Glucose Concentration in Culture Media Changes Proteoglycan Production and Fiber Formation in Tissue Engineered Meniscus

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DISCLOSURES: J. Kim (N), M. C. McCorry (N), N. Springer (N), A. Plaas (N), J. Sandy (N), L. J. Bonassar (3B; Fidia Pharmaceuticals, Histogenics, Inc., and 3DBio Corp. 5; Histogenics)

INTRODUCTION: The meniscus is a fibrocartilaginous tissue, mainly consisting of water and collagen with small portions of proteoglycans. The large diameter circumferential collagen fibers interconnected by radially oriented tie fibers give rise to the stable mechanical properties of the meniscus which plays a role in load distribution and stability of the knee[1,2]. Therefore, it is crucial to mimic the native meniscal fiber structure in the tissue engineered meniscus. Although glycosaminoglycans (GAGs) are known to support the collagen fiber structure, it has been shown that reducing chondroitin and dermatan sulfate GAG chains using chondroitinase ABC results in the enhancement of tensile strength and fiber production[3,4]. Furthermore, we found that there is a negative correlation between the fiber diameter and the amount of GAGs[5]. In addition, small leucine-rich proteoglycans (SLRPs) composed of protein-substituted GAGs have been shown specifically to be closely associated with the collagen fiber formation. Biglycan, decorin, and fibromodulin have multiple binding sites for collagen with high affinity, and their absence resulted in abnormal fiber formation while their abundance inhibited fibrillogenesis[6,7,8]. Adipose-derived stem cells have been shown to increase the production of GAGs in response to an increase in glucose concentration[9], yet how fibrochondrocytes (FCCs) and mesenchymal stem cells (MSCs) react to a change in glucose concentration and its potential influence on fiber formation has not been studied. Thus, the objective of this work was to investigate the relationships between the glucose concentration of culture media, the production of SLRPs and the resultant fiber formation.

METHODS: FCCs were harvested from the menisci as previously described[10]. MSCs were isolated from the trabecular bone marrow of 1-3 day old bovid distal femur, followed by the expansion in 2D culture until passage 4 as previously described[11]. Constructs were generated using meniscus molds with extracted collagen type I from Sprague-Dawley rat tails mixed with FCCs or MSCs at a final concentration of $25x10^6$ cells/ml and 20 mg/ml collagen gel. Each tissue engineered meniscus was clamped into a polysulfone disk by stacking a stainless steel mesh with a stainless steel bar to mimic a native mechanical boundary condition to direct collagen orientation (Figure 1A). Then, the constructs were cultured in media containing DMEM, 10% FBS, 100 µg/mL penicillin, 100 µg/mL streptomycin, 0.1 mM non-essential amino acids, 50 µg/mL ascorbate, and 0.4 mM L-proline at 37°C and 5% CO^2 for up to 2 or 4 weeks. Media used in this study had five different concentrations of glucose: 4500, 1000, 500, 250, 125 mg/L. Culture media was collected and replenished two times a week. In the end of experiments, each meniscus was weighed and sectioned to acquire samples for biochemical, western blot, histological, and custom fiber analysis.

RESULTS: Amplex Red glucose assay revealed that around 95 percentage of glucose was consumed in the groups with media of the concentrations below 1000 mg/L over the 3 to 4 day culture while only 63% and 21% of glucose in the media of the 4500 mg/L glucose concentration were consumed in the FCCs and MSCs groups, respectively (Figure 1B). The glucose concentration of culture media did not substantially affect the expression level of biglycan and decorin. However, the relative fibromodulin expression level exhibited a logarithmic linear increase in response to the increase in the media glucose concentration (R^2=0.87 and 0.99 for MSCs and FCCs, respectively) (Figure 1C). Fiber analysis based on a custom MATLAB code displayed that both the alignment index and the fiber diameter have peaks at 500 mg/L glucose concentration in both FCCs and MSCs (Figure 1D).

DISCUSSION: There is a positive correlation between the glucose concentration in media and the proteoglycan production in both MSCs and FCCs. Among several SLRPs, the fibromodulin expression displayed the highest sensitivity to changes in media glucose concentration. This correlation eventually links to the fiber formation in tissue engineered constructs. Therefore, the decrease in fibromodulin induced by lowering the media glucose concentration might account for the enhanced alignment index and fiber diameter. However, the highest fiber formation was observed at 500 mg/L concentration rather than the lowest glucose concentration, indicating that there is a sensitive balance between cell metabolism and fiber formation since glucose is the major source of energy. Moreover, western blot analysis revealed that FCCs were more sensitive to changes in glucose concentration than MSCs in terms of SLRPs production, suggesting that the reduction of fibromodulin mainly results from the change in fibromodulin production of FCCs according to the glucose concentrations. These results support the involvement of fibromodulin in fiber formation and the use of low glucose concentration media as a strategy to improve fiber formation in tissue engineered meniscus constructs. Further work will be needed to determine whether the effect of glucose on fiber formation and alignment is transduced via energy (ATP) production per se or changes in glycosylation of SLRPs and/or other matrix components.

SIGNIFICANCE: Altering glucose concentration in construct culture media affects the production of fibromodulin in both FCCs and MSCs. This change in fibromodulin production upon media glucose concentration suggests that it is possible to regulate collagen fiber formation in terms of alignment and diameter of collagen fibers in tissue engineered constructs by changing culture conditions.

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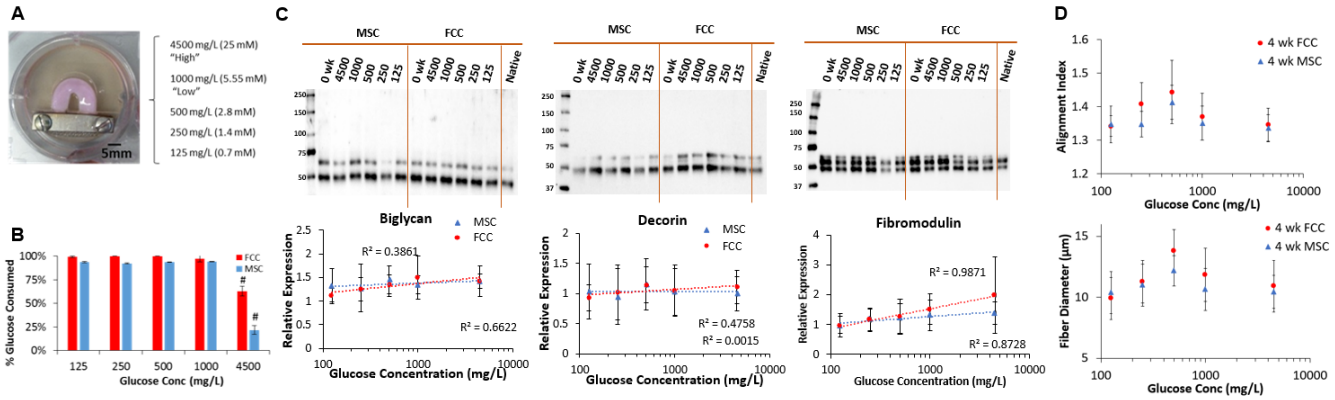

Figure 1: A) Representative image of the clamped construct **B)** Glucose consumption as a function of each construct ($^{\#}p<0.05$, n=4-6) **C)** Western blot analysis for biglycan, decorin, and fibromodulin (n=4) **D)** Alignment index and fiber diameter analysis of each construct (*p<0.05, n=4-6).