Tissue Engineered Intervertebral Discs Show Optimal Fiber Formation and Enhanced Mechanical Properties at Sub-Physiologic Levels of Glucose

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INTRODUCTION: Intervertebral disc (IVD) degeneration is implicated as one of the leading causes of lower back pain and incidences of spinal disease. Tissue engineering of the disc seeks to address damage caused by alterations to the disc's native structure and biochemical properties at the later stages of disease. However, recapitulating the complex fiber organization of the annulus fibrosis (AF) remains an enduring challenge for tissue engineering of the disc. Particularly, engineered constructs are often lacking in mechanical properties which suggests that additional in vitro techniques are necessary to supplement existing approaches. We have previously demonstrated that fiber deposition in engineered meniscal constructs can be controlled by altering glucose in culture media. Fiber formation was enhanced by cellular contractile forces but limited by glycosaminoglycan (GAG) deposition, leading to optimal formation at physiologic to sub-physiologic glucose concentrations[1]. Interestingly, typical IVD culture is performed at high glucose concentrations (~4500 mg/L) which correlate to super-physiologic blood glucose levels. Although such phenomena have been explored in the formation of meniscal fibrocartilage, the extent to which they extend to other types of fibrocartilage such as the AF has never been examined.

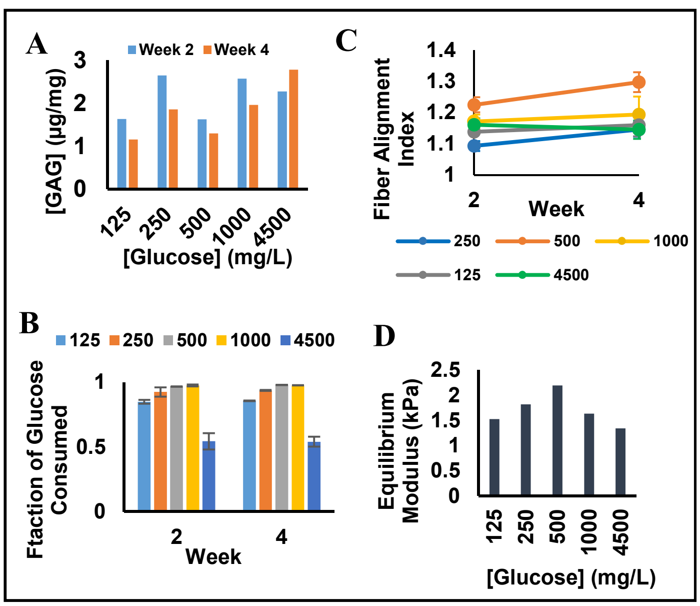
METHODS: A total of 25 tissue engineered IVD's were manufactured as described previously[2], utilizing ovine mesenchymal stem cells (MSC's). Constructs were cultured in DMEM with sub-physiologic (125, 250, 500 mg/L), physiologic (1000 mg/L), or super-physiologic (4500 mg/L) concentrations of glucose for a duration of 4 weeks to enable self-alignment of the collagen matrix. Culture media was collected for analysis of media glucose levels and replenished 3 times a week. Meanwhile representative samples from each group were collected at the end of Weeks 2 / 4 and sectioned for fiber and GAG analysis. Second harmonic generation (SHG) microscopy was used to acquire images of the collagen network as well as cell morphology via autofluorescence. Images were analyzed using a custom MATLAB code to measure fiber orientation index[3]. Constructs were also collected at Week 4 and loaded into a mechanical testing platform, then subjected to a uniaxial stress-relaxation protocol.

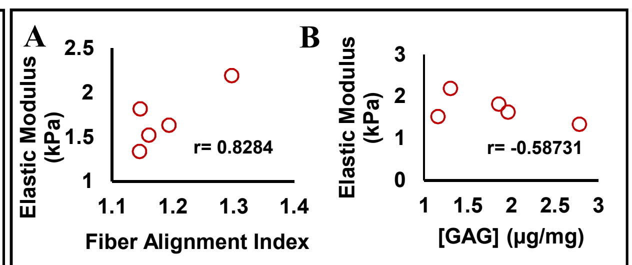
RESULTS: Though at Week 2 there appears to be no significant trend, by Week 4 there was an evident increase in GAG content with increasing glucose concentration (Fig. 1A). Throughout the entirety of the culture period, all groups consumed between 85-98% of the media glucose by each media change, with the exception of the 4500 mg/L group which consumed less than 50% (Fig. 1B). The 500 mg/L constructs exhibited the most aligned collagen bundles as early as Week 2 and again at Week 4 (Fig. 1C), which is also apparent visually (Fig. 3). In comparison, the other three culture groups achieved comparable final fiber orientation indices. Upon completion of the culture period, the 500 mg/L group demonstrated the greatest equilibrium modulus as compared to the other media groups (Fig. 1D); moreover, the moduli exhibit a higher degree of correlation with fiber alignment than with GAG concentration (Fig. 2)

DISCUSSION: The objective of this study was to determine the effect of glucose concentration on the fibril architecture in cell-laden engineered constructs, particularly the implications of traditional culture techniques. The data obtained here is consistent with previous studies showing optimal fiber formation and enhanced mechanical properties at 500 mg/L glucose. These results indicate that saturating the system with glucose may lead to prioritization of metabolic activity such as GAG production rather than fiber organization and maintenance, as all of the groups with the exception of 4500 mg/L demonstrated glucose consumption levels indicative of starvation. Additionally, it is not the presence of GAG's in the system that most directly influences the mechanical properties, but rather the alignment and organization of the collagen fiber matrix.

SIGNIFICANCE/CLINICAL RELEVANCE: Manipulation of glucose in media is a simple way to control fiber formation in engineered fibrocartilage and consequently provide tissue engineered IVD constructs with appropriate mechanical properties.

REFERENCES: 1) McCorry et al., ORS Annual Meeting, 2017 2) Bowles et al., TEA, 2010 3) Boys et al., ORS Annual Meeting, 2018





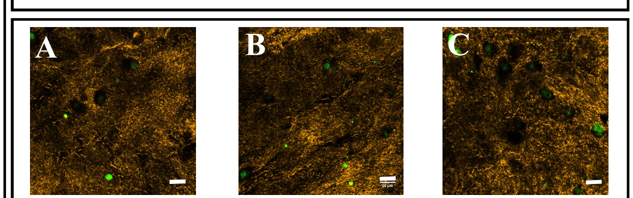


Figure 1. A) GAG concentration as a function of glucose concentration for each group at Week 2 and 4. B) Glucose consumption for each group at Week 2 and 4, n=5. C) Fiber orientation as a function of major: minor axis, n= 5. D) Equilibrium moduli for whole constructs in each group. **Figure 2.** Correlation plots of elastic modulus versus A) fiber alignment and B) GAG concentration for each group at Week 4. **Figure 3.** SHG images of representative samples, scale bar=20µm. A) 250 mg/L. B) 500 mg/L. C) 4500 mg/L. Error bars for all plots are ± SD.