FIELD-FREE PARTICLE SEGREGATION AND EXTRACTION FOR BEAD-BASED ASSAYS IN PLUGS

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ABSTRACT

Bead based assays require the essential steps of particle mixing, segregation, and extraction. In droplet microfluidics, particle concentration techniques typically require either electrical or magnetic fields together with charged or magnetic particles. Here we present a passive, on-chip particle mixing, segregation, and extraction technique based on the interaction between naturally occurring hydrodynamic recirculating flows and particle sedimentation within the plug. The interaction can be quantified using the Shields number (θ), a dimensionless ratio of a particle's drag force to its gravitational force, which scales with plug velocity. Depending on θ, three distinct flow regimes can be defined, and the key steps of particle segregation and resuspension can be performed simply by changing flow velocity. We demonstrate highly efficient concentration (~100%) of 38 µm glass beads in 200 and 500 µm diameter plugs traveling at velocities up to 5 mm/s. Segregated particles are then extracted using a transverse drop splitter. This technique expands the capabilities of plug-based microfluidics to perform particle filtration without on-chip components and thereby could be applied to bead-based screening assays.

KEYWORDS

Particle concentration, bead based assay, plug, droplet microfluidics, sedimentation

INTRODUCTION

The bead-based assay (BBA) is a workhorse protocol in biology and chemistry, routinely used in sample pre-concentration, solid phase extraction, synthesis, and other techniques. The conventional BBA requires 3 steps (**Fig. 1A**): 1) mixing particles with solution, 2) concentrating particles in a local region (pellet), typically using magnets or centrifugation, and 3) removing the supernatant. Implementing a BBA is a challenge in droplet microfluidics because particle concentration and extraction are difficult to implement. While there are a multitude of particle concentration techniques available in single-phase microfluidics [1, 2], only a few are available in plug microfluidics. Existing techniques require external magnetic [3, 4] or electric [5, 6] fields together with magnetic or charged particles, which may be undesirable in many circumstances. This paper demonstrates the 3 key steps of a BBA in a microfluidic plug **(Fig. 1B)**: 1) mixing of particles with sample, 2) high-efficiency segregation of particles in the plug, and 3) extraction of particles from the plug. All are accomplished in a passive chip without on-chip components or fields. The first two steps are accomplished by exploiting the interaction between the particle sedimentation force and the recirculating vortices within the plug [7-8]. We use the non-dimensional Shields number to categorize and choose the appropriate flow regimes for mixing and concentration. The third step is done using a transverse drop splitter.

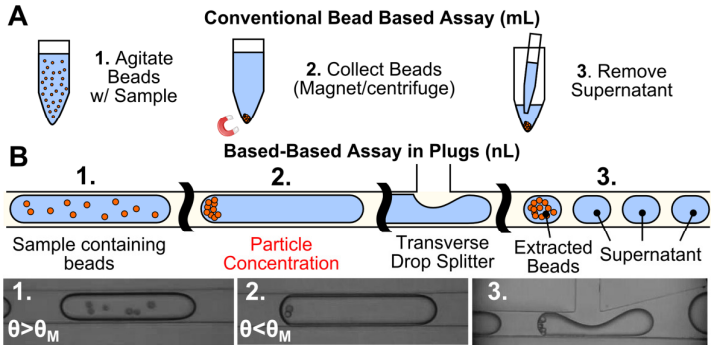

Fig. 1: Comparison of bead based assays in the traditional vs. plug format. The latter is achieved by field-free particle concentration followed by transverse drop splitting.

