Lab on a Chip

Dynamic Article Links ►

Cite this: DOI: 10.1039/c1lc20494g

www.rsc.org/loc

Supporting information

Electronic supplementary information (ESI).

1. The fabrication of microfluidic chip

The microfluidic chips employed for hydrogel bead extraction were fabricated using a conventional soft lithography technique[1]. A replicate for molding of poly(dimethylsiloxane) (PDMS) was obtained by pattering a silicon wafer using photoresist SU-8 2050 (MicroChem Corp, USA). To facilitate the separation between the replicate and molded PDMS component, the replicate was treated by trimethylchlorosilane (TMCS). The PDMS pre-polymer (RTV615, GE Toshiba Silicones Co. Ltd. USA) was mixed in a ratio of 10:1, and subsequently casted on this lithographically patterned replicate. After curing at 80 °C for 2 h, the PDMS component was separated from the replicate, followed by trimming and hole introduction. The finished PDMS component was treated with oxygen plasma (PDC-32G, Harrick Plasma, USA) for bonding onto a glass slide. Finally, the microfluidic chip was treated at 60 °C for one week to make PDMS surface hydrophobic.

2. Reagents and materials

The carrier immiscible fluid, soybean oil (0.92 g/cm^3) was obtained from Beiya Meidcal oil C o.Ltd, China. Sodium alginate (SA) and calcium chloride ($CaCl_2$), were purchased from China National medicines Co. Ltd, and prepared into 2.5% w/w and 1% w/w solutions in deionized (DI) water, respectively. DI water (18.2 MΩ·cm) was made by a water purification system (Direct Q3, Millipore, USA). Prior to the hydrogel bead formation in the microfluidic chip, the solutions are filtered through 0.2 μm syringe filter to remove bacteria and any large clumps of alginate.

3. Calcium alginate (CA) hydrogel beads generation and extraction.

Sodium alginate (SA), calcium chloride ($CaCl_2$) and oil were injected independently into chip by syringe pumps (TS2-60, Longer Precision Pump Co. Ltd, China). The microfluidic device was linked to the syringe pump through ploy(tetrafluoroethylene) (PTPE) tubes. By controlling the flow rates of the inletting hydrogel precursors and oil, SA droplets and $CaCl_2$ droplets were generated and mixed in the following serpentine microchannel prior to their purification. By a gelatinization reaction of SA and $CaCl_2$, calcium alginate (CA) hydrogel beads were generated. After that, the hydrogel beads dispersed in the carrier oil (flow rate 60 μl/h) entered into bead extraction module. Water was continuously infused into bead extraction module through the large branches (150 μm width) with flow rate from 100 μl /h to 500 μl /h resulting in a laminar-like oil/water interface between the branch junctions. Because of hydrodynamic force, carrier oil was squeezed out through small branches (30 μm width) and then CA hydrogel beads were purified from oil into aqueous solution. The efficiency of oil depletion is determined by the relative flow rates between infused water and carrier oil.

4. Cell experiments

Colon cancer HCT116 cells were cultured in DMEM (Hyclone, Thermo-Fisher Scientific (Beijing), Co., Ltd,) with 10% BSA and 1% penicillin and streptomycin. Viability studies on the cells encapsulated in the hydrogel beads were conducted using cell viability assays, i.e., fluorescein diacetate (FDA, Sigma-Aldrich, USA) and propidium iodide (PI, Sigma-Aldrich, USA). HCT116 cells solution and sodium alginate were mixed in 1:2 volume ratio. Then the sodium alginate mixture was injected into the microfluidic chip with a flow rate 3μl /h for cell encapsulated hydrogel beads generation and extraction. For comparisons, oil-off-chip purification method (conventional method) was used to extract hydrogel beads from carrier oil into cell culture media: cell-encapsulated hydrogel beads dispersed in carrier oil were collected at outlet and then washed off chip with rinse of DI water and multiple low centrifugations (1200 rpm for 1 minute). Finally, cell-encapsulated hydrogel beads collected by those methods were dispersed in cell culture media and cultured for 6 hrs in CO_2 incubator (Thermo Forma Series II , Thermo scientific, USA). Cell viability in CA hydrogel beads was assessed by an inverted fluorescence optical microscope after labelling live and necrotic cell populations with FDA and PI independently. Then cell viability was quantified by counting the number of live and dead cells with image processing software and the percentage cell viability was normalized against the total number of cells in CA hydrogel beads.

