

Characterizing Discogenic Cell Based Tissue Engineered Disc Replacements

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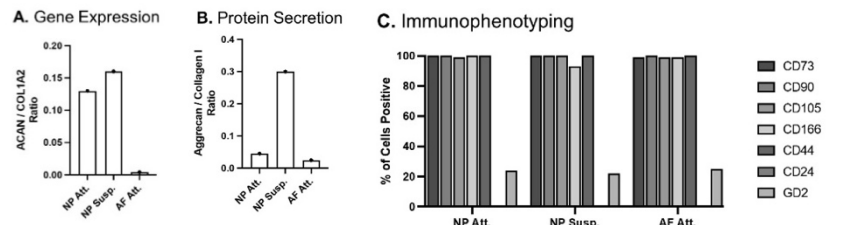


Figure 1: (A) Relative (normalized to HPRT1) gene expression for ACAN / COL1A2. (B) Ratio of protein secretion over a 5-day period for Aggrecan / Collagen I. (C) Cell surface expression of markers related to mesenchymal and intervertebral disc cell lineages.

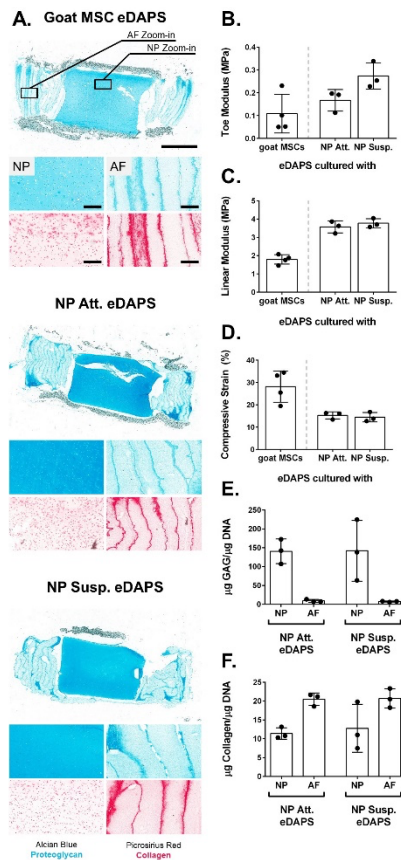


Figure 2: (A) Alcian Blue and Picrosirius Red histology staining (scale = 3mm for entire eDAPS, 300 µm for zoom-ins), (B) toe modulus, (C) linear modulus, and (D) compressive strain. (E) GAG content per DNA and (F) collagen content per DNA for the NP and AF regions of Discogenic eDAPS.

SIGNIFICANCE: Discogenic Cells are a promising development in the translation of tissue engineered therapies that remove the many hurdles of working with native human disc cells. Discogenic Cells were shown to be safe in clinical trials for patients with mild to moderate lumbar disc degeneration and produced clinically meaningful improvements in low back pain, function and quality of life. This work further advances translation by culturing tissue engineered discs at a scale appropriate for human cervical disc replacement using a translatable human NP and AF cell source.

REFERENCES: [1] Gullbrand+ *JOR Spine*, 2018. [2] Gullbrand+ *Sci Transl Med*, 2018. [3] Martin+ *Sci Rep*, 2017. [4] Kim+ *Acta Biomater*, 2020. [5] Silverman+ *The Spine Journal*, 2020. [6] Gullbrand, *Acta Biomater*, 2018. [7] Sakai+ *Nat. Commun*, 2012. [8] Kim+ *Acta Biomater*, 2015. [9] Buckley+ *Tissue Eng. Part A*, 2012.

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INTRODUCTION: Current treatments for back pain associated with late-stage intervertebral disc (IVD) degeneration may temporarily relieve pain through fusion. However, they do not restore biological and mechanical function to the disc. The use of living tissue engineered constructs to replace degenerated discs has the potential to overcome these limitations and has been investigated in vitro by a number of groups.¹ Our group developed endplate-modified disc-like angle ply structures (eDAPS) that mimic the native structure and function of the disc.²

Our previous work seeding eDAPS with either animal-derived mesenchymal stromal cells (MSCs) or animal-derived annulus fibrosus (AF) and nucleus pulposus (NP) cells revealed that both MSCs and native cells result in eDAPS that are compositionally and functionally similar to native, but only when produced at small size scales.^{3,4} However, this comparison has not been validated at human size scales. This challenge of physically scaling the eDAPS is compounded by the challenges of working with human native disc cells, including the limited endogenous cell population and the difficulty of obtaining cells from healthy donors, which previously made translation of these findings insurmountable. DiscGenics has developed a manufacturing process utilizing intervertebral disc material obtained from human organ donors to produce Discogenic Cells.⁵ This study sought to evaluate the compositional and functional maturation of eDAPS seeded with Discogenic AF and NP cells obtained via DiscGenics' manufacturing process, compared to eDAPS seeded with goat bone-marrow derived MSCs.

