Supplementary Information (SI) for Lab on a Chip.

Supporting Information

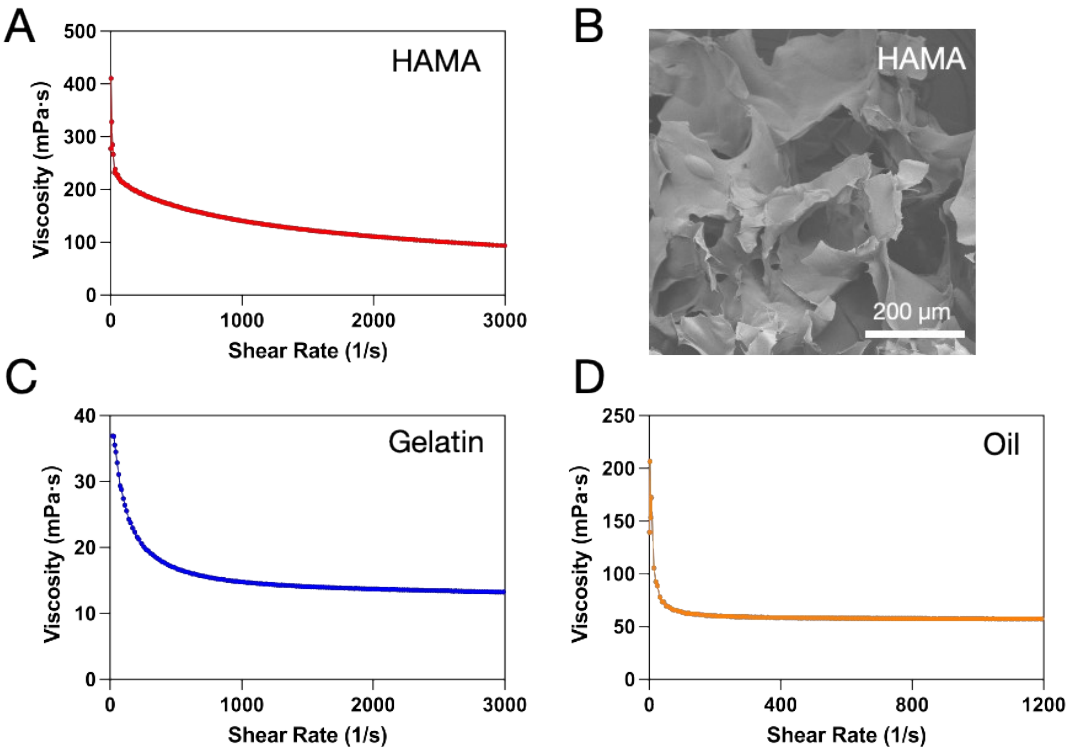


Figure S1. Physical properties of the materials used in HCSM fabrication. (A) Strain yielding properties of HAMA. (B) SEM picture of HAMA. (C-D) Strain yielding properties of Gelatin and Oil.

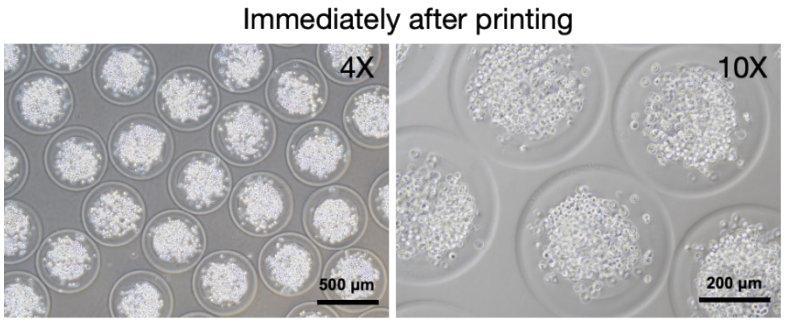
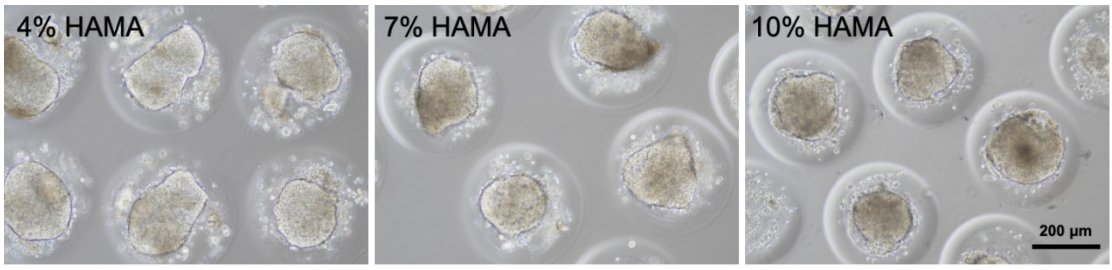


