A BEAD-IN-DROPLET SOLUTION EXCHANGE SYSTEM VIA CONTINUOUS FLOW MICROFLUIDIC RAILING

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ABSTRACT
A continuous flow, microfluidic railing system has been developed for the rapid and autonomous exchanges of bead and bead-in-droplet formations. Fluidic encapsulations of microparticles (e.g., microbeads and living cells) inside individual microdroplets provide various possibilities for chemical and biological applications. This work extends the bead-in-droplet technology from a single type droplet to a multiple-stage, different droplet solutions system. A micropost array railing system has been demonstrated to passively: (i) guide an array of bead-in-droplet in a “first” droplet solution to different liquid flows and release the microbeads from the droplets, and (ii) re-encapsulate the released microbeads in droplets containing a different, “second” droplet solution. Experimental results revealed successful continuous flow solution exchanges for water-in-oil droplets with size of about 60.2µm in diameter containing microbeads of 15µm in diameter.

INTRODUCTION
Microdroplet technologies could be the ideal platforms for advanced biological applications (e.g., genomics, cellular studies, and point-of-care diagnostics) due to a variety of possible benefits such as rapid mixing and low background noises [1-3]. On the other hand, microbeads have been used as substrates in microfluidic assays to take advantages of their unique properties in high surface-to-volume ratios in geometry, easy handling in microfluidic channels, and many possible functionalization schemes as a variety of diverse molecular detection probes [4-6]. Therefore, the integration of microbeads and microdroplets as the bead-in-droplet architecture could be a power tool to significantly advance microfluidic applications [7, 8].

In the state-of-art microfluidic devices, researchers have developed automated on-chip microfluidic systems for rapid assays [9-10]. A number of on-chip droplet handling techniques have also been developed, such as simple formation of droplets [11-14] and fusion of droplets with reagents to initiate chemical reactions inside droplets [15-17]. Some recent works show that the continuous flow methodology could enable high-throughput processing of microdroplets, including demonstrations of the continuous flow railing systems for the formation and multi-stage reactions of droplets, microbeads and cells [18-22].

One caveat, however, is that multi-step assays with suspended microparticles often require complete exchange of the solutions. In a multi-stage reaction system, particles (beads) in the droplets must be released and transferred into droplets of different solutions. However, it is difficulty to retrieve the beads inside droplets and execute solution exchange routines. Specifically, researchers often perform complex and time-consuming off-chip processes, such as centrifuge to the exchange processes. Therefore, a rapid, on-chip solution exchange system for the multi-stage, bead-in-droplet process could advance the state-of-art microfluidic technologies.

Previously, our group has demonstrated the micropost array railing technique to accomplish droplet ‘lysis’ (i.e., the release of droplet contents via droplet destabilization) and the retrieval of the contents of the droplets such as microbeads under continuous flow conditions [14]. The droplet ‘lysis’ was accomplished by continuously moving microdroplets from oil suspension to water flow (after washing the surfactant from the surface of the droplets). Here we advance this technique to accomplish the rapid bead-in-droplet solution exchange processes by breaking the droplets, transferring the microbeads to a different solution, and forming new droplets containing the initial microbeads in the second solution.

METHOD

Concept
Figure 1 illustrates the concept of the bead-in-droplet solution exchange system which consists of three major components: (i) microposts in railing patterns, (ii) four microfluidic flow channels in green, grey, yellow and blue colors, respectively, corresponding to continuous loading of water-in-oil droplets containing microbeads suspended in oil with surfactant (green), washing oil without surfactant (grey), washing water (yellow), and a second solution (blue), and (iii) a T-junction at the outlet for new droplet formations. Micropost array railing techniques are utilized to guide both droplets [19, 20] and microbeads [21, 22] into distinct, adjacent fluids of flows (e.g., oil and water). First, loaded micro bead-containing droplets are passively guided by the arrayed microposts from the initial oil suspension (green) into the surfactant-free washing oil (grey) at the first junction. After the micropost array guides the droplets to the second flow channel, the washing oil removes the surfactant on the surface of the droplets to allows the droplet ‘lysis’. At the second junction between the washing oil and washing water (yellow) channels, bead-in-droplets are broken apart and their inner contents (microbeads) are released in to the washing water channel. The released microbeads are further guided by microposts to the second solution channel (blue) and its outlet port. During the process, microbeads are washed in the washing water channel to remove initial solution on
Conceptual illustrations of the continuous flow bead-in-droplet solution exchange system. Loaded droplets containing microbeads and a “first” solution (red) are passively guided into adjacent flow streams via a micropost array rail technique. The droplets are washed by oil without surfactant (grey) to enable microbead-release into the washing water (yellow). Subsequently, released microbeads are transported into the “second” solution (blue). A T-junction is then used to form droplets containing both microbeads with the second solution. Lastly, a T-junction is used to form new droplets containing both the microbeads and the second solution. The bead-in-droplets in the second solution can be used to form new droplets containing both the microbeads and the second solution. The bead-in-droplets in the second solution can be collected at the outlet for experiments and assays or to go through another stages of solution exchanges depending on the system reaction requirements. Based on the proved configuration, droplet solutions for the bead-in-droplets system can be continuously altered.

