Supplementary Information

***Stroke Volume Analog on a Chip - In Vitro* Hydrodynamic Model of Cardiac Pumping Efficiency**

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MTF Kinematics

MTF film kinematics were based on our previously described thin film deflection model[28]. Briefly, the thin film's motion was reconstructed during contraction based on three assumptions. First, that the curvature of a MTF can be described using Stoney's equation for anisotropic thin film bilayer deformation. This implies contraction along a cylinder, well describing the motion of perpendicularly aligned ($\alpha = 0°$) MTFs. Second, to account for angled hysteresis, we assumed that the cantilever's bending state is the linear combination of a straight and angled bending state defined by the isotropic relaxation of the PDMS and anisotropic contraction of the muscle tissue respectively. Finally, to account for the diminished contractile strength exhibited by angled films, we assumed that total contractile strength scales proportionally to the area of contributing muscle tissue (which is reduced as surfaces become pinned in the angled state). Based on these we were able to the approximate thin film kinematics.

These assumptions yield the following governing equations for thin film deflection. First, Stoney's modified equation, which has previously been used to measure contractile force in other MTFs.[36–38]. This is given by:

$$\sigma_c = \frac{\bar{E} S_b{}^2}{6 S_f \left(1 + \frac{S_f}{S_b}\right) R} \tag{Eq. S1}$$

where σ_c, is the tissue contractile stress, R is the radius of curvature for a cylinder created by the contractile film, $\bar{E}$ is the Young's modulus of the elastic thin film, S_f is the film thickness and S_b is the thickness of the cardiomyocytes. This served to model perpendicularly aligned thin films ($\alpha = 0°$), but did not encompass the hysteresis observed in angled films ($\alpha \neq 0°$). To account for this, we reasoned that cantilever deformation could be approximated as a linear combination of

isotropic relaxation provided by the PDMS and anistropic contraction by the muscle tissue, such that

$$\chi_c(t) = \big(1 - T(t)\big) \cdot \chi_\alpha(t) + T(t) \cdot \chi_0(t) \qquad \text{(Eq. S2)}$$

Where χ_c is the set of vector coordinates describing the observed (combined state), while χ_α is the set of vector coordinates for the muscular contractile state, and χ_0 is the set of vector coordinate for isotropic state. Given this, T(t) is then a function describing how to transfer between these two states, or the relative percentage of each state at a given time, t . To approximate the transfer function, T(t), here we used the square of the first derivate of the radius of curvature function, $R(t)$, as this captured the normalized rates of curvature change.

Finally, we accounted for the region of muscle tissue which was pinned, and could not contribute to muscular contraction. Previous FEM models indicated that diagonally aligned MTFs presented a compressed segment at the base of the MTF which was 'pinned'[35], and therefore unable to contribute to cantilever deformation. To account for this pinned segment, contractile forces were reduced proportionally to the amount of pinned area.

Thrust Produced by Biohybrid Thin Films

Thrust was approximated based on previous approaches proposed by Noca et al.[43] and as previously applied to vibrating polymer thin films[44-46]. Neglecting viscosity, gravity, and compressibility, thrust in the horizontal direction, τ_x ,is given by:

$$\tau_x = \rho_f \frac{\partial}{\partial t} \int_S u_x \, \partial\Omega + \rho_f \int_{\partial S} u_x (u_x \cdot n_x) \, \partial l + \int_{\partial S} P \cdot n_x \, \partial l \qquad \text{(Eq. S3)}$$

Where P is pressure, t is time, u_x is the fluid velocities' horizontal component, and n_x is the horizontal component of the unit vector tangent to the control surface. Applying this to our computational models, each term can be explicitly determined within the simulation, however this

becomes more challenging for experimental measurements. In particular, quantify pressure fields

with the local specificity needed is difficult to do directly. To approximate these pressure fields,

we used pressure reconstruction methods as previously outlined by Dabiri et al.[64,65] (**Fig 2.f**) ,

which allowed for the evaluation of the total thrust generated by the thin films.

