Evaluation of a Human-Scale Tissue Engineered Intervertebral Disc in a Large Animal Model

Sarah E. Gullbrand[1,2]
Beth G. Ashinsky[1,2,3]
Edward D. Bonnevie[1,2]
Dong Hwa Kim[1,2]
Lachlan J. Smith[1,2]
Dawn M. Elliott[4]
Thomas P. Schaer[1]
Harvey E. Smith[1,2]
Robert L. Mauck[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 (i.e., 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 native structure and function of the 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 implants functionally mature *in vivo*, recapitulating many of the characteristics of the native disc.[4] The rat tail is a fraction of the scale of the human intervertebral disc, however, and so translation of this technology towards clinical use will require evaluation of human-sized implants in a larger animal model. We anticipate that the first human application of the eDAPS implant may be in the human cervical spine, and so here evaluate the structure, composition, and mechanical function of the eDAPS after 4 and 8 weeks *in vivo* in a goat cervical disc replacement model.

Methods

eDAPS sized for the goat and human cervical disc space (9 mm height, 16 mm diameter) were fabricated as previously described [5] and seeded with allogeneic goat (caprine) bone-marrow derived mesenchymal stem cells. eDAPS were cultured for a total of 13-17 weeks in a chemically defined media with TGF-β3 prior to implantation. With IACUC approval, 9 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. Animals were euthanized at 4 weeks (n=4) or 8 weeks (n=3) for analyses. In the 8 week group, quantitative T2 mapping was performed. Following MRI, the eDAPS implanted motion segment and the adjacent native cervical motion segment were isolated and subjected to 20 cycles of compression, where the applied compressive stress was equivalent to that in the human cervical discs due to the weight of the head (0 to −25 N, 0.084 MPa). eDAPS implanted motion segments were then fixed, decalcified and processed through paraffin for histology. Histologic analyses were conducted on samples from the 4 and 8-week groups. Sections were stained with alcian blue (proteoglycans) and picrosirius red (collagens). Second harmonic generation imaging (SHG) was also utilized to visualize organized collagen at the eDAPS-vertebral body interface. Significant differences ($p<0.05$) in quantitative outcomes were assessed via Kruskal-Wallis with a Dunn's multiple comparison test.

Results

eDAPS composition and structure were maintained at or above pre-implantation levels after 4 weeks *in vivo* (Figure 1A, B). After 8 weeks *in vivo*, there was an increase in collagen matrix deposition within the PCL endplates and the annulus fibrosus, accompanied by slight reductions in proteoglycan staining within the NP region compared to 4 weeks. SHG images also revealed the deposition of organized collagen within the initially acellular PCL endplates, resulting in nascent integration of the eDAPS with the vertebral bodies at 4 weeks that further matured after 8 weeks (arrows, Figure 1B). T2-weighted MRIs demonstrated improved signal intensity within the eDAPS after 8 weeks *in vivo,* compared to pre-implantation levels, suggestive of improved construct water content (Figure 1C). Compressive mechanical testing showed significant maturation of eDAPS mechanical properties from pre-implantation values after 8 weeks *in vivo* (Figure 2A). While toe and linear region moduli (Figure 2B) of the eDAPS implanted motion segments trended higher than native goat cervical disc moduli, the transition and maximal strains (Figure 2C) were significantly reduced from pre-implantation levels at 8 weeks, and were not significantly different from the native cervical motion segment.

Discussion

In this study, we demonstrate the feasibility of a total disc replacement with a human-scale, tissue engineered disc in a large animal

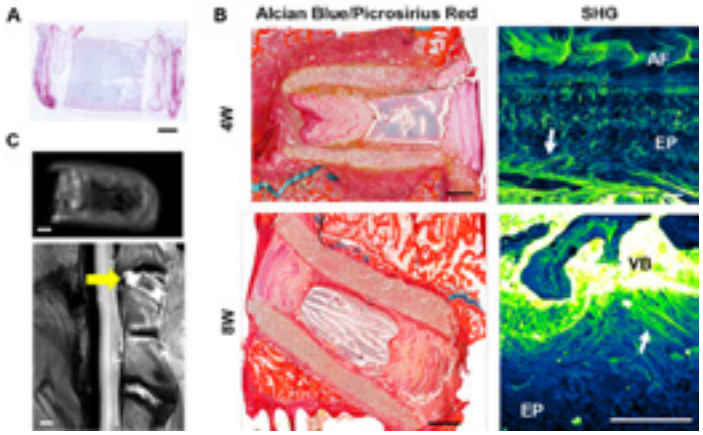

Figure 1. (A) Alcian blue and picrosirius red stained histology of eDAPS prior to implantation, scale = 2mm. **(B)** Histology (scale = 2mm) of whole eDAPS and SHG images (scale = 200μm) of the EP-vertebral body interface 4 and 8 weeks post implantation. (C) T2 weighted MRI of eDAPS before (top, scale = 2mm) and after (bottom, scale = 5mm) 8 weeks of implantation.

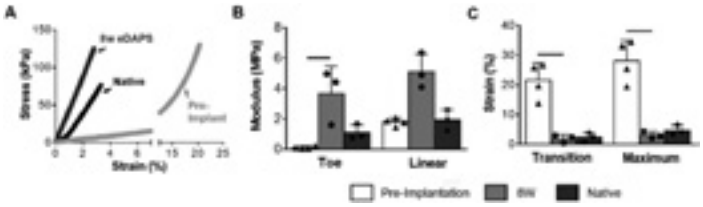

Figure 2. (A) Representative compressive stress-strain curves for each experimental group. **(B)** Toe and linear region moduli, and **(C)** transition and maximum strain for each experimental group. Bars denote significance, $p<0.05$.

model. Composition in the EP and AF regions of the eDAPS generally matured with increasing duration of implantation as the eDAPS progressively integrated with the native spine, while NP composition was maintained or slightly reduced from pre-implantation levels. Significant improvements in eDAPS compressive mechanical properties were observed after implantation, reaching near native levels after 8 weeks. Ongoing and future work is focused on further translating the eDAPS towards clinical use by optimizing the NP region for improved *in vivo* performance, implanting eDAPS for longer durations within the goat cervical spine, and exploring the effects of fixator removal to restore physiologic loading to the construct *in vivo*.

Significance

This work demonstrates for the first time the function of a human-scale tissue engineered disc following *in vivo* implantation in a large animal model. Development and translation of tissue engineered total disc replacements has the potential to significantly expand treatment options for symptomatic disc degeneration, restoring structure and function via a living implant.

References

1. Bowles RD, Gebhard HH, Härti R, *et al.* Tissue-engineered intervertebral discs produce new matrix, maintain disc height, and restore biomechanical function to the rodent spine. *Proc Natl Acad Sci USA* 2011; 108(32): 13106-11.

2. Nerurkar NL, Sen S, Huang AH, *et al.* Engineered disc-like angle-ply structures for intervertebral disc replacement. *Spine (Phila Pa 1976)* 2010; 35(8): 867-73.

3. Martin JT, Gullbrand SE, Kim DH, *et al.* In vitro maturation and in vivo integration and function of an engineered cell-seeded disc-liked angle ply structure (DAPS) for total disc arthroplasty. *Sci Rep* 2017; 7(1): 15765.

4. Gullbrand SE, Kim DH, Bonnevie E, *et al.* Towards the scale up of tissue engineered intervertebral discs for clinical application. *Acta Biomater* 2018; 70: 154-64.