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A Multifunctional Microfluidic Platform for Generation, Trapping and Release of Droplets in a Double Laminar Flow

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Abstract

Droplet microfluidics, involving micrometer-sized emulsion of droplets is a growing subfield of microfluidics which attracts broad interest due to its application on biological assays. Droplet-based systems have been used as microreactors as well as to encapsulate many biological entities for biomedical and biotechnological applications. Here, a novel microfluidic device is presented for the generation, trapping and release of aqueous including hydrogel droplets in a double laminar oil flow. This platform enables the storage and release of picoliter-sized droplets in two different carrier oils by using hydrodynamic forces without the need of electrical forces or optical actuators. Furthermore, this design allows droplets to be selectively and simultaneously exposed to two different conditions and collected on demand. Successful encapsulation of hepatoma H35 cells was performed on-chip. Viability of cell-laden droplets was performed off-chip to assess the potential applications in 3D encapsulation cell culture and drug discovery assays.

Keywords

Microfluidics; Hydrogel Droplet; Cell-laden droplets; Hydrodynamic force

Introduction

Droplet microfluidics is a well-known technology in which an individual droplet can be generated, transported, stored (1), fused (2–4), split (5, 6), sorted (7, 8) and analyzed (9) with minimum sample loss, minimum cross-contamination, low undesired concentration gradient and fast diffusion. It offers new routes for chemical and biochemical processes (10–16).

By using droplet technology, solid beads can be produced inside a microchannel (17). Hydrogel, such as alginate (18), has been widely used to form microbeads (19). However, alginate is made of microfibers with diameters of 10–100 microns which is significantly

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different in surface interaction, porosity, and fiber concentration from native extra cellular matrix. In order to culture cells in mimicked 3D microenvironment, fibers must be significantly smaller than cells. Therefore, in this study PuraMatrix, synthetic peptide nanofiber hydrogel, was chosen as it mimics the main aspects of the in vivo environment for cells to proliferate, differentiate, migrate, and rapidly create their own microenvironments (20~24). The peptide of PuraMatrix consists of 4 repeats of RADA with 99% water. Its gelation is initiated by salt concentrations of 1mM or higher, resulting in nanofiber scaffold with the fiber diameter and pore size of ~10 nm and 50–200 nm respectively (25). In this study, the gelation of PuraMatrix was initiated by the ion containing dye in one of the laminar flows.

Previously reported methods for droplet trapping and storing have been using a single oil carrier flow (26, 27). For example, traps in an array format (28, 29) were connected to serpentine channels and the trapping mechanism was based on flow resistance on the microchannel. Other studies applied electrical fields for the storing and release of droplets (30). Limitations on the use of electrical forces might arise when using cells as samples or when a simple set-up is required. Using droplets handled by double oil flows to perform chemical reactions have been recently reported (31). Transferring droplets between flows have also been successfully applied to handle beads in a reliable manner (32, 33). However, these methods require either external forces to manipulate the droplets or complicated manufacturing features. The device presented here uses hydrodynamic forces and the geometry of the channels exclusively to form droplets. Therefore, it allows biological operations and its monitoring over time with minimum interference from undesirable factors on biological specimens.

Materials and Methods

All chemicals were purchased from Sigma-Aldrich except when mentioned specifically. Hydrogel microdroplets were generated by hydrodynamic focusing by introducing the sample in “Sample inlet” and oil in “Oil 1 inlet” as shown in Figure 1. Formed droplets continued to “Main oil channel”, which had a double laminar flow, and came into contact with the second oil laminar flow from “Oil 2 inlet” containing the gelating agent. Both oil were co-flowing along the main channel. Gravity driven flow was used to inject the samples. By adjusting the relative heights of open syringes connected to sample/oil inlets, droplet production rate and size could be controlled. Syringe heights were balanced so that droplets were produced at a desired rate and size. Meanwhile the droplets would be dragged towards the trap due to the branched-out flow at the trap opening leading to the trap neck and the neighbor main channel with counter-directional flow by hydrodynamic forces, but trapped droplets could not pass through the narrow trap neck (Fig. 1). Once the droplets were caught into the traps, the two laminar oil flow pressures were balanced so that the main oil channel was filled completely by the dye-containing oil, leading to an acceleration of the gelation process. Droplets remained in the traps without the need of stopping the flow in the main channel.