METHODS: Adult IVD tissue was procured from a consented organ donor. The tissue was dissected into separate NP and AF regions followed by digestion with collagenase. The cells were expanded and passaged in attachment culture (Att.). Cells were then grown in a 3D suspension culture (Susp.) using a viscous media to allow growth of cell clusters. RNAs were isolated for gene expression, followed by real-time PCR. Protein expression was determined by seeding cells in a micromass-based assay for 5 days and measured in the supernatant by ELISA. Immunophenotyping was performed by incubating cells with fluorescently conjugated antibodies and determining positive cell expression via flow cytometry. eDAPS were seeded with Discogenic Cells as previously described.⁶ The AF analog of the eDAPS was seeded with AF cells grown in attachment culture. NP analogs were seeded with NP cells grown in either attachment (Att. eDAPS) or suspension (Susp. eDAPS) culture. eDAPS were cultured for 10 weeks with constant mechanical agitation in a chemically defined chondrogenic medium containing TGF-β3. The AF and NP analogs of control eDAPS were seeded with goat MSCs and cultured for 12 weeks under the same conditions. Following culture, eDAPS were fixed, processed for paraffin histology, and stained with Alcian Blue, Picrosirius Red, and Hematoxylin/Eosin (n=3/group). Remaining eDAPS (n=3/group) were subjected to 20 cycles of 48N compression followed by a 10 minute 48N creep load. NP and AF components of these eDAPS were then separately digested in proteinase K for quantification of DNA, glycosaminoglycan (GAG), and collagen content.

RESULTS: The ratio of gene expression for ACAN / COL1A2 was higher in NP cells from Att. or Susp. culture relative to that of AF cells from Att. culture (Figure 1A). Measurement of secreted protein showed that NP cells grown in suspension culture had a greater aggrecan / collagen I ratio than either AF or NP cells grown in attachment culture (Figure 1B). All cell surface markers tested showed similar expression between the AF and NP cells (Figure 1C). eDAPS cultured with attachment- or suspension-derived Discogenic NP cells were histologically, mechanically, and biochemically similar. Att. or Susp. NP cells deposited more proteoglycans in the NP region of the eDAPS than the goat MSCs (Figure 2A). Discogenic eDAPS were mechanically distinct from MSC eDAPS and had increased toe modulus (Figure 2B), increased linear modulus (Figure 2C), and decreased compressive strain (Figure 2D), when compared to goat MSC eDAPS. Both Discogenic eDAPS were mechanically similar, and both Discogenic NP cells produced the same amount of GAG (Figure 2E) and collagen (Figure 2F). Discogenic AF cells produced a minimal amount of GAG.

DISCUSSION: Characterization of Discogenic Cells prior to seeding indicated that all cells embodied a progenitor phenotype, expressing extremely low levels of CD24.⁷ Discogenic NP cells cultured in suspension initially produced greater amounts of aggrecan than NP cells cultured via attachment, but eDAPS cultured with both cell types were similar in every outcome measured. It is likely that both cell populations performed similarly once transitioned to 3D culture as NP cells expanded in monolayer have been shown to develop normal NP phenotypes following 3D culture.⁸ Unfavorable diffusion gradients often create hostile environments for cells toward the center of large implants that result in heterogeneous matrix deposition,⁹ as was observed in MSC-seeded eDAPS. Critically, the deposition of proteoglycans in the NP was more homogeneous in eDAPS seeded with Discogenic NP cells, indicating that Discogenic Cells more readily thrived in the unfavorable environment of a human-sized disc. Additionally, the NP and AF regions of Discogenic eDAPS were biochemically distinct, whereas goat MSC eDAPS regions were less defined with increased collagen deposited along the outer edges of the NP. Future work will involve the creation of human MSC eDAPS as well as in vivo evaluation of Discogenic eDAPS in our established large animal model.