

Supplementary Information

Engineering Anisotropic Cardiac Monolayers on Microelectrode Arrays for Non-invasive Analyses of Electrophysiological Properties

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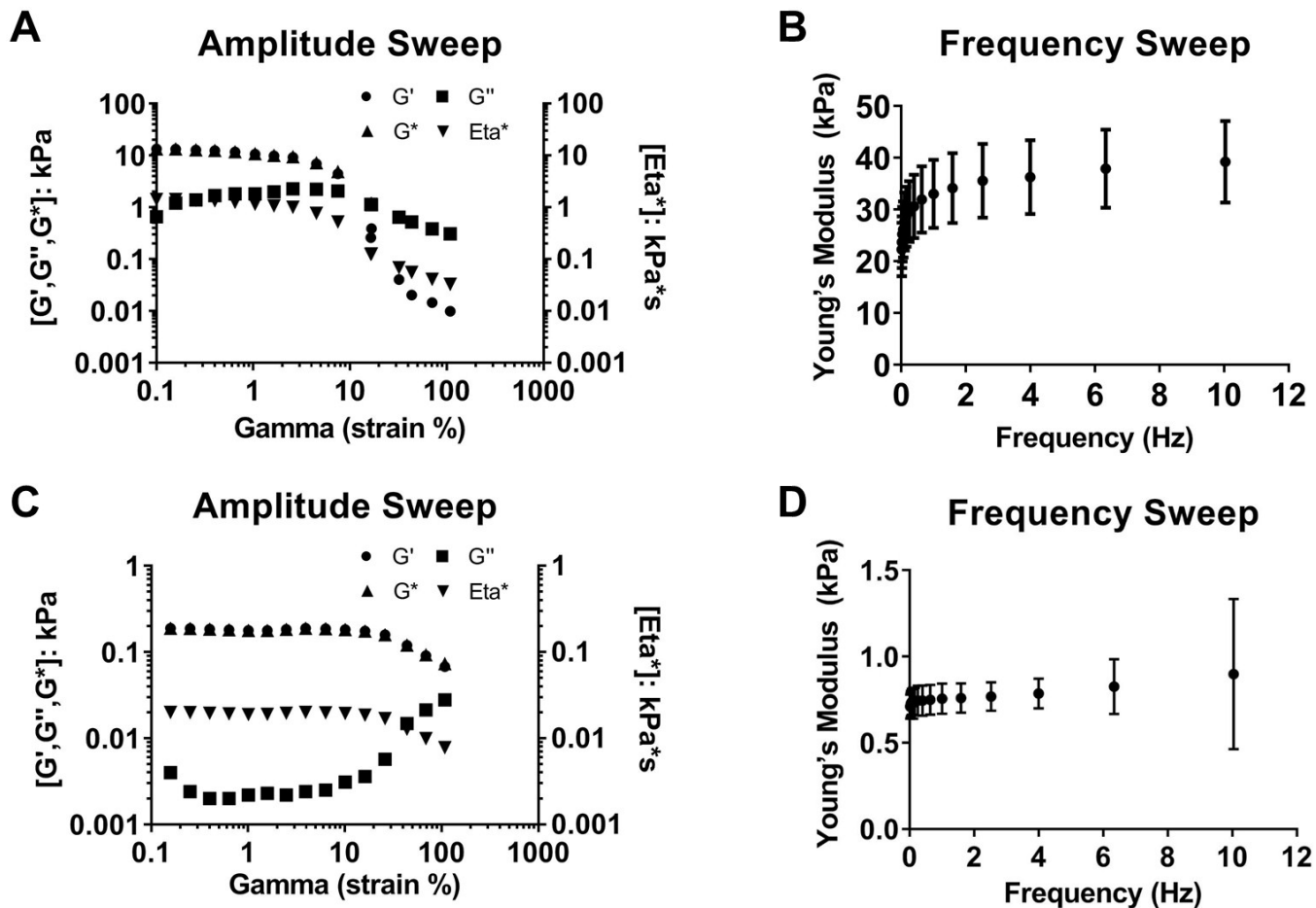
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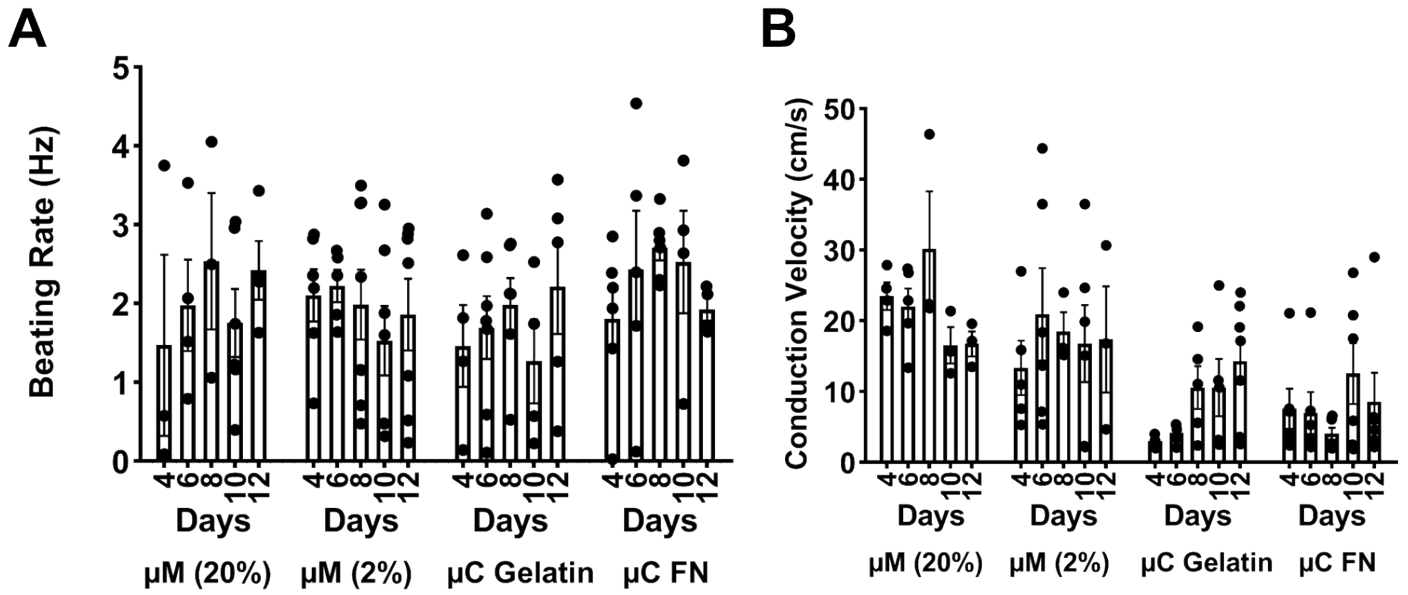
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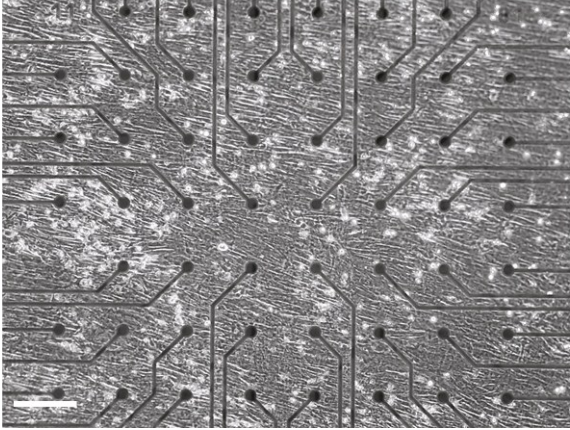