Dose-Response Curve Fitting

Sigmoidal curve fitting function was given as:

$$S(c) = j \frac{e^{k(c-c_0)}}{1 + e^{k*(c-c_0)}} + i \qquad \text{(Eq. S4)}$$

where c is the dosage concentration, c_0 is the effective dosage and, $j, i,$ and k are empirically

determined fitting parameters. These we found to be as follows ($c_0 = 10^{-6.61}$ $M, j=1.42$,

$i=6.86$, and k $= 2.97$) for WT hiPSCs treated with isoproterenol.
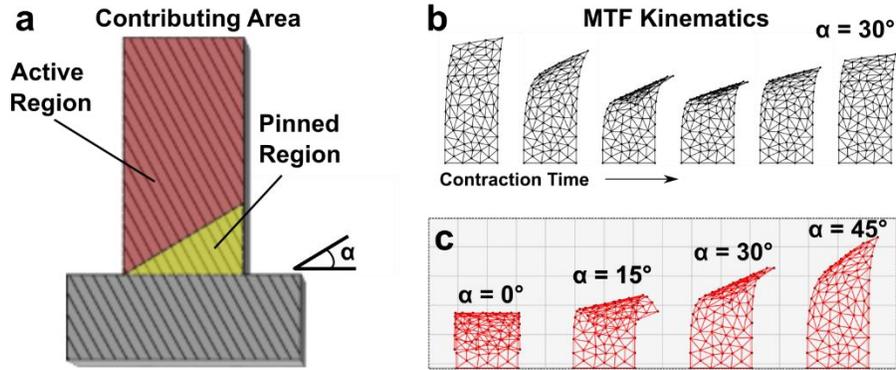
Supplemental Figures
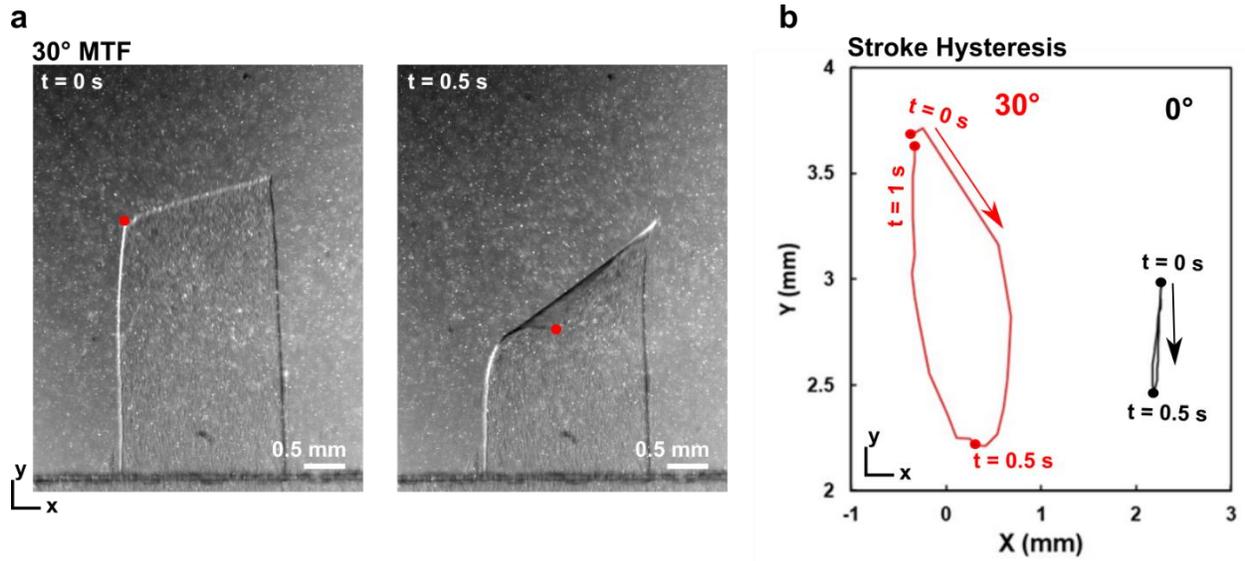
Supplemental Fig. 1 MTF Angled Stroke Hysteresis. (**a.**) Brightfield micrographs of an angled MTF (α=30°) taken at two different timepoints (t = 0s – left, t = 0.5s -right), with the upper left free-edge highlighted in red. (scale bars 0.5 mm) (**b**) Path of travel for