Figure S2. Bright-field images of hiPSCs evenly distributed in HCSM immediately after printing.



Core: 6 μL min^{-1}	Core: 10 μL min^{-1}	Core: 10 μL min^{-1}
Shell: 6 μL min^{-1}	Shell: 10 μL min^{-1}	Shell: 10 μL min^{-1}
Oil: 60 mL h^{-1}	Oil: 60 mL h^{-1}	Oil: 60 mL h^{-1}

Figure S3. Bright-field images of hiPSCs self-assembled into spheroids within different HCSM.

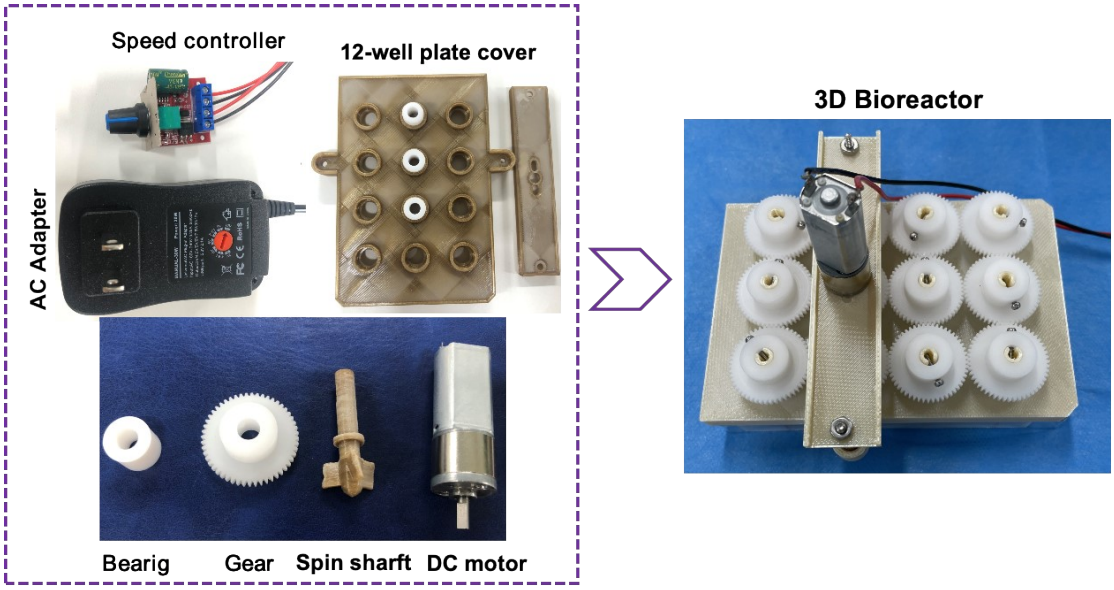


Figure S4. Real images of the stirring speed controllable 3D bioreactor and its main components.

