USING STRUCTURED MICROFLOWS TO SYNTHESIZE FUNCTIONAL PARTICLES

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ABSTRACT

Diverse patterns of flows in microfluidic channels have been utilized with great interests in the field of mixing, separation, and flow cytometry for high throughput analysis of chemical reagent or biomolecules. In this paper, we describe a new approach, hydrodynamic focusing lithography (HFL), in which the structuring of fluids in microflows combined with lithography is exploited to create large arrays of functional microparticles. Contrary to our prior flow lithography method, here the flows are stacked in multiple dimensions. We also demonstrate the utility of this process in a number of assays ranging from patterning of proteins to reinforced gel particles.

KEYWORDS: Hydrogels, Lithography, Multifunctional materials, Flow focusing

INTRODUCTION

Biocompatible multifunctional particles hold great promise for biomedical applications such as drug delivery and bar-coded particles for rapid screening of biomolecules. For these applications, particle design is at least as important as size and requires a high-throughput fabrication technique with precise control over shape and chemical patchiness. Methods currently used to generate anisotropic multifunctional particles include photo resist-based lithography, and the PRINT method. For the multilayer lithography, the use of photoresist materials renders this approach suboptimal for biological applications. While the PRINT method has its strength in producing small sub-μm particles, to date multiphasic particles beyond a 1-D stripe have not been synthesized. In this paper, we introduce a new method called hydrodynamic focusing lithography (HFL) that harnesses flow focusing to create stacked flows in two-layered channels for particle synthesis. Contrary to our prior methods to create multilayered particles, here the fluid interface can be perpendicular to the UV light propagation direction and precise mask alignment at the interface is no longer needed. This change in geometry also allows us to polymerize 2-D arrays, compared to 1-D in the prior method, which can increase throughput dramatically. In HFL, multiple monomer streams can be simultaneously stacked in both the z- and y-direction leading to more complex particles than before. Furthermore, through the use of inert tuning fluids, HFL can be used to adjust heights of particles, providing on-the-fly alteration of particle height. Finally, we demonstrate that this process can be used to generate particles with capture proteins on selected layers or reinforcements.

EXPERIMENTAL

Figure 1: Synthesis of anisotropic layered microparticles. Scale bars are 20μm(D), and 50μm(C and E).

Figure 1A shows a typical channel used for particle synthesis. Narrow channels (40 μm) were used to minimize mixing as stacked streams were introduced, providing stable layered flows in the z-direction. Further along the channel, the flows are widened up to 1 mm for particle synthesis. To mass-produce layered particles in the two-layered PDMS channels, we used stop flow lithography (SFL). This automated, cyclic process allows flow stoppage, then particle polymerization and subsequent flow. During multifunctional particle synthesis, traditional SFL requires precise mask alignment across the interface and each synthesis step polymerizes only 1-D rows of particles. The change in orientation of the fluid interface in HFL allows for production of 2D arrays in each step. With a circular polymerization region of radius D and a particle dimension L, synthesis throughput per cycle is approximately πD^2/4L^2 for a 2D array of particles in comparison to D/L for a single row. In our current setup, D is approximately 2 mm and taking a particle dimension of 5 μm, the throughput is increased by more than 200×. Furthermore, resolution of layer heights is now controlled by automated flow rather than manual mask alignment. Using this technique, we synthesized bifunctional, triangular polyethylene glycol particles
comprised of an upper layer with rhodamine-acrylate and a bottom layer containing 200 nm green fluorescent beads (Fig. 1B-C). The relative thickness of each chemical region, H₁ and H₂, could be readily controlled by adjusting the ratio of inlet pressures. We developed a simple hydrodynamic model to predict H₂/H₁. The prediction from the model is shown as the dashed curve in this graph and compares well with the experiments (Fig. 1D). Multiple flows can be also stacked by increasing the number of inlets entering sequentially from the bottom layer of the device. Using such multilayer stacking, we synthesized triangular particles containing three layers (Fig. 1E).

Figure 2: Synthesis of dual-axis layered microparticles. Scale bars are 10 μm (B), 40 μm (C), and 50 μm (E, F, and G).

We also show that multiple monomer streams can be simultaneously stacked in both the z- and y-directions (Fig. 2). The process can be used for high-throughput synthesis of dual-axis multifunctional particles with mask-defined morphologies, and extends the degree of freedom for chemical anisotropy in a particle to two dimensions. Such particles have not previously been made in microfluidic devices. We generate a 2-D flow focusing geometry by first co-flowing monomers F3 (PEGDA with Rhodamin-A) and F2 (PEGDA with 100 nm blue fluorescent beads) using two inlets of top channel. This flow is then stacked on monomer F1 (PEG-DA with 200 nm green fluorescent beads), which enters from the bottom channel (Fig. 2A). Using a transparency mask with a single row of features, we synthesized cross-shaped particles with dual-axis functionality at the interface of flows (Fig. 2B-C). Using this process, virtually any number of flows can be stacked. To demonstrate this, we generated particles which contain in their center side-by-side stacked monomers which are bounded on the top and bottom by a third monomer stream (Fig. 2D). To achieve this, Flow 1 was introduced in at both the top and second bottom channel, while the monomers contained in the middle layer were combined at the first bottom inlet. Using a mask of rectangular shapes with rounded corners, we synthesized sandwich-like multifunctional particles at the interface of the two flows in the middle layer (Fig. 2D and 2G). As shown in Figure 2E-F, the sandwich particles had green fluorescent top and bottom layers (Fig. 2E) with red and blue fluorescent layers comprising the middle (Fig. 2F). For such dual-axis particles, chemical anisotropy in the y-direction can be controlled by mask alignments at the flow interface.

