Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

Multidimensional Controllable Fabrication of Tumor Spheroid Based on a Microfluidic Device

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Experimental Procedures

Reagents: HCT116, A549, HepG2, and NIH3T3 were obtained from Cancer Institute & Hospital Chinese Academy of Medical Science (China). Polydimethylsiloxane (PDMS) and initiators were purchased from Dow Corning (USA). SU-8 2050 negative photoresist and developer were purchased from Microchem Corporation (USA). HFE-7500 was purchased from 3M Novec 7500 Engineered Fluid (USA). Sodium Alginate and 5-fluorouracil were purchased from Sigma-Aldrich (USA). Perfluorinated polyether-polyethylene glycol (PFPE−PEG) was purchased from Raindance Technologies (USA). Calcium chloride was purchased from Solarbio (China). 1*H*,1*H*,2*H*,2*H*-Perfluorooctyl trichlorosilane, 1*H*,1*H*,2*H*,2*H*-perfluoro-1-octanol, and EDTA-2Na solution (PFO) were purchased from Macklin (China). 200 nm green fluorescent particle solution was purchased from ThermoFisher Scientific (USA). Dulbecco's Modified Eagle Medium (DMEM), trypsin, penicillin, and streptomycin were obtained from Solarbio (China). Fetal bovine serum (FBS) was obtained from Gibco Corporation (USA). EZ-Link Sulfo-NHS-LC-Biotin was purchased from ThermoFisher Scientific (USA). Streptavidin was obtained from Solarbio (China). CellTracker Green CMFDA and CellTracker Red CMTPX were obtained from Invitrogen (USA). EliKine Human VEGF ELISA Kit was purchased from Abbkine (USA). Live/dead assay kit (CalceinAM/PI) and Cytotoxicity assay (CCK-8) kit were obtained from Dojindo Laboratories (Japan). Stroke-physiological saline solution, 4% paraformaldehyde fix solution, QuickBlock blocking buffer for immunol staining, primary antibody dilution buffer, and secondary antibody dilution buffer for immunofluorescence were purchased from Beyotime (China). Hoechst 33342, E-cadherin monoclonal antibody, and $F(ab')_2$-goat anti-mouse IgG (H+L) secondary antibody were obtained from ThermoFisher Scientific (USA). FITC conjugated avidin was purchased from Boster Biological Technology (China).

Cell culture: All cells were cultured in DMEM supplemented with 10% FBS, 100 μg mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin. The cells were cultured in 75 cm^2 culture flasks at 37℃ in a humidified atmosphere of 5% CO_2. The cells were recovered every 2-3 days to keep subconfluently.

FITC-conjugated avidin modification of cells: Dissolved FITC-conjugated avidin solution in PBS solution for a concentration of 100μg mL^{-1}. Covered the adherent biotinylated cells with the FITC-conjugated avidin solution for 15 min at 37℃. Then, remove the solution and wash the cells with PBS 3 times. The

cells were observed using a Zeiss LSM780 inverted confocal laser scanning microscope. After 24h of culture, wash the cells with PBS 3 times, and then observed.

Sample preparation and UPLC-MS/MS condition: The gradient elution conditions of chromatography were as Table S1, flow rate maintains at 0.3 mL min^{-1}. For sample preparation, 100μL culture medium was added into a tube and mixed with 200μL. Then centrifuged the sample at 15000 rpm for 15 minutes. The supernatant was diluted with ultrapure water at a volume proportion of 1:9 to obtain a sample for MS analysis.

Table S1 The gradient elution conditions of chromatography

Time	Mobile phase A (ultrapure water, %)	Mobile phase B (acetonitrile, %)
0.25	95	5
2.5	5	95
3.5	5	95
4	95	5

Statistical analyses: All numerical results were expressed as means ± standard errors of the means of at least three independent experiments, and the two-way analysis of variance was used for statistical comparisons. Significance was tested at p-values 0.05 (*), 0.01 (**), and 0.001 (***). Scientific plots were generated in Origin2021 software.

