SB³C2023
Summer Biomechanics, Bioengineering and Biotransport Conference
June 4-8, Vail, CO, USA

# ACTIVE MICROTISSUE ARRAYS FOR PROBING TISSUE RESPONSE TO DYNAMIC CONDITIONING

**William P. Cortes (1), Kalyn R. Younger (2), Thao D. Nguyen (2), and Daniel H. Reich (1)**

(1) Department of Physics and Astronomy, Johns Hopkins University, Baltimore, MD, USA
(2) Department of Mechanical Engineering, Johns Hopkins University, Baltimore, MD, USA

## INTRODUCTION

The structure of soft collagenous tissues is highly dynamic and mechanoresponsive. Identifying how an applied load induces changes in tissue-level mechanical and structural properties remains challenging. Various active cellular processes occur concurrently with collagen deformation and reorganization in the extracellular matrix (ECM), and these processes and their interactions can depend on loading conditions [1]. Such structural changes in the ECM are widely relevant in physiological, pathological, and bioengineering contexts [1,2]. Thus, a more complete understanding of how loading directs the underlying processes of collagen growth and remodeling is important.

Deconvolving the micromechanisms implicated in collagen growth and remodeling and their interactions calls for a versatile, high-throughput experimental platform. Previously, Reich and coworkers developed a magnetic microtissue tester (MMT) to probe the mechanical behavior of sub-millimeter cell/ECM constructs [3,4]. In this device, arrays of cell/ECM microtissues are suspended between pairs of flexible pillars, whose deflection reports the microtissues' contractile force. A magnetic bead affixed to one pillar for each microtissue enables actuation with a magnetic tweezer (Fig. 1A-C) allowing measurement of individual tissues' mechanical properties under dynamic loads. However, due to the construction of the magnetic actuation apparatus, these experiments were constrained to minutes to hours, which limits the ability to study collagen growth and remodeling. Here, we describe the integration of the MMT arrays with a system that provides long-term mechanical stretch of the entire array (Fig. 1D,E), enabling loading conditions compatible with studying growth and remodeling processes (Fig. 1F).

## METHODS

**MMT**. MMT arrays are fabricated in poly(dimethylsiloxane) (PDMS) and consist of over 100 400 µm x 800 um wells, each containing two flexible posts. An individual microtissue is formed between the posts of each well and contractile forces exerted on the

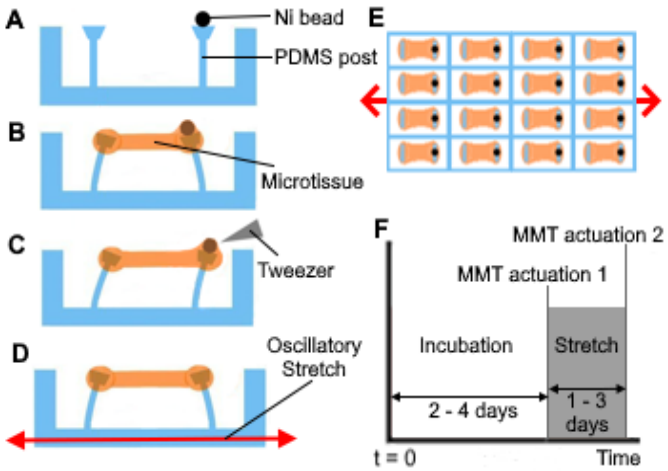


**Figure 1. Scheme for long-term loading and mechanical characterization experiment. A) An empty MMT well is seeded with B) a cell-ECM microtissue and C) subjected to a magnetic field via tweezer. D&E) The entire membrane is then subjected to stretch over times much longer than the magnetic measurement. F) Time-course of experiment.**

posts are determined from observations of the displacements of the pillar heads. Nickel beads (dia. ~100 µm) enable magnetic actuation.

