A 3D Culture System to Model the Ligamentum Flavum in Health and Disease

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INTRODUCTION: Lumbar spinal stenosis (LSS) is a prevalent and disabling cause of low back pain (LBP), affecting an estimated 30 million persons in the United States. Laminectomies are among the most common procedures for LSS, indicating the prevalence of ligamentum flavum hypertrophy (LFH). LFH may be described as a form of fibrosis caused by increased expression of collagen I (COL1), collagen III (COL3), concurrent with a decrease in elastin (ELN) expression.[1] Decades of work in tissue engineering has demonstrated the benefits of 3D systems for recapitulating physiologic cell responses over 2D culture. Our goal in this study is to validate a 3D system for the modeling of LF tissue and hypertrophy. While TGFβ1 has been used in tendon and ligament 3D engineering as an anabolic factor, it has also been shown to induce hypertrophy in LFH. We sought to compare the effect of TGFβ1 with a well-recognized inflammatory factor, IL1β, on LF cells cultured in 2D or 3D culture to determine what role TGFβ1 plays in a 3D LF model.

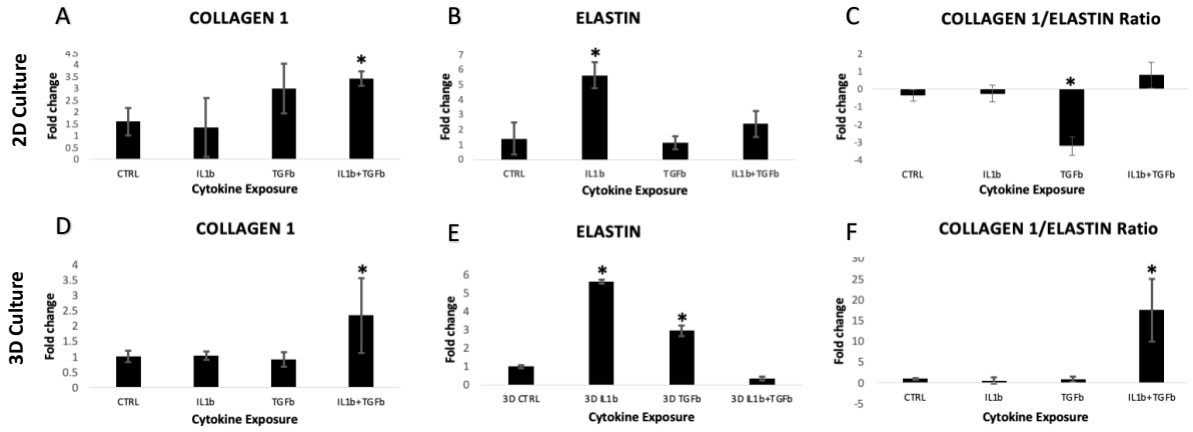
METHODS: Human LF cells were enzymatically harvested from surgical waste tissue following laminectomy according to an IRB-approved protocol. Cells were expanded to the second passage before they were encapsulated within a 0.5% (w:v) Collagen I hydrogel (BioMatrix) at 1 x 10^6 cells/ml and deposited into vacuum-actuated molds within Tissue-Train™ (FlexCell) plates to form static tensile 3D constructs.[2] Samples were matured in Basal medium (BM; DMEM, 10% FBS, P/S/F) for 7 days before exposure to inflammatory cytokines TGFβ1 (10 ng/ml; hypertrophic cytokine), IL1β (10 ng/ml) or both in BM for 24 hours. Expression of COL1, Col3 and ELN was analyzed by RT-PCR.

HYPOTHESIS: We hypothesized that (1) 3D cultures would produce more matrix than 2D culture, and (2) that inflammatory conditions (IL1β) would decrease the COL1/COL3 expression ratio and reduce ELN expression, a hallmark of the LF hypertrophy as compared to controls.

RESULTS: Constructs comprised of 1 x 10^6 human LF cells in bovine tail Collagen 1 hydrogels formed ligament-like structures over the 8-day duration of the experiment. RT-PCR demonstrated 3D cultures produced higher levels of COL1, COL3 and ELN than 2D cultures in control, unstimulated conditions. (data not shown; DNS). In 2D culture, TGFβ1 increased COL1 (Fig 1A) and COL3 (DNS) expression but had no effect on that of ELN (Fig. 1B), while IL1β greatly increased ELN expression almost 5-fold (Fig. 1B). In 3D culture, TGFβ1 did not increase COL1 (Fig. 1D) or COL3 (DNS) expression while modestly but significantly increasing ELN expression as IL1b continued to stimulate ELN expression (Fig 1E). The combination of TGFβ1 and IL1β enhanced COL1 expression in both 2D (Fig 1A&B) and 3D cultures (Fig. 1D&E) while suppressing the stimulatory effect of IL1β on ELN expression and TGFβ1 on COL3 (DNS). The COL1/COL3 ratio was significantly increased in 2D and 3D cultures (approximately 5-fold, DNS), while the COL1/ELN ratio was elevated by almost 10-fold only in the 3D cultures only (compare Fig. 1D and 1F).

DISCUSSION: In this study, TGFβ1 and IL1β stimulated different responses in human LF cells: TGFβ1 increased COL1 and COL3 expression [3] while IL1β, to our surprise, increased ELN expression. If ELN is considered a hallmark of healthy LF tissues, this may seem contradictory. Elastin, however, is also produced by fibroblasts during wound healing, and may be a part of a repair process. Interestingly, the combination of TGFβ1 and IL1β greatly reduced both COL3 and ELN expression while maintaining a high COL1 gene expression level. This resulted in an elevated COL1/COL3 ratios in both 2D and 3D cultures and a much elevated COL1/ELN ratio in 3D cultures, a metric often used in histomorphometric analysis of LF hypertrophy.[4] A dramatic decrease in Elastin protein is a hallmark of LF hypertrophy.[4] The observation that the 3D culture of LF cells can report this important feature clinically evident LF hypertrophy provides evidence that the 3D collagen 1 hydrogel can provide a more physiologically relevant microenvironment than culture on 2D plastic.

SIGNIFICANCE/CLINICAL RELEVANCE: The use of 3D models of tissues is known to report more physiologically relevant outcomes than 2D cultures for the study of tissue development, homeostasis, disease modeling and therapeutic testing. Demonstrating the culture of LF cells in 3D and their response to hypertophic and inflammatory cytokines represents a first step toward use of this model in testing the ability of novel therapeutic such as miR29a to prevent or treat LF hypertrophy.[5]



LEGENDS: Fig 1. Gene Expression Analysis of Ligament Tissue Markers in 2D and 3D cultures. Human ligamentum Flavum cells cultured for 7 days in 2D and 3D in basal media were exposed to TGFβ1 and/or IL1β and analyzed for Collagen 1 (A&D), elastin (B&E) and calculated ratios (C&F). All groups are normalized to their respective unstimulated controls. Group size is 6 per condition. The asterix represents a value less than 0.05, where only the significance of a measurement to control, untreated values (CTRL) is shown.

REFERENCES: [1] Sun C et al. (2020) FASEB J 34(8):9854-9868; [2] Yang G et al. (2013) Biomaterials 34(37):9295-306; [3] Wang S et al. (2023) Exp Mol Med 55(7):1413-1423. [4] Zheng ZY et al (2021) Orthop Surg 13(8):2457-2467; [5] Wawrose RA et al. (2023) Eur Spine J. doi: 10.1007/s00586-023-07671-y.