5. Observation and analysis system

An inverted fluorescence optical microscope (IX71, Olympus Optical Co. Ltd. Japan) with a charge coupled device CCD camera (DP72, Olympus Optical Co. Ltd. Japan) was used to observe and record CA hydrogel beads generation and extraction process. In the proof-of-concept studies, homemade Fe_3O_4 nanoparticles (dark), oil-soluble Sudan III(red) and food dye (blue) were respectively spiked into SA solution, oil and perfusion water to help visualization of the process of extracting hydrogel beads at the laminar-like oil/water interface generated in the downstream bead extraction module. Video images were analyzed by an image analysis software IPP (Imaging Pro Plus 5.1, Media Cybernetics Inc. USA).

6. The process of calcium alginate beads generation and extraction.

A schematic diagram of the microfluidic device is presented in Figure S1. The microfluidic channel is composed by beads generation part and extraction part. For beads generation module, the middle inlet channels of the two flow-focusing channels (Figure S1-a and S1-b) are 200 μm in width and taper to 50 μm near the junction, and the side channels taper to 80 μm. The 200μm long orifice is first 50 μm in width and then expands to 80μm in width. Synthesizing channels (Figure S1-c) are composed by a straight line channel with two circular expansion chambers and a serpentine channel. The upstream channels of the T channels are 80 μm wide, and the downstream channel is 120 μm wide. The diameters of the circular expansion chambers are 250μm and 300μm separately. Sodium alginate (SA), calcium chloride ($CaCl_2$) and oil were injected independently into chip by syringe pumps. By the flow control of microfluidic chip, the sizes and morphology of droplets could be altered systematically[2-3]. Sodium alginate droplets and $CaCl_2$ droplets mixed in latter synthesizing channel (Figure S1-c). By a gelatinization reaction of sodium alginate and $CaCl_2$, Calcium alginate (CA) hydrogel beads were generated in upstream beads generation module and purified into aqueous solution in downstream beads extraction module(Figure S1-d).After purification, CA hydrogel bead was dispersed in aqueous solution and could be collected at outlet (Figure S1-e). Oil depletion efficiency was quantified by the following steps: (1) Set up the device and let it work for 3 hours, collecting continuous phase from main outlet at the same time. (2)The injected oil volume (V) could be calculated from syringe pump and main outlet oil volume (V1) could be measured through centrifugation step. (3) So oil depletion efficiency was (V-V1)/V.

7. Cells viability comparison test

By using such a new microfluidic design, viable cells (i.e., HCT116, colon cancer cell line) can be encapsulated in the alginate hydrogel beads and purified into a cell culture media (Figure S2-a and S2-c). Cell viability was evaluated by using both Fluorescein diacetate (FDA) and Propidium iodide (PI). Normally, FDA is a cell-permeant esterase substrate that can serve as a viability probe to measure both enzymatic activity and cell viability. PI is an intercalating agent and a fluorescent molecule with red emission fluoresces. Following standard operation protocol suggested by the data sheet, FDA powder was previous dissolved in acetone to get 5mg/ml solution and diluted to 5 μg/ml for final cell viability test while PI was dissolved in water (500 μg/ml, w/w) and diluted to 5 μg/ml for final cell viability test. Fluorescent photos (Figure S2-b and S2-c) revealed that significantly improved cell viability was achieved when immiscible oil phase was removed. Cell viability was quantified by counting the number of live and dead cells with image processing software (Imaging Pro Plus 5.1) and the percentage cell viability was normalized against the total number of cell in CA hydrogel beads.

