

Intermediate Concentrations of TGF- β 1 Enhance Fiber Formation and Alignment in Tissue Engineered Meniscus

Jongkil Kim¹, Lawrence J. Bonassar, PhD¹

¹Cornell University, Ithaca, NY

Jk2787@cornell.edu

DISCLOSURES: J. Kim (N), L. J. Bonassar (3B; Fidia Pharmaceuticals, Histogenics, Inc., and 3DBio Corp. 5; Histogenics)

INTRODUCTION: Menisci exhibit a distinct collagen fiber orientation, giving rise to its mechanical functions, such as load transmission or shock absorption^{1,2,3}. Therefore, it is important to mimic the native meniscal fiber structure in tissue-engineered meniscus constructs. Our group formerly demonstrated that a mechanical anchoring culture system mimicking the native meniscus horn attachment increases collagen fiber diameter and alignment resembling native meniscus, leading to enhanced mechanical properties⁴. However, the tensile modulus of the clamped construct was orders of magnitude lower than that of the native meniscus, suggesting additional supporting approaches are further required. It has been reported that transforming growth factor (TGF)- β 1 increases the contraction of a fibrochondrocyte (FCC)-mediated contractile forces and glycosaminoglycan (GAG) deposition⁵. Previously, we have noted that fiber formation is enhanced by cellular traction forces⁴ but weakened by GAG deposition⁶. Interestingly, TGF- β 1 increases both cellular traction forces and GAG synthesis^{5,7}. The net effect of TGF- β 1 at different concentrations on fiber formation within tissue engineered meniscus is unknown. Therefore, the aim of this work was to elucidate the effect of TGF- β 1 on fiber formation and alignment of high-density collagen tissue engineered meniscal constructs containing FCCs.

METHODS: FCCs were harvested from the menisci as previously described^{8,9}. A total of 24 tissue engineered meniscus constructs were generated and clamped to mimic a native mechanical boundary condition to direct collagen orientation⁴. Then, the constructs were divided into four groups and cultured in complete DMEM media at 500 mg/L glucose containing TGF- β 1 (0, 0.1, 0.5, and 10 ng/ml) at 37°C and 5% CO₂ for up to 2 weeks. Culture media was collected and replenished every three days while gross images were taken and normalized to each initial area to calculate construct contraction using ImageJ. At day 15, each meniscus was weighed and sectioned to obtain samples for GAG content and custom fiber analysis. To evaluate fiber formation, second harmonic generation images of the constructs were obtained using a Zeiss LSM 880 confocal/multiphoton inverted microscope using a 40x/1.2 C-Apochromat water immersion objective.

RESULTS: Constructs cultured with TGF- β 1 at 0.5 and 10 ng/ml began to contract after day 6 while constructs with TGF- β 1 at 0.1 ng/ml started contracting after day 9. The contraction was persistent until day 15, reaching a plateau at ~40% of their initial area. In contrast, non-treated constructs showed minimal contraction over a period of culture (Figure 1A). Wet weights of all TGF- β 1 treated groups significantly decreased compared to the non-treated group (Figure 1B). The release of GAG in media and the GAG production within meniscal constructs increased with the increasing TGF- β 1 concentrations, producing a dose-dependent response (Figure 1C and 1D). Especially, the treatment of TGF- β 1 at 10 ng/ml concentration resulted in a significantly increased GAG production compared to the other groups. SHG images of each construct showed that the non-treated group exhibited relatively thin collagen fibers compared to the TGF- β 1 treated groups. While the collagen fibers became thicker and more oriented in response to TGF- β 1 concentrations, the highest concentration of TGF- β 1 (10 ng/ml) led to a dense and compact, but unorganized fiber structure indicated by white arrows (Figure 1E). Intermediate concentration of TGF- β 1 (0.1 and 0.5 ng/ml) produced large bundles of organized collagen fibers.

DISCUSSION: The aim of this study was to investigate the optimal concentration of TGF- β 1 for tissue engineering the meniscus. One of the major ECM components of the meniscus is GAGs, accounting for compressive resistance of the meniscus. Therefore, enhancing GAG production within a meniscal construct is essential. The 10 ng/ml TGF- β 1 treated group showed the significantly increased GAG production but resulted in the compact fiber formation, indicating that high amount of GAG may disturb a native like collagen fiber formation. In contrast, 0.5 ng/ml also increased GAG production, but guided the formation of an organized collagen network.

SIGNIFICANCE: Our overall results suggest that while GAG production is important to provide the meniscal construct with proper mechanical properties, excessive production of GAGs inhibits the fiber formation, and thus the use of the intermediate concentration of TGF- β 1 would be optimal for tissue engineering the meniscus.

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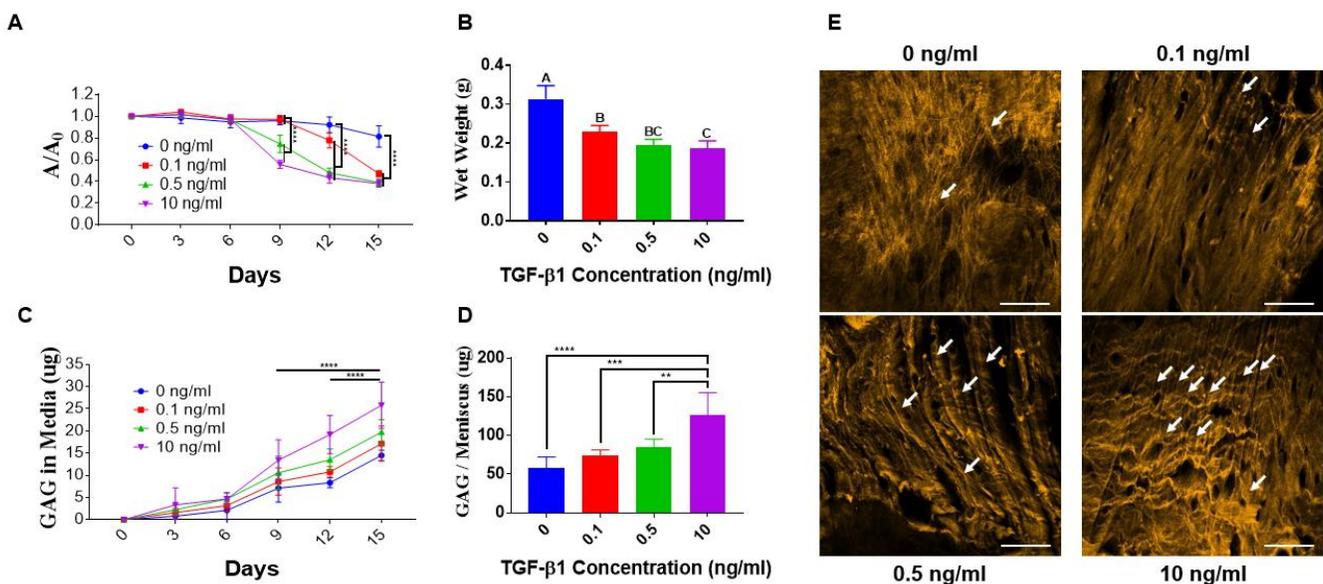


Figure 1: A) Percent area of meniscal constructs normalized to their initial areas (n = 6, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001). B) Wet weight of each construct (n = 6, data with different letters are significantly different (p < 0.05) C) GAG content in media and D) GAG content in each construct. E) Representative SHG images of each group. All statistical Error bars are \pm std (Scale bar = 50 μ m)