Supplementary Figure 1. Rheological properties of gelatin hydrogels in two different concentrations (20% and 2%). A) The amplitude dependence of G' , G'' , G^* and Eta^* of 20% gelatin hydrogels. The linear viscoelastic range of 20% gelatin hydrogels was determined from amplitude sweep test. B) Young's modulus of 20% gelatin hydrogels was determined from frequency sweeps performed at a constant strain of 0.5% ($n = 6$, mean \pm SEM). C) The amplitude dependence of storage modulus (G'), loss modulus (G''), complex shear modulus (G^*) and viscosity (Eta^*) of 2% gelatin hydrogels. The linear viscoelastic range of 2% gelatin hydrogels was determined from amplitude sweep test. D) Young's modulus of 2% gelatin hydrogels was determined from frequency sweeps performed at a constant strain of 0.5% ($n = 4$, mean \pm SEM).

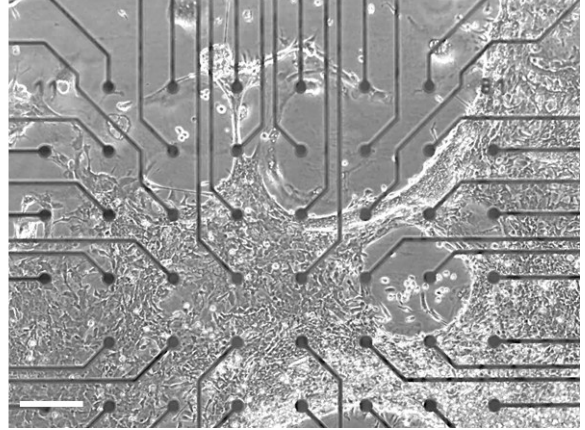


Supplementary Figure 2. Individual observation for each substrate. A) Beating rate (Hz) differences between the different days for each substrate. B) Conduction velocity (cm/s) differences between the different days for each substrate.

