Cyclical Physiological Strain Blunts TGF-β Induced Myofibroblast Activation in a Human Tendon-on-a-Chip

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INTRODUCTION: With increasing emphasis on new approach methodologies (NAMs), tissue chips have emerged as powerful platforms to generate human-relevant data that complement, and in some cases reduce reliance on, animal models. We previously engineered a human tendon-on-a-chip (hToC) featuring fibroblast-laden collagen hydrogels and a vascular flow channel separated by an ultrathin nanomembrane[1]. While this system successfully recapitulated aspects of tendon fibrosis driven by TGF-β, the rigid plastic architecture did not allow cyclic loading, a critical limitation because passive controlled motion (PCM) therapy, delivering low-amplitude, protected tendon excursions, is widely regarded as the most effective intervention to mitigate peritendinous adhesions after flexor tendon repair[2], albeit with mechanisms that remain debated. The cytokine transforming growth factor-beta (TGF-β) is known to be a key regulator of the transitioning of fibroblasts to a diseased myofibroblast state[3]. These myofibroblasts are the primary mediators of fibrotic scarring and are known to secrete excessive amounts of collagen and other extracellular matrix (ECM) components that lead to abnormal tissue stiffness and function[4]. To elucidate the mechanobiology of fibrosis in tendon, we developed a stretchable human tendon-on-a-chip (stretchToC) that can be leveraged to directly interrogate the changes in the diseased phenotypes of fibroblast laden collagen hydrogels. We hypothesize that physiological strain (4%) will ameliorate fibrosis by blunting TGF-β1induced myofibroblast activation and adhesion-like remodeling within the construct.

METHODS: The stretchToC devices were fabricated from polydimethylsiloxane (PDMS) as a monolith consisting of 6 arrayed wells, each containing two pillars between which fibroblast-laden hydrogels are tethered (*Fig. 1A*). Flanking the wells on either end are continuous vacuum chambers that connect via tubing to a vacuum pump, allowing the compliant PDMS to be actuated (*Fig. 1B, C*). A calibration curve relating the vacuum pressure applied and the resultant axial strain was established by tracking the pillar deflections in videos using a custom MATLAB code. Collagen hydrogels (~3mg/mL) were seeded with 500,000 cells/mL of human tendon fibroblasts, derived from tenolysis surgical tissue waste at the University of Rochester Medical Center under approved IRB protocols. The hydrogels are then cultured for 7 days at 37 °C and 5 % CO_2 with or without TGF-β1 (10 ng/mL) and with or without daily cyclical physiological strain (4% strain, time period = 10 sec, 1 hour duration). Contraction percentages were determined by tracing the outline of the well and the hydrogels in FIJI. After 7 days the hydrogels were fixed and stained for nuclei (Hoechst), F-actin (Phalloidin), and alpha-smooth muscle actin (α-SMA). Immunofluorescent (IF) images were captured on a spinning-disc confocal microscope and the number of, and intensity of α-SMA+ cells was determined using the image segmentation software Imaris. The alignment of the fibroblasts within the tissue was determined using a custom MATLAB code through directionality functions.

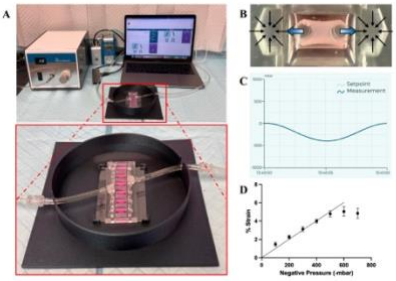

Figure 1: *(A) Experimental setup consisting of a Fluigent vacuum pump and controller, and computer interface connected to the stretchToC device housed within a culture chamber (Inset). (B) Close-up of a single well showing pressure and contraction of vacuum chambers and resultant pillar strain, (C) Example of waveform for cyclic strain (D) Calibration curve for applied pressure vs. axial strain.*

Statistics: A two-way analysis of variance (ANOVA) with multiple comparisons was used to determine differences between the four different strain/TGF-β1 combinations (-/-, -/+, +/-, and +/+), with Bonferroni-corrected multiple comparisons.

