Supplementary information

Reflow-Molded Deep Concave Microwell Arrays for Robust and Large-Scale Production of Embryoid Bodies

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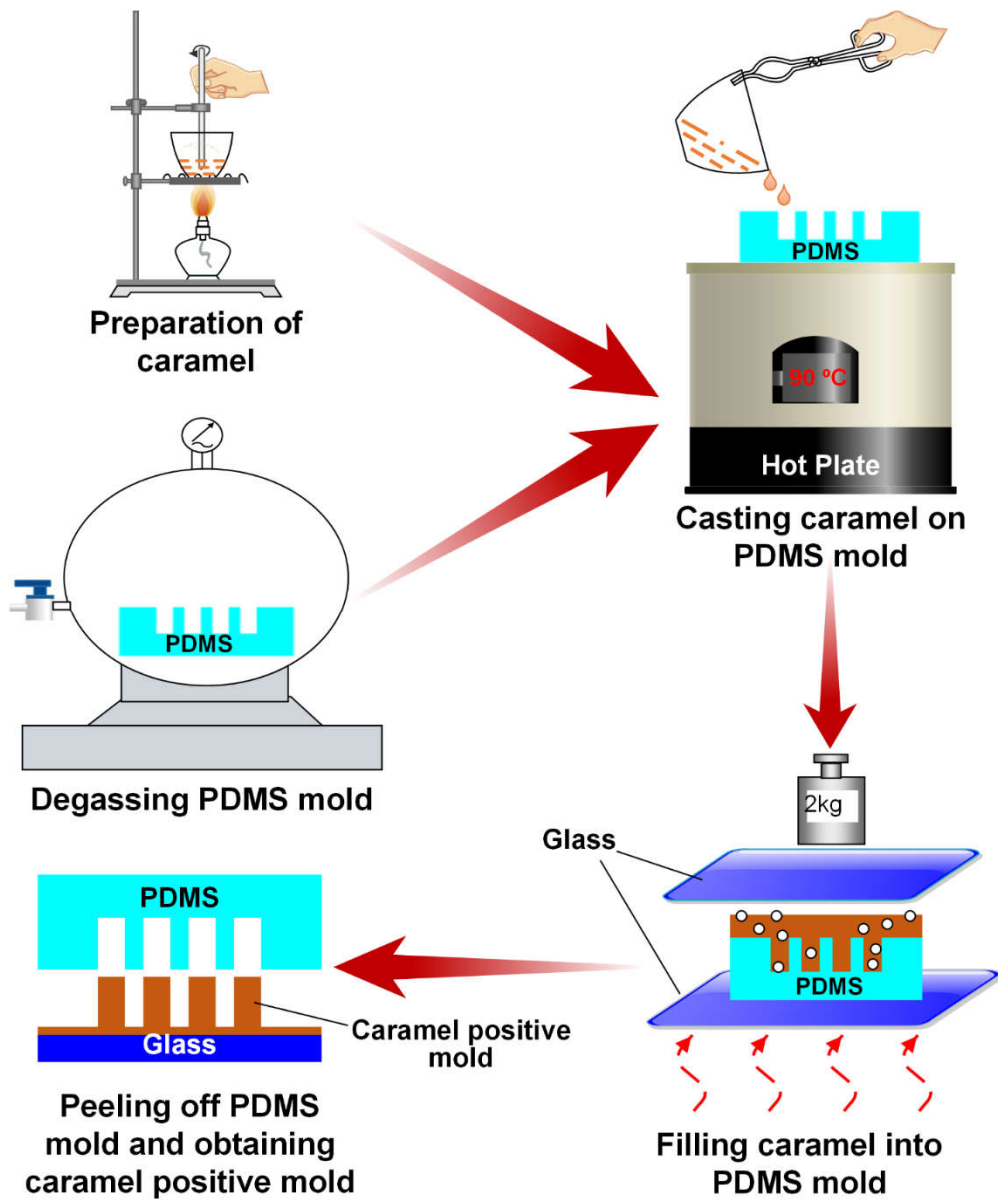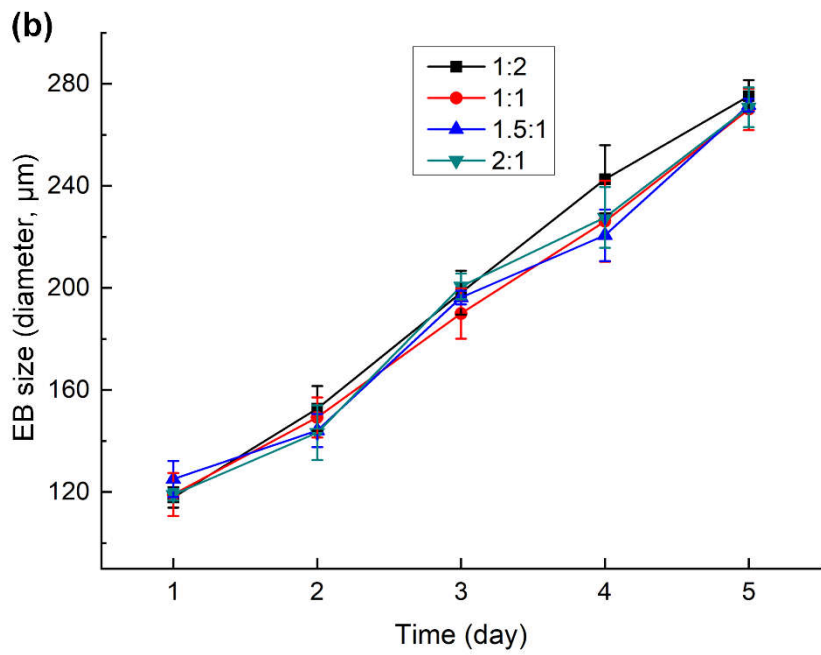*Email address: qiangzh@cqu.edu.cn (Q. Zhao); gang_li@cqu.edu.cn (G. Li)

