SI Appendix

Device Characterization. Cantilever dimensions were measured for 40 cantilevers (20 each from 2 independent substrates) for each geometry under differential interference contrast (DIC) microscopy using a 20X Plan-Apochromat objective on a Zeiss Axiovert 200M (Carl Zeiss MicroImaging, Inc.). The elastic modulus of the PDMS was measured via uniaxial tensile testing on an Instron 5848 Microtester equipped with a 50 N load cell (Instron, Canton, MA). 3 strips each from 3 independent preparations were measured using digital calipers, preloaded to 0.5 N and then stretched at a strain rate of 1%/second. The elastic modulus was calculated via a linear fit of the stress vs. strain curves over the region of 10-20% strain. These measurements were then used to numerically estimate cantilever spring constants and variability under linear bending assumptions (SI Fig 1A,B). To confirm our numerical estimations, we experimentally measured the spring constants from 3 sequential deflections of 20 cantilevers of each geometry (10 each from 2 separate substrates) using calibrated glass micropipettes (see supplementary methods for micropipette calibration). Our experimental measurements fell within the range of values predicted by linear beam bending with an average spring constant of 0.098 ±0.017 μN/μm for the flexible geometry and of 0.397 ± 0.039 μN/μm for the rigid geometry (SI Fig 1C). For deformations spanning the range of those exerted by microtissues, all plots were linear with an average R^2 value of 0.96 and 0.99 for the flexible and rigid geometries respectively. Spring constant estimates from sequential deflections of individual cantilevers had an average repeatability of 5% (standard deviation). The inherent uncertainty of each force measurement was incorporated into all statistical comparisons using standard propagation of error formulas.

Micropipette Calibration. Glass micropipettes (World Precision Instruments, Sarasota, FL) were calibrated by hanging thin wire weights (0.538-6.000 mg) from the needle tip. Needles were mounted horizontally with a micromanipulator on a microscope stage. A second micromanipulator was used to position the weights on the end of the needle tip. The needle tip was imaged with and without the weights in place. The difference in focal planes between the images of the loaded and unloaded tip was used to determine the amount of needle bending. All measurements were repeated a minimum of three times and the average values were used to generate load vs. displacement curves, which were then fit using linear regression. Two separate needles (k=0.3474 μN/μm, R^2=0.996 and k=0.0609 μN/μm, R^2=0.999) were calibrated in order to measure the stiffness of PDMS cantilevers with different geometries.

Bio-Chemo-Mechanical Model of Microtissue Contractility. We envisage a two-dimensional tissue, thickness b, lying in the $x_1 - x_2$ plane on top of cantilever posts with its normal along the x_3-direction (Fig. 3B). A bio-chemo-mechanical model has been devised that captures the formation and dissociation of stress fibers, as well as the associated generation of tension and contractility (1). The stress fiber formation is initiated by a nervous impulse or a biochemical or mechanical perturbation that triggers a signaling cascade within the tissue. We model this signal as an exponentially decaying pulse having level C (which may be thought of as the concentration of Ca^{2+} or Rho) given by

$$C = \exp(-t_i / \theta), \qquad (1)$$

where θ is the decay constant and t_i is the time after the onset of the most recent activation signal. The formation of stress fibers is parameterized by an activation level, designated η ($0 \le \eta \le 1$), defined as the ratio of the concentration of the polymerized actin and phosphorylated myosin within a stress fiber bundle to the maximum concentrations permitted by the bio-chemistry. The evolution of the stress fibers at an angle ϕ with respect to the x_1 axis (Fig. 3B) is characterized by a first-order kinetic equation

$$\dot{\eta}(\phi) = \left[1 - \eta(\phi)\right] \frac{C\bar{k}_f}{\theta} - \left(1 - \frac{\sigma(\phi)}{\sigma_0(\phi)}\right) \eta(\phi) \frac{\bar{k}_b}{\theta}, \tag{2}$$

where the overdot denotes time-differentiation. In this formula, $\sigma(\phi)$ is the tension in the fiber bundle at orientation ϕ, while $\sigma_0(\phi) \equiv \eta \sigma_{\max}$ is the corresponding isometric stress at activation level η, with $\sigma_{\max}$ being the tensile stress at full activation ($\eta = 1$). The dimensionless constants $\bar{k}_f$ and $\bar{k}_b$ govern the rates of stress fiber formation and dissociation, respectively. In turn, the stress σ is related to the fiber contraction/extension rate $\dot{\varepsilon}$ by the cross-bridge cycling between the actin and myosin filaments. The simplified version of the Hill-like equation (2) employed to model these dynamics is specified as

$$\frac{\sigma}{\sigma_0} = \begin{cases} 0 & \dfrac{\dot{\varepsilon}}{\dot{\varepsilon}_0} < -\dfrac{\eta}{\bar{k}_v} \\ 1 + \dfrac{\bar{k}_v}{\eta}\left(\dfrac{\dot{\varepsilon}}{\dot{\varepsilon}_0}\right) & -\dfrac{\eta}{\bar{k}_v} \le \dfrac{\dot{\varepsilon}}{\dot{\varepsilon}_0} \le 0 \\ 1 & \dfrac{\dot{\varepsilon}}{\dot{\varepsilon}_0} > 0 \end{cases} \tag{3}$$

where the rate sensitivity coefficient, $\bar{k}_v$, is the fractional reduction in fiber stress upon increasing the shortening rate by $\dot{\varepsilon}_0$.

