milliPillar: a platform for the generation and real-time assessment of human engineered cardiac tissues

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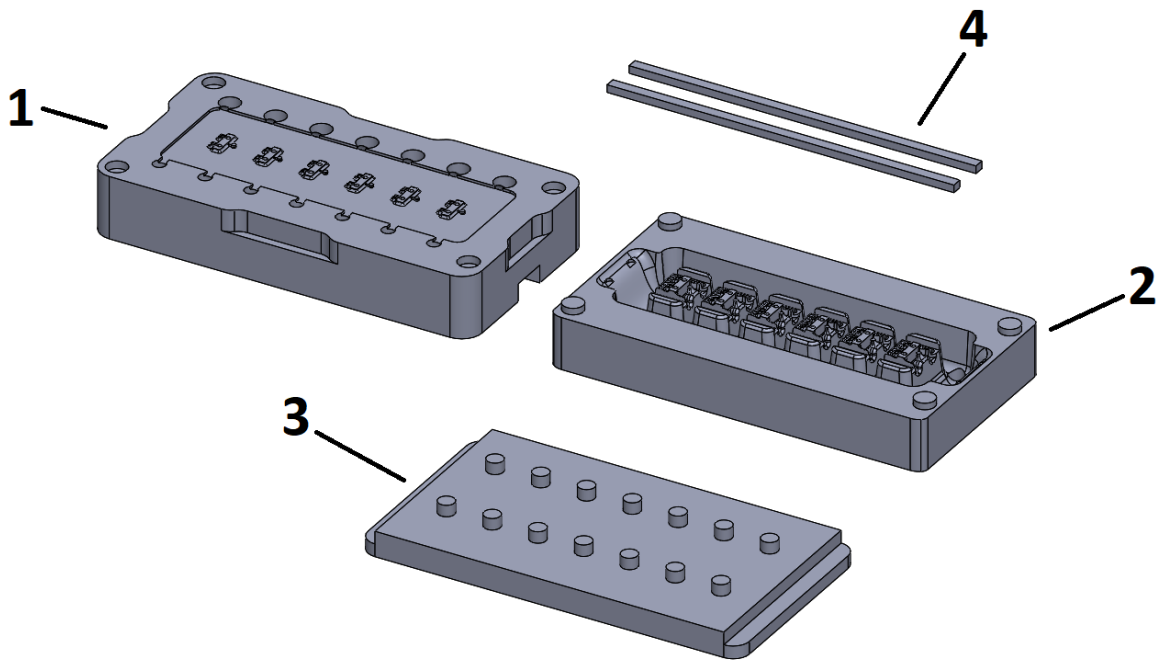
Supporting Information



1	Mold top half (“core” with filling and clamping features)
2	Mold bottom half (“cavity” with holes for casting ejection)
3	Mold ejection hole plugs
4	Carbon (graphite) electrodes

Figure S1. milliPillar molds. Visual representation of the different components of the mold as labeled in the CAD file.