RESULTS: A univariate regression model established the relationship between the applied negative pressure and hydrogel axial strain, yielding a linear calibration between 0 and –400 mbar, corresponding to 0–4% strain (*Fig. 1D*). Overall, no statistically significant differences in fibroblast alignment were observed, although alignment appears to be primarily driven by TGF-β1 treatment (*Fig 2A-D, E*). However, the contraction of the hydrogels was significantly increased by TGF-β1 treatment but attenuated by strain. Static hydrogels contracted by 28.9% (no TGF-β1) and 40.7% (with TGF-β1), whereas strained hydrogels contracted by 11.0% (no TGF-β1) and 26.5% (with TGF-β1) (*Fig. 2F-I, J*). The proportion and fluorescence intensity of cells expressing α-SMA was increased by TGF-β1, but the application of cyclic 4% strain reduced the normalized α-SMA staining intensity. These findings support the hypothesis that cyclic physiological strain blunts TGF-β–induced myofibroblast activation.

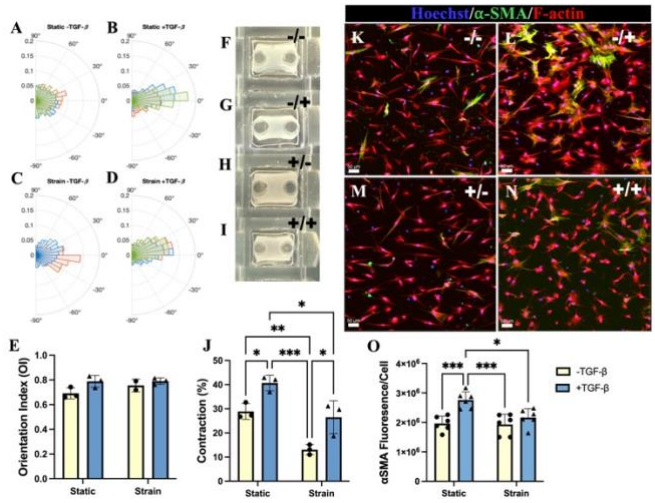

Figure 2: *Effects of cyclic strain and TGF-β on fibroblast alignment, hydrogel contraction, and myofibroblast activation. (A-D) Rose plots showing the orientation of cells, (E) Fibroblast Orientation Index, (F-I) Representative images of hydrogel contraction under different stimuli (Strain/ TGF-β 1) (J) Percent contraction of hydrogels, (K-N) Representative IF images for α-SMA (green), F-actin (red) , scale bar = 50 μm, (o) Quantification of α-SMA⁺ normalized fluorescence intensity.*

DISCUSSION: This study establishes the stretchToC as a platform for investigating the mechanobiological mechanisms of fibrotic tendon healing. Our findings demonstrated that daily cyclic mechanical strain reduces both the extent to which tenocytes are undergoing the myofibroblast transition and the hydrogel contraction, consistent with the reduced activation of the highly contractile myofibroblasts. Our findings support the hypothesis that cyclical mechanical strain exerts a protective effect against tissue fibrosis. However, the current model does not yet incorporate all the cellular and molecular components of the myofibroblast microenvironment (MME), including resident and infiltrating macrophages and the pathologic vasculature linked to fibrotic and inflammatory states[1]. Future versions of the stretchToC will incorporate the crosstalk between these cellular components to enable a more comprehensive investigation of the impact of mechanical strain on the MME. Ultimately, these studies could provide a mechanistic understanding of the well-documented clinical benefits of physical therapy after tendon injury.

SIGNIFICANCE/CLINICAL RELEVANCE: An understanding of the biological underpinnings of the mechanobiology of tendon healing could provide mechanistic insights into how controlled passive motion physical therapy reduces fibrotic remodeling and peritendinous adhesion following tendon repair. By recapitulating this effect in a human tendon-on-a-chip, the study offers a NAM platform to design clinically effective rehabilitation strategies.

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