Supplementary Figures

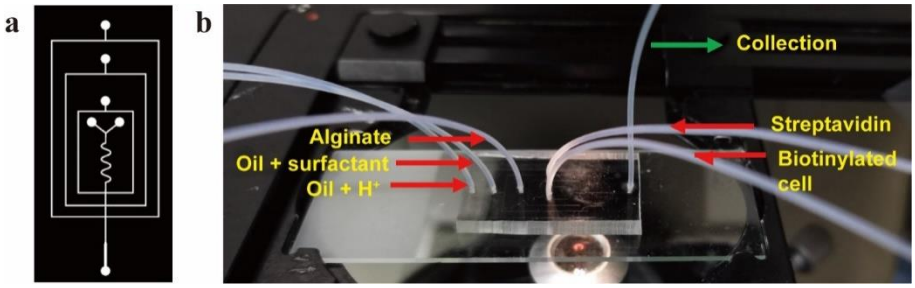

Fig. S1. Design and operation of the microfluidics device. (a) The mask design of the microfluidics device. (b) Device diagram for microcapsules generation.

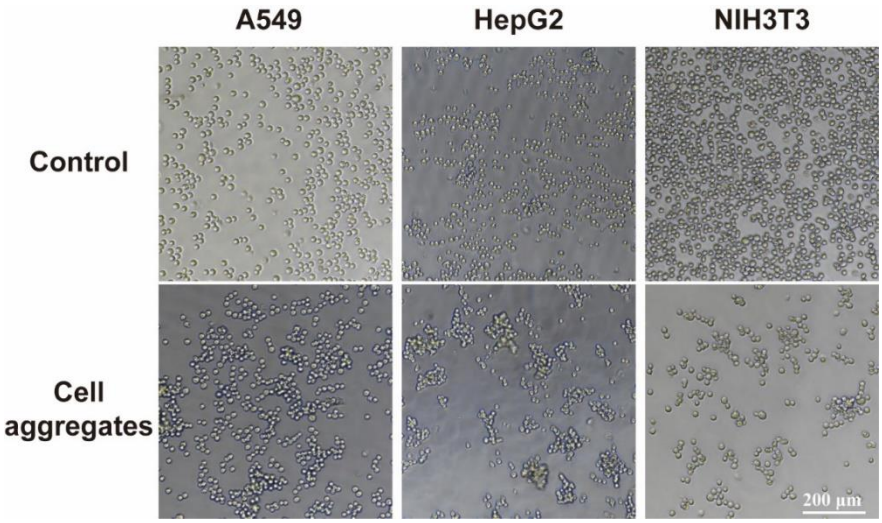

Fig. S2. Brightfield images of biotinylated A549, HepG2, and NIH 3T3 before and after mixing with streptavidin solution using the microfluidics device. Cell aggregates were formed after mixing.