the indicated free-edge over a single stroke, for representative angled (α=30°, red) and perpendicular (α=0°, black) MTFs, showing increased stroke hysteresis in the angled thin film case. Path of travel given as the 2D projection in the x-y plane.
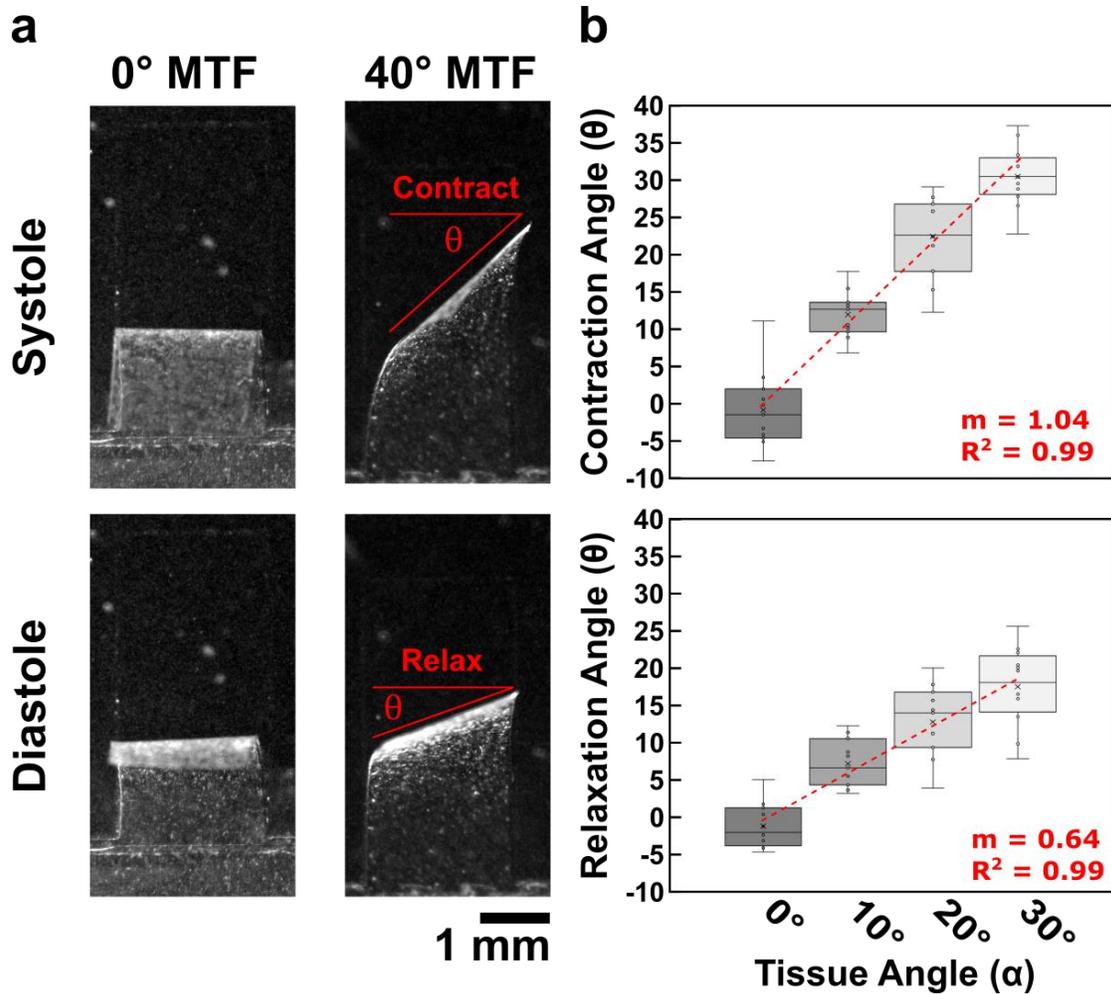
**Supplemental Fig. 2 Systolic and Diastolic Angles Kinematics**. (**a.**) Example darkfield micrograph of perpendicularly aligned (α=0°, left) and angled (α=40°, right) MTFs during peak contraction (upper) and mid relaxation (lower). Red highlighted region shows example measurements of contraction and relaxation angles, θ. (**b.**) Corresponding ensemble measurements of contraction (upper) and relaxation (lower) angles as a function microcontact printed tissue angle, α. Red dotted line denotes a linear fit