DROPLET-BASED MICROFLUIDIC DEVICES FOR MULTIPLE-DROPLET TRAPPING, STORING, AND CLUSTERING EMPLOYING GUIDING TRACKS AND FORWARD/BACKWARD FLOWS

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ABSTRACT
We present a double-layered microfluidic device integrating multiple-function as droplet producing, manipulating, trapping, guiding, storing, clustering and demonstrated triple-droplet clustering in a simple controlled manner, using guiding tracks and simple forward/backward flows for improving trapping/storing efficiency. With this method, large array of different multiple-droplets could be manipulated, stored and monitored simultaneously. We expect the proposed strategy will be valuable to study transfer of molecules across the droplets, enzymatic reactions and high throughput bioassay reaction.

KEYWORDS: Droplet-Based Microfluidics, Trapping, Multiple-Droplet Storing, Track

INTRODUCTION
Droplet-based microfluidic has proven to be a useful tool to investigate heterogeneous reactions that occur between multiple phases thanks to the reproducible flow patterns, effective and well controlled mass transport between phases. Nowadays, Droplet-based microfluidic are showing its potential for systematic study of the transport across interfaces between different micro reactors since the surfactant layers around micro droplets are in principle permeable to small molecules. In order to study such bio/chemical assays and medium transfer from one droplet to another, droplet paring system in continuous-flow channels and even static arrays has been developed and demonstrated [1]. However, a more desirable but more difficult unit operation for complex assays is to cluster multiple-droplets containing different reagents/samples for various biological and chemical experiments. Therefore the main objective of our research was to design a device that could be used to cluster multiple-droplets.

THEORY
The device consists of two PDMS layers (Fig. 1). The lower layer is used for trapping droplets. The upper layer, including guiding tracks [2] and storing chambers, is used for droplet guiding, storing, and clustering. When a droplet flows through the trapping structure, it is easily captured due to the fact that the width (15 μm) of the exit is too small for a droplet to pass through (Fig. 2a). As soon as a droplet is captured it blocks the exit resulting in the termination of liquid flow within the trap and preventing a second droplet from entering the trapping structure. Subsequent droplets have to move through the bypass.

After all the trapping sites are occupied (Fig. 3a), the flow direction is reversed (Fig. 3b). The trapped droplets are supposed to be released from the trapping well and subsequently move toward their corresponding storing well. However, because the flow rate in the bypass is bigger than the one through the trapping structure (Fig. 2d); droplets tend to pass
outside the storing well. In order to overcome the problem, droplets guiding tracks are implemented (Fig. 2a, b, c) in upper PDMS layer and the height (35 μm) of the micro-channel in lower PDMS layer is reduced to less than the size of the droplet (60 μm- 100 μm). So droplets are confined after the formation. A consequence of the vertical confinement on the droplets is that they become sensitive to depth modulations of the channel.

For a droplet of constant volume, this interfacial energy is minimal in a spherical shape and it increases as the drop flattens into a pancake shape. Under the track, flattened droplet releases its interfacial energy by partially entering into the cavity of the track. Therefore, confined droplets prefer to follow the tracks (Fig. 2b) which lower their interfacial energy.

For multiple-droplets clustering, a chamber is developed and deployed above the storing well in each trapping structure (Fig. 2a) for permanently storing the droplets. When a confined droplet move through the chamber, due to the interfacial energy of the droplet stored and higher density of the oil (HFE-7500) compared to water, both of them would generate forces on the droplets to float-up out of the confining channel to the super stratum of the chambers. The change in interfacial energy transforms the flattened droplets into spherical ones that have the minimal interfacial energy (Fig. 2b). After that, the second and third sets of droplets are allowed to enter the device and be clustered in the same manner (e.g., repeated trapping/storing by forward/backward flows) (Fig. 2c, d, e, f). Former stored droplets will remain in the chambers permanently even when forward/backward flow flows rapidly in the channel because of lower hydrodynamic stress on the droplet in the chambers (Fig. 2c).

**Figure 2.** Working principle of the proposed track and chamber. (a) Top view. (b)-(c) Cross-sectional view from a’-b’ in (a). (b) Confined droplet follows the track toward its corresponding chamber. (c) Droplet stays in the chamber under forward flow because the hydrodynamic stress on the droplet in the chamber is much weaker than in the channel ($W_a < W_b$). (d)-(e) COMSOL simulation results for velocity distribution in trapping array and chamber.

**EXPERIMENTS/RESULTS**

COMSOL® simulation was carried out to optimize the experiment conditions. With the optimized structure, we ensure that only one droplet would be trapped (Fig. 2d) and the stored droplet would not escape from the chamber (Fig. 2e). Fig. 3 shows experimental results for triple-droplet clustering. The guiding tracks have proven to be effective in guiding droplets with enhanced trapping/storing efficiency (Fig. 4 and Table 1). However, the accumulated clustering efficiency was reduced due to non-uniform size and shrinkage of droplets. This led the droplets to escape or vanish from the chambers. Nevertheless, this problem can be solved by using good surfactant and device modification.
CONCLUSION
A simple and effective device to trap, store, and cluster multiple droplets have been demonstrated. With this method, large array of different multiple droplets could be manipulated, stored and monitored simultaneously. We expect the proposed strategy will be valuable to study medium transfer and multiple bioassays across the clustered droplets.

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REFERENCES

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Table 1. The proposed device shows high efficiency in droplet trapping and storing. However, the accumulated clustering efficiency was reduced due to non-uniform size and shrinkage of droplets.

<table>
<thead>
<tr>
<th>Step</th>
<th>Trapping rates</th>
<th>Storing rates (per single step)</th>
<th>Clustering rates (accumulated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1\textsuperscript{st} step</td>
<td>100%</td>
<td>96.77%</td>
<td>96.77%</td>
</tr>
<tr>
<td>2\textsuperscript{nd} step</td>
<td>91.6%</td>
<td>98.89%</td>
<td>76.34%</td>
</tr>
<tr>
<td>3\textsuperscript{rd} step</td>
<td>100%</td>
<td>97.32%</td>
<td>66.67%</td>
</tr>
</tbody>
</table>

Figure 3. Photographs of multiple-droplet trapping, storing and clustering with time lapse.

Figure 4. Droplets stored without the help of tracks. Lower storing efficiency (78.65%) was demonstrated compared to the device with the tracks.