Annulus Fibrosus Cells as a Potential Cell Source for Nucleus Pulposus Tissue Engineering

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Introduction: Intervertebral disc degeneration is characterized by altered mechanical function and a loss in proteoglycans, decreasing the osmotic pressure [1-3]. It is believed that disc degeneration originates in the nucleus pulposus (NP) due to the disc's avascular environment and limited nutrient diffusion [4]. Tissue engineering methods are being widely investigated to recapitulate the mechanical and structural properties of various musculoskeletal tissues. Although autologous cells may be the optimal cell source for tissue regeneration, cells from degenerated tissue may not be able to reproduce the properties of the healthy native tissue. Cells from the outer annulus fibrosus (AF) may provide a potential cell source for growing NP tissue replacements. Therefore, the objective of this study was to culture AF cells in a 3D hydrogel scaffold to promote chondrogenic-like behavior and to evaluate the effect of supplementing the culture media with TGF-β_3.

Materials and Methods: Intervertebral discs were obtained from adult mongrel dogs after euthanasia was performed for reasons unrelated to this study. The AF was dissected from the disc with a scalpel and cells were allowed to crawl out of the tissue for expansion in serum media with growth factors [5]. Passaged cells were cast into a slab of 2% w/v agarose (Sigma, Type VII) at a concentration of 30 x 10^6 cells/mL. Cylindrical samples were cored from the slab (dimensions: ϕ = 4 mm; thickness = 2.34 mm) and cultured in serum-free chondrogenic media supplemented with 10 ng/mL of TGF-β_3. At day 14, TGF-β_3 was removed from the culture media for half of the samples (release group), while the other half continued to receive growth factor (continuous group). The compressive Young's modulus, glycosaminoglycan (GAG) and collagen content were measured every two weeks (n = 5 for each time point). Transient application of TGF-β_3 was examined, as this protocol has been shown by our laboratory to enhance cartilaginous tissue development in engineered tissues using immature chondrocytes.

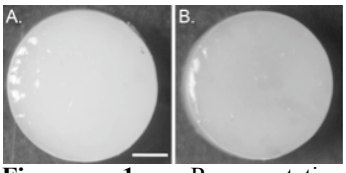

Figure 1: Representative constructs from the (A) continuous and (B) release groups. Bar = 1 mm.

Results and Discussion: The presence of growth factor in the media greatly influenced the mechanical and biochemical properties. By day 42, the constructs were uniformly opaque (Figure 1), suggesting uniform matrix deposition [5]. The Young's modulus (E_Y) of constructs cultured with continuous TGF-β_3 supplementation increased throughout the six-week culture; however, the Young's modulus of the release group dropped after transient application of TGF-β_3 in the media (Figure 2A). These results are comparable to those previously observed for adult canine chondrocytes and immature bovine NP cells [5-6]. The biochemical properties corresponded to the observed increases in mechanical properties. At day 42, the GAG content in the continuous group was 1.80 ± 0.36% and was 5X greater than the release group (Figure 2B). The collagen content in the continuous group was 1.98 ± 0.42% and was 2X greater than the release group (Figure 2C).

Conclusion: Non-degenerated NP tissue is composed primarily of proteoglycans and water, while the fibrous AF tissue has very high levels of collagen [7]. The GAG/collagen production from the AF cells in the 3D scaffold was ~1.0 in the continuous group, suggests that AF cells may provide a suitable cell source for NP tissue engineering. Future work will focus on optimizing the 3D scaffold to better mimic the mechanical properties of the native tissue [8], while maintaining an environment conducive for NP matrix production.

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References: [1] Nachemson+ *Spine*, 1979; [2] Johannessen+ *Spine, 2006;* [3] O'Connell+ *Spine,* 2011; [4] Soukane+ *J Biomech*, 2007; [5] Ng+ *Tissue Eng Part A,* 2010*;* [6] Reza+ *Biotech & Bioeng,* 2009 [7] Eyre *Int Rev Connect Tissue,* 1979; [8] Cloyd JM+ *Eur Spine J*, 2007.

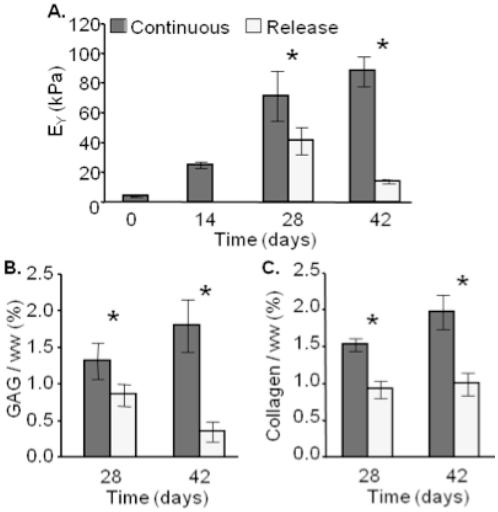

Figure 2. A) Young's modulus (E_Y) and B) GAG and C) collagen content normalized by wet weight. * denotes $p < 0.01$.