RESULTS AND DISCUSSION

Figure 3: Applications of hydrodynamic focusing lithography. (A and B) On-the-fly alteration of particle heights. (C,D and E) reinforced barcoded particles. (F,G,H, and I) protein conjugation on selected regions.

An application of flow layering in flow lithography is the use of outer inert tuning fluids to easily and rapidly vary particle height in a channel with a fixed height. To demonstrate the adjustment of particle height, we used inert PEG (n=400) as a tuning fluid in the top and bottom streams of a three-layer flow (Fig. 3A). The thickness of the tuning layers was adjusted by varying the inlet pressures of the streams. Using the process, we prepared particles with a height of ~ 18 μm which was around 50 % of the height of particles generated without tuning fluids (Fig. 3B). We also reduced particle heights up to ~ 8 μm. Importantly, the use of inert tuning fluids extends the process of flow lithography to devices that are impermeable to oxygen (such as glass channels) and does not necessitate using polymerization chemistries which are quenched by oxygen.
Another application of this technique can be the production of reinforced barcoded particles for detection of large biomolecules. Highly porous barcoded particles that are prepared by low concentration (≤ 10%) of poly(ethylene glycol) diacrylate (PEGDA) monomer can offer sensing capability to detect large targets, allowing diffusion of the biomolecules in the gel networks. However, the soft barcoded particles cannot have been scanned in a microfluidic device, showing the mechanical instability that results in the bending or folding of the particles (Fig. 3D). HFL allows us to combine the usually orthogonal characteristics of an open porous capture region for biomolecule detection with strong structural properties that resist deformation in flow. For the proof of the principles, we synthesized trilayered reinforced barcoded particles. In the particles, two soft porous layers were comprised of < 10% PEGDA while one hard supporting layer consisted of > 40% PEGDA. As shown in Figure 3E, the supporting layer prevented the distortion of soft porous layers.

Finally, we demonstrate that particles prepared by HFL can be patterned with proteins on a specific layer. These “caps” can be used to restrict target capture to specific particle faces (Fig. 3F-I). To achieve protein capturing, we first synthesized biotin-PEG-acrylate (biotin-PEGA) by preparing a 1:1 molar mixture of biotin hydrazide and PEGA-succinimidyl ester in commercial 1X phosphate buffered saline (PBS) and used this as an anchor to attach streptavidin. This allows us to directly copolymerize biotin in a selected region of the particle. The trilayer flow is shown in Figure 3F, and the acrylated biotin is in the center flow. The resulting synthesized triangular particles are shown in Figure 3G. Next, streptavidin-Cy3 was incubated with the particles at 37°C for 30 min (Fig. 3H). The streptavidin-Cy3 will strongly associate with the biotin. As the size of streptavidin (~ 5 nm) was bigger than the porosity size of the hydrogel networks, the proteins could not penetrate the gel structures, resulting in the coatings on sides of particles. In Figure 3I, the fluorescence pattern indicates that proteins were not bound to top and bottom layers. Furthermore, the resulting specific association shows that the biotin is still active after UV polymerization, akin to our prior work with nucleic acids. The short UV exposure dose required for synthesis is the most likely reason that bioactivity is retained.

CONCLUSION
We have presented a new technique called hydrodynamic focusing lithography (HFL) that combines flow-stacking and microfluidic particle synthesis. In HFL, the layered flows can be used to introduce chemical anisotropy in z-direction of particles. For certain applications, the approach can increase the throughput of multifunctional particle synthesis over 200 times when compared to traditional stop-flow-lithography. We have also demonstrated that dual-axis layered particles can be produced by HFL. Through the use of inert tuning fluids, HFL has been used to generate particles with various heights on-demand. Moreover, we prepared new classes of reinforced gel particles for diagnostic applications. Lastly, we showed that particles generated by HFL were patterned with proteins on a specific layer and remained active. HFL is a compelling method as the technique is compatible with other flow lithographic methods. For example, the combination of HFL and Lock Release Lithography (LRL) can lead to chemical patterning in all dimensions as LRL can provide chemical anisotropy of particles in x–y dimension. We have believed that HFL can provide a powerful way to reach new complex particles.

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