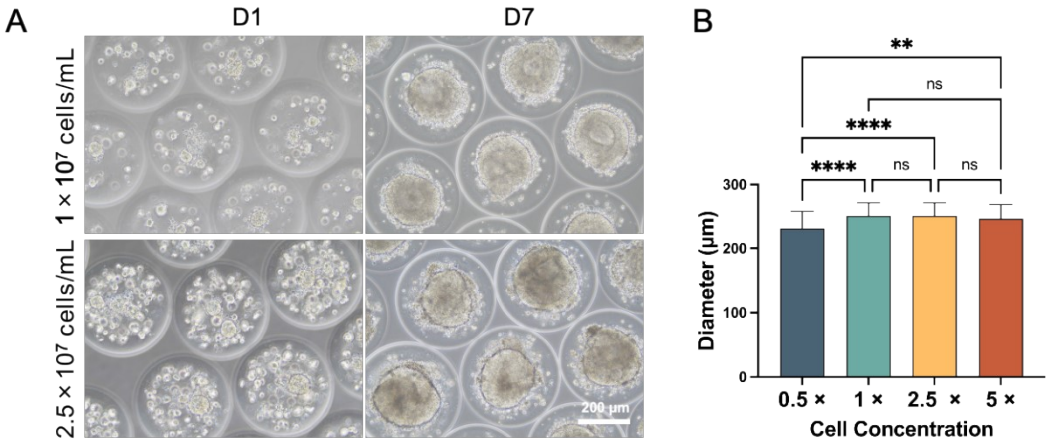


Figure S5. Formation of hiPSC spheroids at different cell printing concentrations. (A) Bright-field images of hiPSC spheroids formed at different cell printing concentrations in static culture. (B) Comparison of the diameters of hiPSC spheroids formed at different printing concentrations on day 7 of static culture. All data were presented as the mean ± SD (n = 3). ***p*<0.01, *****p*<0.0001, ns denoted no significance.

