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

Controlled Assembly of Heterotypic cells in a Core-Shell Scaffold: Organ in a Droplet

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1. Fabrication of Microfluidic device

The microfluidic device was designed by Auto CAD 2012 software and fabricated by soft lithography as follows.[1] 100 μm SU-8 2050 photoresist was spin-coated on a silicon wafer at 1000 rpm for 45 s. After soft bake at 95℃ for 45 min, the coating was masked by a droplet-maker design and exposed under UV for 27 s. The patterns were then developed with SU-8 developer (MicroChem, MA, USA) after post exposure bake (5 min). The patterns of the photoresist on the silicon wafer were replicated by PDMS stamp. After curing at 65℃ for 1 hour, PDMS stamp was peeled off from the wafer and bonded onto a glass slide after surface treatment by oxygen plasma. Before each experiment, the microchannels of the PDMS device were chemically treated to be hydrophobic using commercial Aquapel solution (Aquapel Glass Treatment, PA, USA).

2. Generation of core-shell spheroids

Water-in-water-in-oil (w/w/o) double emulsions are generated using the microfluidic device. The inner phase was cell culture media, the middle phase was 1.7% MVG-alginate (medium viscosity high-guluronic acid alginate, viscosity > 200 mPa·s; PRONOVA, MA, USA) containing 100 mM Ca-EDTA as cross-linking precursor, and the outer phase was HFE-7500 (3M, MN, USA) with 1% surfactants (Perfluorinatedpolyethers-polyethyleneglycol; RainDance Technologies, MA, USA). Co-flows of the inner and middle phases were sheared by the oil phase at the cross-junction, forming monodisperse droplets of aqueous core and hydrogel shell. Different sizes of double emulsions were generated by changing the flow rates.

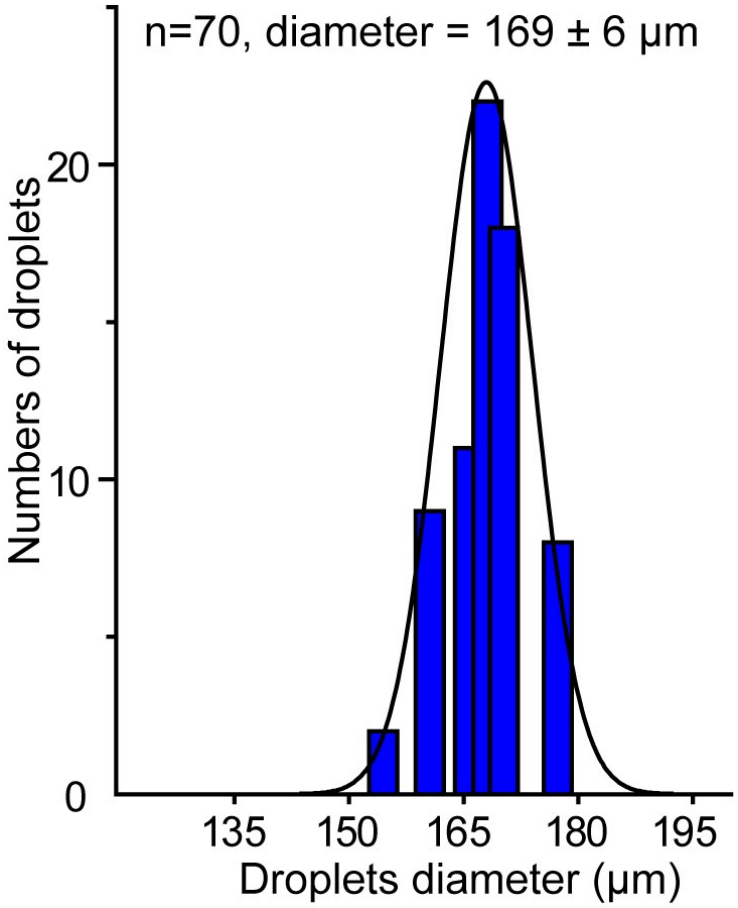


Figure S1. The narrow size distribution of monodisperse w/w/o double emulsions.