Preparation of caramel

Degassing PDMS mold

Casting caramel on PDMS mold

Hot Plate

90 ℃

PDMS

2kg

Glass

PDMS

Filling caramel into PDMS mold

PDMS

Glass

Caramel positive mold

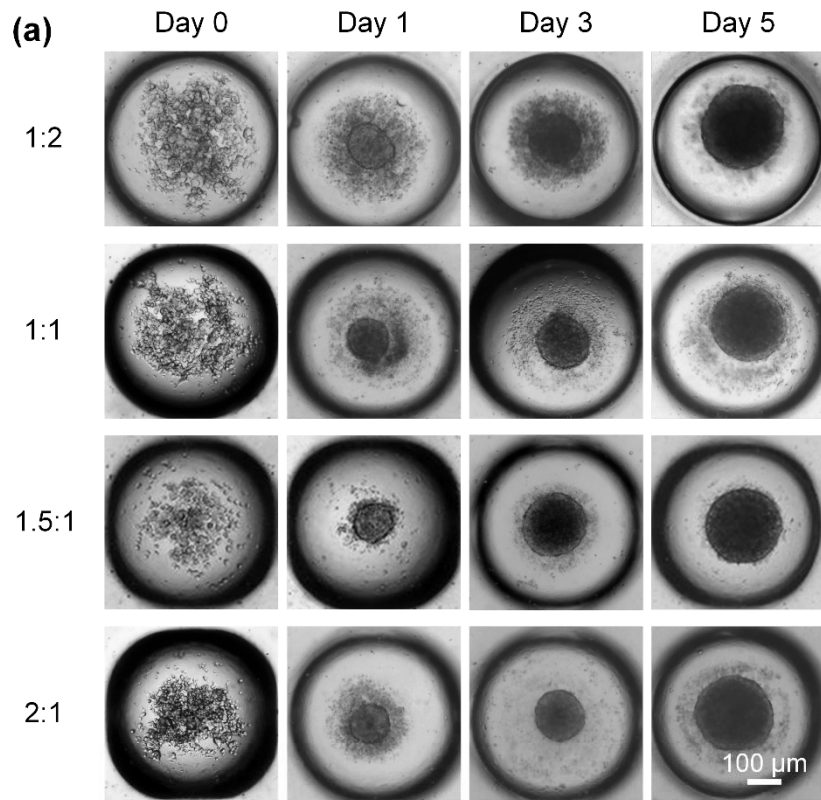**Peeling off PDMS mold and obtaining caramel positive mold**