8. Laminar-like oil/water interface simulation

Prior to testing the microfluidic chip for hydrogel bead formation and purification , a laminar-like oil/water interface between the branch junctions in bead extraction module has been simulated by using commercial COMSOL multiphysics software (COMSOL Inc., Burlington, MA, USA). The numerical simulation results, as shown in Figure S3, depict the operation mechanism in downstream bead extraction module. Simulation model is featured with a extraction channel (100 μm width) with large branches (150 μm width) on one side and small ones (30 μm width) on the opposite side. Water (blue) is continuously infused into the bead extraction module through the large branches, resulting in a laminar-like oil/water interface. The relative flow rates between infused water and carrier oil affect the location of laminar-like oil/water interface in the bead extraction module. At a lower infusion water flow rate (Figure S3-a), the oil/water interface is located closer to downstream side of the extraction module. As a result, it is very likely that the oil cannot be completely captured by the small branched microchannels, leading to fluctuated interface and compromised oil depletion efficiency (25%). Gradually improved oil depletion efficiency can be achieved at a higher infusion water flow rate (Figure S3-b and S3--d) with a clear laminar-like oil/water interface in bead extraction module(Figure S3-c). Specifically ,98% oil depletion efficiency can be achieved at high water infusing flow rate(Figure S3-e).The simulation of whole oil depletion process confirm that oil depletion efficiency is affected by the relative flow rates between infused water and carrier oil, which could be match well with experimentally validated data.

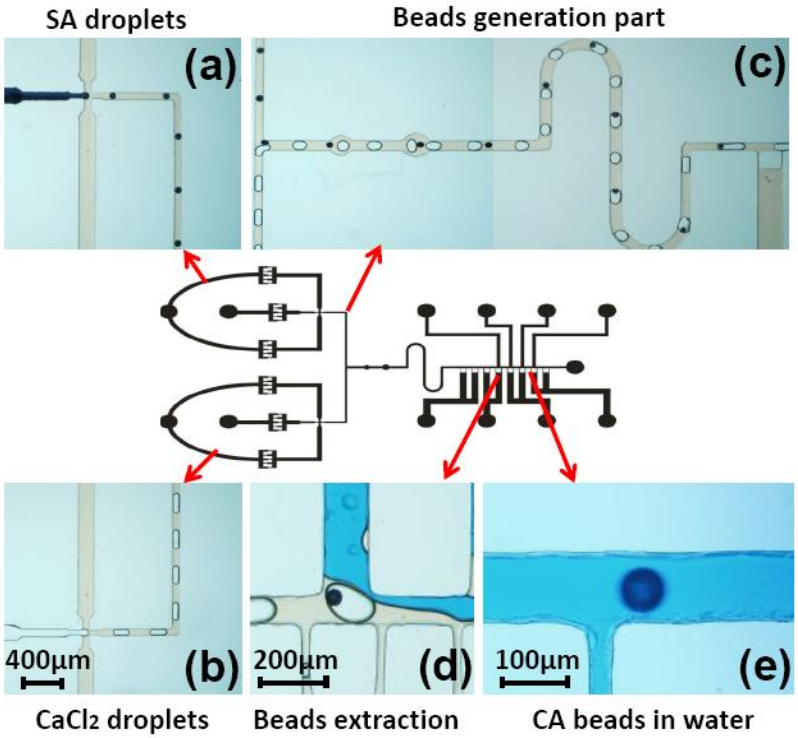

Figure S1. Snapshot micrographs depict the process of hydrogel beads generation and extraction. (a-b) : SA droplets and $CaCl_2$ droplets were generated by flow focusing channels (SA flow rate 3 μl /h, $CaCl_2$ flow rate 15 μl /h, oil flow rate 30 μl /h). (c) CA hydrogel beads generation part: SA droplets and $CaCl_2$ droplets mixed in serpentine synthesizing channel. (d) Hydrogel beads extraction module: carrier oil could be removed by the squeezing flow of water. CA hydrogel bead transferred through laminar-like oil/water interface. (e) After extraction, CA hydrogel bead was dispersed in aqueous solution.

