ACOUSTIC FOCUSING OF MICROPARTICLES IN TWO-PHASE SYSTEMS – TOWARDS CELL ENRICHMENT OR MEDIUM EXCHANGE IN DROPLETS

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ABSTRACT

We present a method to first laterally position microparticles inside droplets by acoustic forces and then split the droplet into three daughter droplets to achieve a 2+ fold enrichment of microparticles inside the center droplet. We show that acoustic forces can be applied to both manipulate polystyrene beads (5 µm) and red blood cells inside droplets. The presented technology opens up for development of droplet operations used for medium exchange and particle concentration in droplet-based cell assays.

KEYWORDS: Droplet microfluidics, Integrated acoustics, Ultrasound

INTRODUCTION

Droplet microfluidics has emerged as a valuable tool to miniaturize biological reactions and allows for high-throughput analysis of single cells. Efficient methods to exchange buffer in cell-containing droplets is, however, a missing functionality for the full integration of many standard biological assays in droplet-based platforms [1]. In earlier work acoustics have been used to manipulate microparticles and cells in one-phase systems [2], here we show that acoustic forces can be successfully applied in two-phase systems as well. The benefit of using acoustic forces lies in that it is a non-contact method, and has been shown to be gentle to cells [3]. In this work acoustophoresis in droplets is combined with a trident shaped droplet split to achieve enrichment of microparticles in droplets. The proposed concept is illustrated in figure 1.

EXPERIMENTAL

A microfluidic device for droplet generation and splitting was etched in silicon yielding rectangular channels (cross section 165x435 µm), and sealed with a glass lid. The channels were silanized to render the walls hydrophobic. Monodisperse aqueous droplets were generated with olive oil as the continuous phase, and microparticles were encapsulated in the droplets. The chip was actuated using a piezoelectric transducer to create half wavelength resonance across the width of the channel, with a pressure node at the center. Polystyrene beads (5 µm) were used to validate the system. The particles are affected by the acoustic forces based on their acoustic properties. Both polystyrene beads and red blood cells have a posi-
tive acoustic contrast factor in water and are expected to be moved to the node of the standing wave (i.e. the center of the microfluidic channel). After acoustic focusing and the trident shaped droplet split, the daughter droplets were collected and the beads were counted using a Coulter Counter. The results were compared between the center and side daughter droplets to quantify the concentration factor and recovery. Red blood cells were encapsulated and acoustically manipulated to show the possibility to manipulate cells inside droplets.

RESULTS AND DISCUSSION

At the resonance frequency (1.8 MHz) the polystyrene beads were positioned at the center of the droplets as expected. Further down the microfluidic channel the droplets were split into three daughter droplets of approximately the same size (25-28 nl). When ultrasound was applied the majority of the beads were directed to the center daughter droplet during the splitting yielding an enrichment of microparticles in the center daughter droplets, see figure 2.

![Figure 2: Acoustic focusing of polystyrene beads during controlled splitting in a trident shaped droplet split. Left: Without ultrasound. The beads are distributed in the entire main droplet, resulting in an even distribution of beads in the daughter droplets. Right: With ultrasound. The beads are positioned to the center of the droplet, directing the beads to the daughter droplets in the center outlet.](image)

The experiments were performed at two different start concentrations of beads, and the enrichment was found to be 2.2±0.3 and 2.4±0.1 times for the low and high start concentration respectively, see figure 3. This indicates that the initial start concentration is not a critical parameter for successful performance of the system. The recovery of polystyrene beads in the center droplets was 81% and 89% for the low and high start concentration respectively, when 64% of the original droplet volume was removed.

![Figure 3: Concentration factor (n=3). The original concentration was 7.5x10⁵ beads/ml and 6x10⁶ beads/ml, respectively. Error bars represent the standard deviation.](image)
To demonstrate the suitability of described system for droplet-based cell assays, red blood cells were used. At application of ultrasound the encapsulated red blood cells were positioned to the center of the droplets as shown in figure 4.

Figure 4: Acoustic manipulation of red blood cells inside a droplet. Left: Without ultrasound. The cells are randomly distributed in the droplet. Right: With ultrasound. The cells are focused to the center of the droplet. It can be noted that the droplet is deformed by the ultrasound due to the difference in acoustic properties of the two phases.

The described system opens up for medium exchange in droplet-based assays where less than 5% of the original droplet volume would remain after three consecutive splits with intermediate refilling.

CONCLUSION

We have successfully shown the removal of approximately 2/3 of the droplet volume with in average 85% recovery of beads by combining integrated acoustics with droplet microfluidics. Our results show proof-of-principle of a droplet microfluidics unit operator for medium exchange or particle concentration in droplets, which has been lacking for many years.

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REFERENCES


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