

Quantitative Assessment of Cardiomyocytes Mechanobiology through High-Throughput Cantilever-Based Functional Well Plate Systems

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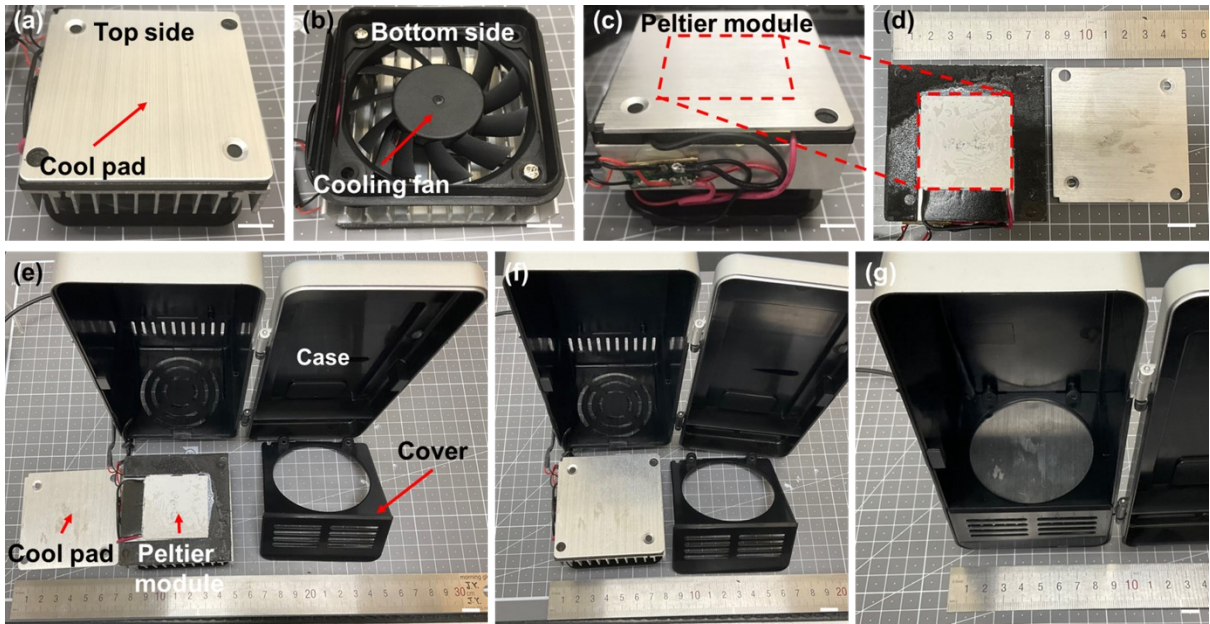


Figure S1. Manufactured USB type Peltier refrigerator; (a) upper cooling pad; (b) lower cooling fan; (c) Peltier module and 5 V USB circuit; (d) Peltier module refrigerator case and culture medium storage (e-g) Peltier refrigerator. (Scale 1 cm in white color)

