Dynamically Tunable Substrates to Probe Tendon Pathophysiology

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Introduction: With the FDA's recent move to phase out mandatory animal testing in preclinical trials, the demand for translatable *in vitro* models has never been greater. Yet some tissue-on-a-chip platforms still fall short, particularly musculoskeletal tissue like tendon, where no reliable, translatable models exist. Tendon mechanics are central to both healthy function and disease, with elastic moduli averaging ~300 kPa *in vivo* and shifting dramatically with pathology, yet these changes remain poorly understood. To address this, we developed two magnetorheological elastomer (MRE) formulations and a protocol designed to model tendon pathology. MREs are biocompatible and uniquely powerful: when exposed to a magnetic field, they undergo tunable, reversible changes in modulus, offering a dynamic platform to replicate the mechanical environment of diseased tendon and to test potential therapies in ways static models simply cannot. Importantly, our MREs match native tendon elastic modulus (~300 kPa) and can be shifted ±100 kPa on demand, allowing us to ask: how does altering modulus beyond (above or below) the physiologic range reshape tenocyte phenotype and genotype?

Methods: Two materials were generated to have an elastic modulus achieving a 300 kPa elastic modulus: low-range (LR) to range from 200-300 kPa and high-range (HR) to range from 300-400 kPa. MREs were fabricated by mixing 527 polydimethylsiloxane (PDMS), 184 PDMS, carbonyl iron particles (CIPs), and toluene. 5g of prepolymer was poured into a 35mm dish and cured at 60 °C for 16.5 and 16hrs. Modulus was altered by exposing MREs to various magnetic field strengths with 0.25-inch thick, 1.25-inch diameter N52 disc permanent magnets. Microindentation was performed using a 0.9 mm radius ruby probe to determine elastic moduli; 20 mm parallel plate rheology for shear and loss moduli at 0.1% strain between 0.2 and 20 rad/s. Strain sweeps confirm 0.1% strain is within the linear region.

ScxGFP tenocytes were isolated from 3-week-old healthy mouse tails. Cells were taken from male and female mice (2 each). While cells from different mice were not mixed, sex-dependent differences were not observed. Tails were digested in 2 mg/ml collagenase I at 37 °C for 45min filtration with a 40 µm nylon mesh. Tenocytes were expanded in hypoxia before the first passage. Before cell seeding, MREs were sterilized and functionalized with 100 µg/mL collagen type I. To probe cell morphology dynamics, cells were seeded at 2,000 cells/cm^2. After 24hrs of pre-conditioning at 300 kPa, the elastic modulus was altered by ± 50 or 100 kPa. Tenocytes were fixed with 4% paraformaldehyde at 0.25, 0.5, 1, 2, and 3hrs. Cells were stained with Phalloidin and DAPI and imaged using an upright microscope with a 10× objective. A custom CellProfiler pipeline was developed to objectively quantify cell morphology (**Fig. 1**).

Results: To enable controlled modulation of substrate modulus, MRE formulations were fine-tuned to produce two distinct ranges: a low-range (LR) dynamic modulus of 200–300 kPa and a high-range (HR) dynamic modulus of 300–400 kPa. These ranges were selected to approximate physiologically relevant moduli experienced by tendon cells in healthy versus fibrotic or healing tissues. The formulations are shown in **Fig. 2A**. For each, 1 part toluene was added per 5 parts PDMS (by mass) to reduce prepolymer viscosity and promote uniform dispersion of carbonyl iron particles (CIPs). Mechanical characterization confirmed that LR MREs achieved elastic moduli of 206.9 ± 8.2, 251.9 ± 28.1, and 297.2 ± 21.9 kPa when fabricated with 0, 1, and 3 0.25-inch-thick magnets. HR MREs reached 298.9 ± 14.3, 351.5 ± 25.7, and 399.6 ± 28.5 kPa using 0, 2, and 3 magnets (**Fig. 2B**, n=27). Corresponding storage moduli were 58.3 ± 1.9, 80.4 ± 1.2, and 94.0 ± 0.6 kPa for LR MREs, and 107.8 ± 2.0, 120.3 ± 0.8, and 132.8 ± 3.2 kPa for HR MREs (**Fig. 2C**, n=3).