Fig. S1. Schematic diagram of the caramel replica moulding process.
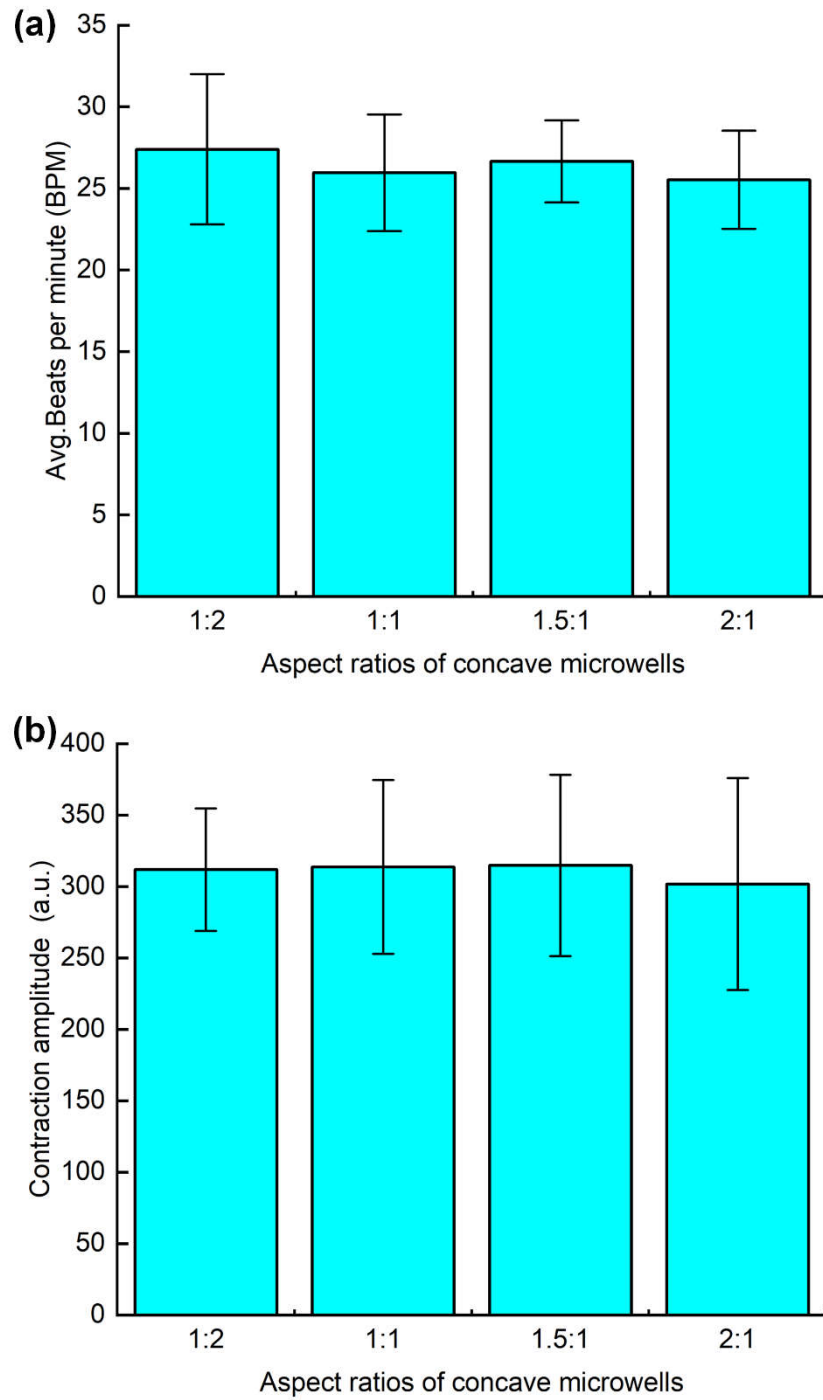
Fig. S2. Formation and growth of EBs in concave microwells of different ARs. (a) Time-lapse photographs recorded for the formation of EBs in concave microwells of different ARs. (b) Growth curves of EBs cultured in concave microwells of different ARs.
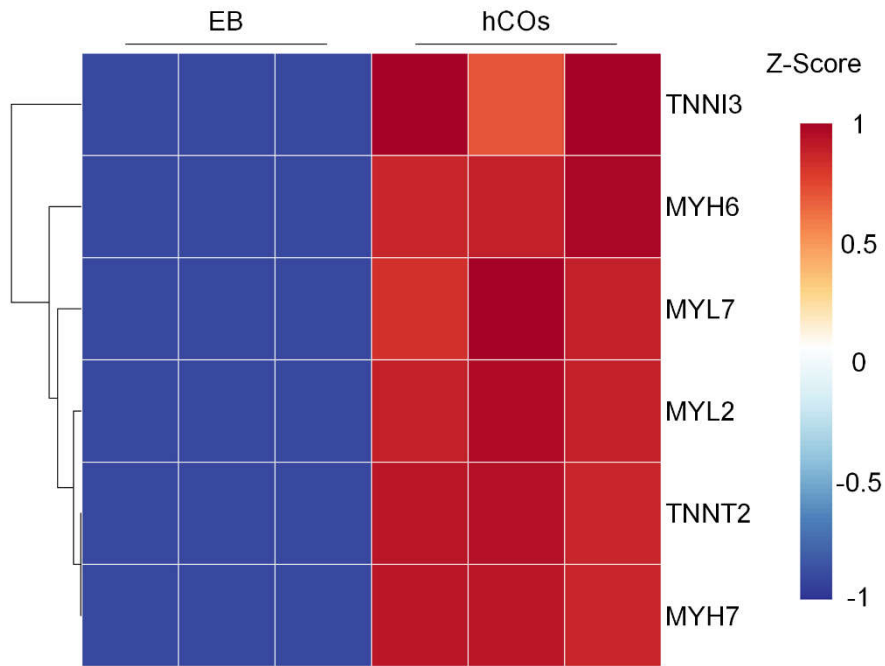
Fig. S3. Heat map analysis of cardiac structural gene expression between undifferentiated EBs and hCOs.