of the average measured angle, with slope m (m = 1.04, R^2=0.99 & m = 0.64, R^2=0.99 respectively) (n = 10 cantilevers for each test condition).
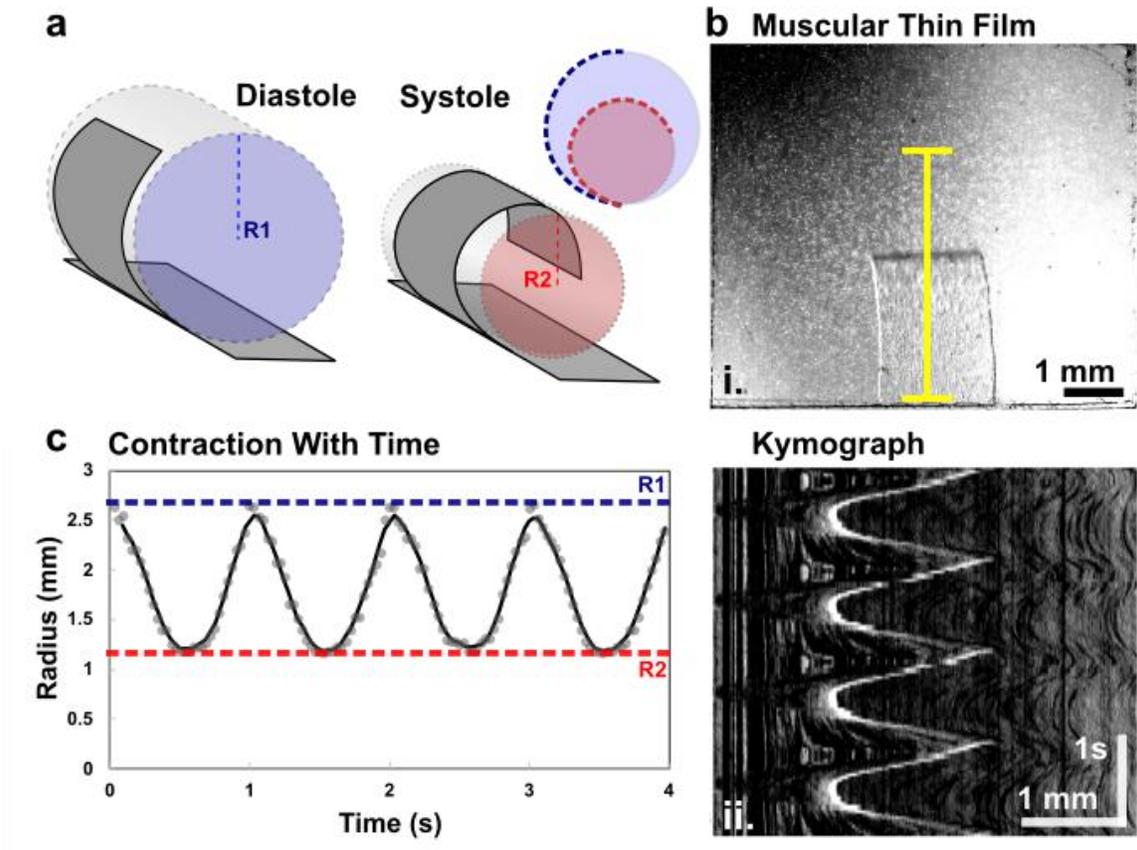
Supplemental Fig. 3 Extracting Curvature Kinematics. (a) Schematic depicting muscular thin film (MTF) contraction. MTFs project their length onto an imaginary cylinder of changing radii during contraction, with a larger cylinder during diastole (R1) and a smaller cylinder during systole (R2). (**b**) Example brightfield micrograph of a perpendicularly aligned MTF (**i.**) ($\alpha=0°$.) during contraction. with corresponding kymograph (**ii.**) projected from the highlighted region of interest (yellow) (**c**) Contraction curvature as a function of time for the example MTF as determined by the kymograph.

