The Influence of TGF-β3 on the Maturation of Tissue Engineered IVDs Containing Highly Contractile Human MSCs

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Introduction: Tissue engineered intervertebral discs (TE-IVDs) for whole organ replacement have become an increasingly popular research target. Whole TE-IVDs are designed to replace severely damaged discs by mimicking native form and function [1–3]. We have previously developed a composite TE-IVD made from primary ovine NP and AF cells suspended in alginate and type I collagen respectively [4,5]. Mesenchymal stem cells (MSCs) are an attractive, clinically relevant cell source and have the ability to differentiate into a variety of cell types [6]. Previous studies have demonstrated the feasibility of using mesenchymal stem cells (MSCs) in engineering IVD tissue. Human MSC phenotype influences their differentiation into divergent lineages and scaffold materials alone have the ability to drive hMSC phenotype, and therefore differentiation. Further, growth factors such as TGF-β3 are known to be chondrogenic and are thought to support the development of disc-like phenotypes. This study aims to determine the behavior of highly contractile human MSCs and the potential effects on implant maturation by exposure to TGF-β3.

Materials and Methods: *Cell Culture:* Human MSCs (courtesy of RoosterBio, Inc) at population doubling level (PDL) 7-9 were cultured in hBM-MSC High Performance Media (RoosterBio, Inc) to PDL 14-15 before being harvested for TE-IVD manufacture. *TE-IVD Manufacture:* To create the inner TE-NP, 3% alginate was seeded with hMSCs ($25x10^6$ cells/ml) and injected into custom molds. In the chondrogenic group, 10 ng/mL of TGF-β3 was added to the alginate. Each TE-NP was placed in the well of a 24-well plate and 410 µl of type I collagen gel (4 mg/ml) seeded with hMSCs ($1x10^6$ cells/ml) was pipetted around the TE-NP to create the TE-AF. The constructs were cultured for 2 weeks in DMEM media with 10% FBS, 2.5% HEPES, and 1% antibiotics. *Analysis:* The discs underwent stress-relaxation tests in unconfined compression. The equilibrium and instantaneous moduli, and effective hydraulic permeability were determined by fitting the data to a poroelastic model. The AF and NP regions were separated and biochemical analysis was performed using a hydroxyproline assay for collagen content, and modified DMMB assay for glycosaminoglycan (GAG) content. Histology with picrosirius red staining evaluated cell morphology and collagen organization. Immunohistochemistry was performed to look at expression of MHC Class markers 1a and 1b.

Results: *Mechanical and Biochemical Properties:* No differences were seen in the equilibrium or instantaneous moduli between the basal and TGF-β3 treated groups. However, a significant increase in permeability was seen in the TGF-β3 treated group ($P = 0.045$). In the TE-AF region, the GAG content was 51% lower and the collagen content was 24% lower in the TGF-β3 treated group than in the basal group ($P < 0.001$). In the TE-NP region, GAG content was 70% higher in the TGF-β3 treated group. No measurable collagen content was detected in the TE-NP of either group. *Histology and IHC:* All histological images showed the development of an intermediate region of tissue between the alginate TE-NP and the collagen TE-AF. This region has never been seen in any previous TE-IVD formulations and reflects the properties of both manufactured regions. The cell morphology varied between the regions. The hMSCs in the TE-AF were elongated and fibroblast-like while the MSCs in the TE-NP were rounded and chondrocyte-like. Under polarized light, the basal group TE-AF was much more organized and developed than the TGF-β3 group. IHC stains for MHC Class 1a and 1b expression demonstrate less intense staining of the MHC Class 1a receptor and more intense staining of the MHC Class 1b receptor in the basal group than in the TGF-β3 treated group.

Discussion: The results indicate that human MSCs are a promising cell source to use in intervertebral disc tissue engineering. Highly contractile human MSCs allow for the use of higher density collagen gels (4 mg/ml) that increase stiffness 4 fold over previously reported TE-IVDs. Cells in this regime create new tissue growth between the two manufactured regions that acts as a transitional region, more closely mimicking the organization see in native discs and improving integration of the two regions. Treatment with 10 ng/ml TGF-β3 in the alginate TE-NP alters both the mechanical and biochemical properties of TE-IVDs, primarily through down regulation of ECM production in the TE-AF. Additionally, up regulation of immunogenic MHC Class 1a receptors and down regulation of MHC Class 1b receptors indicates that TE-IVDs treated with TGF-β3 would incite a stronger immune response than basal TE-IVDs when implanted *in vivo*. Future work will focus on determining the RNA expression of the two different regions within each treatment group as well the effects of mechanical loading on human MSC TE-IVD maturation.