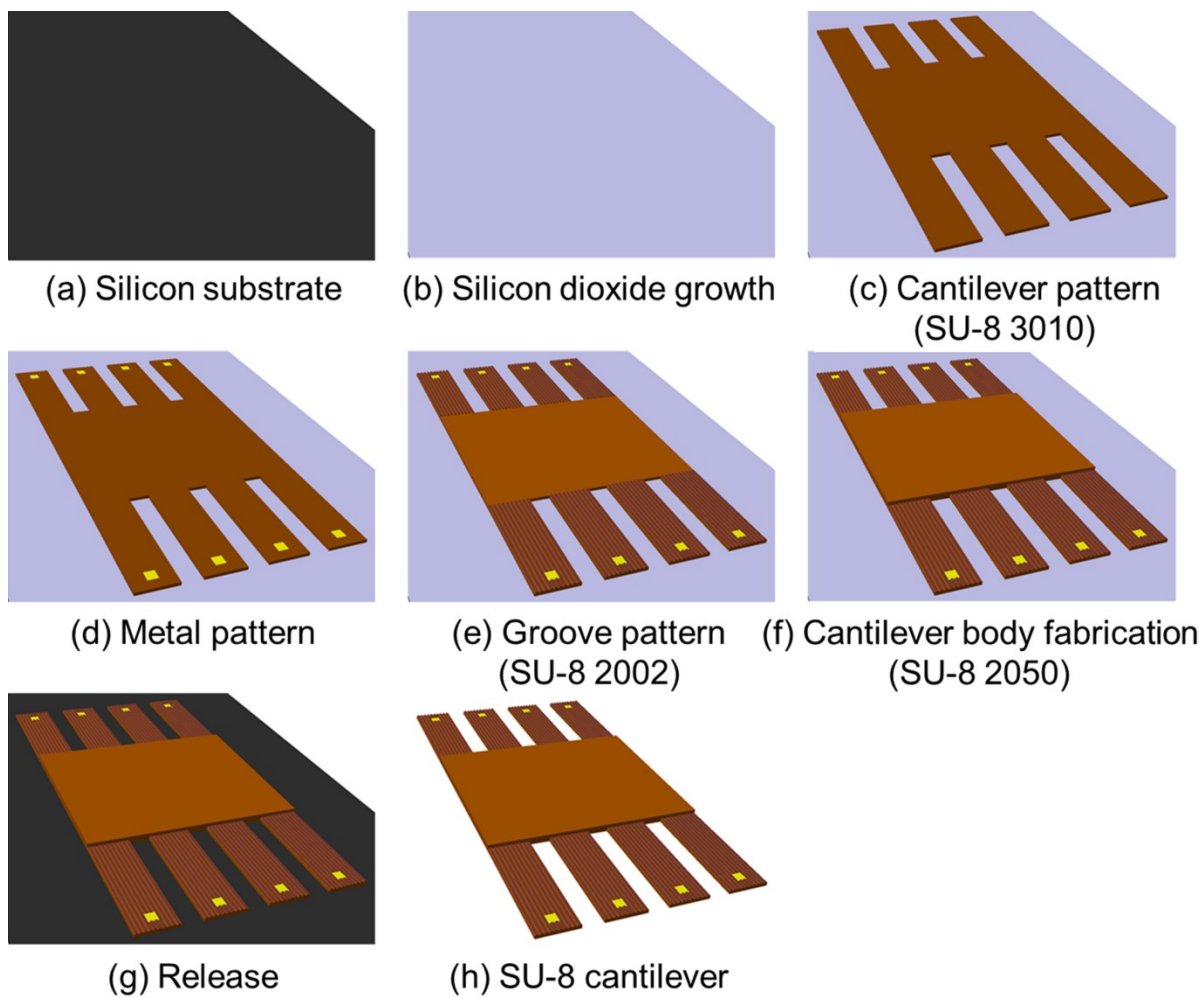


Figure S2. SU-8 cantilever fabrication process, (a) Si wafer, (b) 300 nm thickness SiO_2 sacrificial layer, (c) SU-8 3010 pattern, (d) metal pattern, (e) SU-8 2002 (groove) pattern, (f) SU-8 2050 cantilever body fabrication, (g) release, (h) SU-8 cantilever array

