MAGNETIC MICROPARTICLE BASED DNA EXTRACTION IN A DROPLET MICROFLUIDIC CHIP

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ABSTRACT

We are reporting an efficient method for magnetic microparticle based DNA extraction on a segmented flow microfluidic platform [1]. The droplet splitter and the magnetic setup were optimized separately to achieve highest separation efficiency. The novel asymmetric droplet splitter was developed allowing for dynamic control and arbitrary selection of droplet splitting ratios between 50:50 and 95:5. Further, the magnet position and orientation were optimized via numerical modeling and experiments. The optimized microflidic system was used for microparticle-based DNA extraction, resulting in 97% of microbead separation efficiency, 90% DNA capture efficiency, while removing 90% of original volume in single step.

KEYWORDS: DNA Extraction, Asymmetric Droplet Splitter, Magnetic Microparticle, Droplet Microfluidics

INTRODUCTION

A central step of most detection or quantification methods is the sample preparation, and more specifically, the target molecule extraction and purification. However, in droplet based microfluidics, current methods to separate the particles from the droplet are not very efficient, since only half of the original droplet volume is removed [2,3]. In this work we are reporting an efficient method for a microparticle-based selective DNA extraction protocol in a segmented flow microfluidic chip. The novel extraction approach is based on asymmetric droplet splitting and magnetic setup optimization, to improve the microparticle separation efficiency, and hence, the target extraction efficiency.

THEORY

A droplet-based segmented flow microfluidic tool for extracting DNA target with magnetic microparticles was optimized in several steps. First, novel microfluidic concepts to control the splitting ratio of the droplets were developed together with an improved magnetic setup for the separation of the microparticles. In contrast to the sequential droplet splitting [4], an asymmetric droplet splitter was introduced based on a difference in fluid resistance in the splitting channels. In our case, an additional oil inlet was used in one of the splitting channels to allow for dynamic control of droplet splitting (Fig. 1). To reduce the experimental work, the optimization of splitter design was achieved using a computational fluid dynamics model [5]. The achieved dynamically controlled splitting ratios were from 50:50 to 95:5 of volume fraction. In the next step, the asymmetric splitting design was integrated with a magnetic particle extraction process. The magnet position and orientation at the T-junction split were optimized in order to get the best separating efficiency. A detailed three-dimensional model of the magnetic field in proximity of a permanent magnet was used to calculate the magnetic force on the superparamagnetic particles.

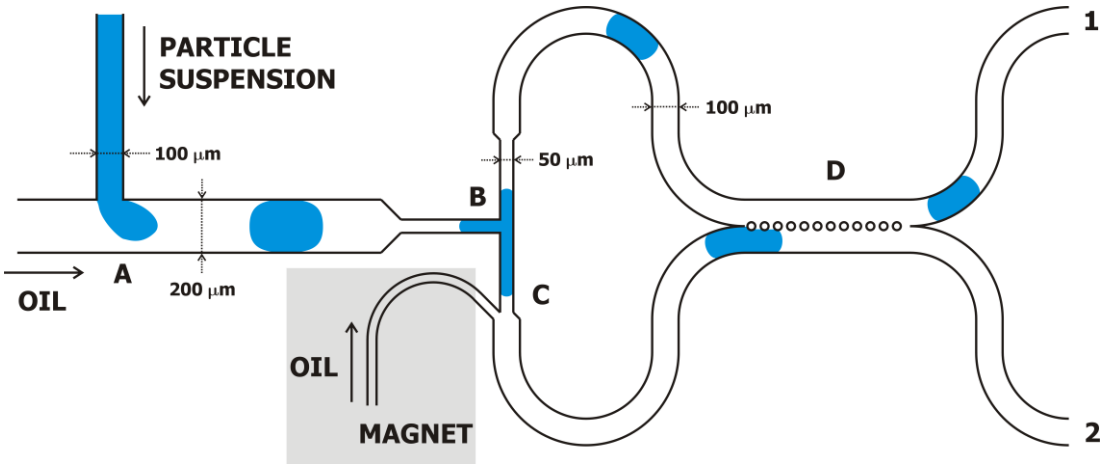

Figure 1: The microfluidic design: (A) the droplet formation T-junction, (B) narrow droplet splitting T-junction, (C) extra oil inlet for splitting control, (D) pressure equilibration, magnet position is denoted by a grey square.