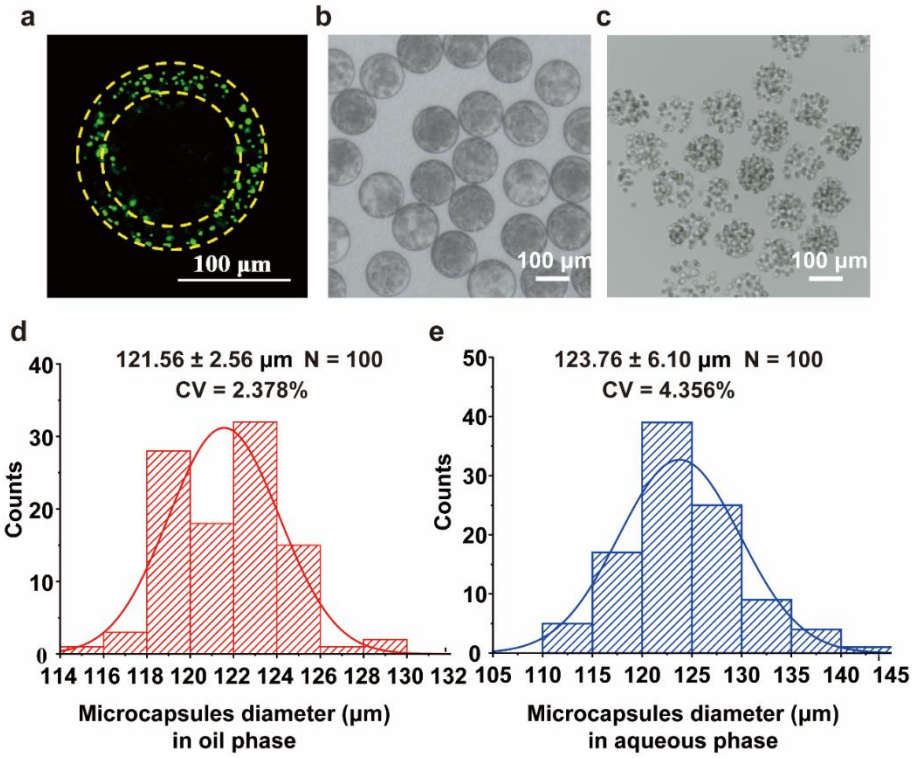

Fig. S3. Morphology of the cell-laden microcapsules. (a) Confocal fluorescence images of the microcapsules. 5% (v/v) 200 nm green fluorescent particle solution was mixed with the alginate phase to visualize the alginate phase. (b) and (c) showed the bright field of cell-laden microcapsules in the oil phase and in the aqueous culture medium respectively. (d) Size distribution of microcapsules produced at flow rates of 100/50/500/500 µl h^{-1} in the oil phase. (n = 100) (e) Size distribution of microcapsules produced at flow rates of 100/50/500/500 µl h^{-1} after being transferred into an aqueous culture medium. (n = 100)

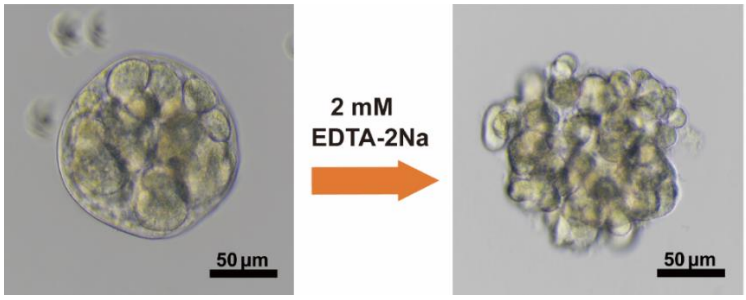

Fig. S4. Spheroids released from the microcapsules in the culture medium containing 2 mM EDTA-2Na.

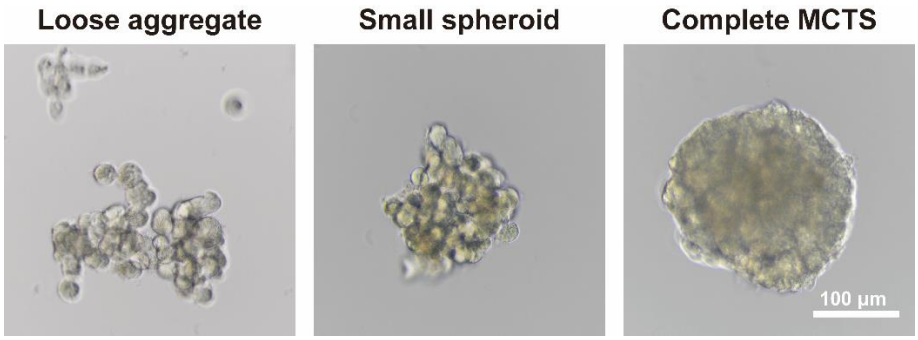

Fig. S5. Brightfield images of cell clusters released from microcapsules at different stages of spheroid formation.

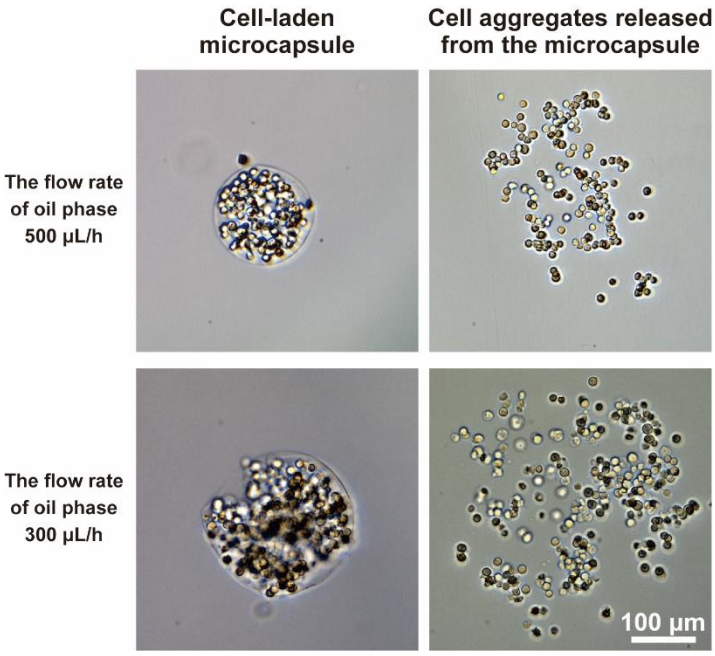

Fig. S6. Brightfield images of initial cell morphology in cell-laden microcapsules. In-situ cell release was carried out immediately after the microcapsules were harvested.