Table S1. Temperature distribution of manufactured Peltier refrigerators

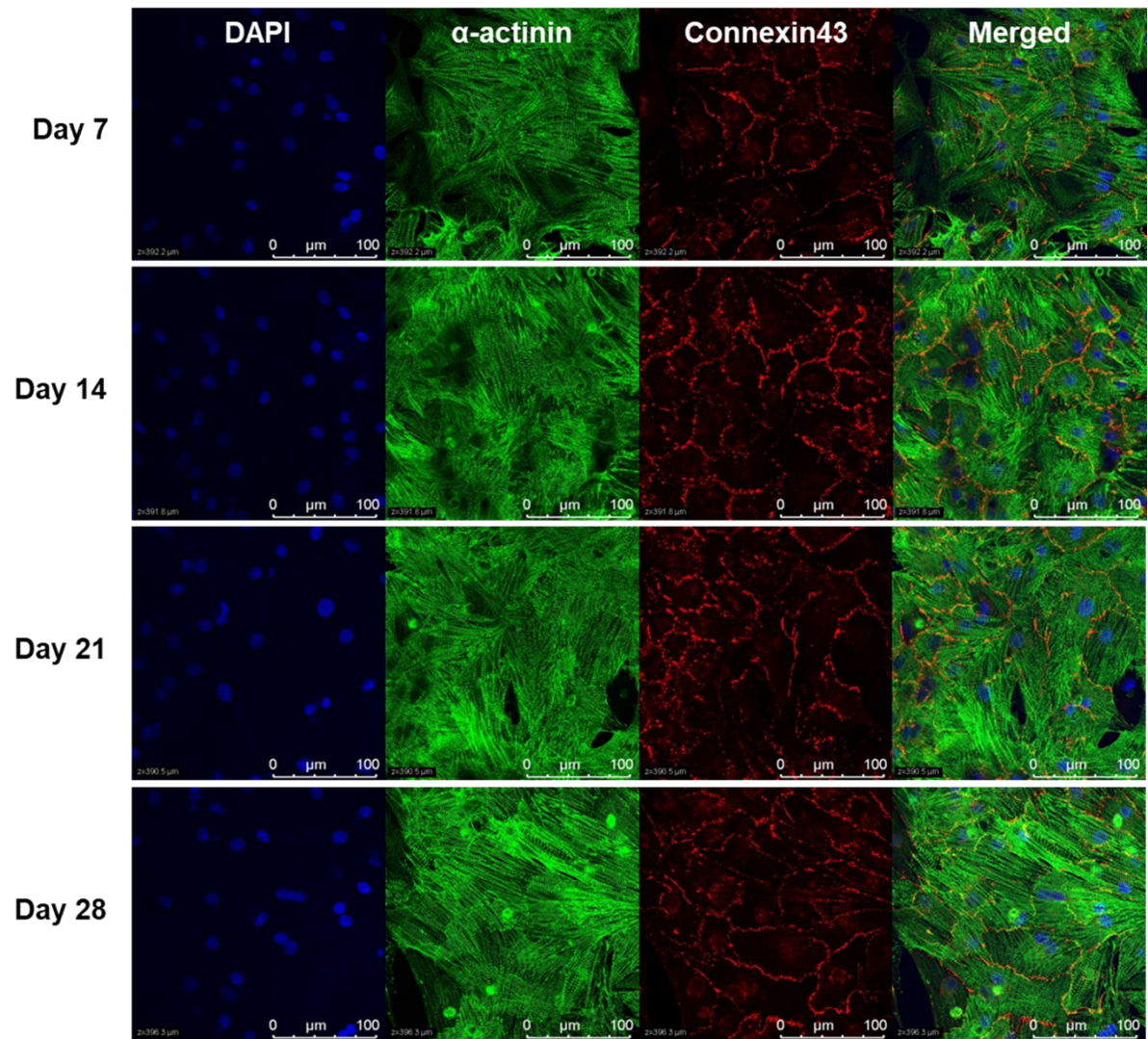
Time \ Temperature (°C)	Bottom	Left	Center	Right	Top
5 min	22.4	22.2	21.2	22.3	22.1
10 min	16.2	15.9	15.5	15.9	16.1
30 min	14.2	12.5	12.1	13.1	12.5
1 h	12.2	11.6	11.2	12.5	11.8
2 h	9.8	8.7	8.5	9.6	8.8
6 h	8.8	7.9	7.2	8.8	8.0
12 h	7.6	6.2	5.6	6.8	6.3
24 h	7.1	5.7	5.5	6.2	5.6
48 h	6.9	5.8	5.2	6.3	5.8

Table S2. Changes in pH of cell culture media by temperature and CO₂ concentration

Sr. No.	Without cell	Fresh media	1 h	12 h	24 h	36 h	48 h
1	Warm bath (37 °C)	7.52	7.52	7.53	7.63	7.64	7.7
2	5 % CO ₂ incubator (37 °C)	7.52	7.52	7.5	7.42	7.4	7.4
3	Peltier refrigerator (6 °C)	7.52	7.52	7.51	7.52	7.52	7.51
4	Room temperature (25 °C)	7.52	7.52	7.53	7.56	7.6	7.66

(a)

