A MEMBRANELESS CONTINUOUS-FLOW FILTER FOR HIGH-THROUGHPUT SEPARATION AND ENRICHMENT OF PARTICLES AND CELLS

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ABSTRACT

We describe a new microfluidic-based filtration method capable of performing simultaneous size-based separation and enrichment. Instead of forcing a particle or cell-laden suspension through a microporous membrane, microchannels are constructed with embedded weir-like barriers oriented parallel to the flow direction. When this geometry is incorporated into a curved flow path the resulting centrifugal forces act to push the suspended components across the barrier from the inner wall to the outer wall, with only those smaller than the barrier gap able to pass across. In addition to demonstrating extremely high selectivity, clogging effects are minimized because the primary flow acts to sweep aggregates downstream. We also describe a new lithography-free microfabrication approach to construct these barrier structures.

KEYWORDS: Separations, Microfabrication, Etching, Inertial Flows

INTRODUCTION

There is a critical need for advanced filtration methods adaptable for separation of particles, cells, and cell-sized components from complex fluid mixtures—specifically those offering the capability to rapidly process large sample volumes (> mL/min flow rates). Microfluidic technologies provide a natural platform to address these challenges, but most of these efforts have yet to advance past the proof of concept stage.

THEORY

We have developed a new approach that uniquely overcomes the key limitations of previous filtration methods investigated for size-based separations of particles in the size range of blood cell components. Instead of forcing a cell-laden suspension to flow through tiny pores in a membrane filter, we are able to construct a filter oriented along the centerline of the microchannel so that it creates a barrier between the left and right hand sides (Fig. 1a). When this geometry is incorporated into a curved flow path, the resulting centrifugal forces that arise due to fluid motion [1] act to push the cells across the centerline barrier from the inside wall to the outside wall, with only the cells smaller than the barrier gap able to pass across. The harnessing of curvature induced forces to drive the filtration process is a distinct advantage of this approach, as compared with conventional cross-flow filtration methods where devices are specifically designed to suppress these flow effects [2, 3]. Consequently, this filtration method does not impose an excessive pressure drop because the barrier is oriented parallel to the flow direction rather than perpendicular to it. Moreover, this approach is most effective at high flow rates because the magnitude of the curvature-induced transverse flow is maximized under these conditions, making it ideally suited for high-throughput analysis of large sample volumes.

Figure 1: (a) Overview of membraneless filter design. (b) A mixture of 3 µm (1.68 x 10^6 particles/ml) and 10 µm (4.55 x 10^4 particles/ml) fluorescent beads is injected along the inside wall of a U-shaped microchannel (300 µm width, 1000 µm radius of curvature) at 1.2 ml/min. Only 3 µm beads are collected in outer outlet, while enriched and concentrated 10 µm beads are collected from the inner outlet.
EXPERIMENTAL

To construct microchannel networks incorporating embedded centerline barriers, we have developed a new microfabrication approach that harnesses specific biochemical interactions between an enzyme and a biodegradable substrate to enable localized and precisely controlled etching. This bio-sculpting process is based on the enzymatic activity of proteinase K, a serine protease capable of cleaving peptide bonds, especially alanine-alanine bonds. Etching is initiated when proteinase K is transported from bulk solution to the surface of a PLA substrate, after which enzyme-substrate complexation catalyzes surface reactions yielding low molecular weight cleavage products that subsequently become hydrolyzed and are released back into the bulk along with dissociated proteinase-K (Fig. 2a). When an aqueous solution of proteinase-K is directed through a microchannel template, the ensuing enzymatic degradation imprints a replica of the flow path into a PLA substrate comprising the channel floor (Fig. 2b). Characteristic etching rates of 1–10 µm/h are achievable (Fig. 2d). Etching depth increases monotonically with enzyme concentration up to ~0.1 mg/mL, above which a saturation condition is attained whereby the majority of surface complexation sites become occupied (Fig. 2c). The etching rate also reflects the temperature-dependent enzymatic activity, occurring approximately 7 times faster at 37 °C than at room temperature (Fig. 2e).

Complex cross-sectional topologies can be fashioned by establishing a lateral gradient in enzyme concentration to spatially regulate the etching process, as illustrated when parallel streams containing the proteinase-k solution and an aqueous buffer are co-injected into a microchannel template (Fig. 2f). To more sharply define the etched zone, we replaced the buffer stream with a 1% w/v aqueous solution containing bovine serum albumin (BSA) (Fig. 2g,h). In addition to providing a convenient mechanism to localize the surface reaction, the macromolecular nature of both BSA and proteinase-k also acts to limit lateral diffusion as compared with conventional chemical etchants so that the resulting microchannel topology can be maintained far downstream (Fig. 2i).