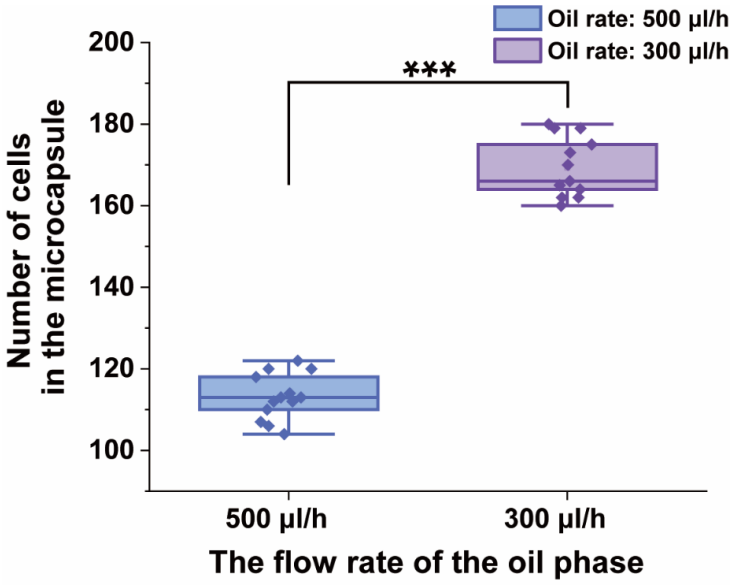

Fig. S7. Quantification of initial cell loading of different sizes of cell-laden microcapsules. The cell-laden microcapsules were generated at the flow rate of the oil phase fixed at 300 μl h^{-1} and 500 μl h^{-1} respectively. (N=13, ***P < 0.001).

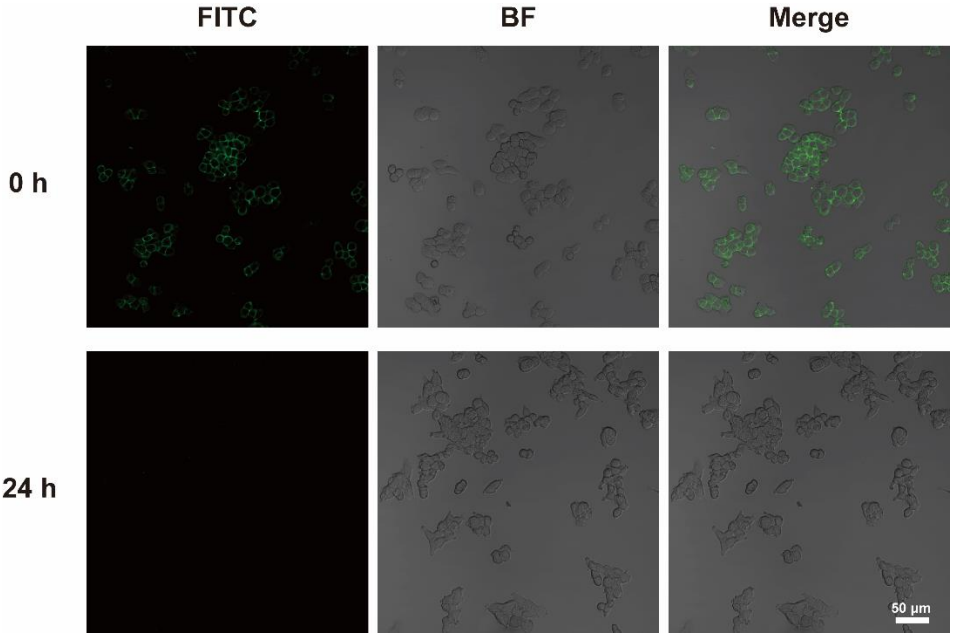

Fig. S8. Confocal fluorescence images and bright field images of biotin and FITC-conjugated avidin modified adherent cells. The pictures were taken at 0h and 24h after modification.