Fig. S4. Comparison of the contractile properties of hCOs differentiated from EBs cultured in concave microwells of different ARs. (a) Beating frequency. (b) Contraction amplitude.

Table S1. The physical parameters used for the COMSOL CFD modeling.[1,2]

Diffusion coefficient of glucose	8×10^{-11} m^2/s
Diffusion coefficient of lactate	1.1×10^{-10} m^2/s
Maximum consumption rate of glucose	3.4×10^{-7} mol/m^2/s
Consumption constant of glucose	0.58 mol/m^3
Average flow rate	1~5 mm/s
Initial concentration of glucose in fresh medium	25 mol/m^3
Initial concentration of lactate in fresh medium	0 mol/m^3
Initial concentration of lactate in microwells before applying fresh medium	30 mol/m^3
Diameter of concave microwells	500 μm
Diameter of spheroids	250 μm

Table S2. List of primers used in this study.

Gene	Forward 5′-3′	Reverse 3′-5′
Oct3/4	GACAGGGGGAGGGGAGGGAGCTAGG	CTTCCCTCCAACCAGTTGCCCCAAAC
Nanog	CAGCCCCGATTCTTCCACCAGTCCC	CGGAAGATTCCCAGTCGGGTTCACC
Pax6	ACCCATTATCCAGATGTGTTTGCCCGAG	ATGGTGAAGCTGGGCATAGGCGGCAG
Brachyury	GCCCTCTCCCTCCCCTCCACGCACAG	CGGCGCCGTTGCTCACAGACCACAGG
AFP	GAATGCTGCAAACTGACCACGCTGGAAC	TGGCATTCAAGAGGGTTTTCAGTCTGGA
β-Actin	ACCCCGTGCTGCTGACCGAG	TCCCGGCCAGCCAGGTCCA
TNNT2	TTCACCAAAGATCTGCTCCTCGCT	TTATTACTGGTGTGGAGTGGGTGTG
TNNI3	GGAGGACACCGAGAAGGAAAAC	TCAAACTTTTTCTTGCGGCCC
MYH6	TCAGCTGGAGGCCAAAGTAAAGGA	TTCTTGAGCTCTGAGCACTCGTCT
MYH7	TCGTGCCTGATGACAAACAGGAGT	ATACTCGGTCTCGGCAGTGACTTT
MYL2	GCAGGCGGAGAGGTTTTC	AGTTGCCAGTCACGTCAGG
MYL7	CTTGTAGTCGATGTTCCCCG	TCAAGCAGCTTCTCCTGACC
GADPH	GGGAAGGTGAAGGTCGGAGT	GGGGTCATTGATGGCAACA

Table S3. Measured shape parameters of 60 EBs randomly collected from a deep concave microwell array for 5 days.