![Figure 2: Mechanism and parameters affecting the enzymatic etching process. (a) Illustration of surface degradation cycle. (b) Schematic representation of etching process and SEM image of etched channel (scale bar, 200µm). (c) – (e) Profilometry analysis of etched channels in different etching conditions. (c) Effect of enzyme concentration. (d) Etching depth versus time. (e) Effect of reaction temperature. (f)-(h) Flow images in a Y-shape device (scale bar, 500 µm) and the profile of etched channels achieved using different combinations of proteinase K and BSA. (f) Proteinase K and buffer. (g) Proteinase K and 1% BSA (w/v). (h) proteinase K and 10% BSA (w/v). The flow channel is filled with different color dyes for visualization. (i) Schematic of a Y-shape elastomer microchannel template with co-flowing proteinase K (red) and BSA (blue) solutions. Proteinase K-BSA complexation (green) occurs at the interface between streams, confining etching only to the PLA surface in contact with the proteinase K stream.

More intricate surface features can be fashioned by sequentially varying the composition and flow conditions applied in each stream (Fig. 3). We demonstrate this by employing a microchannel template with three independent inlets to construct a cross-sectional profile incorporating a weir-like barrier oriented parallel to the flow direction. First, BSA solution is injected into the center stream while proteinase-k is introduced into both outer streams, etching the PLA substrate to a depth of 10 µm on either side of the central zone protected from enzymatic degradation by the BSA. Next, BSA is infused into both the center stream and one of the outer streams after which etching is continued yielding different depths on each side of the microchannel. Finally, proteinase-k is injected into all 3 inlets to define the height of the centerline barrier. When completed, this process produces a cross-sectional profile incorporating three different heights (17, 7, and 35 µm from left to right)—a topology that is preserved along the entire length of the microchannel.

RESULTS AND DISCUSSION

Filtration capability is evaluated by injecting a mixture of fluorescent polystyrene beads of diameter 3 and 10 µm into the inner inlet of the microchannel design shown in Fig. 1 at a flow rate of 1 ml/min, after which samples were collected at outlets positioned at the inner and outer sides of the centerline barrier and analyzed by microscopy. The inner outlet contained the 10 µm beads that were unable to pass across the barrier, while the outlet from the outer microchannel wall contained only 3 µm beads—the only species able to cross the barrier (Fig. 1b).
Figure 3: Fabrication of microchannels incorporating membraneless filtration barriers along the centerline. Alternating flows of proteinase-k (etchant, red) and BSA (inhibitor, blue) are introduced into a microchannel with 3 inlets (bar = 1 mm). In step 1, a proteinase-k solution is pumped into the side channels while a BSA solution flows in middle channel to locally block etching, yielding 2 parallel channels in the PLA substrate. In step 2, the proteinase-k solution in the left side channel is replaced with BSA to block further etching of the substrate, yielding a deeper right side channel. In step 3, proteinase-k solution is introduced into all 3 inlets and etching is continued to define the gap between the center barrier. The resulting cross-sectional profile is maintained at different positions from upstream to downstream due to the laminar flow profile during etching (channel length = 1 cm; all flow rates are equal).

We also investigated the ability to collect dilute large-sized particles dispersed within a more concentrated suspension of small-sized particles (i.e., mimicking blood cells). Mixtures of fluorescently labeled beads were injected, after which the eluted fractions from the inner and outer streams were collected and analyzed using a flow cytometer. In preliminary tests using 3 and 10 µm beads, we have been able to isolate the 10 µm beads with >98% normalized efficiency at a concentration ratio of 10³ (Fig. 4). Considering that our current device design is unoptimized and intended only to demonstrate proof of concept for the proposed filtration method, we believe this indicates great potential to achieve the selectivity and isolation efficiency needed to meet a variety of demanding analytical requirements.

Figure 4: Flow cytometry results for (a) the injected mixture of 3 and 10 µm beads at concentrations of 1.68x10⁶ and 4.55x10² particles/ml, respectively; (b) effluent from the inner outlet; and (c) effluent from the outer outlet.

CONCLUSION

The enzymatic bio-sculpting process described here uniquely addresses a critical need for convenient methods to fashion topologically complex microfluidic environments, as illustrated by construction of a membraneless filter based on a microchannel geometry that would be challenging to construct using conventional microfabrication methods. This micromachining technique holds promise to help enable construction of microfluidic networks incorporating the structured microenvironments needed in advanced chemical and biological analysis applications.

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REFERENCES


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