EXPERIMENTAL

The optimized microfluidic setup was tested for microparticle-based DNA extraction (Fig. 2). The superparamagnetic microparticles coated with streptavidin were mixed with excess of biotinylated capture probes and incubated to allow the immobilization of the probes. The microparticles and a sample containing ssDNA targets were injected into droplets on the microfluidic chip. The droplet was transported through the channel for one minute, to allow mixing and hybridization of microparticles and targets. At the T-junction splitter, the microparticles were magnetically separated from the droplet, extracting the target DNA from the sample. Part of the droplet sample stayed with the particles.

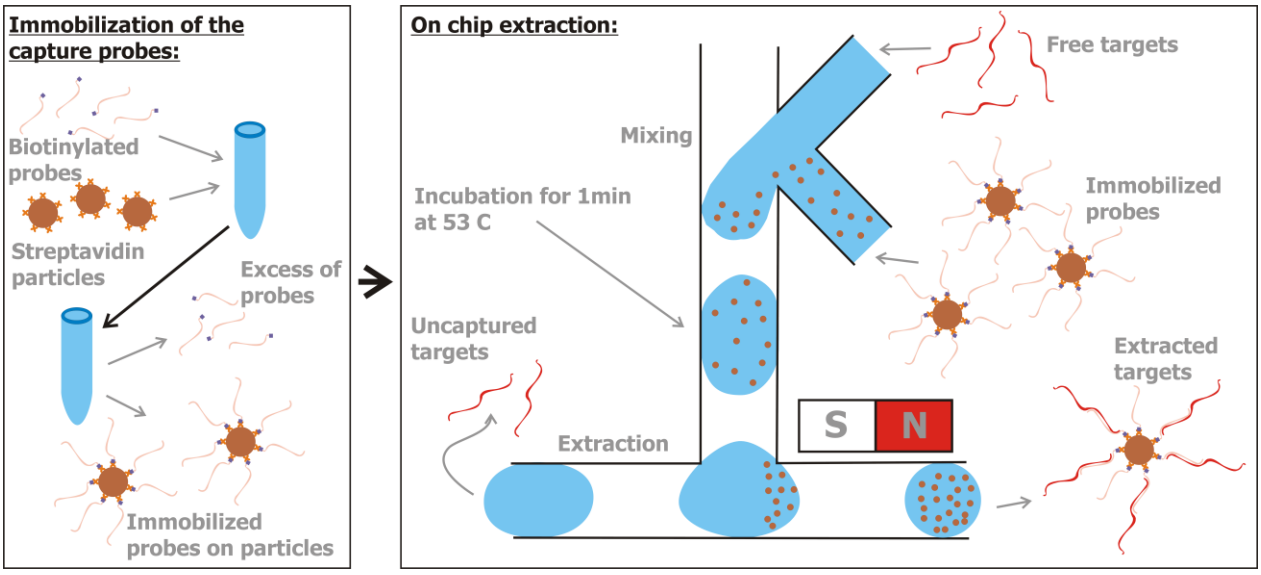

Figure 2: Overviev of the DNA extraction assay. Left: Immobilization of the capture probes off chip. Right: On chip extraction.

RESULTS AND DISCUSSION

Splitting the droplets at ratios between 60:40 and 90:10 resulted in high particle separation efficiencies of 97% (Fig. 3), and very good target recovery capabilities. Using the hybridization assay in the segmented flow setup resulted in a lower or similar loss of target DNA strands (10±4%) compared to the off-chip reference methods, confirming the good choice of oil, surfactants and surface coating.

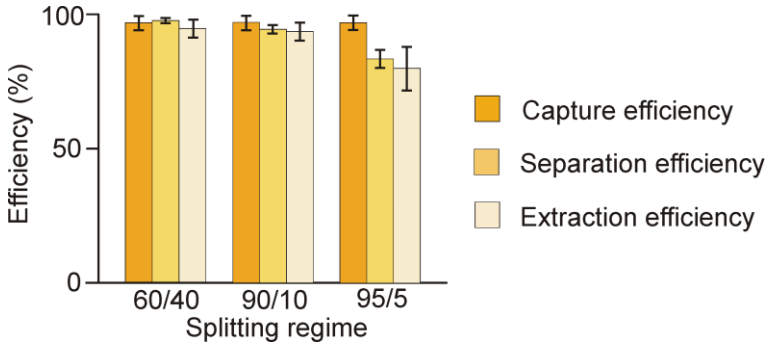

Figure 3: Target capture efficiency, magnetic microbeads separation efficiency and target extraction efficiency at three different splitting regimes.

CONCLUSION

The novel microfluidic and magnetic setup showed the potential to separate superparamagnetic particles from the sample droplet, while removing 90% of the original volume in a single step. The novel separation system allows for better on-chip quantification when coupled to quantification techniques, such as ELISA or qPCR.

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