A μM (2%)



B μC Fibronectin



Supplementary Figure 3. Bright field images on day 12 for A) μM 2% hydrogel, and B) μC fibronectin to check delamination and pick only the electrodes that were covered with cardiomyocytes for recording. Scale bar represents 200 μm .

The Beating Rate (Hz) for 12 Days

A

Day	μM (20%)			μM (2%)			μC Gelatin			μC Fibronectin		
	Average (Hz)	SEM	Number of MEA	Average (Hz)	SEM	Number of MEA	Average (Hz)	SEM	Number of MEA	Average (Hz)	SEM	Number of MEA
Day 4	1.47	0.48	3	2.10	0.17	6	1.85	0.15	4	1.89	0.10	6
Day 6	1.98	0.17	4	2.29	0.07	5	1.58	0.25	7	2.51	0.13	5
Day 8	2.17	0.26	3	1.99	0.12	8	1.98	0.13	6	2.71	0.24	6
Day 10	2.07	0.15	6	1.50	0.05	7	1.27	0.26	4	2.57	0.22	4
Day 12	2.42	0.14	4	1.88	0.06	7	2.40	0.17	5	1.92	0.21	4

The Beating Interval (s) for 12 Days

B

Day	μM (20%)			μM (2%)			μC Gelatin			μC Fibronectin		
	Average (s)	SEM	Number of MEA	Average (s)	SEM	Number of MEA	Average (s)	SEM	Number of MEA	Average (s)	SEM	Number of MEA
Day 4	0.60	0.01	3	0.79	0.01	5	2.78	0.47	3	2.38	0.28	5
Day 6	0.44	0.02	5	0.51	0.01	7	1.52	0.26	5	0.68	0.05	6
Day 8	0.57	0.02	2	1.03	0.02	6	0.54	0.02	4	0.48	0.01	5
Day 10	3.02	0.22	3	2.00	0.04	6	4.02	0.40	4	0.51	0.04	4
Day 12	2.50	0.50	3	1.17	0.02	3	1.05	0.04	5	1.44	0.14	5

The Coefficient of Variation (%) for 12 Days

C

Day	μM (20%)			μM (2%)			μC Gelatin			μC Fibronectin		
	Average (%)	SEM	Number of MEA	Average (%)	SEM	Number of MEA	Average (%)	SEM	Number of MEA	Average (%)	SEM	Number of MEA
Day 4	21.4	11.4	3	16.4	6.5	5	130.0	15.1	3	73.9	59.8	5
Day 6	67.5	24.8	5	45.2	40.3	7	145.4	10.0	5	102.5	52.2	6
Day 8	49.2	17.0	2	38.7	19.3	6	83.0	4.9	4	55.2	6.3	5
Day 10	59.4	12.1	3	22.9	5.1	6	135.0	21.5	4	119.3	8.6	4
Day 12	45.5	10.8	3	29.9	1.0	3	81.8	21.0	5	175.6	5.5	5

The Conduction Velocity (cm/s) for 12 Days

D

Day	μM (20%)			μM (2%)			μC Gelatin			μC Fibronectin		
	Average (cm/s)	SEM	Number of MEA	Average (cm/s)	SEM	Number of MEA	Average (cm/s)	SEM	Number of MEA	Average (cm/s)	SEM	Number of MEA
Day 4	23.5	0.19	4	25.4	5.70	6	3.1	0.72	3	6.9	2.98	6
Day 6	24.1	5.91	5	26.9	5.23	7	15.8	3.23	7	6.4	1.66	6
Day 8	30.2	2.93	3	18.5	4.96	3	17.3	10.36	7	13.1	3.19	8
Day 10	15.6	0.84	3	21.2	3.78	7	8.1	1.47	6	19.2	4.73	7
Day 12	16.8	0.76	3	17.4	0.47	3	10.9	3.81	8	10.6	3.41	6

Supplementary Table 1. Electrophysiological recordings over 12 days of culture for all conditions. The average readouts on each day and the number of MEA replicates were used for A) the beating rate (Hz), B) the beating interval (s), C) the coefficient of variation (%), and D) the conduction velocity (cm/s).

Supplementary Movie 1: Bright field MEA video of anisotropic cardiac monolayer beating on top of μC fibronectin coating from day 4 of culture.

Supplementary Movie 2: Bright field MEA video of anisotropic cardiac monolayer beating on top μM 2% gelatin hydrogel from day 4 of culture.

Supplementary Movie 3: Field potential propagation across the MEA from μC fibronectin condition on day 4 of culture. Red color in the propagation map denotes early detection of the field potential, and blue color denotes late detection of the field potential.

Supplementary Movie 4: Field potential propagation across the MEA from μM 2% condition on day 4 of culture. Red color in the propagation map denotes early detection of the field potential, and blue color denotes late detection of the field potential.