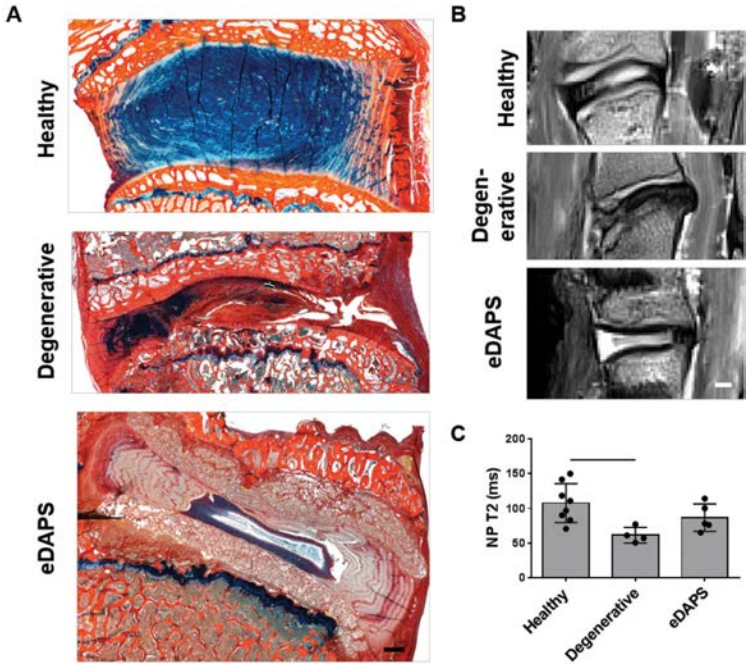

Figure 1. (A) Alcian blue and picrosirius red stained histology of eDAPS 8 weeks post-implantation compared to healthy and degenerative goat cervical discs, scale = 1 mm; **(B)** T2 weighted MRI of eDAPS after 8 weeks of implantation, compared with healthy and degenerative goat cervical discs, scale = 4 mm; **(C)** NP T2 relaxation times for all groups (bars denote $p < 0.05$).

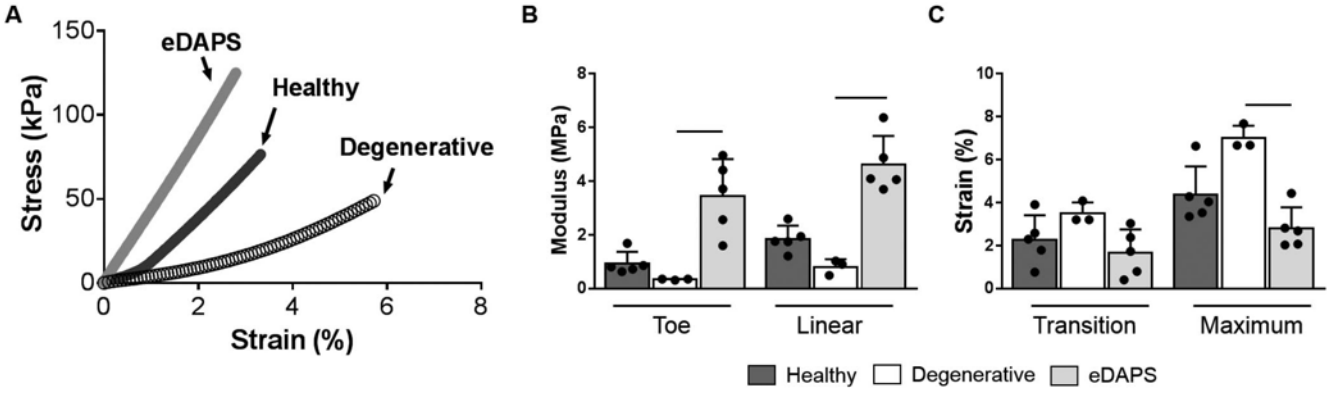

Figure 2. (A) Representative compressive stress-strain curves for each experimental group, from which **(B)** toe and linear region moduli and **(C)** transition and maximum strains were calculated. Bars denote $p < 0.05$.

After two months *in vivo*, eDAPS integrated with the native tissue, and their structure and function recapitulated many features of the native, healthy disc. However, quantitative MRI revealed that the NP T2 of the eDAPS spanned the range of NP T2 relaxation times for both healthy and degenerative discs, and corresponded with low GAG staining and matrix content in the central NP on histology. This is likely due to low cell viability and matrix accumulation in this region during *in vitro* culture[5]. Future work will focus on strategies to enhance cell viability and improve matrix homogeneity within these large human-scale engineered constructs. Ongoing work is also focused on exploring the effects of plate removal to restore anabolic, physiologic loading to the eDAPS *in vivo*, post implantation and integration.

Conclusion

Results from this study demonstrate that a tissue engineered disc replacement can restore native-like structure and function to the disc, compared with degenerative discs. Development and translation of tissue engineered total disc replacements has the potential to significantly expand treatment options for patients with symptomatic advanced disc degeneration, restoring disc structure and function via a living implant.

References:

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