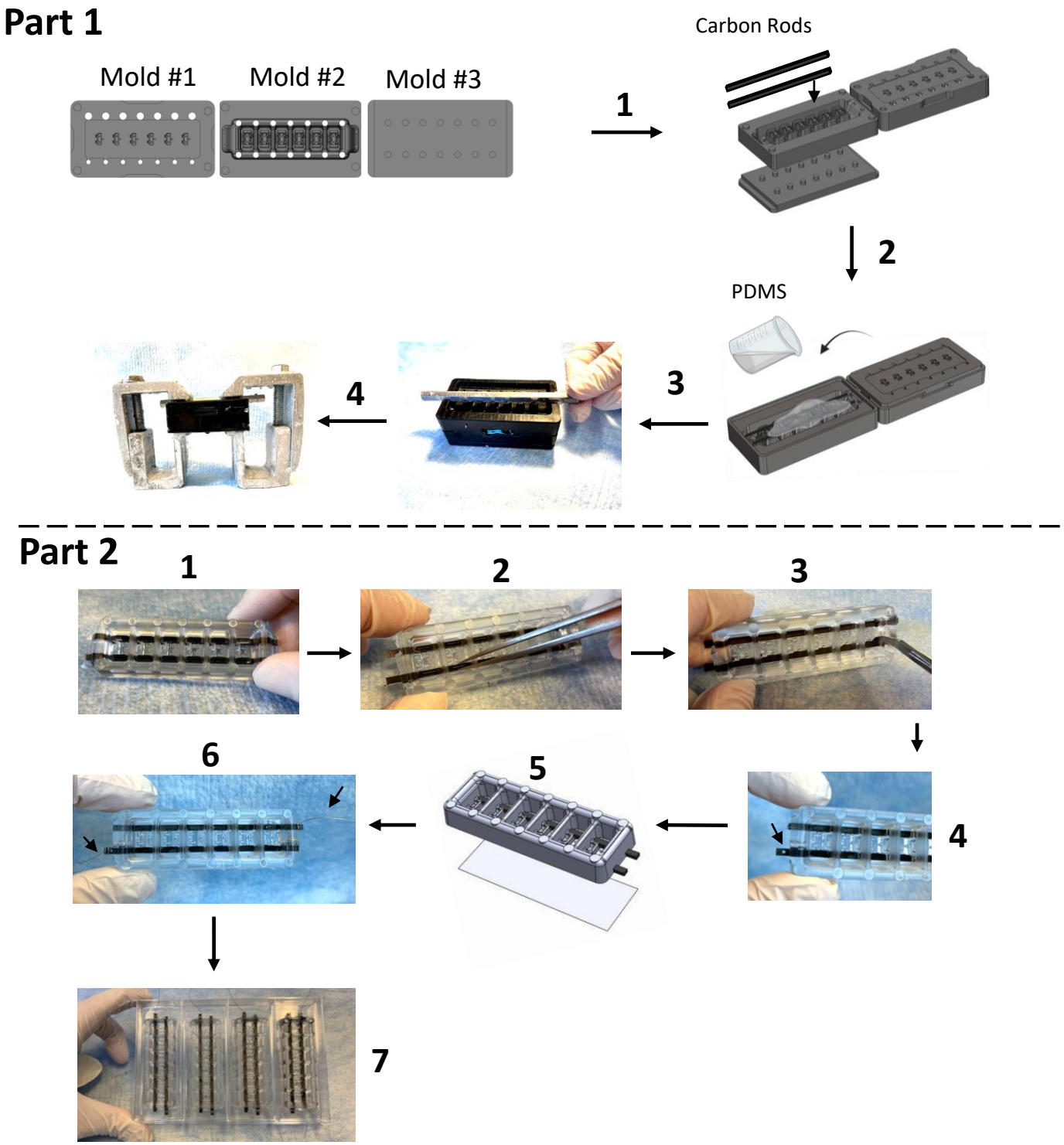


Figure S2. Preparing the milliPillar reactor. (A) Simplified graphical protocol for the milliPillar platform fabrication process; Part #2 and #3 were assembled and carbon rods were placed into designated inserts in part #2 **(1)** Degassed PDMS was used to fill part #2 and after an additional degassing step, it was topped with part #3 (2). A Hex screw was added to the top of the mold **(3)**, and two clamps were used to firmly clasp each end of the hex screw (4). **(B)** Simplified graphical protocol visualizing the PDMS cleanup and carbon rod preparation and exposure. Once the platform was safely excavated from the mold **(1)**, tweezers were used to remove the PDMS tabs to expose electrodes **(2)**. A scalpel was also used to ensure exposure of the inner surface of the rod by scratching off the PDMS film **(3)**. A hole was drilled into the ends of the carbon rod **(4)**. Oxygen plasma treatment was used to bond the bottom of the platform onto a glass slide **(5)**. Platinum wires that connect to the stimulator were tied to the ends of the rods securely **(6)**. Platforms were autoclaved and placed into a 4- well plate.