3. Image analysis and viability test

The process of droplet formation was monitored by a CCD camera (SONY XCDV60, Japan) in an IX2-SLP inverted microscope. We labeled the alginate in the shell with fluorescein and imaged the 3D core-shell structure under confocal microscopy (Leica TCS SP5). LIVE/DEAD® Viability/Cytotoxicity Kit (for mammalian cells) was purchased from Life Technologies, Inc. (USA). Cell viability was tested by live/dead kit (Calcein AM/EthD-1 staining).

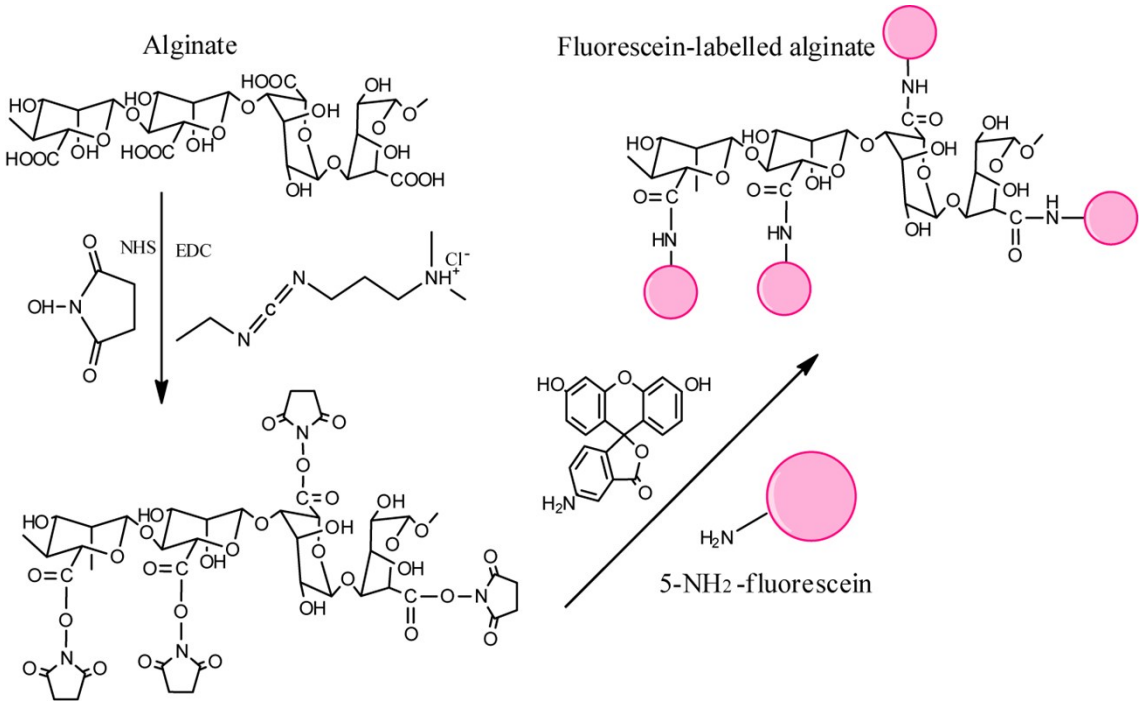
4. Culture of cell lines and heterocellular spheroids

HepG2 cells (cell line of human hepatocellular carcinoma) and NIH-3T3 fibroblasts (cell line of mouse embryonic fibroblast) were cultured in Dulbecco's modified Eagle medium (DMEM; Gibco, NY, USA) containing 10% fetal bovine serum (FBS; Gibco, NY, USA) and 1.0% penicillin-streptomycin at 37℃ and 5% CO_2. The culture medium was replaced every 2-3 days. For each experiment, cells were detached from flasks by trypsin/EDTA solution and re-suspended in DMEM with a density of 1×10^8 cells/ml. After encapsulations, heterocellular spheroids of HepG2 cells in the core and NIH-3T3 fibroblasts in the shell were filtered out by

a cell strainer (40 μm; BD Falcon, USA) and were co-cultured in DMEM with 7.5 mM $CaCl_2$ at 37℃and 5% CO_2.

