Engineering Functional Tendon Scaffolds Via Biochemical and Mechanical Stimulation in a Clip-Rotation System

Lin Xu[1], Zizhao Li[1], Se-Hwan Lee[1], Sung-Han Jo[1], and Su Chin Heo[1]
[1]University of Pennsylvania, Philadelphia, PA
linxu1@seas.upenn.edu

DISCLOSURES: Xu (N), Li (N), Lee (N), Jo (N), and Heo (5-4WEB Medical)

INTRODUCTION: Tendon injuries, caused by trauma, overuse, or aging, are difficult to treat and often result in poor healing. Current repair strategies frequently fail to restore mechanical strength or achieve proper tissue integration. Thus, new approaches that integrate structural, biochemical, and mechanical cues are needed. Gelatin methacryloyl (GelMA) hydrogels support cell viability activity but lack sufficient mechanical strength, while electrospun polycaprolactone (PCL) scaffolds provide structural integrity but limited bioactivity. To overcome these limitations, we developed a novel Clip-Rotation System that integrates GelMA with electrospun PCL scaffolds, preventing shrinkage and promoting alignment [1, 2]. This platform also enables controlled application of biochemical (TGFβ-1) and mechanical (dynamic tensile loading) stimuli. In this study, we investigated how these cues, individually and in combination, influence tenocyte alignment and gene expression within GelMA-PCL scaffolds.

METHODS: 10% GelMA hydrogel was prepared in PBS containing 0.5mM ruthenium (Ru, Sigma-Aldrich) and 5 mM sodium persulfate (SPS, Sigma-Aldrich). Tenocytes (isolated from bovine juvenile Achilles tendons, 6×10^6 cells/mL, P1) were encapsulated within GelMA. Aligned nanofibrous electrospun PCL scaffolds (15 × 25 mm) were secured in a custom separable clip system fabricated from polyether ether ketone (PEEK) for integration (**Fig. 1**). Scaffolds were then fabricated using a BioX bioprinter (CELLINK) by extruding GelMA hydrogel onto aligned electrospun PCL sheets within the Clip-Rotation System under the following parameters: 10 °C, 50 kPa, 0.35 mm/s print speed, and 6 rpm rotation speed, with in situ crosslinking under 405 nm light (**Fig. 1**). Constructs were cultured in either basal medium (BM) or medium supplemented with TGFβ-1 (5 ng/mL) [3]. Samples were collected at days 1, 7, and 10 for F-actin staining, orientation analysis (FiberFit) [4]. RT-qPCR of Col1, Col2, Col3, and TNC were performed on day 10 samples (**Fig. 2**). To evaluate the effects of dynamic mechanical stress, additional scaffolds were cultured in BM for 2 days before being subjected to dynamic tensile loading (4% strain, 1 Hz, 3 h/day for 3 additional days) in a custom bioreactor. Samples were collected and compared to static controls (**Fig. 3**). Statistical analyses were performed using Student's t-test and ANOVA.

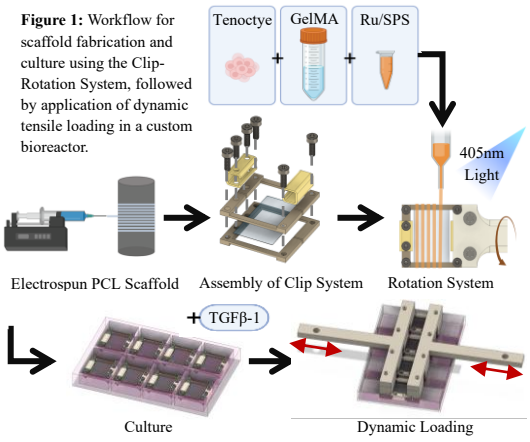

Figure 1: Workflow for scaffold fabrication and culture using the Clip-Rotation System, followed by application of dynamic tensile loading in a custom bioreactor.