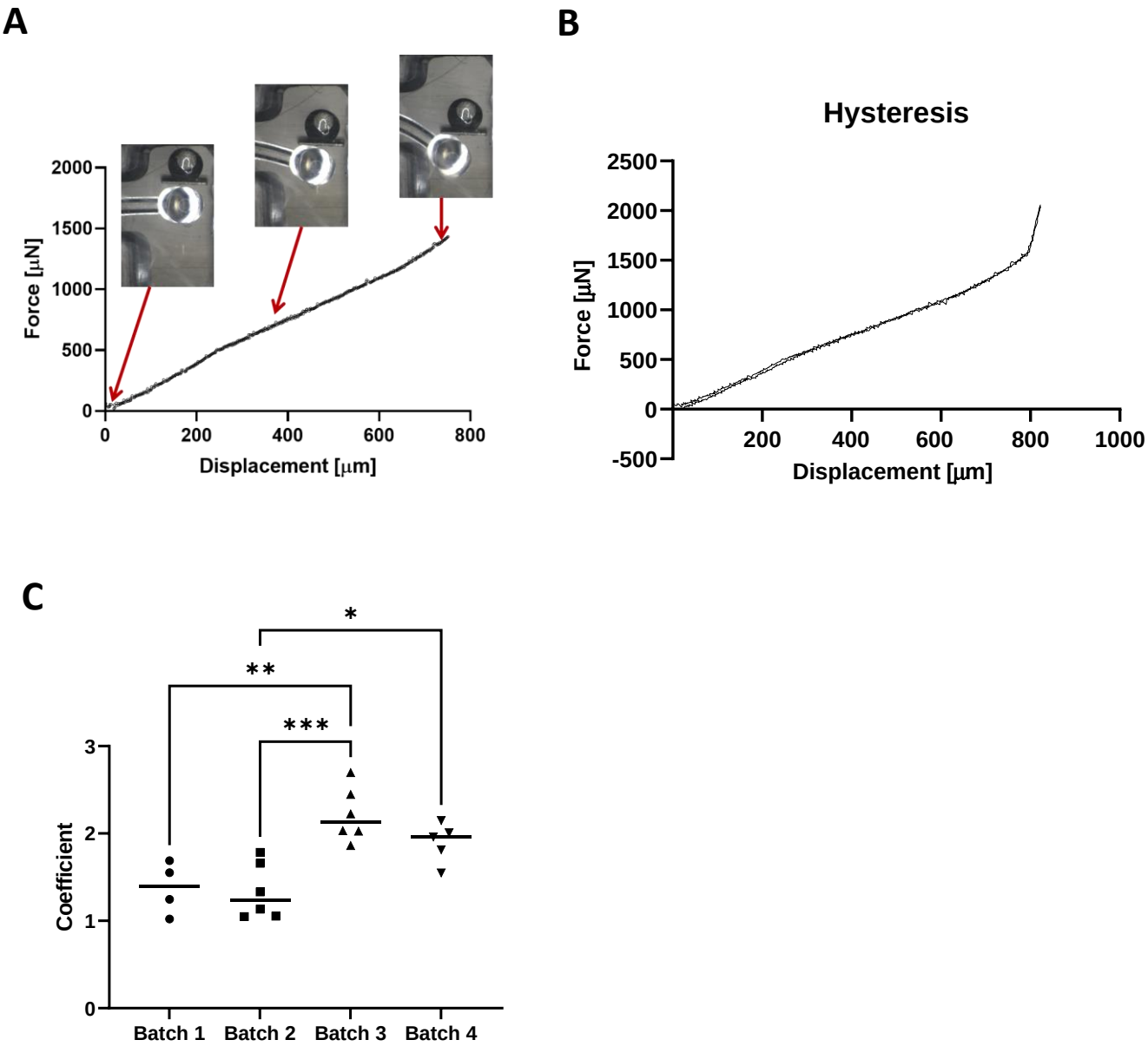


Figure S3: Mechanical testing of milliPillar PDMS pillars. (A) Force as a function of distance coefficients calculated using a microtester. **(B)**Representative force as a function of distance testing curve showing no apparent hysteresis of PDMS pillars. **(C)** Batch to batch variation among four batches of milliPillar platforms, n=4-6.