**Stretching apparatus.** A commercial cell-stretcher (STREX 1400) was adapted for MMT arrays. The STREX stretching chambers are PDMS blocks with a 2 x 2 $cm^2$ well with thin bottom membranes. Cells are cultured on this membrane, and the chamber is mounted between two plates, one of which is linearly actuated to produce stretch

(Fig 2D). The unit is housed in a $CO_2$ incubator. Data are collected by unmounting the chamber and imaging the cells.

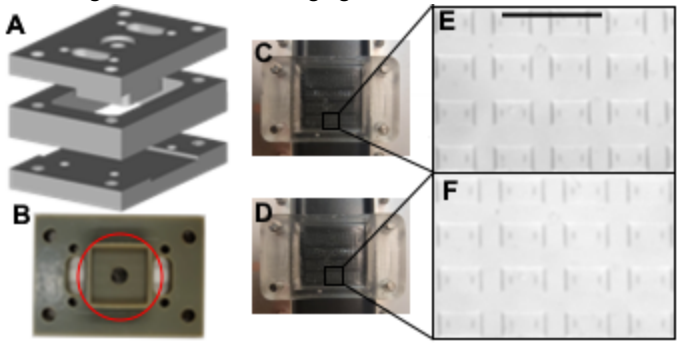


**Figure 2. Fabrication of stretching chamber. A) Molds are 3D printed and B) combined with PDMS negatives (circled in red) to produce stretch chambers. C,D) Stretch chambers on the STREX at 0 and 0.16 strain. E,F) Portions of unstretched and stretched MMT array. Scale bar: 2 mm.**

**Fabrication.** PDMS MMT negative molds were replica molded [5] and macroscopic chamber molds were produced via LCD resin 3D printing (Fig. 2A). Final chamber molds were constructed by mounting the negatives to the 3D print (Fig 2B). The final devices were cast in PDMS, yielding stretching chambers with comparable tolerances to the OEM devices, but with MMT elements patterned along the membrane. Fig. 2C,D shows an MMT chamber mounted on the STREX stretcher.

**Microtissues**. A mixture of Type I collagen ECM-analogue and adult human dermal fibroblasts (nHDFs) was centrifuged into the stretching chamber and incubated following previously described protocols [5]. The cells compacted the ECM, forming dog-boned shaped microtissues after 48 hours incubation.

**Mechanical characterization.** Microtissues were uniaxially strained by applying a triangular magnetic up-down ramp to the magnetic pillar. The tensile stress-strain relationship was determined by observing the pillar displacements and microtissue geometry via microscopy. Tissue elastic modulus was calculated over the initial 0 – 0.1 strain region of the stress-strain curve. Selected microtissues were probed for active recovery for 5 minutes after magnetic actuation.

**Stretching protocol.** Microtissue-seeded chambers were subjected to oscillatory stretch (0.1Hz 2-10%). for 24 hours after 48-hour microtissue formation. Tissue stiffness was measured by magnetic mechanical characterization before and after loading.

## RESULTS

The strain applied to the MMT arrays under stretch was characterized via videos of MMT chambers without microtissues subject to strains up to 0.16 (Fig. 2E,F). The well-to-well variation of the peak strain over the array was < ±0.01 as determined by measuring the displacement of embedded MMT well walls.

This work represents the first characterization of the active dynamics of nHDF microtissues under magnetic stretching in MMTs. The displacements of the pillars under magnetic stretching of such tissues are illustrated in Fig. 3A. An example of the stress and strain response to magnetic actuation is shown in Fig. 3B, along with the resulting stress-strain loop (Fig. 3C). The microtissue stiffness was comparable to other cell-ECM constructs studied on this platform [3,4]. The stress-strain curves displayed hysteresis during actuation arising from tissue viscoplasticity, followed by a longer-term recovery driven by active force generation of the cells in the tissue [3].