THEORY

We use a field-free, hydrodynamic particle concentration technique based on the interaction of particle sedimentation with the recirculating microvortices which occur naturally within a plug [7]. This interaction can be quantified using the Shields number θ, the dimensionless ratio of a particle's drag force to its gravitational force, which scales with plug velocity [8]. The Shield's number is given by $\theta = (9\mu_p V_p)/(a^2 \Delta\rho g)$, where μ_p is the plug viscosity, V_p is the plug velocity, a is

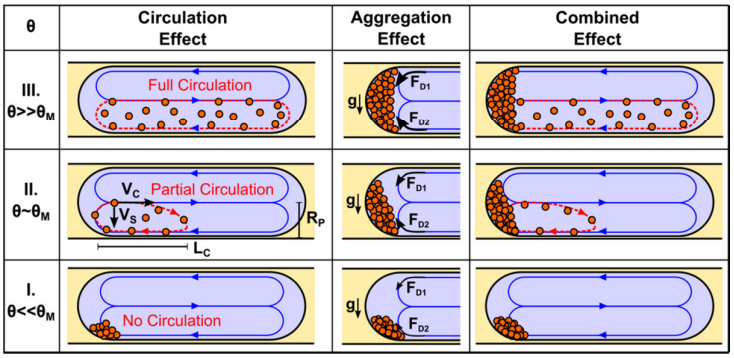

Fig. 2: Theoretical model of particle concentration in plugs (Side View). Three flow regimes are described by the Shields number θ (column 1). In each regime, the circulation effect and the aggregation effect are illustrated in columns 2 and 3, respectively. The combined effect is shown in column 4.

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the particle radius, $\Delta\rho$ is the density difference between the particle and the plug phase, and g is the gravitational constant. Based on the Shield's number, three regimes of particle behavior are identified (**Fig. 2**). Regime I: When θ is less than the movement threshold θ_M, the particle's gravitational force dominates the drag force; hence the particles sediment to the bottom of the plug, while the internal vortices subsequently convect them to the rear cap. This regime provides highly efficient particle segregation within the plug. Regime II: As θ is increased beyond θ_M, particles become suspended in a well-defined circulation zone which begins at the rear of the plug. The length of the zone scales with plug velocity. Regime III: When $\theta >> \theta_M$, the particles circulate throughout the entire plug and the length of recirculation is limited by the plug length. In this regime, the particle's drag force dominates the gravitational force. A second effect, the aggregation effect, causes additional concentration of particles in a stagnation zone at the rear cap. Some of the particles which are dragged towards the rear end become trapped in the independent, co-rotating cap vortices. Particles continue to accumulate, eventually forming a particle-laden stagnation zone. The aggregation effect is also impacted by the Shields number. When θ is large, particles tend to distribute symmetrically within the cap **(Fig. 2)**. This is due to the equal and opposite fluidic drag induced by the internal vortices (F_{D1} and F_{D2}). Conversely, when θ is small, the drag force is not sufficient enough to lift the particles against the gravity; therefore, they segregate toward the lower half of the cap [8].

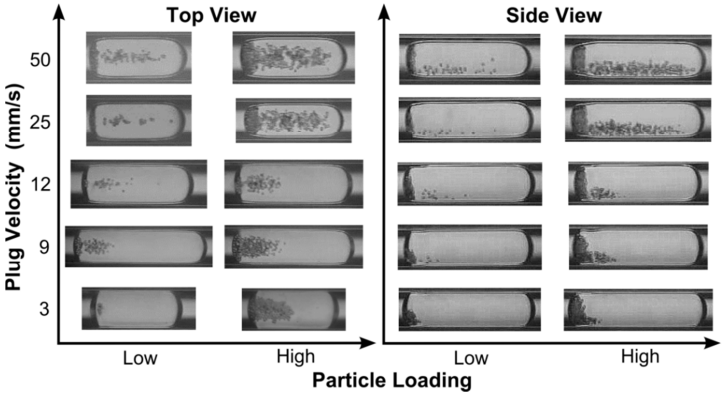

Fig. 3: Particle concentration profiles in a 500 µm ID circular channel. Both the top and side view are shown [8].