A two-dimensional constitutive description for the stress fiber assembly has been derived by noting that the axial fiber strain rate $\dot{\varepsilon}$ at angle ϕ is related to the material strain rate $\dot{\varepsilon}_{ij}$ by

$$\dot{\varepsilon} \equiv \dot{\varepsilon}_{11}\cos^2\phi + \dot{\varepsilon}_{22}\sin^2\phi + \dot{\varepsilon}_{12}\sin 2\phi, \tag{4}$$

The average stress generated by the fibers follows from a homogenization analysis as

$$S_{ij} = \frac{1}{\pi}\int_{-\pi/2}^{\pi/2}\begin{pmatrix} \sigma(\phi)\cos^2\phi & \dfrac{\sigma(\phi)}{2}\sin 2\phi \\ \dfrac{\sigma(\phi)}{2}\sin 2\phi & \sigma(\phi)\sin^2\phi \end{pmatrix} d\phi\,. \tag{5}$$

The constitutive description for the tissue is completed by including contributions from passive elasticity, attributed mainly to the collagen matrix. The passive stresses act in parallel with the active cellular response, whereupon additive decomposition gives the total stress:

$$\Sigma_{ij} = S_{ij} + \left(\frac{E\nu}{(1-2\nu)(1+\nu)}\varepsilon_{kk}\delta_{ij} + \frac{E}{(1+\nu)}\varepsilon_{ij}\right), \tag{6}$$

where δ_{ij} is the Kronecker delta and (for a linear response) E is the effective Young's modulus and ν the Poisson ratio.

The corresponding characterizing parameter for the *actin* (or stress-fiber) orientation imaged in the experiments is not straightforward. Most techniques only image the dominant stress-fibers. The very fine mesh-work of actin filaments is not visible when standard epifluorescence or confocal microscopes are used. Thus, to correlate the observations with the predictions we define a circular variance

$\Gamma = 1 - (\bar{\eta} / \eta_{\max})$, used by (3) that provides an estimate of how tightly the stress-fibers are clustered around a particular orientation ϕ. Here $\eta_{\max}$ is the maximum polymerization level, which occurs at orientation ϕ_S while $\bar{\eta}$ is an average value defined as $\bar{\eta} \equiv 1/\pi \int_{-\pi/2}^{\pi/2} \eta \mathrm{d}\phi$. The value of Γ varies from 0 to 1, corresponding to perfectly distributed and totally aligned distributions, respectively.

Finite Element Method Simulations of Microtissue Contractility. The microtissue contractility model was implemented in ABAQUS (Dassault Systemes) as a user-defined material as described previously (1, 3). The tissue was modeled using membrane elements (M3D4 in ABAQUS) of unit thickness and the response solved for in a finite strain setting. Solutions are presented using an average element size of 1 μm. Mesh sensitivity studies confirmed that reducing the mesh size further did not significantly change the results. Cantilevers were modeled as rigid plates constrained to move in the $x_1 - x_2$ plane. Dimensions of these 2D plates were same as the top face of the cantilevers used in the experiments. The displacement d_i of the plates within the $x_1 - x_2$ plane is constrained by a spring of stiffness k such that the force F_i applied by the tissue is related to d_i via the relation $F_i = kd_i$. Spring elements are modeled using the SPRING1 option in ABAQUS. The values of spring constant *k* were chosen to match with the cantilever stiffness used in the experiments. The contact between the tissue and the 2D plate, representing the top face of the cantilever, is implemented by employing the TIE CONSTRAINT option between the tissue and the periphery of the rigid plate in ABAQUS.

Unless otherwise specified, the reference material parameters used in this study were the same as those previously published in (1). To capture the effects of the collagen matrix density and cantilever stiffness as well as variance in tissue type specific contractility, parametric analysis was conducted for the passive Young's modulus (*E*) and the maximum tension capable of being exerted by the stress fibers ($\sigma_{\max}$); refer to (1) for further details about these parameters. Suitable agreement to experimental results was found using E=5 kPa, 15 kPa, and 25 kPa for microtissues constructed from 1.0, 1.75, and 2.5 mg/ml collagen respectively (SI Table 1). These values gave very close agreement with experimental measurements of microtissue forces under each condition and are in the range of previously published values for tissue-populated collagen matrices (4). Maximum isometric stress, $\sigma_{\max}$, was determined to be 250 kPa. The non-dimensional reaction rate constants are $\bar{k}_f = 10$, $\bar{k}_b = 1$, $\bar{k}_v = 2$ with $\dot{\varepsilon}_o = 3.0 \times 10^{-3}\ \mathrm{s}^{-1}$ and the Poisson ratio $\nu = 0.3$.

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