5. Synthesis of fluorescein-labeled alginate

Fluorescein-labeled alginate was synthesized by conjugating alginate with 5-aminofluorescein based on the EDC-NHS reaction, as schematically shown in Scheme S1. 100 mg alginate was first dissolved in 5.0 mL PBS buffer overnight. 5.0 mg 5-aminofluorescein, 300 mg N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC; Sigma, USA) and 150 mg N-Hydroxysuccinimide (NHS; Sigma, USA) were then successively added into the alginate solution under stirring. After reaction at room temperature for 24 hours, the solution was transferred to a dialysis tubing (MWCO: 3500 Da). The dialysis was then performed in deionized water for 5 days and the water was replaced 2-3 times per day. Therefore, fluorescein-labeled alginate solution of 0.8 % (w/v) was obtained, which was stored at 4℃.



Scheme S1. Synthesis of fluorescein-labelled MVG alginate by EDC-NHS reaction.

6. Spatially assemble different cells in the core-shell spheroids

Using the microfluidic device, different cells were spatially assembled into the core and shell of the spheroid, respectively. HepG2 cells were suspended in the inner aqueous solution with a density of 1×10^8 cells/ml. Due to the lamellar flow and the fast *in situ* cross-linking of alginate in the shell, HepG2 cells dispersed in the inner phase were spatially confined in the core of the spheroid, as shown in Figure S2. Similarly, NIH-3T3 fibroblasts were spatially arranged in the shell by pre-mixing them with the middle alginate solution, as shown in

Figure S3. We simultaneously assembled HepG2 cells in the core and NIH 3T3 fibroblasts in the shell, forming a 3D microtissue in each drop, as shown in Figure S4. A movie was recorded to clearly demonstrate each process, as shown in Movie S1.

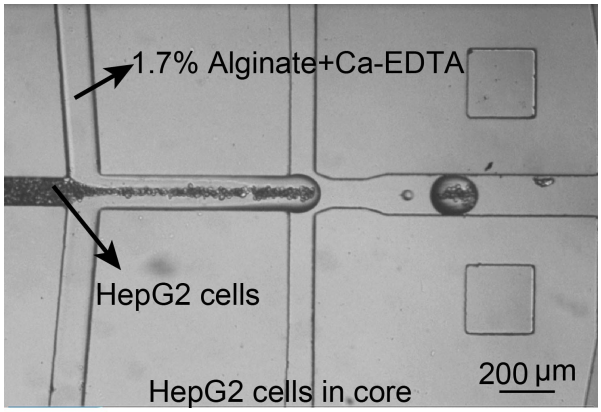


Figure S2. HepG2 cells confined in the core of the spheroid.

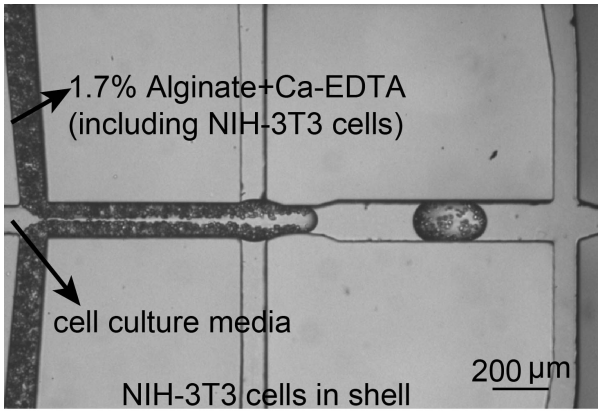


Figure S3. NIH-3T3 fibroblasts distributed in the shell of the spheroid.