EXPERIMENT

Plugs are generated by combining an aqueous bead suspension with oleic acid (μ_C = 27mPaS) in a 500 µm bore PEEK T-junction or a 200x100 µm microfluidic channel. The suspension consists of deionized water containing soda-lime glass beads with mean diameter 38 µm and a 34-42 µm D50 distribution. The flow rates for oil and water range from 0.6-10 µL/s, resulting in plug velocities between 1-50 mm/s. This corresponds to Reynolds number <25 and capillary number <0.1. Videos are recorded with a high speed camera at 1200 frames per second (FPS) with 336x96 pixel resolution, at 600 FPS with 432x192 resolution, and 30 FPS with 1280x720 resolution.

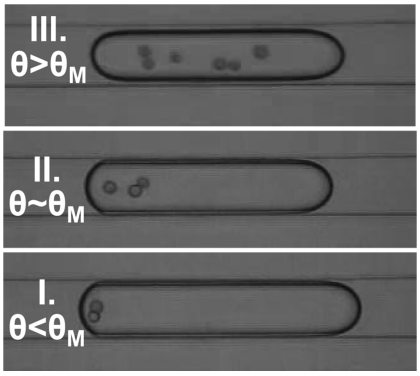

Fig. 4: Particle concentration profiles in a microchannel with 200x100µm cross section. High efficiency particle concentration occurs when $\theta<\theta_M$, and mixing occurs when $\theta>\theta_M$.

RESULTS AND DISCUSSION

Experimental data of particle segregation (**Figs. 3-5**) closely follow the theoretical model. The particle segregation, recirculation/mixing and aggregation phenomena can be explained in terms of Shield's number, particle size, and particle loading.

Effect of Shield's Number/Flow velocity. In our experiments, we vary Shield's number simply by changing the plug velocity. **Figs. 3&4** shows the images of particle recirculation (within the plug) in the 500 µm capillary and rectangular microchannel, respectively. In both cases, the particles are 38 µm soda lime beads. The circulation zone length L_C, scaled by the plug radius R_P, is shown in **Fig. 5**, where 3 distinct regimes can clearly be identified. In region I (small θ), which occurs at low plug velocity (V_p < 5mm/s), the gravitational force on the particle dominates the viscous drag. In such cases, the beads segregate at the rear end due to minimal recirculation as well as aggregation effect. This regime is ideal for high efficiency (~100%) particle concentration. Region II begins when θ exceeds the movement threshold, which is experimentally found to be θ = 10 (V_p ~6 mm/s). In this regime, particles circulate in a well-defined zone which begins at the rear of the plug. The length of the circulation zone scales linearly with flow velocity, increasing from 0.4 to 1.4 mm over plug velocities ranging from 9 to 25 mm/s (θ~15-42). In region III, which occurs at large θ (plug velocity >25mm/s), the particles circulate within the entire length of the plug. This regime is ideal

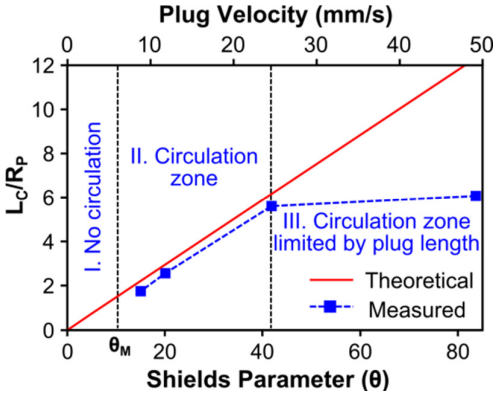

Fig 5: Theoretical and measured lengths of the particle circulation zone graphed vs. the Shields number, which scales with plug velocity. The dotted lines demarcate the 3 flow regimes.

for the vigorous mixing of beads with the sample which can enhances the capture of target molecules. Hence, it is interesting to note that the two key operations of bead based assays, mixing and segregation, can be achieved by simply changing the plug velocity to regions III and I, respectively.