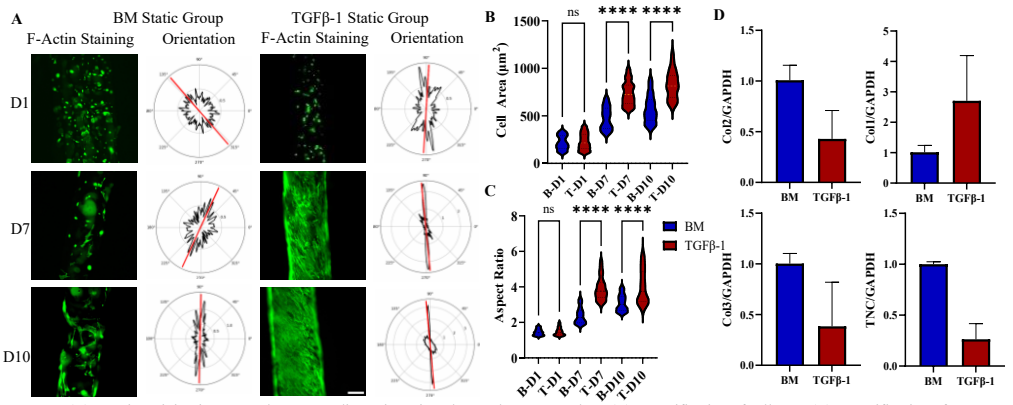

Figure 2: (A) F-actin staining images and corresponding orientation plots at days 1, 7, and 10. (B) Quantification of cell area. (C) Quantification of aspect ratio. (D) Gene expression of Col1, Col2, Col3, and TNC at day 10. (n = 3; ****p < 0.0001). Scale bar = 200 μm.

RESULTS: TGFβ-1 supplementation promoted greater cell spreading, alignment, and elongation compared to BM across all time points (**Fig. 2A–C**). Cell orientation analysis showed that the mean cytoskeletal filament orientation in the TGFβ-1 group at days 7 and 10 was close to 90°, likely reflecting the directionality of the printed hydrogel strands. By day 10, BM constructs also approached a mean orientation near 90°, but with substantially fewer cells and greater variability in alignment. At day 10, RT-qPCR revealed increased Col1 expression in the TGFβ-1 group, while Col2, Col3, and TNC were reduced (**Fig. 2D**). In the pilot loading study, no significant difference in cell orientation was observed between static and dynamically loaded BM groups on day 4 (**Fig. 3A**). However, the loaded group exhibited modest but significant increases in cell area and aspect ratio, consistent with enhanced spreading and elongation (**Fig. 3B**). Gene expression analysis further showed upregulation of Col1, Col3, and TNC in the loaded group compared to static controls (**Fig. 3C**).

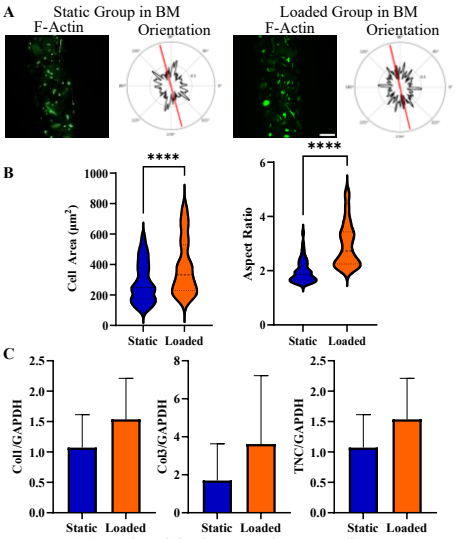

Figure 3: (A) F-actin staining images and corresponding orientation plots with/without loading at day 4. (B) Gene expression of Col1, Col3, and TNC. (n = 2). Scale bar = 200 μm.

DISCUSSION: This study highlights the complementary roles of biochemical and mechanical cues in promoting tenogenic behavior within GelMA–PCL scaffolds fabricated using the newly suggested Clip-Rotation System. TGFβ-1 supplementation enhanced cell spreading, alignment, and elongation over time, while producing a gene expression profile characterized by Col1 upregulation with reduced Col2, Col3, and TNC. This shift suggests promotion of tendon-specific matrix deposition while suppressing fibrocartilaginous and fibrotic responses. In contrast, dynamic loading on day did not significantly alter orientation but modestly increased cell area and aspect ratio, consistent with enhanced elongation under mechanical stimulation. Loading also induced upregulation of Col1, Col3, and TNC, indicating a mechanoresponsive phenotype supportive of extracellular matrix remodeling and tendon maturation. Given the limited a short 4-day loading period, these findings should be considered preliminary, and extended mechanical stimulation will be necessary to fully define effects on cell alignment and matrix organization. Future studies will also investigate the combined application of dynamic loading and TGFβ-1 supplementation to optimize tenogenic outcomes further. Overall, these findings demonstrate that both biochemical and mechanical stimulation contribute to tendon-like phenotypes, and establish the Clip-Rotation System as a versatile platform for integrating hydrogel–fiber scaffolds with controlled cues to guide tendon regeneration.

SIGNIFICANCE: This study introduces a hybrid tendon scaffold platform using the Clip-Rotation System to integrate structural, biochemical, and mechanical cues. TGFβ-1 supplementation and dynamic loading enhanced tenocyte alignment and tenogenic gene expression, demonstrating the potential of this approach to guide functional matrix remodeling and advance scaffold-based strategies for tendon regeneration.

References [1] Li+, J Nanobiotechnol 2023; [2] Gniesmer+, PLoS One 2020; [3] Koo+, Sci Rep 2024; [4] Morril+, BMMB 2016.

ACKNOLEDGEMENTS: This work is supported by the NIH (P50 AR080581) and NSF (CMMI-1548571).