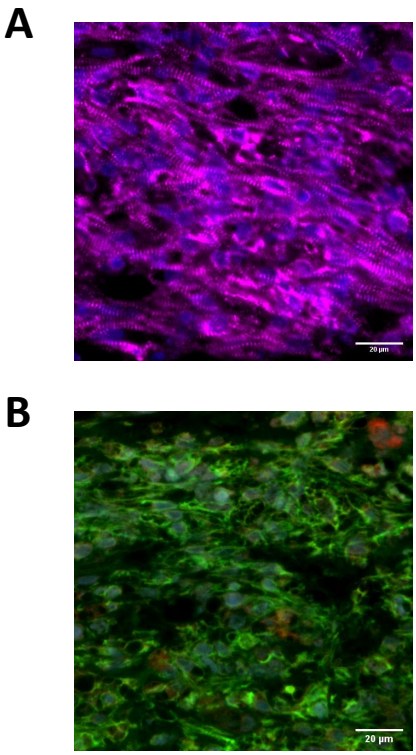
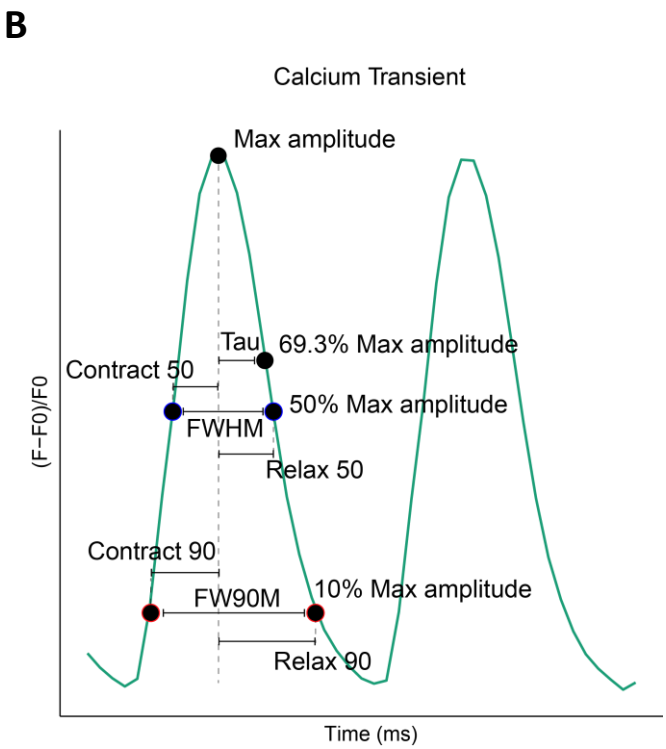


Figure S4. Alignment and ventricular characterization of milliPillar tissues cultured for 100 days.(**A**) Immunostaining of milliPillar cardiac tissues cultured for 100 days sarcomeric α-actinin (magenta).(**B**) Ventricular characterization of milliPillar cardiac tissue by immunostaining for MLC2v (green), MLC2a (red), DAPI (blue).

A

Feature	Measurements
BPM (BF/CI)	beats per minute
Tau [s] (CI)	exponential decay constant; time at which the peak intensity is reduced to 1/e of its maximum value
Full width half max (FWHM) [s] (CI)	time between the two 50% of peak intensity value
Full width 90 max (FW90M) [s] (CI)	time between the two 10% of peak intensity value
Contract 50 [s] (CI)	time between left 50% intensity to full peak intensity value
Relax 50 [s] (CI)	time between full peak to right 50% intensity value
Contract 90 [s] (CI)	time between left 10% intensity to full peak intensity value
Relax 90 [s] (CI)	time between full peak to right 10% intensity value
RR interval [s] (CI)	average distance between peaks
SDRR [s] (CI)	standard deviation of RR intervals
RMSSD [s] (CI)	root mean squared successive RR differences (RMS of change in RR interval)
Post Rest Potentiation Force Generation [uN] (BF)	force generated after 20 seconds of rest following exertion by stimulation at 4 Hz
1 Hz Force Generation [uN] (BF)	average force generated during stimulation at 1 Hz
Max Beating Frequency [Hz] (BF)	maximum beating frequency, regardless of stimulation frequency
Contraction Velocity [ms] (BF)	maximum velocity reached by the tissues during contraction
Relaxation Velocity [ms] (BF)	maximum velocity reached by the tissues during relaxation
Excitation Threshold (ET) [V] (BF/CI)	minimum voltage the tissue can capture and respond to at 1 Hz
Maximum Capture Rate (MCR) [Hz] (BF/CI)	maximum frequency the tissue can capture and respond to at 5 V