No.	Area (μm^2)	Perimeter (μm)	Major diameter (μm)	Minor diameter (μm)	Circularity	Solidity
1	55933.41	887.660	271.235	262.564	0.892	0.960
2	59881.09	909.533	279.771	272.519	0.910	0.965
3	55790.73	866.480	269.679	263.405	0.934	0.967
4	55980.98	919.286	280.502	254.106	0.832	0.953
5	50558.86	837.220	254.435	253.006	0.906	0.964
6	60356.72	919.286	283.380	271.186	0.897	0.965
7	56789.54	887.660	277.091	260.949	0.906	0.966
8	52746.73	845.300	262.973	255.384	0.928	0.970
9	47562.43	807.961	250.473	241.776	0.916	0.964
10	61022.59	923.326	281.816	275.699	0.899	0.965
11	58549.35	903.819	280.156	266.092	0.901	0.961
12	55029.73	876.233	270.165	259.345	0.901	0.955
13	54030.92	881.947	272.895	252.091	0.873	0.961
14	58263.97	894.066	273.894	270.850	0.916	0.967

15	55933.41	874.560	273.194	260.682	0.919	0.966
16	61355.53	915.939	282.827	276.212	0.919	0.970
17	52318.67	845.300	263.337	252.962	0.920	0.966
18	61212.84	940.466	285.773	272.729	0.870	0.958
19	60404.28	921.652	280.824	273.869	0.894	0.967
20	51938.17	856.727	262.731	251.701	0.889	0.965
21	54004.98	860.286	267.065	257.470	0.917	0.963
22	56745.09	896.079	274.039	263.649	0.888	0.954
23	57392.75	890.232	275.138	265.593	0.910	0.957
24	62125.66	936.716	294.543	268.555	0.890	0.957
25	48524.77	816.223	255.806	241.525	0.915	0.963
26	55449.77	878.537	274.143	257.532	0.903	0.959
27	58887.35	924.312	279.964	267.812	0.866	0.956
28	54403.54	888.519	271.838	254.816	0.866	0.950
29	52460.56	856.860	265.537	251.547	0.898	0.960
30	55698.87	862.708	271.154	261.541	0.940	0.971
31	58688.07	894.366	286.193	261.096	0.922	0.965
32	61428.18	944.986	282.233	277.121	0.864	0.958
33	55150.84	870.268	266.017	263.969	0.915	0.961
34	55499.59	878.537	268.719	262.967	0.904	0.960
35	55549.41	890.232	283.678	249.324	0.881	0.957
36	55399.95	874.402	267.121	264.065	0.911	0.962
37	58787.71	908.483	280.543	266.807	0.895	0.957
38	59634.65	910.196	281.792	269.451	0.905	0.958
39	57791.31	888.519	273.799	268.745	0.920	0.964
40	57492.39	900.214	282.063	259.522	0.892	0.959
41	53785.83	864.295	270.970	252.730	0.905	0.960
42	54467.28	868.382	266.027	260.687	0.908	0.963
43	49259.06	826.521	250.930	249.945	0.906	0.959
44	56122.23	874.161	271.366	263.323	0.923	0.964
45	51449.43	844.562	258.678	253.239	0.906	0.958
46	54905.35	870.074	268.036	260.814	0.911	0.960
47	53299.08	871.767	264.865	256.216	0.881	0.955
48	54661.98	862.602	267.617	260.065	0.923	0.964
49	50475.93	824.828	254.973	252.058	0.932	0.968
50	46387.24	792.834	248.645	237.535	0.927	0.965
51	52520.28	840.475	259.532	257.660	0.934	0.967
52	45608.44	787.055	244.407	237.598	0.925	0.963
53	58166.58	888.115	274.559	269.742	0.927	0.966
54	46971.34	806.788	248.604	240.566	0.907	0.957
55	54954.03	864.295	271.368	257.840	0.924	0.965
56	50914.01	836.388	264.553	245.038	0.915	0.959
57	51011.36	828.915	256.232	253.480	0.933	0.966
58	45267.71	791.142	246.346	233.967	0.909	0.959
59	49843.16	820.741	256.800	247.128	0.930	0.965
60	47020.01	805.095	247.344	242.042	0.912	0.961
Avg	54731.09	870.9022	269.1731	258.5318	0.906033	0.961
SD	4284.968	38.93852	11.60325	10.3456	0.020534	0.004
CV	0.078291	0.044711	0.043107	0.040017	0.022663	0.004