Initial stretching experiments up to 24 h duration have shown that the microtissues remain in good contact with the pillars and retain their characteristic morphology under low-frequency oscillatory strain (0.1Hz 2-10%) and are amenable to magnetically actuated mechanical characterization post-loading (Fig. 4). Preliminary results are indicative of stiffness increases compared to unconditioned controls. Further studies of changes in ECM morphology will also be presented. We also note that results to date indicate strain-rate dependence in the dynamic response (Fig. 3C,4), these effects will also be discussed.

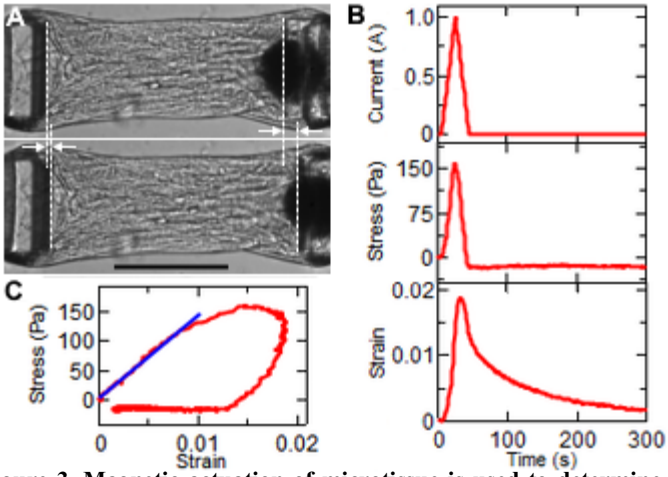


**Figure 3. Magnetic actuation of microtissue is used to determine tissue mechanical properties. A) Pillar displacements used to extract mechanical properties. B) Current pulse applied to magnetic tweezer over 40 s. Shown below are the accompanying stress response and applied strain (Initial strain rate: 0.001/s). C) The resulting stress-strain curve (red) and linear fit (blue) over 0-0.01 strain yield an elastic modulus of 15 kPa. Scale bars: 200 µm.**

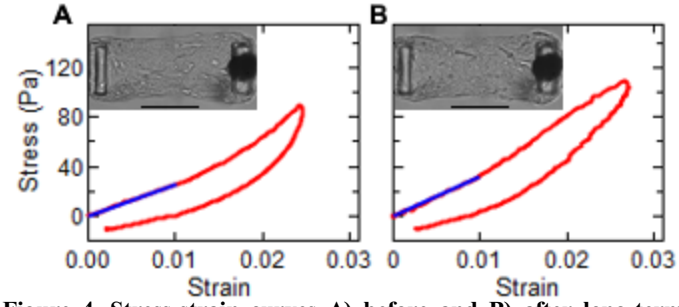


**Figure 4. Stress-strain curves A) before and B) after long-term conditioning, with tissue images inset. The elastic modulus observed was 2.5 kPa and 3.2 kPa respectively (Initial strain rate: $4x10^{-4}$/s). These measurements did not probe the active recovery phase. Scale bars in insets: 200 µm.**

## DISCUSSION

Here, we describe the construction of an apparatus for applying long-term mechanical stimulation to a microtissue array model. At low oscillatory strain rates, MMTs were shown to be compatible with long-term conditioning. The fabrication process described can be used to integrate a variety of bio-MEMs architectures with long-term conditioning, and the compatibility of this platform with many standard cell culture assays allows for a wide range of experimental studies that can provide new input for the development of models that relate cellular function and ECM mechanics to the effects of applied loads.

**ACKNOWLEDGEMENTS** We acknowledge support from the U.S. National Science Foundation (NSF): CMMI-2032922.

**REFERENCES** [1] Vernerey, F. and Farsad, M. J Mech Behav Biomed Mater. 4(8):1683-1699, 2011. [2] Frantz, C. et al., *J Cell Sci*. 123(24):4195-4200, 2010. [3] Zhao, R. et al., *Adv Mater*. 25(12):1699-1705, 2013. [4] Liu, A. et al. *Sci Rep*. 6(1):33919, 2016. [5] Legant, W. et al., *Proc Natl Acad Sci,* 106(25):10097-10102, 2009.