Effect of Particle Size and Loading. Since the phenomena of particle suspension and segregation depends greatly on the sedimentation rate, this technique is best suited for dense and large size particles [8]. To illustrate the importance of particle size, **Fig. 6** compares concentration profiles of 1 µm polystyrene particles (ρ=1.03 g/cm^3) with 30 µm glass beads (ρ=2.52 g/cm^3) traveling at identical flow velocities. In the former case, θ=3.7x10^5, therefore the polystyrene beads are uniformly distributed throughout the plug and there is no sign of particle segregation. In the case of the glass beads, θ~5, and the beads concentrate at the rear end. The particle loading effect influences the aggregation effect. High particle loading favors the formation of the aggregation zone in the rear cap, regardless of flow velocity. High particle loading contributes to a positive feedback effect, where the deposition of particles reduces the local flow velocity, which in turn leads to further aggregation. In these experiments, a high concentration is defined as >10^5/mL, and a low concentration is defined as <10^4/mL.

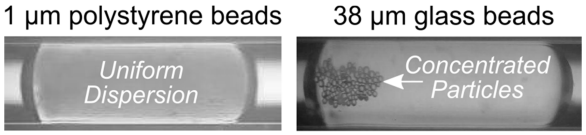

Fig 6: Top view comparison of experimental concentration profiles with small, light particles versus large, heavy particles at an identical flow rate of 3 mm/s in a 500 µm diameter circular channel. Both experiments use a high particle loading of >10^5/mL.

Particle Extraction using a Transverse Drop Splitter. Using the above concentration phenomena, a bead-based assay can be implemented in following steps: 1. Operate the system in Regime III to ensure proper mixing of beads with the sample. 2. Operate the system in Regime I to ensure 100% collection efficiency. (Switching flow regimes is done by simply changing the plug velocity.) 3. Extract the beads from the sample. The last step can be done using the transverse drop splitter. It is a simple T junction (**Fig. 7**) having a tapered outlet to ensure maximum shear at the rear end of the plug where the beads are localized. By controlling the oil flow rate of the 'chopping' channel, plugs are transversely split into multiple droplets. The initial daughter droplets contain supernatant, while the last contains the extracted particles (pellet). Since this extraction must occur in Regime I, the plug velocity is low (0.5 to 5 mm/s) and the ratio of flow rate between the two channels ranges from 2 to 5 depending on the plug length. High separation efficiency can be achieved by using long initial plugs, or repeating the splitting and extraction process.

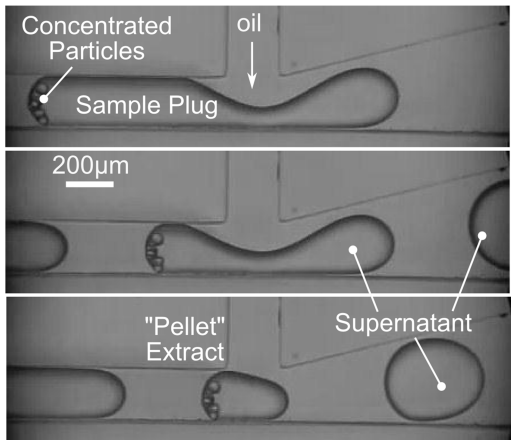

Fig. 7*:* Bead extraction using a transverse drop splitter. The last daughter droplet contains the concentrated particles.

CONCLUSION

This paper demonstrates a field-free, gravitationally driven approach to perform particle suspension, concentration and extraction within the microfluidic plugs. The first notable advantage of this technique is that it is passive, requiring no external components, and the process can be controlled simply by changing the flow velocity. Secondly, our technique can achieve a collection efficiency of nearly 100% as compared to prior approaches which have reported 83% efficiency [6]. This technique can expand the capabilities of plug based microfluidics to perform bead based assays on-chip without external components and therefore could be applied to numerous heterogeneous screening assays.

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