Development of Disc-Like Angle Ply Structures for Total Disc Replacement at Clinically Relevant Size Scales

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Introduction

Replacement of the disc with a viable, tissue-engineered construct that mimics native disc structure and function is an attractive alternative to fusion or mechanical arthroplasty for the treatment of disc pathology. Towards this end, our group has developed disc-like angle ply structures (DAPS) sized for the rat caudal disc space that achieve near native composition and mechanical function with *in vitro* culture.[1] Composite tissue-engineered discs have also been developed by several other groups; however, the average size of constructs reported in the literature remains a fraction of the size of human discs.[2-3] In order to translate tissue-engineered disc replacement towards clinical use, successful fabrication and *in vivo* evaluation of these constructs at larger size scales is critically important. The purpose of this study was to evaluate the maturation of medium (rabbit lumbar disc equivalent) and large (goat/human cervical disc equivalent) sized DAPS constructs over 15 weeks of *in vitro* culture and after 5 weeks of subcutaneous implantation.

Methods

DAPS Fabrication, Culture and Subcutaneous Implantation

DAPS were fabricated in two sizes—medium (3 mm height × 10 mm diameter, NP diameter = 5 mm) and large (6 mm height x 20 mm diameter, NP diameter = 10 mm). The AF region of the DAPS was fabricated by electrospinning aligned sheets of 250-300 μm thick poly(ε-caprolactone) (PCL), and cutting the sheets into strips at a 30 degree angle. The strips were hydrated, coated with fibronectin and seeded with bovine AF cells (3,333 cells/mm^2). Following 1 week of culture in chemically defined media with TGF-β3 (CM+), strips were coupled at opposing fiber angles (+/− 30°), and wound in a custom mold to form the circular AF region. The NP region of the DAPS was fabricated by seeding bovine NP cells in a 2% agarose hydrogel (20 million cells/mL), and culturing for 2 weeks in CM+ prior to combining with the AF region. The combined DAPS were cultured for either 5, 10 or 15 weeks in CM+ on an orbital shaker. After 5 weeks of pre-culture, DAPS of both size scales (n = 6 per size) were implanted subcutaneously (SQ) in athymic rats for 5 weeks.

Viability, Metabolic Activity and Biochemistry

At each *in vitro* and *in vivo* time point (n=4-6 per group), DAPS of each size were bisected. From one half-DAPS, GAG and collagen content were quantified via the DMMB assay and the OHP assay, respectively. From the remaining half DAPS, cell viability of the NP region was assessed via live-dead staining, and metabolic activity of the AF region was assessed via the MTT assay. *MRI and Mechanical Testing:* At each *in vitro* time point (n = 3 per group), sagittal MRI T2 maps of the DAPS were obtained. Mechanical properties of the DAPS in unconfined compression (20 cycles compression, 0.24 MPa) were determined via a bi-linear fit of the stress-strain curve.

Histology

DAPS (n = 2 per group) were processed through paraffin, sectioned in the sagittal plane, and stained with Alcian blue (GAG) or picrosirius red (collagen).

Statistics and Correlation Analysis

Significant differences in quantitative outcome measures were assessed via two- way ANOVA with Tukey's post-hoc test. Correlations between DAPS T2 values, biochemistry and mechanical properties were assessed using the *corr.test* function in R (r-project.org) for each size scale.

Results

In general, medium DAPS outperformed large DAPS with respect to AF and NP cell viability during *in vitro* culture and following subcutaneous implantation (Figure 1A, B). Subcutaneous implantation of the DAPS significantly increased AF cell metabolic activity in the medium DAPS, while NP cell viability was significantly reduced at both size scales compared to pre- implantation values. Analysis of compressive mechanical properties (Figure 1C, D), illustrated that medium DAPS matured more rapidly than large DAPS, as characterized by increases in toe modulus and reductions in transition strain at 10 weeks. AF T2 values significantly decreased from 5 to 15 weeks culture in both medium and large DAPS; NP T2 values were not affected by culture duration. NP and AF GAG and collagen content were significantly higher in medium DAPS compared