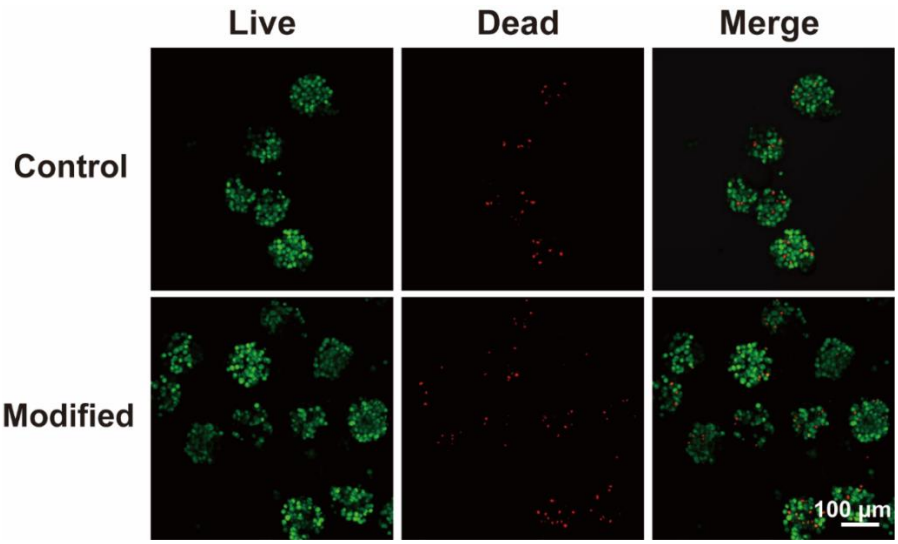

Fig. S9. Confocal fluorescence images of modified or unmodified HCT116 cell-laden microcapsules after 1 day of culture. Calcein-AM/PI staining kit was used to stain the cells**.**

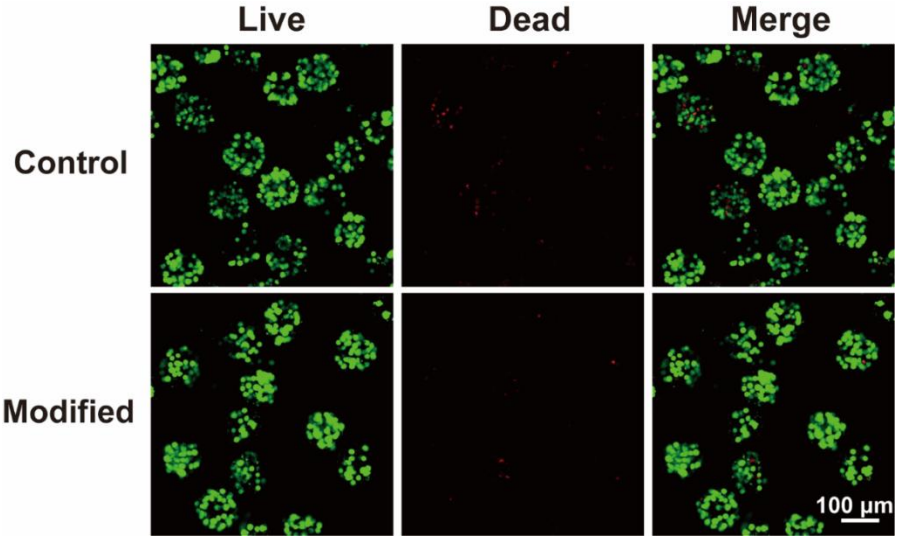

Fig. S10. Confocal fluorescence images of modified or unmodified HepG2 cell-laden microcapsules after 1 day of culture. Calcein-AM/PI staining kit was used to stain the cells.