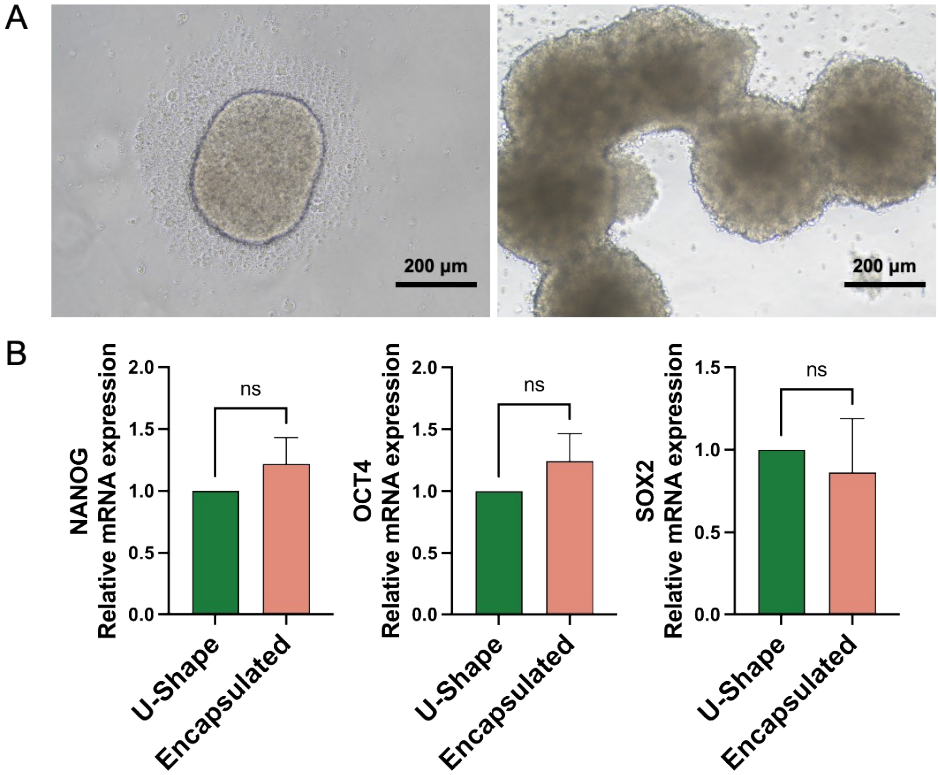


Figure S6. Characterization of hiPSC spheroids cultured in U-shaped 96-well plates. (A) Bright-field images of hiPSC spheroids, in U-shaped 96-well plates (left); fused spheroids (right). (B) Pluripotent gene expression of hiPSCs cultured in U-shaped 96-well plates and encapsulated in HCSM. All data were presented as the mean ± SD (n = 3). Ns denoted no significance.

Video S1: At an oil flow rate of 60 mL h^{-1}, approximately 240 microspheres were generated per minute on this platform. (Video S1 is provided in a separate file in the Supporting Information)

Video S2: Live imaging of hiPSCs at 24 h post-encapsulated in HCSM under microscope. (Video S2 is provided in a separate file in the Supporting Information).

Video S3: Video of the 3D bioreactor being stirred at different speeds. (Video S3 is provided in a separate file in the Supporting Information)

Video S4: Live imaging of uniformly sized hiPSC spheroids at 5 d post-encapsulated in HCSM under microscope.(Video S4 is provided in a separate file in the Supporting Information)

Video S5: Live imaging of beating cardiac organoid derived from hiPSCs at D27 of differentiation in HCSM under microscope. (Video S5 is provided in a separate file in the Supporting Information)

Video S6: Live imaging of beating cardiac organoid at D10 with Fluo-4 staining under fluorescence microscope. (Video S6 is provided in a separate file in the Supporting Information)

Table S1: List of primers used for RT-PCR analysis.

Gene	Forward	Reverse
GADPH	TGTTGCCATCAATGACCCCTT	CTCCACGACGTACTCAGCG
NANOG	CCGGTCAAGAAACAGAAGACCAGA	CCATTGCTATTCTTCGGCCAGTTG
OCT4	GATCACCCTGGGATATACAC	GCTTTGCATATCTCCTGAAG
SOX2	TCAGGAGTTGTCAAGGCAGAGAAG	GCCGCCGCCGATATTGTTATTAT
SOX17	GGCGCAGCAGAATCCAGA	CCACGACTTGCCCAGCAT
AFP	AGTGAGGACAAACTATTGGCCT	ACACCAGGGTTTACTGGAGTC
ALB	GAGACCAGAGGTTGATGTGATG	AGTTCCGGGGCATAAAAGTAAG
CK19	ACCAAGTTTGAGACGGAACAG	CCCTCAGCGTACTGATTTCCT
CYP3A4	TCATTGCTGTCTCCAACCTTCACC	GCTTCCCGCCTCAGATTTCTCAC
CYP2C9	GAGGAGCATTGAGGACCGTGTTC	GGAGCACAGCCCAGGATGAAAG

Table S2: List of primary antibodies used in immunofluorescence staining.

Antigen	Host species	Supplier	Cat number	Dilution
NANOG	Rabbit	abcam	ab109250	1:250
OCT4	Mouse	abcam	ab184665	1:250
SOX2	Rabbit	abcam	ab97959	1:500
Nestin	Mouse	abcam	ab22035	1:500
ACTN2	Mouse	abcam	ab9465	1:500

TNNI	Mouse	Santa Cruz Biotechnology	sc-365446	1:250
CYP3A4	Mouse	Santa Cruz Biotechnology	sc-393860	1:250
CK19	Rabbit	abcam	ab76539	1:500
ALB	Goat	Bethyl	A80-129A	1:250
VIM	Rabbit	MCE	HY-P80371	1:500
E-cadherin	Mouse	abcam	ab231303	1:500
ZO-1	Rabbit	abcam	ab307799	1:500
F-Actin		Beyotime	C2205S	1:200