The tunable MREs provided a platform to examine how tenocytes respond to discrete shifts in modulus. In static controls, higher modulus led to expected changes in cell morphology: increased area (**Fig. 3A**) and aspect ratio (**Fig. 3B**) and decreased form factor (**Fig. 3C**). Under dynamic conditions, cells exposed to softening or stiffening events showed a transient phenotypic shift toward the new modulus before returning to their pre-conditioned morphology, most apparent in cell area (**Fig. 3D**) and aspect ratio (not shown). Interestingly, form factor did not exhibit mechanical memory in the stiffening condition (not shown). Conditions and time points were analyzed from n=9 images.

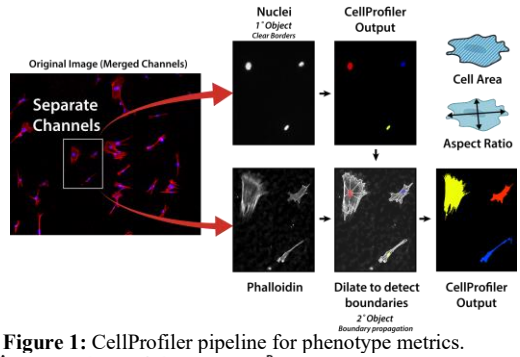

Figure 1: CellProfiler pipeline for phenotype metrics.

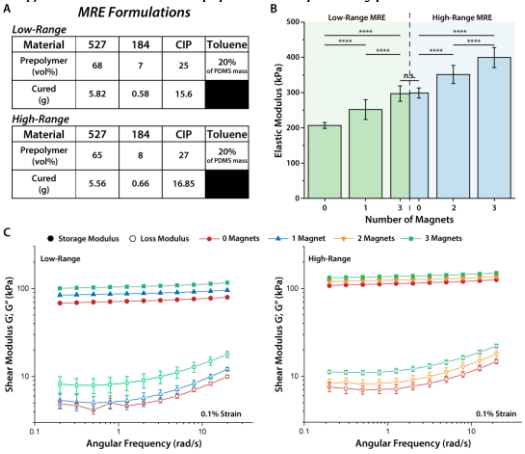

Figure 2: Formulation and mechanical properties of MREs.

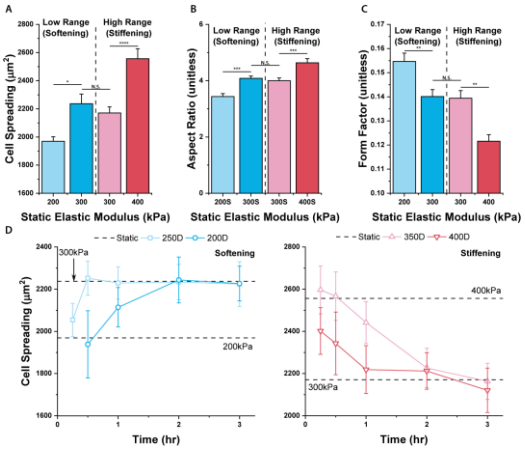

Figure 3: Cell phenotype alterations in response to static and dynamic elastic modulus changes.

Discussion: These results demonstrate that the MRE platform provides a reproducible method to tune substrate modulus within physiologically relevant ranges, enabling controlled transitions between soft and stiff environments. Tenocytes responded to increasing static modulus by spreading, elongating, and reducing their form factor, consistent with established mechanobiological behavior. Under dynamic conditions, cells initially adapted their morphology toward the new modulus before gradually reverting to their pre-conditioned state, revealing a form of mechanical memory. Interestingly, this memory was feature-specific: cell area and aspect ratio both displayed reversible adaptation, while form factor exhibited memory only under softening, not stiffening. Moreso, direction and magnitude of the modulus change seem to generate rate and magnitude differences of the dynamic changes: with area, a 50 kPa stiffening event generates a larger cellular response than the 100 kPa stiffening event. The rate and magnitude changes will be extracted to provide further insights. Together, these findings suggest that tenocytes integrate both current and past mechanical cues, highlighting the importance of mechanical history in regulating tendon cell behavior. Limitations include increased time generating MREs compared to the original formulation and no surface cues, such as patterning, to increase cellular alignment, as seen *in vivo*. Future work includes RT-qPCR and quantification of ECM deposition on the same timeframe. The data from these experiments will provide further insights into how tenocytes remodel their local environment in response to a softening or stiffening event, such as an acute tendon injury.

Significance/Clinical Relevance: These studies have resulted in novel substrates that have tunable and reversible mechanical properties in response to a magnetic field. These substrates have been used to study cellular phenotype dynamics of tenocytes in response to sudden mechanical perturbations.