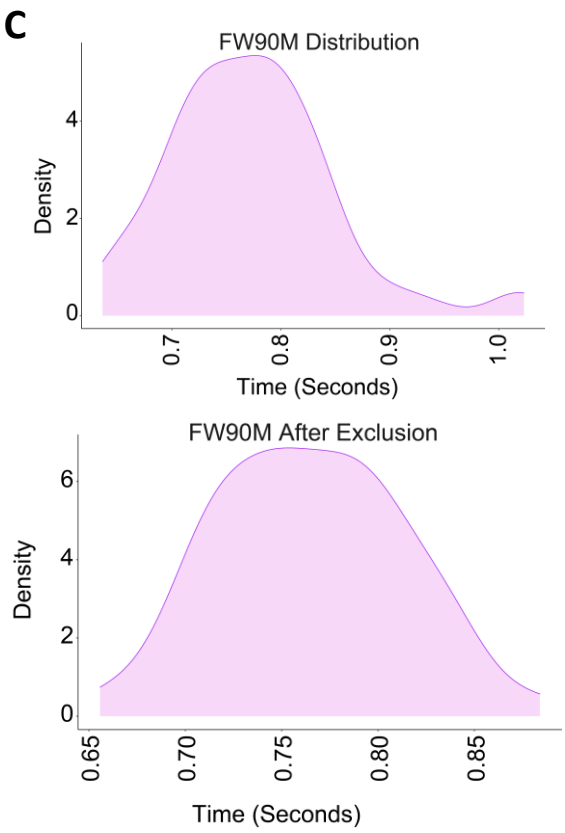


Figure S5. Detailed schematic of functional outputs from custom milliPillar software. (A) Table describing the functional metrics obtained from real-time brightfield and calcium imaging. **(B)** Schematic of a calcium trace with functional metrics labeled. **(C)** Representative example of a distribution curve of tissues generated and excluded using FW90M as a quality control metric. The Top 10% and Bottom 10% were excluded from this batch to remove outliers and create a normally distributed population.

A

Region	Start (s)	Stop (s)	Voltage (V)	Freq (Hz)	Period(s)	First Frame	Last Frame	Duration
Spontaneous	0.00	5.00	0	0		0	99	100
ET	5.00	10.00	5	1	1.000	100	199	100
ET	10.00	15.00	4.5	1	1.000	200	299	100
ET	15.00	20.00	4	1	1.000	300	399	100
ET	20.00	25.00	3.5	1	1.000	400	499	100
ET	25.00	30.00	3	1	1.000	500	599	100
ET	30.00	35.00	2.5	1	1.000	600	699	100
ET	35.00	40.00	2	1	1.000	700	799	100
ET	40.00	45.00	1.5	1	1.000	800	899	100
ET	45.00	50.00	1	1	1.000	900	999	100
ET	50.00	55.00	0.5	1	1.000	1000	1099	100
FFR	55.00	75.00	5	0.5	2.000	1100	1499	400
FFR	75.00	95.00	5	1	1.000	1500	1899	400
FFR	95.00	115.00	5	1.5	0.667	1900	2299	400
FFR	115.00	135.00	5	2	0.500	2300	2699	400
FFR	135.00	155.00	5	2.5	0.400	2700	3099	400
FFR	155.00	175.00	5	3	0.333	3100	3499	400
FFR	175.00	195.00	5	3.5	0.286	3500	3899	400
FFR	195.00	215.00	5	4	0.250	3900	4299	400
PRP	215.00	235.00	0	0		4300	4699	400
PRP	235.00	240.00	5	1	1.000	4700	4799	100