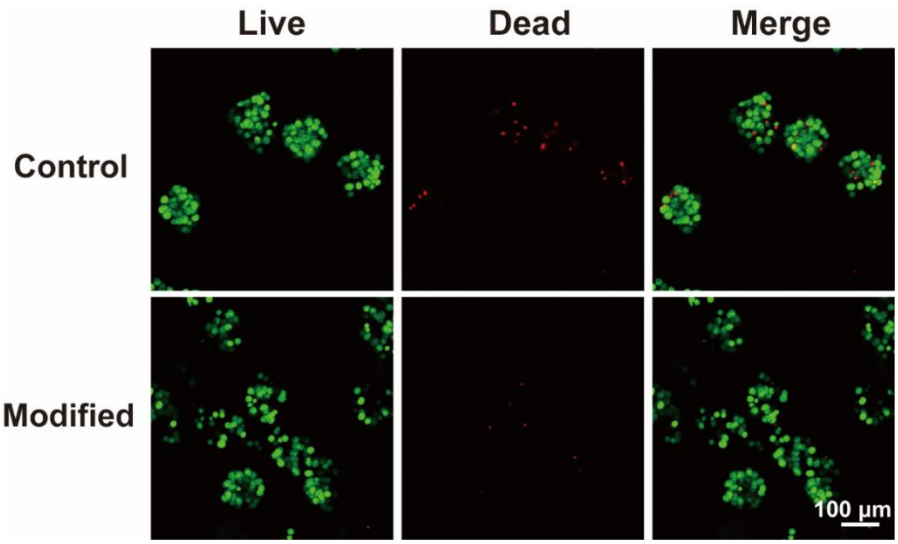

Fig. S11. Confocal fluorescence images of modified or unmodified A549 cell-laden microcapsules after 1 day of culture. Calcein-AM/PI staining kit was used to stain the cells.

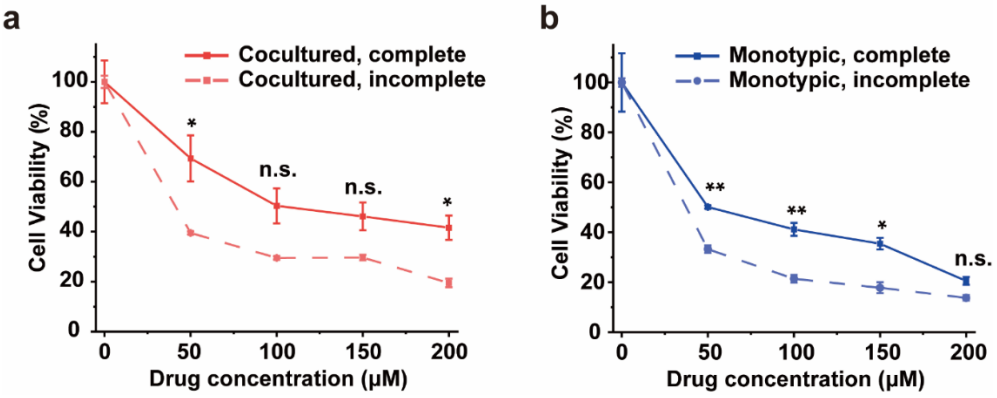

Fig. S12. The cell survival rates of MCTSs after 72h of 5-FU penetration. (*P < 0.05, **P < 0.01). (a) Comparison of cocultured MCTSs' complete and incomplete spheroid drug resistance. (b) Comparison of monotypic MCTSs' complete and incomplete spheroid drug resistance.

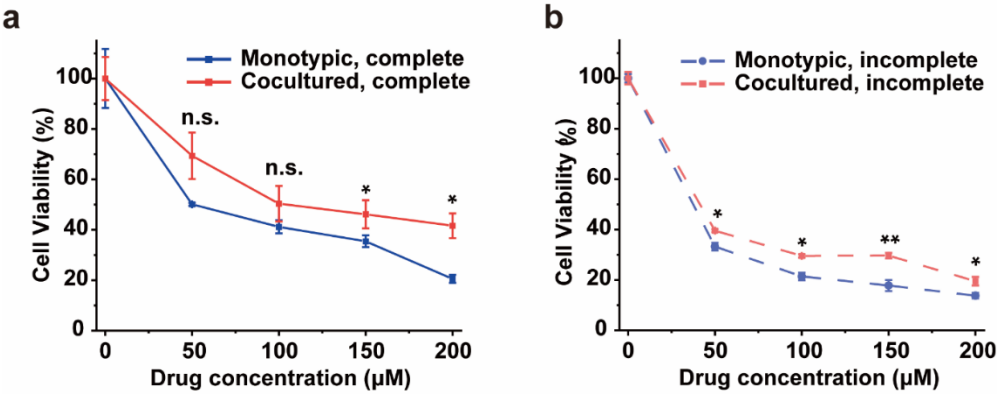

Fig. S13. The cell survival rates of MCTSs after 72h of 5-FU penetration. (*P < 0.05, **P < 0.01). (a) Comparison of complete MCTSs' monotypic and cocultured spheroid drug resistance. (b) Comparison of incomplete MCTSs' monotypic and cocultured spheroid drug resistance.