Supplemental Fig. 4 Angled Kinematic Models. (a) Schematic depicting the contributing and pinned regions of a muscular thin film, as defined by the contact printing angle α. The pinned region, is pushed into the underlying substrate and contributes minimally to the overall deflection generated by the film. **(b-c)** Example reconstruction of MTF contraction for a angled MTF (α = 30º) across different time points (**a,**) and at the same time point (**b.**, peak systolic contraction) for films with different contact printing angles α.
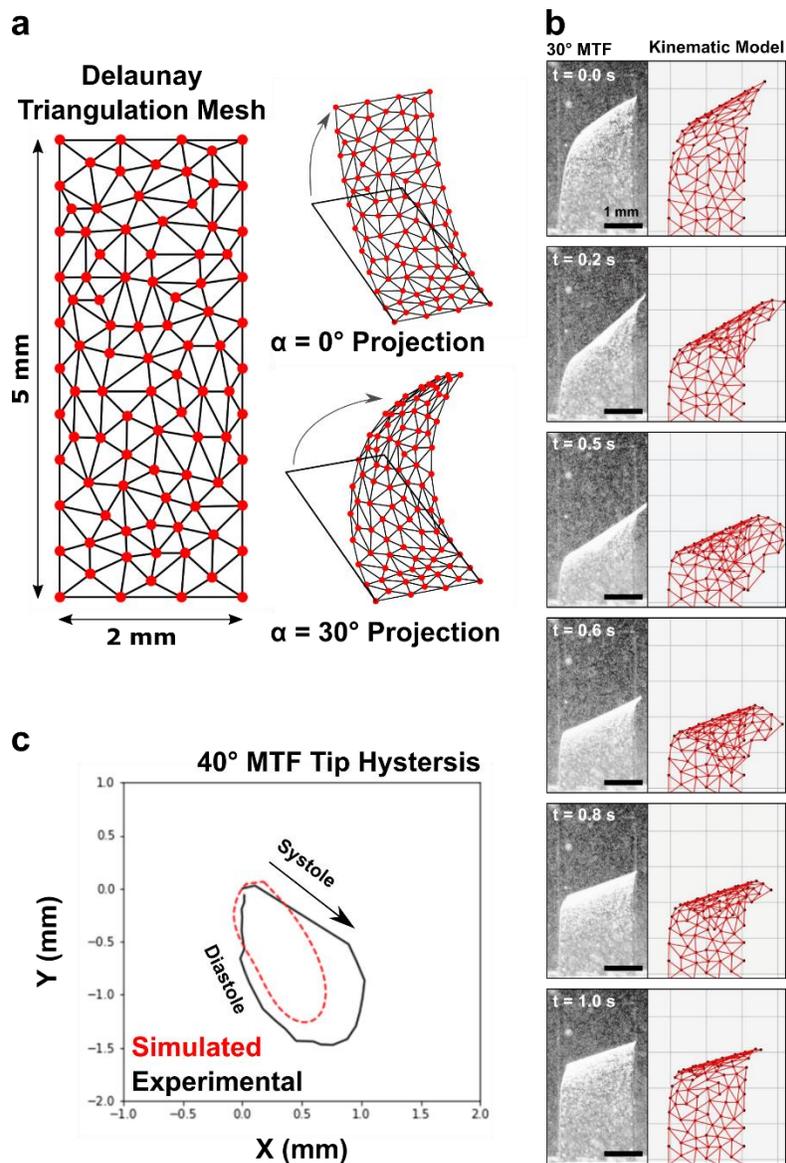
Supplemental Fig. 5 Kinematics Comparison. (a) Vertices mesh of 2x5 mm cantilever formed using Delaunay triangulation. Vertices marked in red, with connecting triangles outlined in black. 3D projection also shown for the perpendicular (α=0°, upper) and angled case (α=30°, lower). **(b)** Brightfield timeseries of an angled MTF contracting (left) with the corresponding analytical kinematic projection (right), showing that the MTF kinematic model approximates the angled motion of the MTFs. **(c)** Path of travel for the free-edge of a MTF over a single stroke, given for a representative experimental angled cantilever (α=40°, black) and for the kinematic model (α=40°, black), showing similar stroke hysteresis in both cases. Path of travel given as the 2D projection.