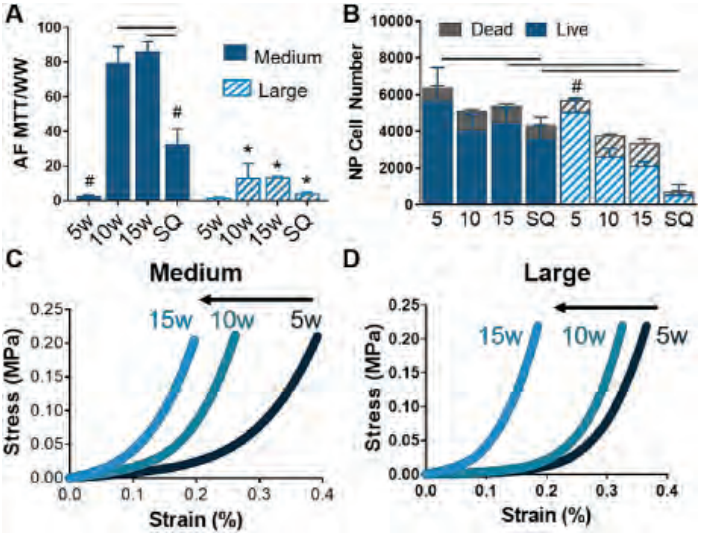

Figure 1. DAPS **(A)** AF cell metabolic activity, **(B)** NP cell viability for all experimental groups, and representative compressive stress- strain curves of **(C)** medium and (D) large DAPS. Bars denote significance, * = $p < 0.05$ compared to medium at the same time point. # = $p < 0.05$ compared to all other time points within a size.

to large DAPS, and reached maximal values after 15 weeks of culture at both size scales (Medium: NP GAG = 3.0%ww, AF GAG = 2.4%ww, NP collagen = 2.0%ww, AF collagen = 1.4%ww; Large: NP GAG = 1.5%ww, AF GAG = 1.2%ww, NP collagen = 1.1%ww,AF collagen = 1.0%ww). NP GAG content was significantly reduced compared to pre-implantation values in both medium and large DAPS following SQ implantation. SQ implantation significantly increased NP collagen content in the medium DAPS, but did not affect AF collagen or GAG content at either size scale. Histology (Figure 2) confirmed quantitative biochemical analyses and further demonstrated the heterogeneity of matrix distribution present in the DAPS, particularly at the large size scale. Correlation analyses illustrated stronger correlations between MRI, biochemistry and mechanical properties in the medium DAPS than in large DAPS.

Discussion

Medium DAPS outperformed large DAPS, maturing more rapidly with more homogenous matrix distribution compared to large DAPS. Subcutaneous implantation was detrimental to

Figure 2. Alcian blue (top) and picrosirius red (bottom) stained sagittal histology section of DAPS in each experimental group (scale = 2 mm).

cell viability and GAG content in the NP region independent of DAPS size, as we have previously observed in small DAPS sized for the rat caudal disc space.[4] In contrast, AF matrix content was maintained at pre-implantation values at both size scales following subcutaneous implantation, potentially due to infiltration of host cells into the outer layers of the AF. *In vitro* matrix distribution and *in vivo* performance of large DAPS could be further improved via the inclusion of nutrient channels, as has been utilized for cartilage tissue engineering, or via culture in a bioreactor.[5-6] Future work will evaluate the DAPS in a large animal model to further the translation of these constructs for the treatment of end stage disc degeneration.

Significance

We have demonstrated the feasibility of scaling up DAPS for total disc replacement to clinically relevant size scales. Clinical translation of tissue-engineered discs will offer an alternative to mechanical disc arthroplasty and fusion procedures, and may change the paradigm of clinical care for patients with disc pathology and associated axial spine and neurogenic extremity pain.

References

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