Mechanosensitivity of Tendon using a Novel *In Vitro* Microtissue Platform

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DISCLOSURES: N/A

INTRODUCTION: The elucidation of isolated cellular pathways is a complex matrix of local and systemic signals. To isolate specific pathways, there exists a need to create accurate models of specific organs and tissues. In tendon, current models are optimal for quantifying protein expression and use for tensile testing but require high cell numbers and long periods of time to contraction. Here, **we hypothesize that** using previously established load-bearing models, we can create a high throughput, translatable model for tendon using a modular chip platform[1]. We find that primary tendon cells isolated from *ScxGFP* transgenic mice can spontaneously form 3D "tendon tissue" and undergo contraction within 3 hours and increase *ScxGFP* reporter activity. Furthermore, we show that modulating the stiffness of the modular chip alters biomechanical properties of the *in vitro* tissue, with the ability to introduce chemical dosing and genetic knockdowns to further determine *in vivo* translatability.

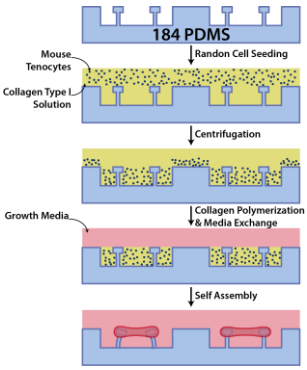

Figure 1: Generalized tendon microtissue seeding protocol.

METHODS: Silicon master molds of cantilever devices were fabricated in nanofabrication facilities using SU-8 negative photoresists. 10:1 184 PDMS (base:curing agent) was applied to the silicon wafer to generate negative cantilever stamps. Positive molds in culture dishes were generated using 5:1 184 PDMS ("stiff") or 15:1 184 PDMS ("soft"). Culture dishes were then sterilized before application of 0.2% (w/v) Pluronic F-127 solution for tissue culture. *Scx*GFP tenocytes were isolated from 3-week-old healthy mouse tails. Tails were digested in 2 mg/ml collagenase I at 37°C with shaking at 400 rpm for 45 minutes before using a 40 μm nylon mesh for cell filtration. Tenocytes were expanded in hypoxia before the first passage. For microtissue seeding, tenocytes were suspended in a rat tail collagen type I solution before being randomly seeded within cantilever wells as illustrated in **Fig. 1**. The solution was polymerized for 10 minutes before a media exchange. Each microtissue well was imaged in brightfield and epifluorescence every 15 minutes for 24 hours.
MicroBundleCompute (MBC) python code was used to generate tissue masks and track pillar deflection[2]. Briefly, binary cantilever masks were generated in FIJI before MBC processing, with cantilever spring constant approximations being used from previously described methods[3]. For *ScxGFP* expression, MBC masks were applied to the epifluorescent image to remove background signal. Custom Matlab code was used to determine tissue architectural metrics, average fluorescent signal, and integrated density. Interaction of force generation and integrated density was determined via 2-way ANOVA with Tukey's Honest Significant Difference test.

RESULTS: Tendon microtissues were tracked over 24 hours in brightfield (**Fig. 2A**) to examine cantilever deflection. Tendon microtissues seeded on stiff cantilevers generated significantly more force over the course of 24 hours compared to the tendon microtissues on the soft condition (**Fig. 2B**). Moreso, microtissues seeded onto the stiff cantilevers displayed increased *ScxGFP* over the 24 hour period, as measured by integrated density (**Fig. 2C**). Lastly, tissues on each condition shared many integrated density values; when binning for integrated density against force generation, 2-way ANOVA results deemed an interaction between *ScxGFP* reporter activity and force generation (**Fig. 2D**). All data shown has a sample size of 8 per condition.

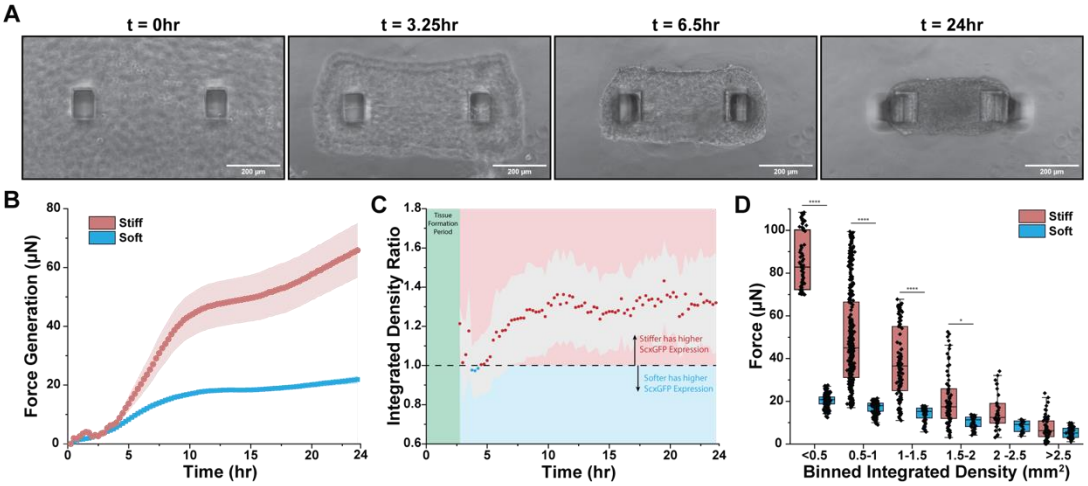

Figure 2: Tendon microtissue preliminary data on translatability. A) Depictions of cantilever deflection between 5 and 24 hr on soft cantilevers. B) Tendon microtissues cultured on stiff cantilevers generated more force over the course of 24 hr. C) Tendon microtissues expressed significantly more *Scx* when experiencing greater biomechanical load on stiffer pillars. D) Tissues binned for integrated density show influence of expression due to force generation. *<0.05, ****<0.0001.

DISCUSSION: Here, we demonstrate preliminary translatability of an updated, high throughput tendon-on-a-chip platform. By utilizing a uniaxial cantilever platform, we allow tendon microtissues to form in a manner aligned to the direction of the applied load. Additionally, we demonstrate the ability to collect real-time force generation data on cantilevers of varying spring constants, which allows us to investigate the molecular mechanisms that regulate mechanosensitive responses in tendon, *in vitro*. Thus far, we have demonstrated translatability through altered *Scx* reporter activity. Our model provides a unique platform to investigate molecular pathways in primary tenocytes using genetic and pharmacological approaches.

SIGNIFICANCE/CLINICAL RELEVANCE: Here, we begin to introduce a high-throughput uniaxial *in vitro* cantilever platform to study tendon development, genetic mutations, and pharmacological interventions to isolate tendon-specific mechanisms of action.

REFERENCES: Kalson, NS (*Matrix Bio*); Kobeissi, H (*arXiv*); Legant, WR (*PNAS*).

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