T-JUNCTION SPLITTING OF DROPLETS FROM NANO LITER TO FEMTOLITER AND MANIPULATION OF SINGLE NANOPARTİCLES ON MICROFLUIDIC CHIPS
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ABSTRACT
In this paper, we got femtoliter volume droplet by splitting of nanoliter droplet at a T-junction channel, and the volume of the daughter droplet was not limited by the channel size. We use this droplet splitting system to entrap several fluorescent nanospheres, which show great potential to manipulate and observe single nanoparticles independently. The influences of fluid flow rate, channel geometry, addition of surfactant on droplet splitting were carefully studied. The droplet splitting in our T-junction chip has a small volume, monodisperse size, and can be produced efficiently, orderly, controllably.

KEYWORDS: Microfluidic Chips, Droplets, Femtoliter

INTRODUCTION
Carrying out chemical and biochemical reactions in compartmentalized containers on droplet-based microfluidic chip has shown great advantages, such as saving reagent, fast, independent and high-throughput[1]. Microreactor containing several molecules or particles offer opportunities for discovering and characterizing new chemical and biochemical phenomena and can greatly improved signal/background ratio in femtoliter-volume droplets[2]. As we know, sizes of water-in-oil droplets always scale with microchannel dimensions in the range 10-100 μm. Liu et al. first reported of a microfluidic platform for splitting off daughter droplets ranging in volumes of 34-523 fL (10^{-15} L) from mother water-in-oil plugs, where the sizes of the droplets were not limited by the channel width of the device[3]. Tang et.al described a novel method of generating monodisperse aqueous droplets with volumes down to 200 aL (10^{-18} L) by means of piezoelectric injection, and use optical tweezers for droplets manipulation and mixing[4].

EXPERIMENTAL
Microfluidic chip design and fabrication

In our work, we designed a microfluidic chip containing a flow-focusing main channel for generation of nanoliter-volume droplet (water-in-oil, mother droplet), and three T-junction side channels for splitting of femtoliter-volume daughter droplet from mother droplet, as shown in Fig. 1A. The main channels are 50 μm in width, and 30 μm in height, the side channels are 25 μm in width. For the orifice-included T-junction chip, the orifice is 50 μm in length, and 25 μm in width, which can obviously facilitate the splitting of mother droplet, and reduce the daughter droplet volume compared with the orifice-avoided T-junction channel.

Soft-lithography was used to fabricate the PDMS chips. Briefly, the designed structure printed on a transparent film was transferred onto a silicon wafer (Institute of Microelectronics of Chinese Academy of Sciences) with 30 mm thick AZ 50 XT photoresist (AZ Electronic Materials USA Corp, USA). After casting about 5 mm thick poly- (dimethylsiloxane) (PDMS, RTV615 GE Toshiba Silicones Co. Ltd) with a 10 : 1 polymer/curing agent ratio, onto the silicon master and waiting until the bubbles disappeared, PDMS was cured at 75 °C for 3 h. Then the cured PDMS was peeled off from the master, and inlet and outlet holes were drilled by a metal pipe. After exposed to a plasma treatment for 1 min, the PDMS was bonded to a glass slide on which a 50 mm thick PDMS film was coated previously. The PDMS chips were baked at 120 °C overnight to get the hydrophobic channels.

Formation of droplets
The two flow streams, soybean oil and water, were pumped constantly by syringe pumps (pump 11 Pico Plus, Harvard Apparatus, USA) into the well-fabricated PDMS hydrophobic PDMS chips, in which soybean oil was used as the continuous phase to wrap water. The flow behavior was observed on an inverted fluorescence microscope (TE2000-U, Nikon Corp, Japan), coupled with a charge-coupled device (CCD, Retiga 2000R, Qimaging Corp, Canada) for the recording process.

RESULTS AND DISCUSSION

At the first T-junction, the mother droplet was split into two daughter droplets, the smaller one was driven into the side channel, and the bigger one continued to move forward in the main channel (as shown in Figure 2), and at the second T-junction, it was split again, so was the third T-junction.

![Figure 2. A single period of splitting of daughter droplet from mother droplet](image)

We studied influences of four different channel geometry on droplet splitting, including orifice-avoided T-junction channel, orifice-included T-junction channel, main channel of 100 μm, side of 50 μm. We found that the introduction of orifice into the T-junction region can obviously reduce the volume of daughter droplet, the smallest volume of daughter droplet generated at the first orifice-included T-junction was 6.5 fL. As the increase of the $U_{oil}/U_{w}$ ($U_{oil}$—flow rate of soybean oil, $U_{w}$—water), the volume of the daughter droplet decreased (as shown in Figure 3).

As the introduction of surfactant, the droplet volume can be reduced to 0.65 fL, also it can prevent small droplet fusing (as shown in Figure 4). We use this droplet splitting system to entrap several fluorescent nanospheres, which show great potential to manipulate and observe single nanoparticles independently (as shown in Figure 5).

![Figure 3. Influence of microchannel geometry on droplet splitting](image)

CONCLUSION

In our work, we generated femtoliter droplets by droplet splitting from nanoliter ones, and studied influences of fluid flow rate, channel geometry, addition of surfactant on droplet splitting. The smallest volume of droplets can be reduced to 0.65 fL by addition of surfactant. Using this droplet splitting system, we realized entrapment of single fluorescent nanospheres.

ACKNOWLEDGEMENTS
We thank the support from the National Basic Research Program of China (973 Program, 2007CB714507), the Science Fund for Creative Research Groups (20621502), the National Natural Science Foundation of China (20875072), and the Ministry of Public Health (2009ZX10004-107 and 2008ZX10004-004).

REFERENCES

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