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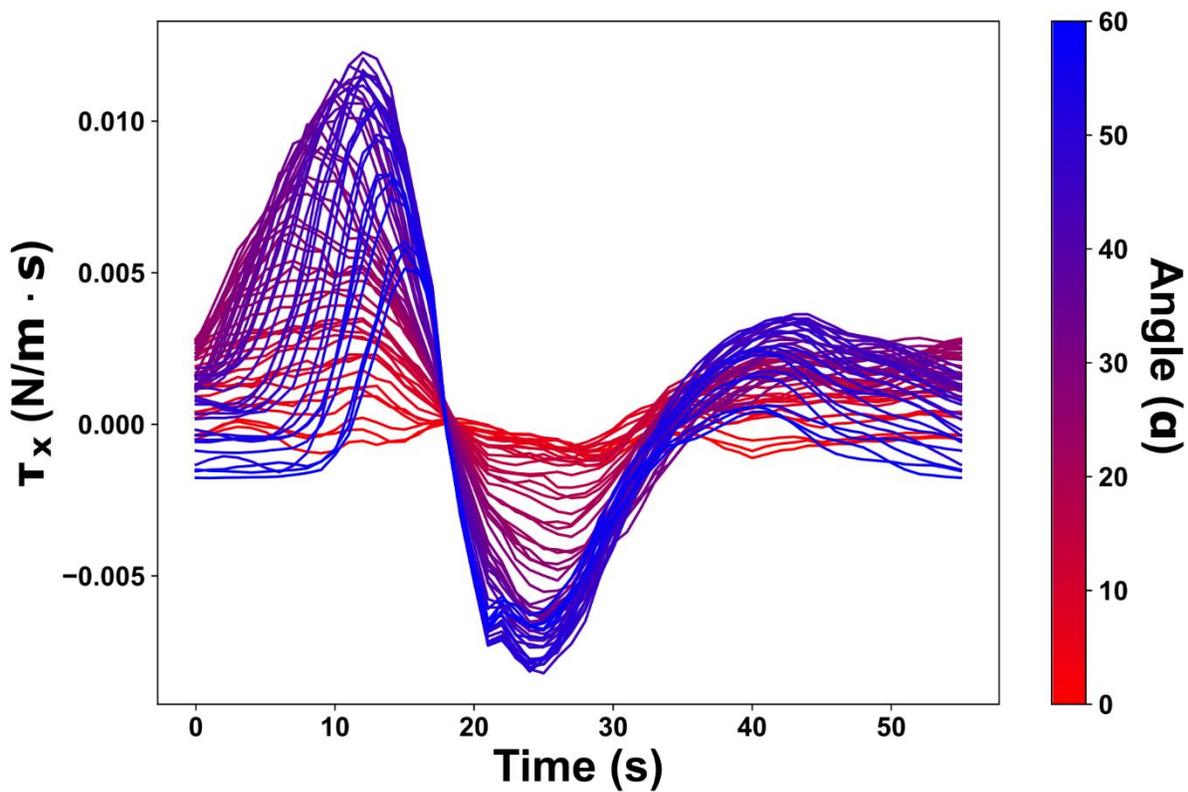
Supplemental Fig. 6 LBM Simulated Horizontal Thrust Generated by Angled MTFs. LBM simulated instantaneous thrust generated in the horizontal direction by angled MTFs during cantilever contraction, given as a function of microcontact print angle. Thrust calculated for angled thin films (α=0-60°) using a control region of interest surrounding the cantilever, and not the entire simulation domain.
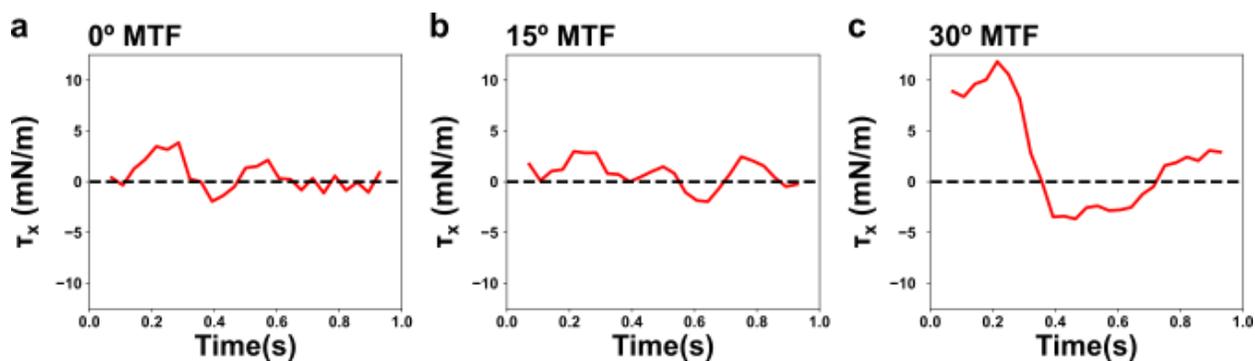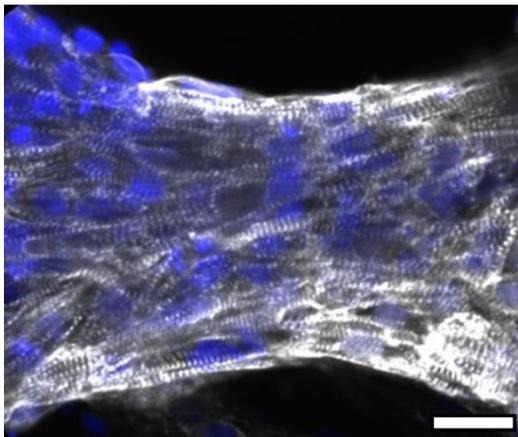