Perfusion



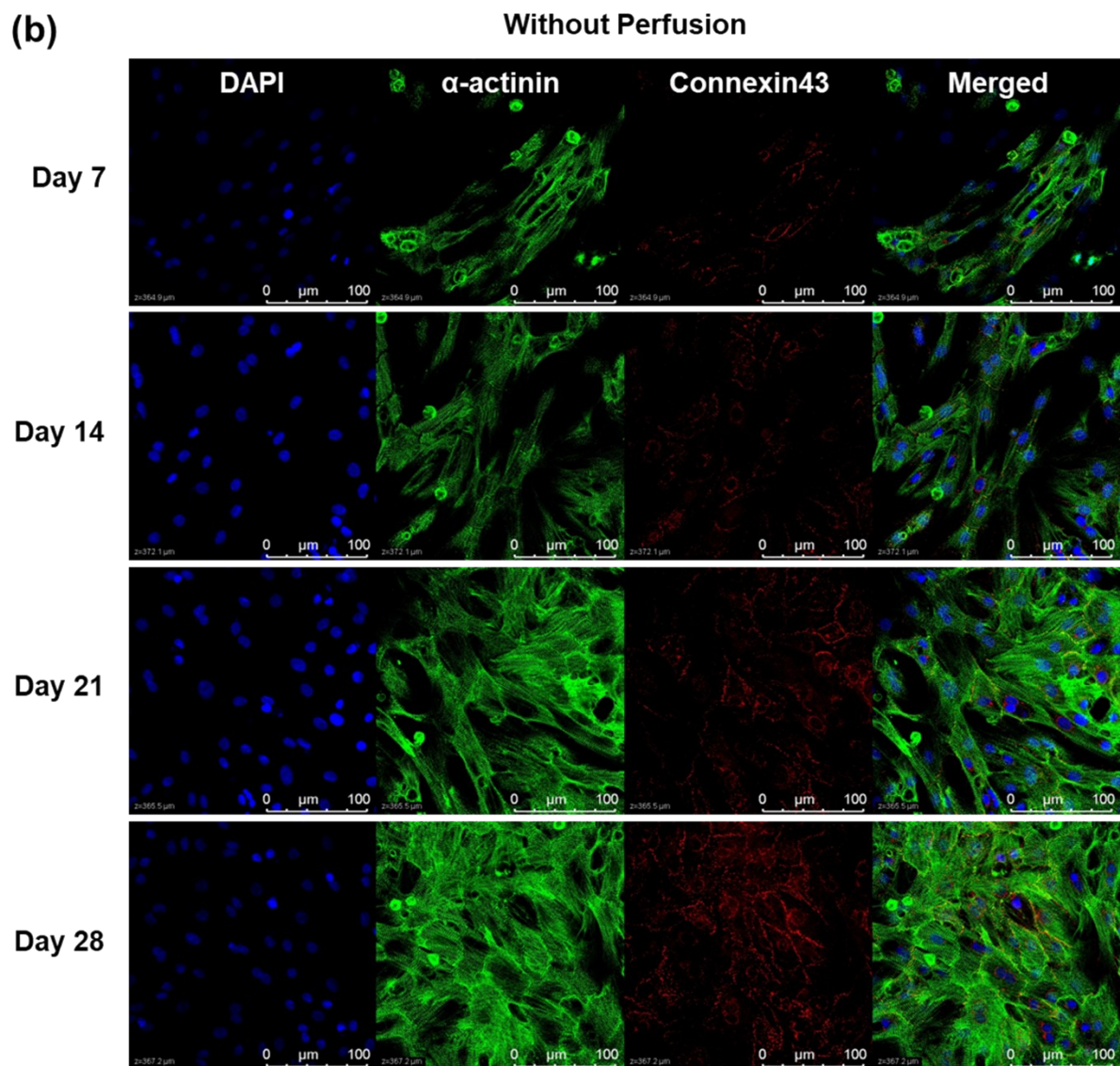


Figure S3. Fluorescent staining images of days 7, 14, 21, and 28 after cardiomyocytes culture; DAPI (blue), α -actinin (green), Cx43 (red) and merged; (a) Perfusion, (b) without perfusion