Design and Fabrication

Figure 2a shows the design details of a prototype system targeting $\phi=15\mu m$ microbeads. Specific design parameters include: $15 \times 15 \mu m^2$ square microposts with 5 $\mu m$ gaps between posts and $1^\circ$ angle for the micropost pattern from the flow direction for successful guidance of bead-in-droplets. The micro flow channels are 200$\mu m$ in width with 50$\mu m$-wide inlets and outlets. The walls of these micro channels are 10 $\mu m$ in width and open junctions between the micro flow channels are 3000 $\mu m$ in length, which is required to maintain the $1^\circ$ angle pattern of the microposts. A 50 $\mu m$-wide T-junction design at the final outlet is used to generate new droplets for the microbeads. The conventional soft lithography process is used to fabricate SU-8 mold inserts with a thickness of 18 $\mu m$. The bead-in-droplet solution exchange systems are then constructed by pouring poly (dimethylsiloxane) into the mold insert with a curing process. Afterwards, the structure is permanently bonded to glass substrates via an oxygen plasma treatment. Figure 2b shows optical images of the fabrication results.
RESULTS

Experimental results revealed that the proposed system can successfully exchange bead-in-droplet solutions under continuous flow conditions. First, bead-in-droplets were generated with water as the droplet solution containing \( \sigma = 15 \mu m \) polystyrene microbeads at a T-junction preceding the inlet. Specifically, red-dyed deionized (DI) water was used as the first droplet solution, and Hexadecane as an oil phase with 5 wt% of Span 80 as surfactant (Fig. 3a). The average diameter of the bead-in-droplets was 60.2 \( \mu m \). These bead-in-droplets were transported into the system (Fig. 3b). All flow movements were controlled by using syringe pumps, such as the formation of those initial bead-in-droplets and all flows in all micro flow channels. Furthermore, the boundaries at various junctions between the micro flow channels required good pressure control or their shapes would be distorted such that pressure controllers were used in additional to flow rate controls.

These bead-in-droplets were guided by the micropost array to the second flow channel of Hexadecane flow for the surfactant washing stage (Fig. 4a). While guided by microposts array during the washing step, it was observed that some of the adjacent droplets merged together. This is a good indication that the surfactant on the surface of droplets is removed. After the droplet washing process, the bead-in-droplets were guided into the washing water flow (yellow dyed DI water) to fully release the microbeads while ‘lysing’ the droplet (Fig. 4b). Subsequently, the released microbeads were washed with the yellow water flow and then transferred into the second droplet solution flow (blue dyed DI water) (Fig. 4c). After wards, the T-junction section enabled the formation of microdroplets containing both the microbeads and the second droplet solution (Fig. 4d).

Average diameter of the second droplets was 84.7 \( \mu m \). The size of the second droplets can be controlled by changing the flow rate of oil flow at the T-junction. Additionally, the configuration of these bead-in-droplets is compatible with the existing droplets sorting methods. Therefore, the continuous flow separation of droplets can be easily integrated to increase the collection efficiency of the second bead-in-droplets at the outlet [23, 24].

These results suggest that this rapid, easy-to-operate bead-in-droplet solution exchange system could be applied to numerous droplet-based platforms that require multiple fluidic reactions, thereby offering a simple, yet powerful solution for lab-on-a-chip applications, including genomics, cellular studies, and point-of-care diagnostics.

CONCLUSION

We have developed a rapid solution exchange system for bead-in-droplets via continuous flow micropost array railing techniques. The prototype system was successfully demonstrated to accomplish: (i) the retrieval of microbeads in water-in-oil droplets by the ‘lysis’ of the droplets, (ii) transfer of the released microbeads into a second solution, and (iii) the formation of new water-in-oil droplets containing the original microbeads and a different, second droplet solution.

![Figure 3. Sample preparation. (a) Bead-in-droplets containing \( \sigma = 15 \mu m \) microbeads were formed with a T-junction. Red dyed DI water was used as the first droplet solution and Hexadecane as the oil phase flow. (b) Generated droplets and bead-in-droplets were infused into the system with syringe pumps and pressure controllers.](image)

![Figure 4. Demonstration of the solution exchange process with \( \sigma = 15 \mu m \) microbeads. Infused bead-in-droplets were successfully transferred by the microposts railing system to (a) Hexadecane flow to wash surfactant, (b) yellow-dyed water for lysis of droplets and releasing the microbeads, (c) blue-dyed water as the second solution, and (d) T-junction for the formation of bead-in-droplets containing microbeads and the second droplet solution.](image)
In this work, the presented system was successfully demonstrated for the exchange of DI water droplets containing $\sigma=15\mu m$ polystyrene microbeads. Furthermore, the presented technique could be tailored to exchange solutions of droplets containing a wide range of chemicals and particles. For example, living cells or functionalized microbeads could be used to conduct cell lysis for high-throughput polymerase chain reaction or bead-based assays for point-of-care diagnostics screenings. Thus, the autonomous system could be adapted for a variety of assays with multiple chemical reactions to potentially play an important role in advanced biological research and industrial biomedical applications.

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