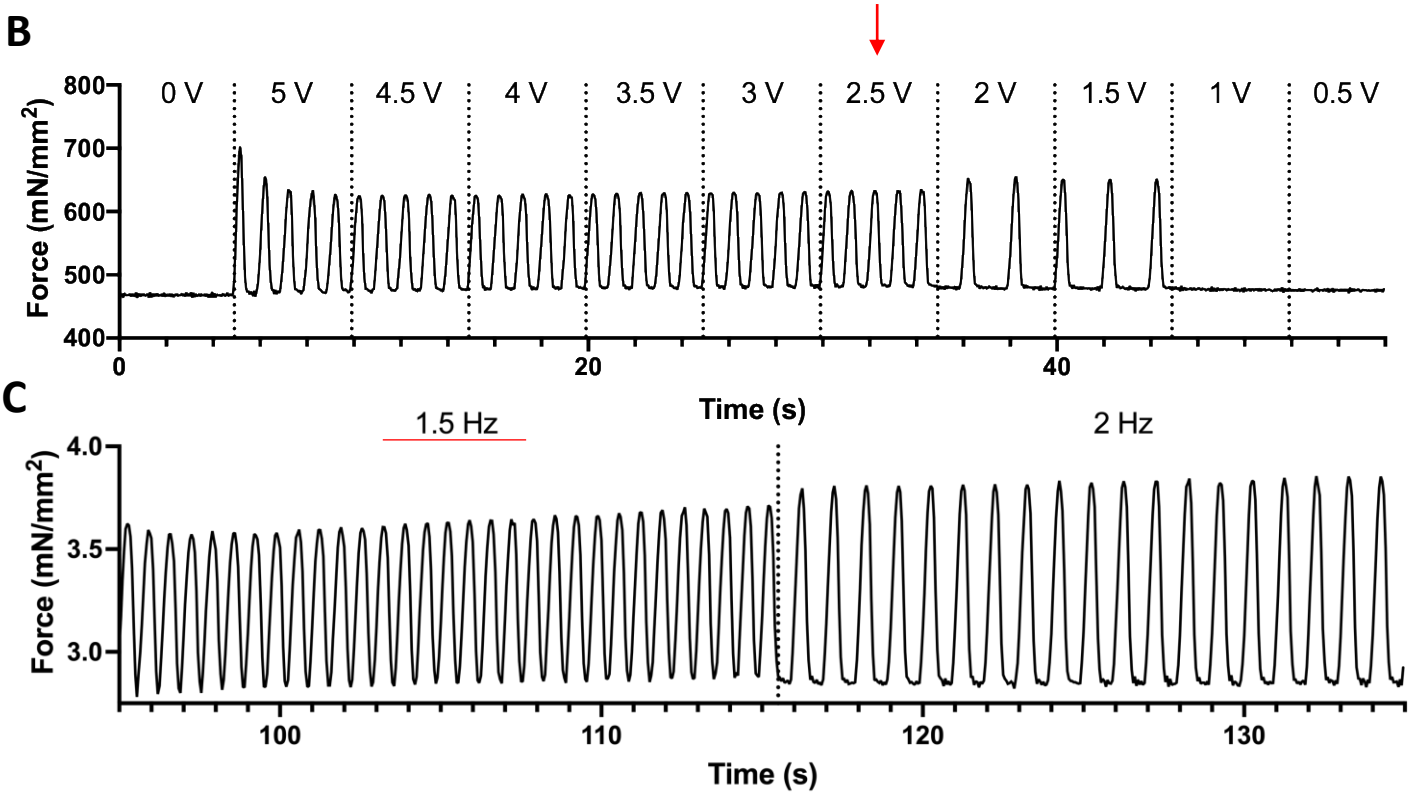


Figure S6. Detailed schematic of ET/MCR stimulation regimen. (A) Table describing the timestamp, voltage, and frequency which tissues are stimulated at during analysis. **(B)** Schematic of the ET segment of the analysis indicating when the tissue stops responding to the 1Hz stimulation (red arrow). **(C)** Schematic of the MCR segment of the analysis indicating when the tissue stops responding to the stimulation at 5V (red underline).