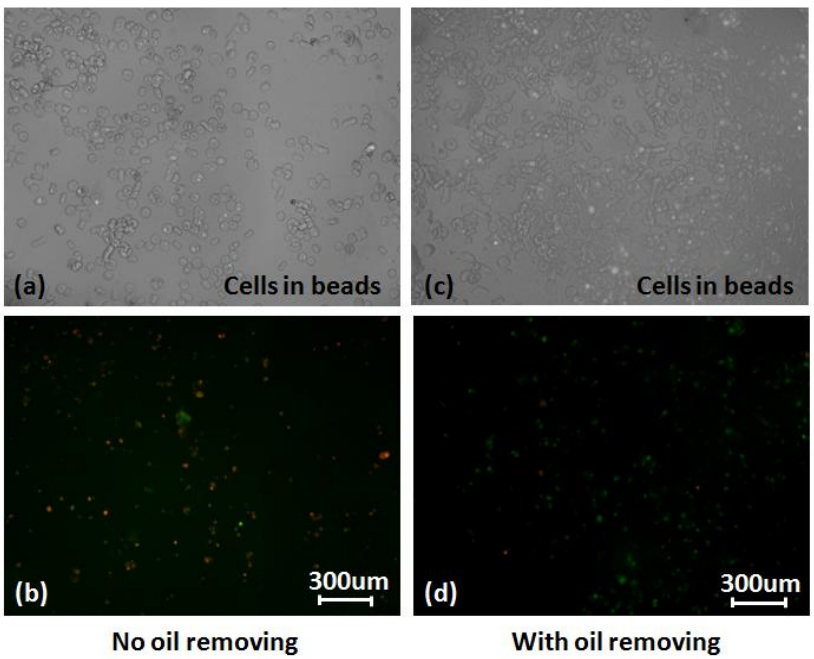

Figure S2: Experiment data for cell viability comparisons test. (a) Bright filed photo shows cell encapsulating hydrogel beads without oil removing. (b) The corresponding dark field of fluorescence photo. Viable cells excitation fluorescence were green while dead cells excitation fluorescence were red. Because no oil was removed, cell viability was low. (c-d) Photos show cell encapsulating hydrogel beads after oil depletion. Significantly improved cell viability was achieved.

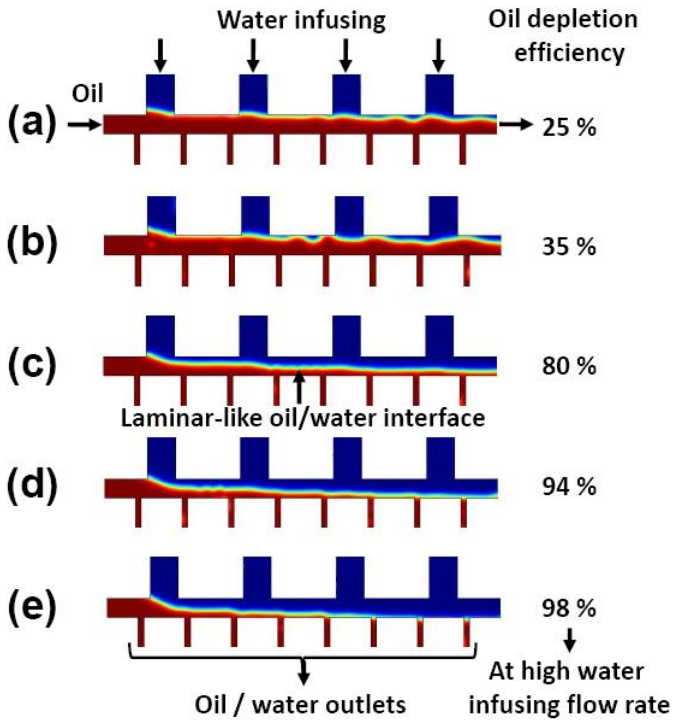

Fig S3. Simulation results show the relative flow rates between infused water and carrier oil affects the location of laminar-like oil/water interface in the bead extraction module. (a) At a lower infusion water flow rate the oil/water interface is located closer to downstream side of the extraction module with low oil depletion efficiency. (b-c) Gradually improved oil depletion efficiency can be achieved at a higher infusion water flow rate. (d-e) High oil depletion efficiency is achieved at high water infusing flow rate.

Notes and references

1. D. C. Duffy, J. C. McDonald, O. J. A. Schueller and G. M. Whitesides, *Anal. Chem.*, 1998, **70**, 4974-4984.
2. K. Liu, H. J. Ding, J. Liu, Y. Chen and X. Z. Zhao, *Langmuir*, 2006, **22**, 9453-9457.
3. K. Liu, H. J. Ding, Y. Chen and X. Z. Zhao, *Microfluid. Nanofluid.*, 2007, **3**, 239-243.