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Short communication

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.

1. Introduction

Droplet microfluidics is a well-known technology in which an individual droplet can be generated, transported, stored (Shi et al., 2008), fused (Ahn et al., 2006a; Fidalgo et al., 2007; Wang et al., 2009a), split (Link et al., 2006, 2004), sorted (Ahn et al., 2006b; Fidalgo et al., 2008) and analyzed (Srisa-Art et al., 2007) with minimum sample loss, minimum cross-contamination, low undesired concentration gradient and fast diffusion. It offers new routes for chemical and biochemical processes (Pipper et al., 2008; Zheng et al., 2003; Chan et al., 2005; Belder, 2005; Xu et al., 2005; Fernandez-Nieves et al., 2005; Nie et al., 2005).

By using droplet technology, solid beads can be produced inside a microchannel (Utada et al., 2005). Hydrogel, such as alginate (Haeberle et al., 2008), has been widely used to form microbeads (Braschler et al., 2005). However, alginate is made of microfibers with diameters of 10–100 μm which is significantly 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 (Semino et al., 2003; Wang et al., 2008; Chen et al., 2013; Holmes et al., 2000; Semino et al., 2004). The peptide of PuraMatrix consists of 4 repeats of RADA with 99% water. Its gelation is initiated by salt concentrations of 1 mM or higher, resulting in nanofiber scaffold with the fiber diameter and pore size of ~10 nm and 50–200 nm respectively (Zhang et al., 1993). 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 (Huebner et al., 2009; Srisa-Art et al., 2010). For example, traps in an array format (Tan and Takeuchi, 2007; Nagai et al., 2002) 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 (Wang et al., 2009b). 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 (Deng et al., 2016). Transferring droplets between flows have also been successfully applied to handle beads in a reliable manner (Kantak et al., 2011; Zhang et al., 2011). 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.

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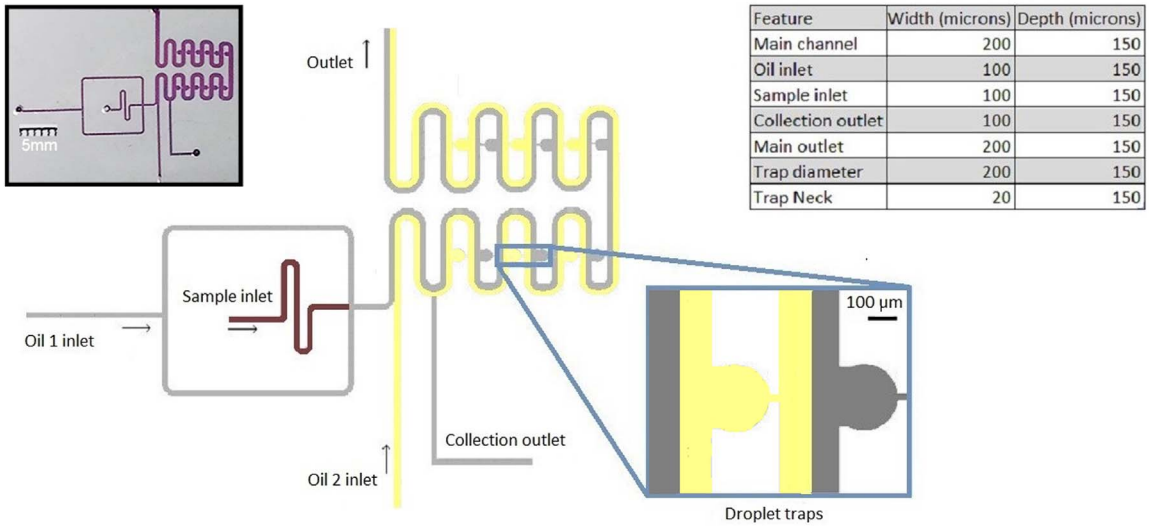

Feature	Width (microns)	Depth (microns)
Main channel	200	150
Oil inlet	100	150
Sample inlet	100	150
Collection outlet	100	150
Main outlet	200	150
Trap diameter	200	150
Trap Neck	20	150

Fig. 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.

2. 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 Fig. 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 min to ensure gelation. Fig. 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 Fig. 2D.a–f for water droplets.

3. 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 Fig. 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 Fig. 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 effi-