The droplet microfluidic device was designed using AutoCAD 2010 software (Autodesk) and constructed using conventional soft lithographic techniques with Polydimethylsiloxane

(PDMS), Sylgard 184 (Dow Corning), using 1:10 ratio between its curing agent and PDMS. The main oil carrier had light mineral oil containing 1–2% (w/w) Span 80 (Fluka) as a stabilizer. The second laminar flow had the same composition as the main one but containing 1 g/ml Sudan dye as gelation reagent for PuraMatrix (BD Biosciences). Water with blue food color, PuraMatrix of 0.25% and 0.5%, and 1% alginate were used in this study. In alginate experiments, calcium carbonate 3% was added to the alginate sample and acetic acid 1% was added to the second laminar flow to allow its solidification by internal gelation. When hydrogel was used, droplets were kept in storage conditions inside traps on the chip for at least 60 minutes to ensure gelation. Figure 2A–C are representative examples of trapped droplets on chip from three materials. After an hour of exposure to the second flow, trapped droplets were released by reversing the flow direction and collected through “Collection outlet” (Fig. 1). The reversed flow was applied by gravity pressure driven flow via an open syringe connected to “Outlet”. Specifically, the flow direction was from “Outlet” to “Collection outlet” during the droplet collection as shown in Figure 2D.a–f for water droplets.

Results and Discussion

Trapping efficiency and behavior for different droplet production rates using our microfluidic droplet device were investigated at 10–20 droplets/min and results are shown in Figure 3. In general, higher droplet production rates (not greater than 20 droplets/min) favored the trapping efficiency (Fig. 3). For 100 μm diameter droplets, the optimal trapping efficiency was achieved at 20 droplets/min in 0.25% PuraMatrix samples (Fig. 3A). For 150 μm droplets, the optimal trapping efficiency (100%) was obtained at 20 droplets/min in 0.25% and 0.5% PuraMatrix as well as 15 droplets/min in 0.25% PuraMatrix (Fig. 3B). For 200 μm droplets, various combinations of conditions achieved 100% of trapping efficiency (Fig. 3C). It is concluded that if a low droplet production rate is not a concern, bigger droplets will increase the trapping efficiency. As shown in Figure 3D at 20 droplets/min production rate, the bigger droplets are, the higher trapping efficiency is for 0.5% PuraMatrix and 1% alginate. Interestingly, it is opposite for 0.25% PuraMatrix, where small sizes (100, 120, 150 and 180 μm) of droplets have 100% trapping efficiency while the trapping efficiency of big droplets of 200 μm is less than 100%. The balance of two counter-directional flow around a droplet trapper, surface tension, size and stiffness of a droplet determines if a droplet would be captured and confined in a trapper. PuraMatrix at 0.25% is so soft that its droplet trapping is a result of balance between deformation caused by the pushing force of flow through the trapping neck and surface tension of droplets to maintain the spherical shape. Surface tension is inversely proportional to sphere diameter, leading to the lower trapping efficiency of 200 μm 0.25% PuraMatrix droplets than the smaller sized ones. On the other hand when stiffness of materials cannot be neglected, for the cases of 0.5% PuraMatrix with storage modulus of 2.5 kPa (34) and 1% alginate with compressive modulus of around 10 kPa (35), bigger droplets of 200 μm had higher trapping/confining efficiency than the smaller ones and less chances to pass a 150 μm (height) $\times$ 20 μm (width) trapper neck.

Different oil flow rates were also tested to study the influence of the flow rates on the trapping efficiency. Figure 4A, B and C show results of experiments measuring the number

of trapped droplets under different positions of the oil flow interface for 0.25% PuraMatrix, 0.5% PuraMatrix and 1% alginate respectively. The trapping efficiency increased with the size of droplets for all oil flow conditions (Figure 4A, B, C). Trapping efficiency and gelation time were determined by both the oil flow rate condition and the droplet size. Bigger droplets were produced (leading to efficient droplet trapping) for both PuraMatrix concentrations when “oil flow 1 < oil flow 2”. For alginate droplets, a higher trapping efficiency was obtained for “oil flow 2 < oil flow 1”. This is expected as alginate droplets needed longer gelation time and this oil flow condition favored by the increased surface contact between droplets and gelating agent. Comparison of dotted lines representing “oil flow 1 < oil flow 2” condition in Figure 4A~C reveals that droplet trapping efficiency increased with the size of the droplet and droplet production rate.

H35, a rat hepatoma cell line, was used in this study as a model cell line to demonstrate hydrogel droplet formation with cells, trapping and releasing as well as encapsulated cell viability after off-chip culture. Figure 5 includes phase-contrast images of two example droplets collected at the collection outlet using H35 cells encapsulated in 1% alginate (Fig. 5A) and 0.5% PuraMatrix (Fig. 5B). Live/death assay results in Figure 5D demonstrated the high viability of cell encapsulated in droplets using this device and the potential of this platform for an efficient cell encapsulation using droplet techniques.