	Tissue Volume	Cells per Tissue	Force Generation (Measured at 1-1.5 Hz Stimulation)	Well Volume	Independent Culture Wells	Throughput	Charge Injected Reported	Electrical Stimulation During Tissue Culture	Electrical Stimulator Commercially Available	Analysis Tool in Public Repository	Platform Available in Public Repository
Turnbull et al, 2014	100 µL	1×10^6	0.57 ± 0.07 mN/mm^2	NA	**Yes**	Low	No	No	No	No	No
Mannhardt el al, 2016	100 µL	1×10^6	~0.12 mN (0.4 mN/mm^2)*	500 µL	**Yes**	Medium	No	No	No	No	No
Jackman et al, 2016	70 µL	3.75×10^5	23.2 ± 1.6 mN/mm^2	1.5 mL	No	Low	No	No	No	No	No
Tiburcy et al., 2017	500 µL	$1x10^4$ - $15x10^6$	6.2 ± 0.8 mN/mm^2	NA	No	Low	**Yes**	No	NA	No	No
Ronaldson-Bouchard et al, 2018	200 µL	$2x10^6$	~2.5 mN/mm^2	30 mL	No	Low	No	**Yes**	No	**Yes**	No
Dostanić, et al 2019	3 µL	$4.7x10^4$	~0.081 mN (0.33 mN/mm^2)*	200 µL	**Yes**	High	No	No	No	No	No
Zhao et al, 2020	2 µL	$1.1x10^5$	0.051 ± .025 mN/mm^2	NA	No	Low	No	**Yes**	No	No	No
Thavandiran, et al, 2020	12 µL	$7.5x10^4$	NA	300 µL	**Yes**	High	No	No	No	No	No
milliPillar, 2021	15 µL	$5.5x10^5$	3.22 ± 0.17 mN/mm^2	400 µL	**Yes**	**Medium**	**Yes**	**Yes**	**Open Source**	**Yes**	**Yes**

Table S1. Comparison of design principles and properties of engineered heart tissue models. * Indicates force generation approximated using available information from published data and a cross sectional area calculated by the radius from IHC images. Cross sectional area was calculated by assuming a cylindrical tissue shape (Area= πr^2).

Supplementary Videos

Video S1. Spontaneously beating BS2 milliPillar tissue with NHDFs. A representative video of a spontaneously beating milliPillar tissue made with 75% BS2-derived cardiomyocytes and 25% NHDFs. Brightfield videos were recorded for clear visualization of pillar movement and force calculation.

Video S2. WTC11 milliPillar tissue paced at 1 Hz with iPSC-CFs. A representative video of a milliPillar tissue made with 75% WTC11-derived cardiomyocytes and 25% iPSC-derived cardiac fibroblasts. Tissues were stimulated at 1 Hz using 5 V/cm and 20 nC/mL charge per pulse for recording. Brightfield videos were recorded for clear visualization of pillar movement and force calculation.

Video S3. WTC11-GCaMP6f milliPillar tissue paced at 1 Hz with NHCFs. A representative video of a milliPillar tissue made with 75% WTC11-GCaMP6f-derived cardiomyocytes and 25% Normal Human Ventricular Cardiac Fibroblasts. Tissues were stimulated at 1 Hz using 5 V/cm and 20 nC/mL charge per pulse for recording. Brightfield videos were recorded for clear visualization of pillar movement and force calculation.

Video S4. Pillar tracking of milliPillar. Custom analysis software can track the location of the pillar heads throughout the analysis stimulation regimen.

Video S5. milliPillar tissue pre-stimulation. A representative video of a milliPillar tissue 7 days post-fabrication (pre-stimulation) stimulated at 1Hz using 5V/cm and 20 nC/mL charge per pulse for imaging and analysis. Brightfield videos were recorded for clear visualization of pillar movement.

Video S6. milliPillar tissue post-stimulation. A representative video of a milliPillar tissue 28 days post-fabrication (21 days post-stimulation, Ramp protocol) stimulated at 1Hz using 5V/cm and 20 nC/mL charge per pulse for imaging and analysis. Brightfield videos were recorded for clear visualization of pillar movement.

Supplementary Files

File S1. milliPillar molds CAD design. A .STEP file that includes the design of the milliPillar Platform

File S2. milliPillar stimulator resources. A .zip file that includes the design and software for the milliPillar Stimulator

File S3. milliPillar analysis software. A .zip file that includes the brightfield and fluorescent calcium analysis software.