Supplementary Information

Model Cancer Metastasis using Acoustically Bio-printed Patient-Derived 3D Tumor Microtissues

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Solutions

1x Penicillin/Streptomycin in PBS: (1X PBS / 1x Penicillin/Streptomycin) 1ml 100x Penicillin/Streptomycin was added to 100ml 1X PBS and mix well.

1% BSA solution: (1X PBS / 1% BSA) 5.0g of bovine serum protein was added to 500ml of 1X PBS, mixed evenly, and then filtered through a 0.22um filter to obtain the 1% BSA solution.

Blocking buffer: (1X PBS / 5% regular serum / 0.3% Triton ™ X-100) To prepare 10 ml, add 0.5 ml of normal goat serum and 30 µl of Triton ™ X-100 to 9 ml of 1X PBS and mix well.

Antibody dilution buffer: (1X PBS / 1% BSA / 0.3% Triton™ X-100) To prepare 10 ml, add 30 ul Triton ™ X-100 to 9.70 ml 1% BSA solution.

Tissue digestive enzymes: (4.7ml RPMI / 200ul enzyme H / 100ul enzyme R / 25ul enzyme A) The corresponding amount of enzyme were added to serum-free medium RPMI, mix the liquid with a pipette dozens of times and mix well.

Preparation:

Miltenyi Human tumor dissociation kit:

Prepare Enzyme H by reconstitution of the lyophilized powder in each vial with 3 mL of RPMI 1640 or DMEM. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw cycles. Store aliquots at –20 °C. This solution is stable for 6 months after reconstitution. For cell culture experiments subsequent to tissue dissociation, Enzyme H should be sterile filtered prior to aliquoting.

Prepare Enzyme R by reconstitution of the lyophilized powder in the vial with 2.7 mL RPMI 1640 or DMEM. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw cycles. Store aliquots at –20°C. This solution is stable for 6 months after reconstitution. ▲Note: Make sure to thoroughly mix this suspension immediately before withdrawing the required reaction volume!

Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL of Buffer A supplied with the kit. Do not vortex. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw cycles. Store aliquots at –20°C. This solution is stable for 6 months after reconstitution.
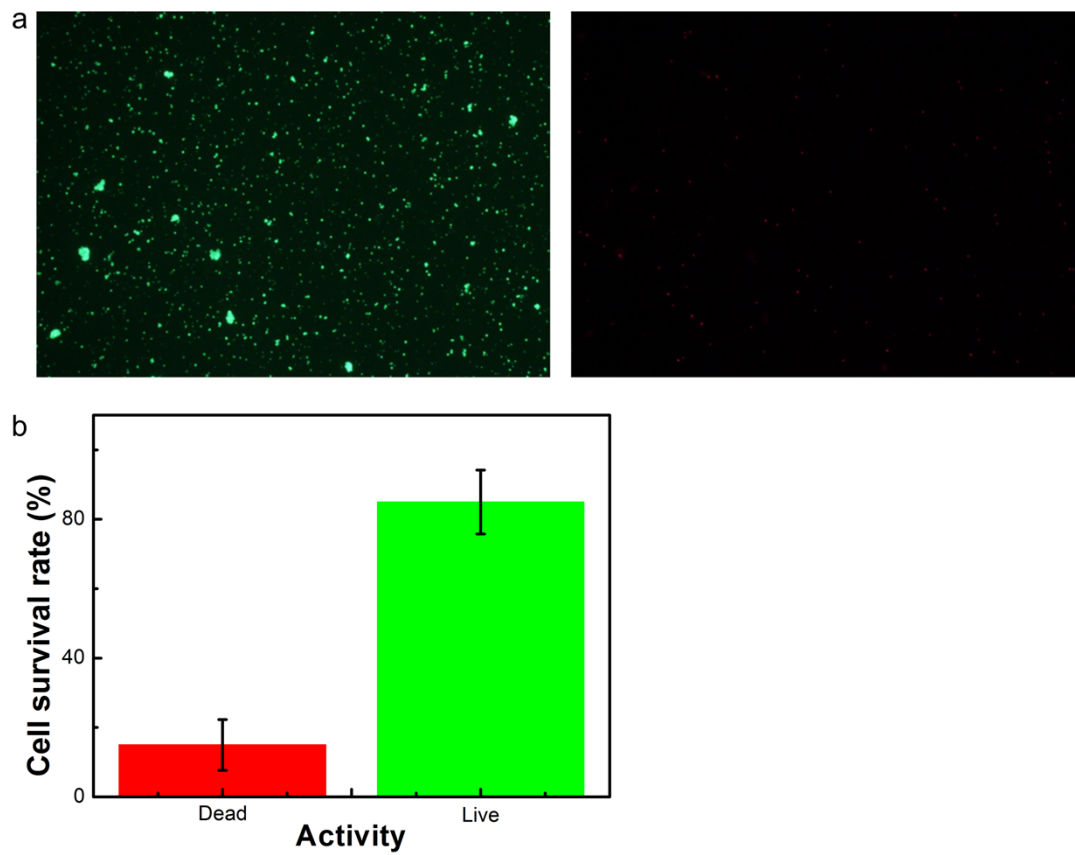
Fig. S1 (a) Characterization of the survival rate of cells isolated from human tissues. Red means dead cells, green means live cells. (b) Quantification of cell viability.
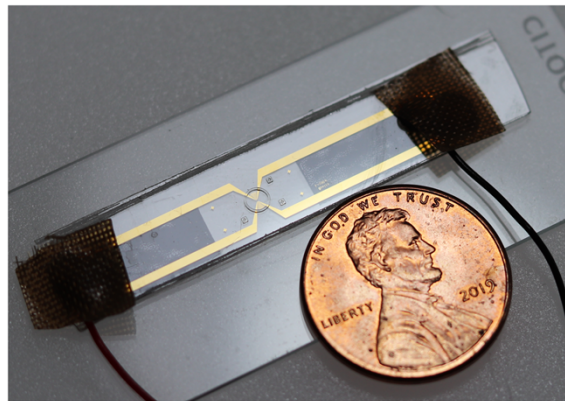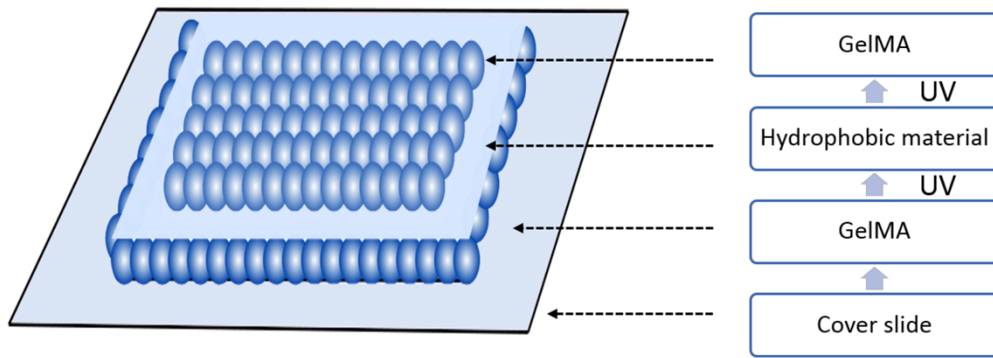
Fig. S2 (a) Schematic diagram of the construction of a 3-dimensional model of acoustic 3D printed devices. (b) Physical image of acoustic 3D printing device.
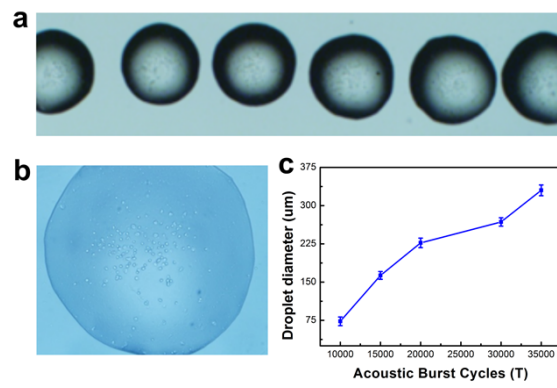
Fig. S3 (a) Acoustic 3D printing devices perform droplet placement and (b) manipulation of cell-containing droplets. (c) the size of the acoustic droplet changes with the acoustic signal.
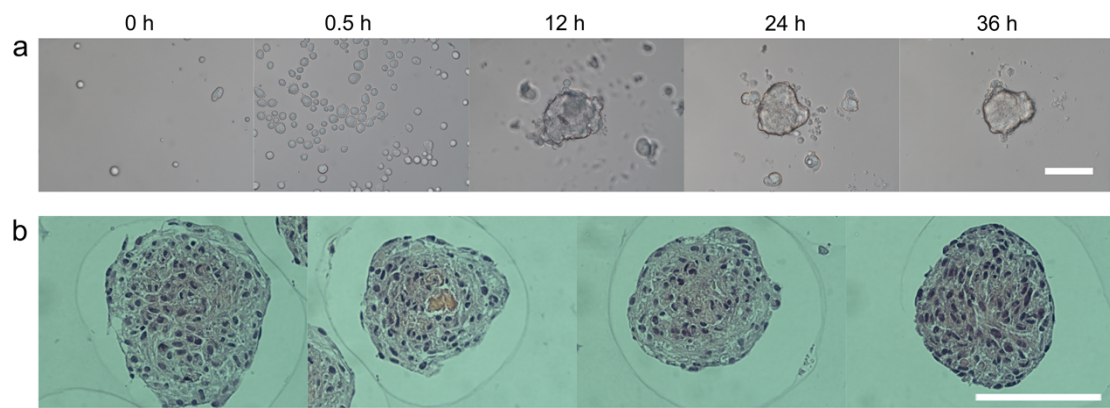
Fig. S4 (a) The patient-derived cells were quickly cultured into tumor micro-tissues within 36 hours. (b) HE section of patient-derived tumor microtissue. The scale bar is 100um.
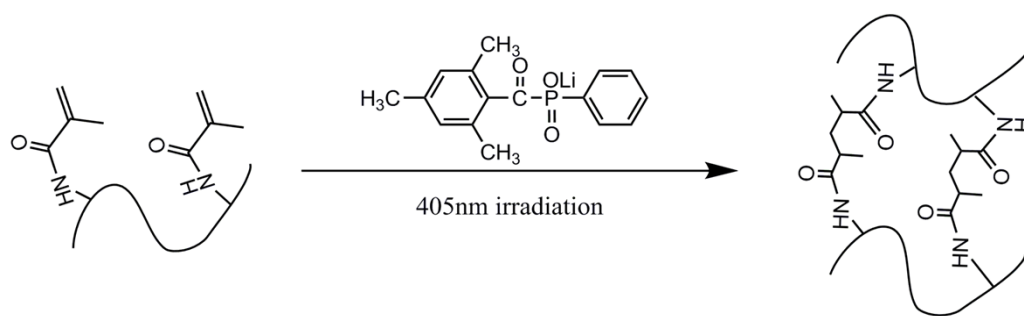
Fig. S5 The GelMA curing process.