Table S4. Measured shape parameters of 20 hCOs randomly collected from a deep concave microwell array for 25 days.

No.	Area (μm²)	Perimeter (μm)	Major diameter (μm)	Minor diameter (μm)	Circularity	Solidity
1	395188.8	2486.826	777.968	646.774	0.803	0.974
2	402334.3	2547.117	756.333	677.305	0.779	0.962
3	373442.2	2572.090	819.452	580.244	0.709	0.934
4	387500.8	2489.297	743.185	663.874	0.786	0.959
5	356717.7	2513.150	734.268	618.557	0.710	0.946
6	369908.2	2567.477	701.419	671.470	0.705	0.936
7	414145.3	2713.59	795.163	663.142	0.707	0.933
8	361259.2	2472.034	746.284	616.346	0.743	0.954
9	356578.2	2518.718	758.137	598.849	0.706	0.938
10	386353.8	2565.567	745.245	660.079	0.738	0.946
11	336505.7	2385.024	746.463	573.977	0.743	0.956
12	438542.4	2807.683	777.477	718.181	0.699	0.936
13	426095.9	2637.156	783.033	692.847	0.770	0.955
14	328042.7	2213.774	681.874	612.542	0.841	0.976
15	340024.2	2439.350	750.541	576.827	0.718	0.954
16	364576.2	2336.033	726.996	638.508	0.84	0.974
17	331421.7	2236.277	687.666	613.640	0.833	0.973
18	331483.7	2326.480	696.717	605.782	0.770	0.963
19	316278.1	2254.891	696.491	578.181	0.782	0.966
20	360143.2	2323.614	702.419	652.813	0.838	0.966
Avg	368827.1	2470.307	741.357	632.997	0.761	0.955
SD	34205.2	158.262	38.271	41.739	0.050	0.014
CV	9.2%	6.4%	5.1%	6.5%	6.6%	1.5%

References

[1] W. Gu, X. Gaeta, A. Sahakyan, A. B. Chan, C. S. Hong, R. Kim, D. Braas, K. Plath, W. E. Lowry and H. R. Christofk, *Cell stem cell*, 2016, **19**, 476-490.

[2] J. Pfeuffer, I. Tkáč and R. Gruetter, *J. Cereb. Blood Flow Metab.*, 2000, **20**, 736-746.

Legends of Videos

Movie S1: Outer flow-induced motion of EBs in a concave microwell with 1:2 aspect ratio.

Movie S2: Outer flow-induced motion of EBs in a concave microwell with 1:1 aspect ratio.

Movie S3: Outer flow-induced motion of EBs in a concave microwell with 1.5:1 aspect ratio.

Movie S4: Outer flow-induced motion of EBs in a concave microwell with 2:1 aspect ratio.

Movie S5: Beating of hCOs without drug treatment.

Movie S6: Beating of hCOs after treatment with 0.01 µM isoproterenol.

Movie S7: Beating of hCOs after treatment with 0.03 µM isoproterenol.

Movie S8: Beating of hCOs after treatment with 0.1 µM isoproterenol.

Movie S9: Beating of hCOs after treatment with 0.01 µM verapamil.

Movie S10: Beating of hCOs after treatment with 0.03 µM verapamil.

Movie S11: Beating of hCOs after treatment with 0.1 µM verapamil.