In summary, a droplet-trapping approach was successfully demonstrated to perform trapping and release droplet operations under controlled exposure to a second flow. The second oil flow containing the gelating agent plays a vital role for the successful gelation and trapping of the hydrogel beads without the need of complicated droplet transfer operations. In a single step it is possible to generate hydrogel beads and store them on demand before releasing them. This could be advantageous for kinetic studies and measurements where tracking of cells or molecules is involved. The ability of transferring droplets through different flows has been of interest for many biochemical applications. In this paper no transfer of the droplet is needed to control the chemical reaction of gelation but the flow rates of the two laminar oil flows, which simplifies the process significantly. This work opens a door for an on-chip cell culture platform where cells can be monitored regularly for an on-demand time period using a much simplified droplet device.

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Highlights

- A microfluidic droplet device is presented for generation, trapping and release of picoliter-sized peptide hydrogel droplets in a double laminar oil flow.
- This platform enables the storage and release of droplets using hydrodynamic forces and geometry without the need of electrical forces or optical actuators.
- This design allows droplets to be selectively and simultaneously exposed to two different conditions and collected on demand.
- Successful encapsulation of hepatoma H35 cells in hydrogel was performed on-chip and cell viability was high.

Figure 1. Schematic drawing of the droplet trapping and release microfluidic device and main channel dimensions. Droplets were generated by hydrodynamic focusing and fed by open syringes connected to sample/oil inlets/outlets. Total dimension of the device is 32×42 mm. The left-top inset shows a real device with food dye to illustrate features of the device.

Figure 2.

Example frames extracted from different trapped droplet samples of (A) blue dyed water, (B) 0.5% PuraMatrix, and (C) 1% alginate. Reversed flow for collection of the droplets (D) from an early stage (a) of reversed flow to a late stage (f). In the panel (a), all droplets were pushed out of their traps after starting of the reverse flow. During the reverse flow, droplets were pushed out of the device and collected through the collection outlet showing in the panels (b) to (f). The Moticam camera was used to record images at 30 frames per second. To characterize the droplet trapping, number of trapped droplets was counted as a function of the oil flow rate and volume using Image J software.

Figure 3.

Images extracted from recorded videos of different droplet production rates and samples were analyzed. The number of trapped droplets were counted over consecutive experiments ($n > 20$ for each setting and recording of each experiment was at least 5 minutes) to show the percentages of trapped droplets for different droplet production rates and droplet sample. Graphs show droplet trapping efficiency of different droplet production rates and samples for (A) 100 µm diameter droplets, (B) 150 µm diameter droplets and (C) 200 µm diameter droplets; (D) Comparison of droplet trapping efficiency of different sizes of droplets at a droplet production rate of 20 droplets/min. The repeatability of experiment data was 100% at the same experimental setting. Because the number of trapped droplets remained the same per condition over repeated experiments error bars of data points equal to zero.

Figure 4.

Images extracted from recorded videos with different droplet sizes were produced and oil flow conditions applied were analyzed. Graphs show droplet trapping efficiency for different droplet sizes and oil flow conditions in the case of (A) PuraMatrix 0.25%, (B) PuraMatrix 0.5%, and (C) Alginate 1%; “oil flow 1” represents flow from “oil 1 inlet” in Figure 1 while “oil flow 2” is from “oil 2 inlet”. When the oil flow rates were adjusted so that the interface was set in the middle of the microchannel the condition was stated as “oil flow rate 1= oil flow rate 2”. Position of the oil interface was measured on screen. When the interface of the two oil laminar flows indicated that oil flow 1 was 50 microns width, the experiments were under “oil flow 1<oil flow 2”. When the interface of the two oil in the microchannel was such that oil flow 2 was 50 microns width, then experiments were under condition “oil flow 1>oil flow 2”. Because the number of trapped droplets remained the same per condition over repeated experiments error bars of data points equal to zero.

Figure 5.

Phase contrast images of extracted and gelated 1% alginate droplet (A) and 0.5% PuraMatrix droplet (B) containing H35 cells. Phase contrast (C) and fluorescence images (D) of live/dead assay of the same 0.5% PuraMatrix droplet containing H35 cells. Approximate size of the droplets is 100 μm in diameter. H35 cells were cultured in DMEM with 10% FBS and 1% penicillin-streptomycin in a 5% CO_2 incubator at 37°C . H35 cell suspension in 10% sucrose solution was made at a concentration of 10 million cells/ml. One volume of the cell suspension was then mixed with one volume of 1% stock PuraMatrix, so that the final PuraMatrix concentration in droplets was 0.5%. The cell/gel mixture was fed to “Sample inlet” on our chip and the same droplet formation procedure was followed. The droplets were left for 60 minutes in the traps on chip to ensure gelation, released, collected and cultured for three days in a petri dish. Viability of cell/gel droplets was evaluated using 4 μM Calcein AM. Live cells became fluorescence green and images were captured using a Zeiss AxioObserver microscope.