Supplemental Fig. 7 Horizontal Thrust Generated by Angled MTFs. Instantaneous thrust generated in the horizontal direction by angled MTFs during cantilever contraction. Thrust given for representative angled thin films (**a** $\alpha=0°$, **b** $\alpha=15°$, **c** $\alpha=30°$) by integrating over a control region of interest surrounding the cantilever. Thrust is only measured in the XY directions and is given in units of N/m, where integrating the thrust over the z-depth of the image would provide the total thrust generated. Thrust is shown to be generated as a function of microcontact print angle.
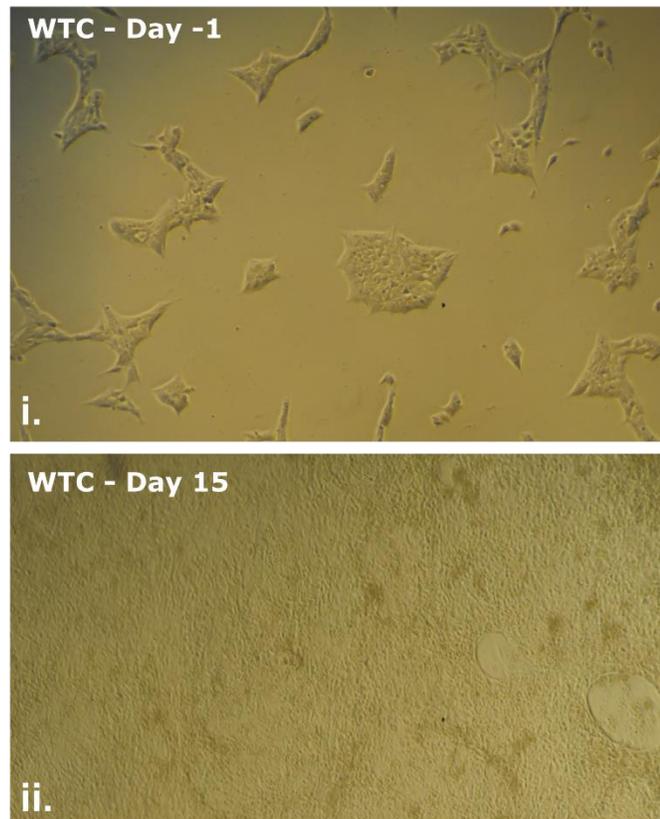
a Differentiation Protocol