Significance: Together, these data show that while human MSCs are a promising cell source to use in disc tissue engineering, TGF-β3 is not ideal for enhanced maturation of implantable TE-IVDs. This study demonstrates that highly contractile human MSCs respond to the differential environments of native mimetic TE-IVD regions with or without growth factor treatment. Additionally, the cells produce a novel transitional region which has not been previously observed in any other TE-IVD formulations. In this model, TGF-β3, considered a potent chondrogenic mediator, serves to down regulate ECM production and impede collagen alignment in the adjacent TE-AF region. TGF-β3 treatment also stimulates expression of the immunogenic MHC Class 1a receptor increasing the risk of an immune response *in vivo*.

Acknowledgements: This research is supported by the Cornell Swanson Fellowship, AO Foundation, AO Spine of North America, HHMI Med into Grad Fellowship, NSF GK-12 Fellowship, and NYSTAR **References:** 1. Nesti LJ et al., Tissue Eng Pt A (2008) 14(9):1527–1537 2. Nerurkar NL et al.Spine (2008) 33(25):2691–701 3. Park S-H et al.Tissue Eng Pt A (2012) 18(5 and 6):447–458 4. Bowles RD et al. PNAS (2011) 108(32):13106–13111 5. Bowles RD et al. NMR Biomed (2011) 25(3):443–451 6. Pittenger MF et al. Science (1999) 284(5411):143–147

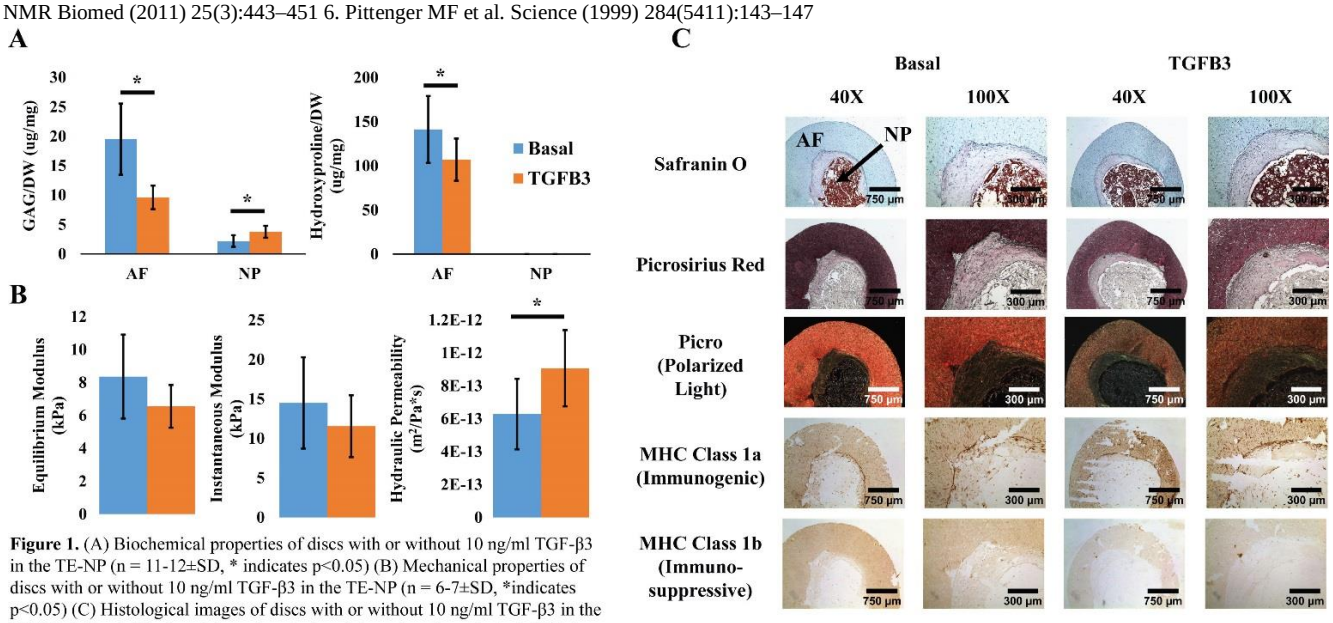

Figure 1. (A) Biochemical properties of discs with or without 10 ng/ml TGF-β3 in the TE-NP (n = 11-12±SD, * indicates $p<0.05$) (B) Mechanical properties of discs with or without 10 ng/ml TGF-β3 in the TE-NP (n = 6-7±SD, *indicates $p<0.05$) (C) Histological images of discs with or without 10 ng/ml TGF-β3 in the TE-NP. Safranin O, picrosirius red, and picrosirius red under polarized light show the distribution of ECM components, cell morphology, and collagen organization. IHC staining shows MHC Class 1a and 1b receptor expression.