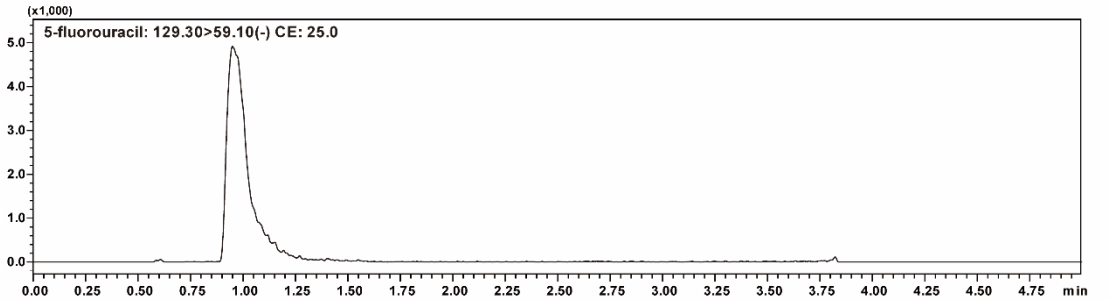

Fig. S14. Chromatograms of the 5-fluorouracil standard sample measured with elution mobile phase was (A) ultrapure water and (B) acetonitrile, which was applied as gradient elution conditions in Table S1.

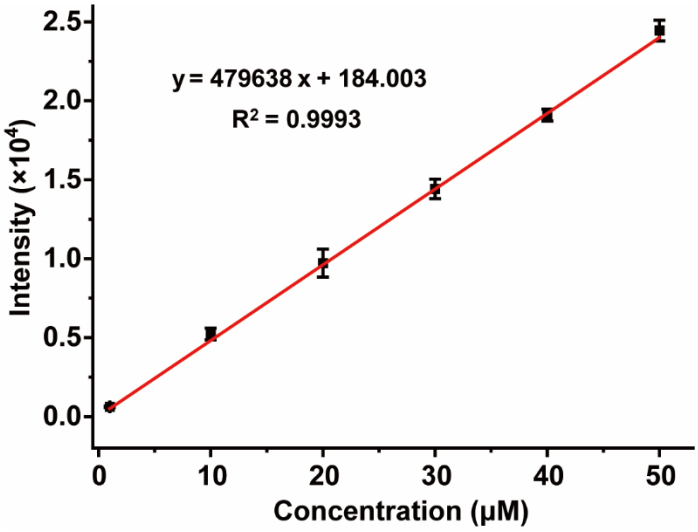

Fig. S15. Calculated standard curves for quantitatively analyzing 5-fluorouracil in the DMEM culture medium.

Supplementary Table

Table S2 Comparison with other hydrogel microdroplets for 3D tumor model

Culture Model	Droplet module	Hydrogel	Size (μm)	Versatility	Controllable parameters	Ref
MCTS	scaffold-free microcapsule;	Alginate (removable)	120-180	HCT116, HepG2, A549, and NIH3T3 (fibroblasts)	Size, initial cell adhesion, cell loading ratio, formation period	Present work
Cell colonies	Scaffold-based microparticle;	PEG-fibrinogen	850	MCF-7, MDA-MB-231	-	Seeto et al. **2022**[1]
MCTS	Scaffold-free microcapsule;	Alginate	75	MCF-7	-	Sun et al. **2018**[2]
MCTS	Scaffold-free microdroplet	-	100-130	Glioblastoma	Size	Lee et al. **2020**[3]
MCTS	Scaffold-based microparticle	type I collagen & methylcellulose	200	MCF-7, MDA-MB-231	Matrix	Rasouli et al. **2021**[4]
MCTS & tumor organoid	Scaffold-based microparticle	Gelatin & cellulose nanocrystals	100/300	MCF-7, patient-derived cancer cells	Size, drug dose	Prince et al. **2021**[5]
tumor organoid	Scaffold-based microparticle;	Matrigel	>400	patient-derived cancer cells	-	Jiang et al. **2020**[6]

References

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