Day	
Day 0	Chir
Day 2	-
Day 4	IWR-1
Day 6	-
Day 8	-
Day 10	
Day 12	Condit. — Sodium
Day 14	Purge — Lactate

b

c

WTC - Day -1

i.

WTC - Day 15

ii.

Supplemental Fig. 8 Stem Cell Differentiation. (**a**) Schematic depicting the protocol for differentiation stem cells into cardiomyocytes. Protocol uses sequential modulation of Wnt signaling activity, using an agonist (Chir) and then a Wnt/β-catenin inhibitor (IWR-1). From there cells are returned to regular growth media, before a sodium lactate condition step, follow by a subsequent glucose purge. (**b**) Immunofluorescent stain of α-actinin (gray) positive WTC cells after 15 days of differentiation (Dapi -blue, scale bar, 100 μm). (**c**) Brightfield micrograph of the differentiation process, depicting small stem cell islands at the beginning of the process (**i.**), which form into a syncytium of differentiated myocytes after 15 days (**ii.**).

Supporting Movie Captions

Movie S1. Angle Dependent Contraction. Example of MTFs contracting, (**a&b**) showing angle dependent contraction dynamics (NRVM, 1Hz field stimulation). (**c**) High magnification view of aligned cardiomyocytes cultured on the MTF surface (NRVMs, spontaneous contraction).

Movie S2. Simulating Angled MTFs. (**a**) Simulated kinematics model of different angled MTFs contraction ($\alpha=0^{\circ}$-60°). (**b**) Comparison of stroke kinematics between experimental (left) and simulated (right) angled MTF. (**c**) Simulated fluid velocity fields for different angled MTFs, showing a top-down view (upper) and side-on view (bottom) (red indicates cantilever triangle meshes).

Movie S3. Measuring MTF Stroke Volume. (**a**) Example brightfield video of a angled MTF contracting ($\alpha=45^{\circ}$, NRVM, 1Hz field stimulation), with corresponding fluorescent beads suspended in solution. (b) PIV for example different angled MTFs, with corresponding brightfield (upper) and fluid velocity field reconstruction (lower) (NRVM, 1Hz field stimulation).

Movie S4. hiPSC-CM Angled MTFs. (**a**) hiPSCs showing contraction after 15 days of differentiation (hiPSC-CM, spontaneous). (**b**) Example of hiPSC-CMs cultured on aligned substrates, showing contraction after being transferred to MTFs (hiPSC-CM, spontaneous). (**c**) Example of a whole MTF chip with four corresponding angled MTFs showing coordinated contraction (hiPSC-CMs, 1 Hz field stimulated, $\alpha=30^{\circ}$). (**d**) Fluid-dynamic readout from an example hiPSC-CM angled MTF (hiPSC-CMs, 1 Hz field stimulated, 10^{-4} M isoproterenol,

α=30º), with brightfield, fluorescent beads, PIV fluid velocity reconstruction and corresponding mass flux.