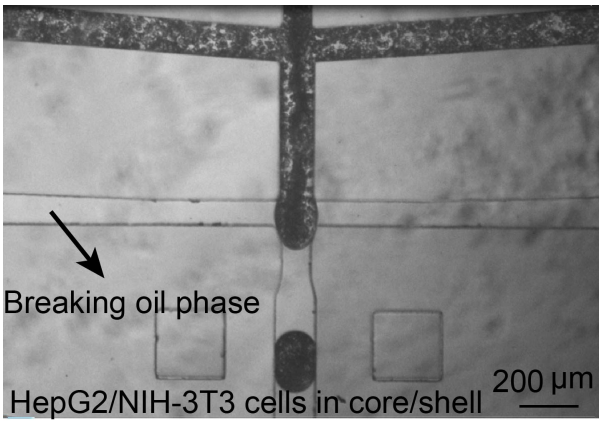


Figure S4. Assembling HepG2 cells and NIH-3T3 fibroblasts into the core and shell, respectively.

Movie S1. *In situ* monitoring the fabrication of core-shell spheroids containing spatially confined cell ensembles.

7. SEM characterization of core-shell spheroids

The morphology of core-shell spheroids was characterized by scanning electron microscopy (SEM; Ultra FESEM, Zeiss). The spheroids maintained their original structure after freeze-drying and were imaged by SEM at room temperature, as shown in Figure S5. To visualize the inner structure, Cryo-SEM was used to observe the cross section of the spheroids (data shown in the paper).

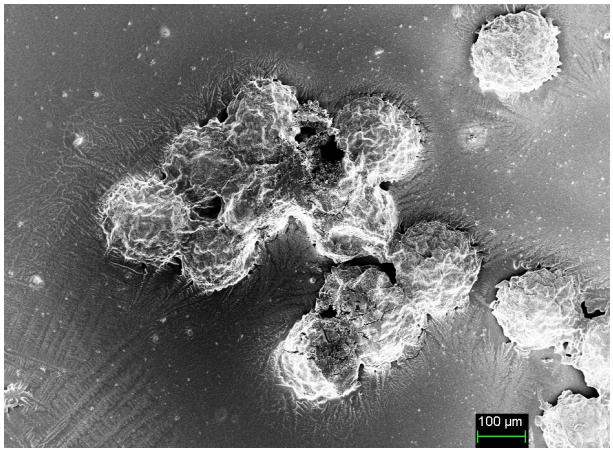


Figure S5. SEM image of freeze-dried core-shell spheroids.

8. Measurement of albumin secretion and urea synthesis

In our experiments, the albumin secretion was quantitatively analyzed by a commercial albumin blue fluorescent assay kit (Active Motif, Inc., USA). This kit simplifies the albumin quantification by the intensity of the fluorescent signal of highly sensitive detection reagents. In a typical procedure, we first set up an albumin standard curve in duplicate using the following concentrations: 200, 100, 50, 25, 12.5, 6.3, 3.2 and 0 µg/mL. To determine the concentration of albumin in each samples, a series of dilutions are prepared with Buffer B. 25 µL of each concentration is mixed with 150 µL dye reagent working solution in a separate well. After incubating the samples 5 minutes at room temperature (25°C) with gentle shaking, the fluorescence intensity was measured at 620 nm with excitation at 560 nm. Each experiment is performed in duplicate.

The urea synthesis was quantitatively analyzed by a commercial urea colorimetric assay kit (BioVision, USA). In the assay, urea reacts as substrate with compounds in the presence of enzymes to form a product that reacts with the OxiRed probe to generate color (λ_{max} = 570nm). The optical density of produced color has a direct relationship with the urea concentration in the solution. In a typical procedure, we first diluted the urea standard to 0.5 mM. Add 0, 2, 4, 6, 8, 10 µL of 0.5 mM urea standard into separate wells. Adjust the total volume of each well to 50 µL with assay buffer to generate the urea standard of 0, 1, 2, 3, 4, 5 nmol/well. 25 µL

samples were collected from cell culture meadium and mixed with 25 μL assay buffer. Therefore, the sample to be tested have the same total volume as that of urea standard. 50 μL reaction mix was added to each well containing the urea standard and the samples. After incubating the reaction protected from light for 60 min at 37°C, the O.D. was measured at 570 nm using a micro plate reader.

9. Reference:

[1] Q. Chen, J. Wu, Y. Zhang, Z. Lin, J.-M. Lin, *Lab Chip*, **2012**, 12, 5180.