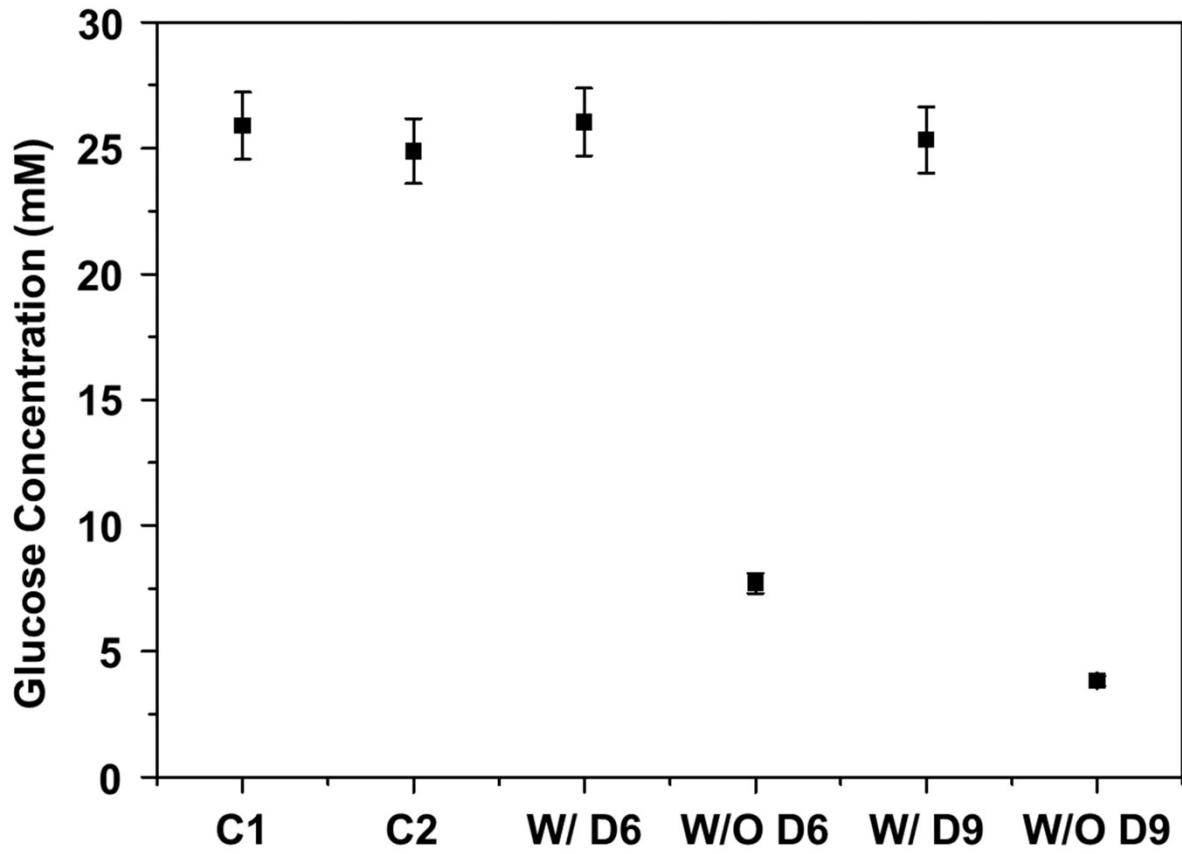


Figure S4. Changes in glucose concentration of cell culture medium according to perfusion