Table S1. Pulse signal parameter setting.

Amp	Frequency	Trig	Burst	Gain
100-140mv	38.13 MHz	2.0s	25000	<25w

Reagents

Table S2. Reagents used in the tissue digestion and cell culture.

Reagent	company	Cat.NO	Final concentration
Tumor Dissociation Kit, human	Miltenyi	130-095-929	Instruction concentration
Primocin	Invivogen	Ant-pm-1	50 mg·ml^{-1}
DMEM/F-12	GIBCO	C11330500BT	1X
Y-27632	Abmole	Y-27632	10 uM
Penicillin/Streptomycin	Invitrogen	15140-122	100U·ml^{-1}/100mg·ml^{-1}
D-PBS	Gibco	14190250	1X
PBS	Gibco	10010023	1X
FBS	Gibco	16140071	10%
BSA	Sigma	A1933-5G	1mg/ml
Triton ™ X-100	Sigma	X100	0.3%
RPMI	Gibco	72400047	1X
DMSO	Sigma	94563-10ml	10%
Paraformaldehyde	Sigma	158127	4%
Trypsin	Gibco	25200056	0.25%

Table S3. The antibodies used for Immunofluorescence were listed below:

Primary antibodies	Company	Cat.NO	Dilution
EGF Receptor (E746-A750del Specific) (D6B6) XP® Rabbit mAb#2085	Cell Signaling Technology	2085S	1:250
CD44 (156-3C11) Mouse mAb#3570	Cell Signaling Technology	3570S	1:400
CD133 (D2V8Q) XP® Rabbit mAb#64326	Cell Signaling Technology	64326S	1:400
Ki67	ZSGB-BIO	ZM0166	1:100
Secondary antibodies	**Company**	**Cat.NO**	**Dilution**
Anti-rabbit IgG (H+L), F(ab')2 Fragment (PE Conjugate)#8885	Cell Signaling Technology,	8885S	1:250
Anti-mouse IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 488 Conjugate)#4408	Cell Signaling Technology,	4408S	1:250

Table S4. Patients' baseline characteristic.

Patient number	Sex	Age (years)	TNM stage	Tumor location	Depth of tumor invasion	Differentiation	Invasion ability
P(V)1	male	69	pT3N0Mx	sigmoid colon	Subserosal layer	moderate	No clear vessel and nerve invasion
P(V)2	female	59	pT3N2bMx	Near the junction of straight and second, the size is about 5cm×5cm	Subserosa fibrofatty tissue	moderate	Visible vascular tumor thrombus, no clear nerve invasion
P(V)3	male	71	pT3NoMx	Mid rectum	Serosa fibrofatty tissue	moderate	No clear vessel and nerve invasion
P(V)4	female	49	pT4bN1Mx	Ascending colon	Serosal layer	moderate	No clear vessel and nerve invasion
P(V)5	male	58	ypT3N0Mx	Peritoneal reflex	Serosal layer	G2 (moderate)	No clear vessel and nerve invasion
P(V)6	female	44	ypT3N2Mx	Anterior peritoneal reflex	Extramuscular fibrous tissue	moderate	Vascular infiltration: visible; nerve infiltration: visible
P(V)7	male	66	pT3N2bMx	Upper rectum	Extramuscular fibrofatty tissue	Poor	Vascular infiltration: visible; nerve infiltration: visible