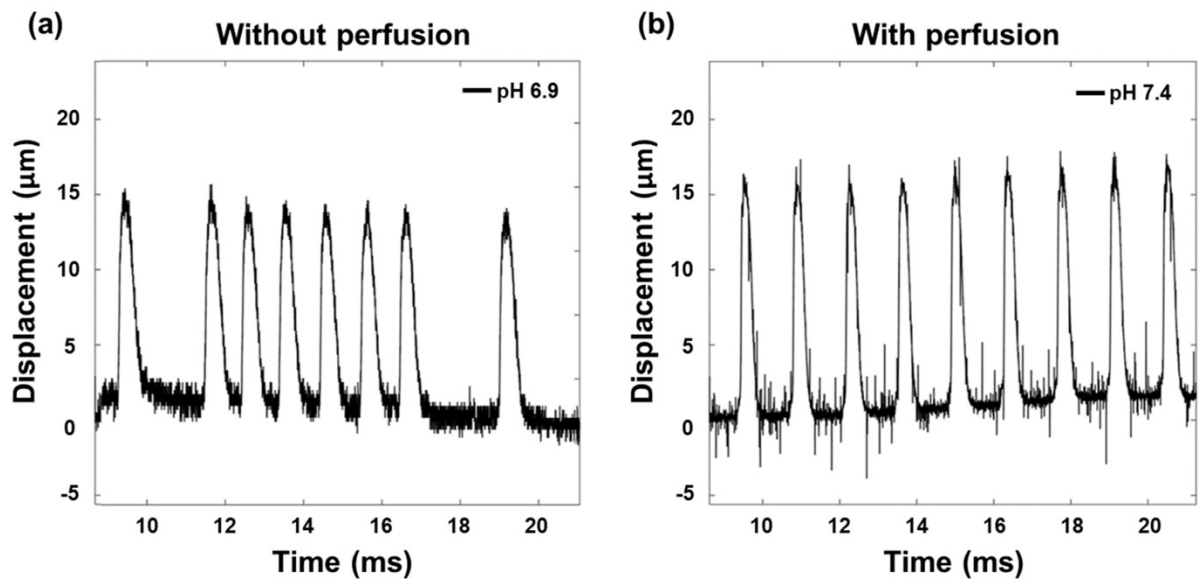


Figure S5. Irregular heartbeat at pH 7.0 or below; (a) without perfusion, (b) with perfusion.

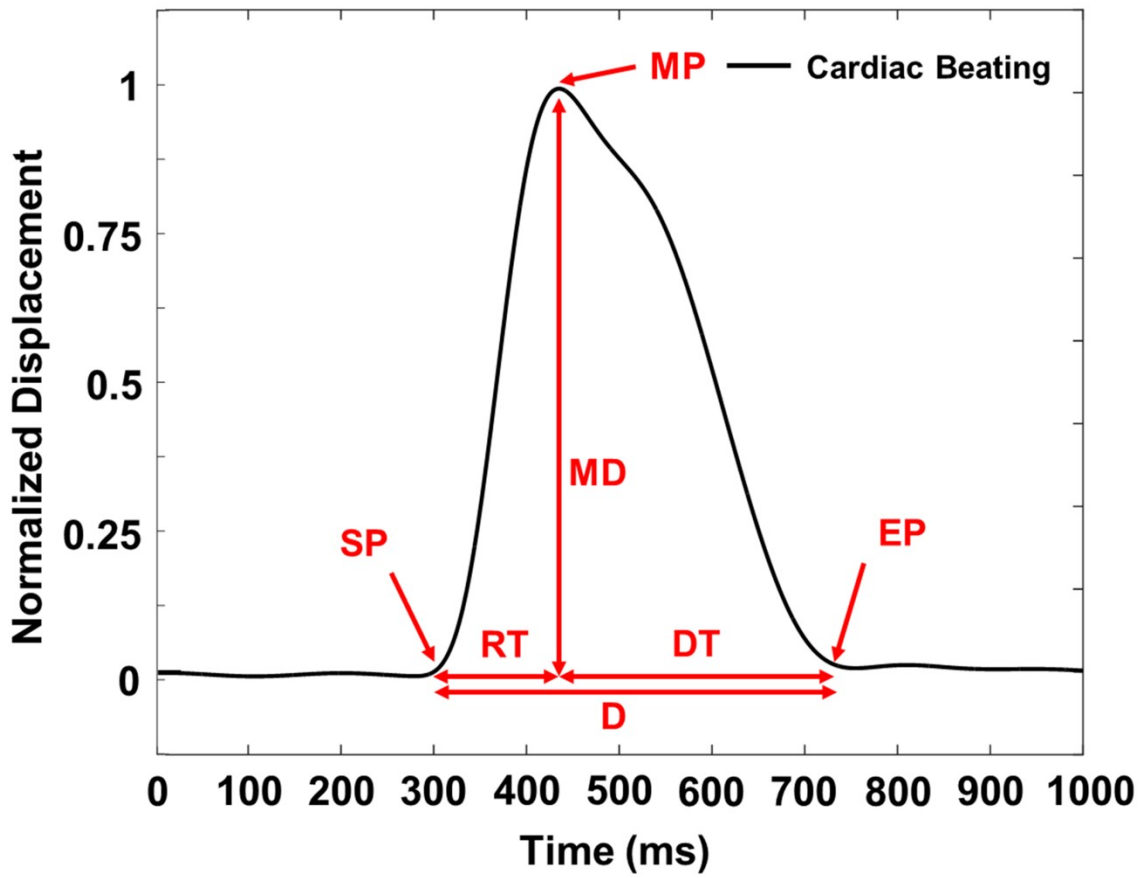


Figure S6. Beating characteristics of cardiomyocytes; Maximum Displacement (MD), Duration (D), Start Point (SP), End Point (EP), Maximum Point (MP), Rise Time (RT), Decay Time (DT)

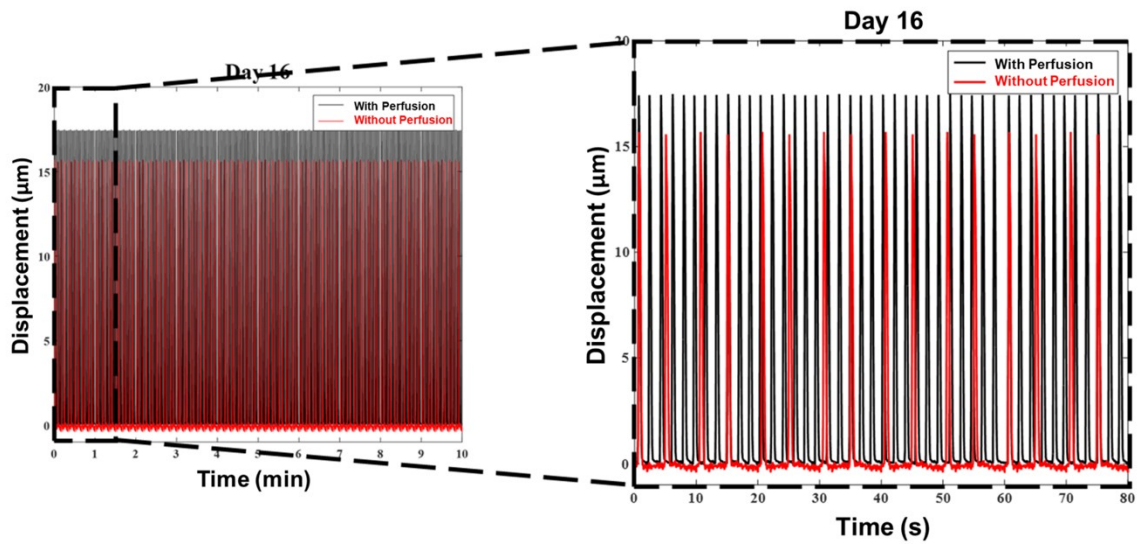


Figure S7. Contraction characteristic on day 16th according to with and without culture media perfusion.

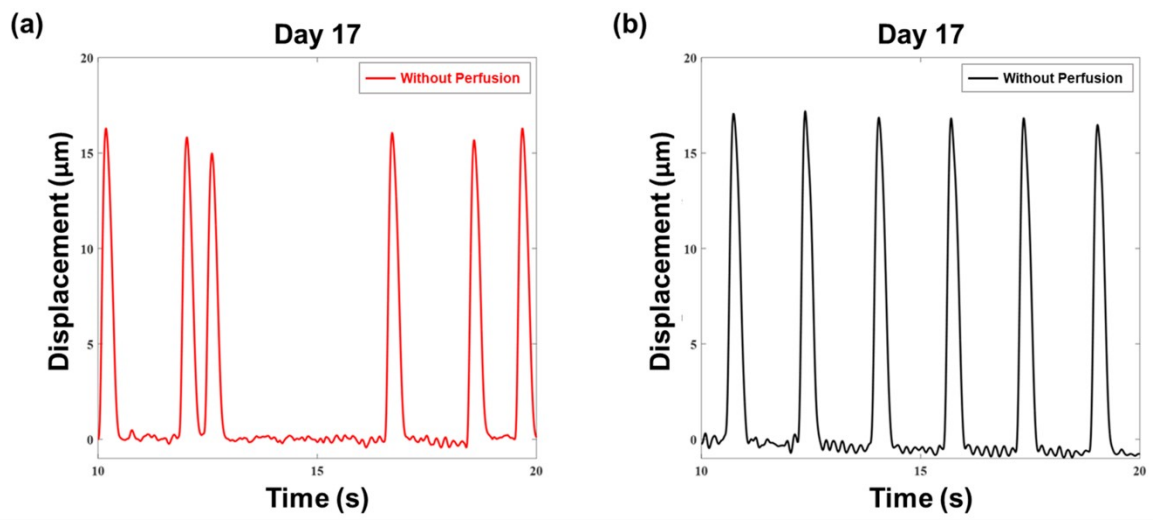


Figure S8. Contraction characteristic on day 17th according to with and without culture media perfusion.; (a) without perfusion, (b) with perfusion.

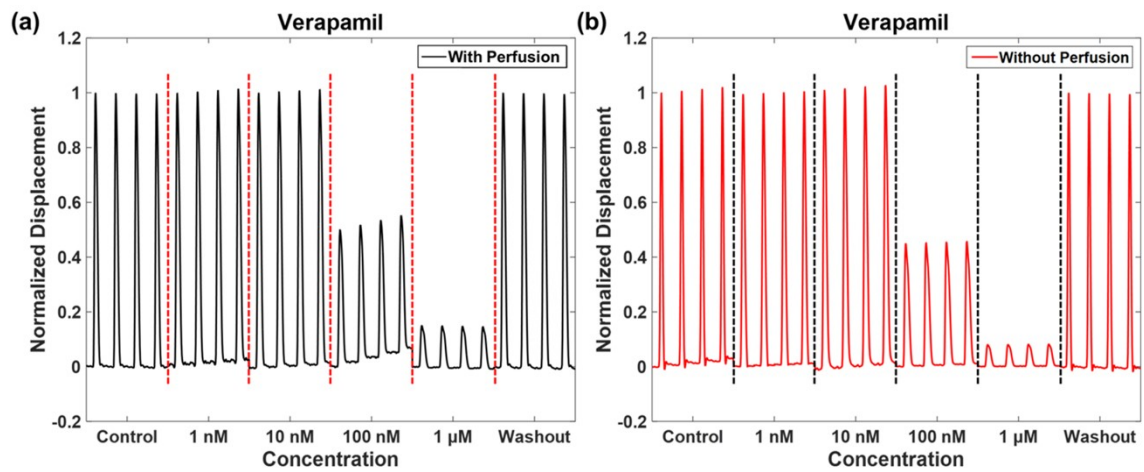


Figure S9. Effect of ion channel-related cardiac drug (verapamil) on the contractility of cardiomyocytes. (a, b) Real-time traces of cardiomyocytes cultured with and without perfusion at different verapamil concentrations.

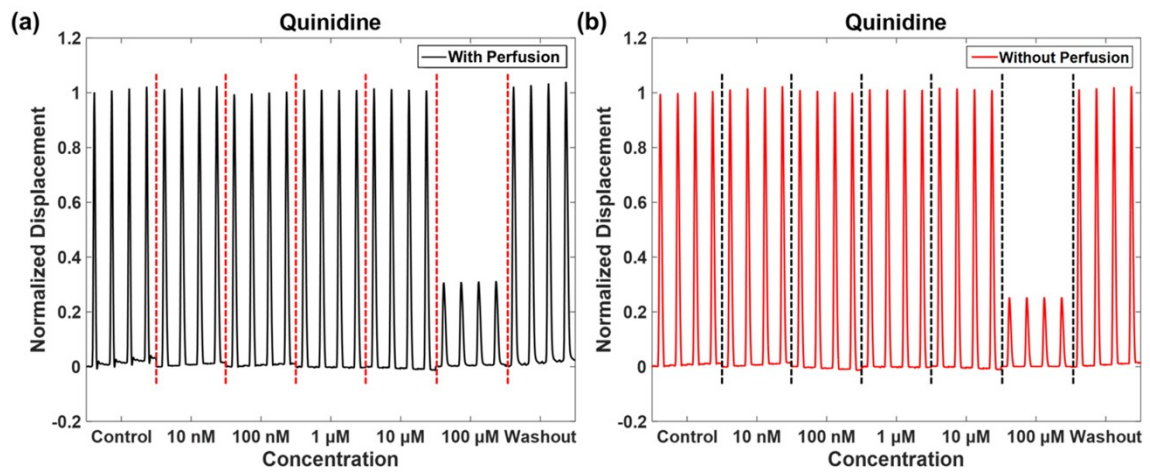


Figure S10. Effect of ion channel-related cardiac drug (quinidine) on the contractility of cardiomyocytes. (a, b) Real-time traces of cardiomyocytes cultured with and without perfusion at different quinidine concentrations.