

Cell and Particle Concentration on the Chip

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ABSTRACT

2 METHODS

Lab-on-a-chip devices are increasingly targeting cell analysis for scientific and clinical applications. Although several techniques for cell analysis have been already implemented at the microscale, preliminary cell sample preparation steps are still performed off the chip, using standard, macro-scale techniques. One example is the centrifugation of cell suspensions that is usually performed in ubiquitous lab-centrifuges. While the miniaturization of centrifuges would be hard to implement and integrate in microfluidic devices, alternative approaches for sample pre-concentration have to be considered. Here we report the use of a microstructured membrane for increasing the concentration of sparse cell suspensions up to three orders of magnitude. The presented design could provide an alternative for centrifugation and would be easy to integrate with existing microfluidic cell analysis techniques.

Keywords: centrifugation, microstructured membrane, lab-on-a-chip, cell suspension, sample pre-concentration

1 INTRODUCTION

Lab-on-a-chip devices for cell processing and analysis are increasingly sophisticated and targeting a wider spectrum of scientific research and clinical diagnostic applications. Despite recent advances however, many sample preparation steps, common for macro-scale techniques, have not yet been implemented in microfluidic systems. One example is centrifugation, which is commonly employed for increasing the concentration the chip. Here we present a new microfluidic design that enables the handling of suspensions of eukaryotic cells and the increase of cell concentration. In a significant departure from existent elastomeric microscale valves [1-3], our approach allows the integration of multiple structures on the same membrane, and the execution of complex sequential procedures using only a limited number of control steps. Through the use of such microstructured membranes we were able to achieve up to three orders of magnitude cell suspension concentration increases.

Standard microfabrication methods were employed to fabricate a two-layer elastomeric device on top of chrome micropatterned glass. One thin, network layer and one thick, control layer were fabricated by casting polydimethyl siloxane (PDMS) on a photopolymer mold. The two layer were bonded together to form a microstructured membrane with an actuation chamber (Fig.1). The assembly was then bonded on top of a chrome patterned glass slide to define the inlet and outlet channels. The chrome pattern was necessary in order to avoid the irreversible bonding of the microstructured membrane to the underlying glass and preserve its motility. The actuation chamber was connected to a syringe employed as a source of vacuum. Mammalian cells were grown in standard cell culture conditions, introduced in the inlet channel as a suspension, and their displacement through the device followed using light microscopy.

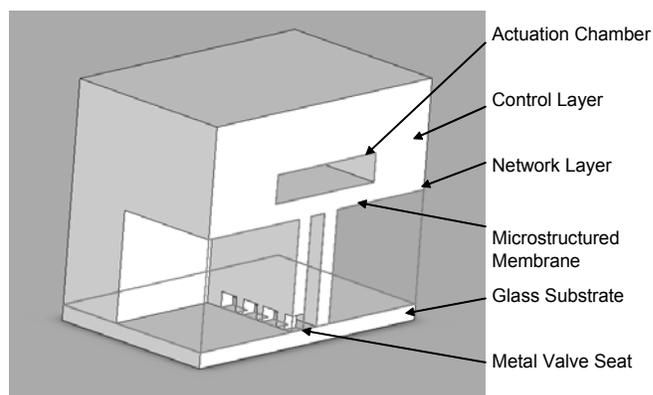


Figure 1: Schematics of the microstructured membrane for cell pre-concentration in lab-on-a-chip devices.

3 RESULTS AND DISCUSSION

The proposed design, allows to reversibly decouple the flow of fluids and the displacement of eukaryotic cells in suspensions, through the use of microstructured membranes that are pneumatically actuated. While a sieve alone in a microchannel could trap many cells while allowing the fluid to go through, hence increasing the concentration of

cells in front of the barrier, the sieve would eventually become clogged by the captured cells, blocking the flow. In our design, a mobile sieving barrier in closed position could trap cells mechanically, and could be subsequently opened to allow the trapped cells to pass into an outlet channel (Fig.2).

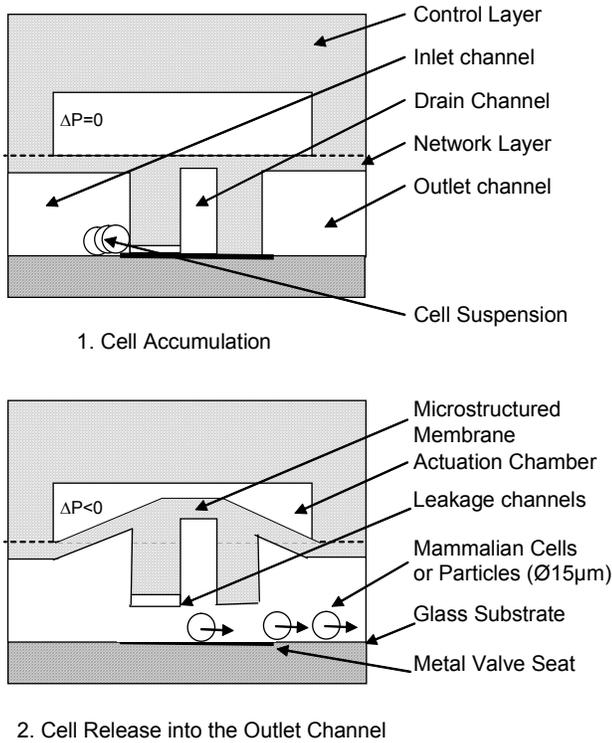


Figure 2: Functioning of the cell concentrator. (1) A microstructured membrane is used to trap cells from a cell suspension while allowing the fluid to be drained into a separate channel. (2) Upon pneumatic actuation, the microstructured membrane is deformed and cells allowed to pass into the outlet channel.

Simultaneous with the cell trapping, the suspension fluid was allowed to pass through the $3 \times 10 \mu\text{m}$ openings in the sieve. Subsequently, the fluid was diverted from the outlet channel however, and guided into a drain channel through which it was removed from the cell suspension. This was accomplished by the use of a second barrier, functioning as a valve and completely sealing the outlet channel (Fig.3). The two barriers, the sieve and the valve, were part of the same microstructured membrane and by their simultaneous lifting followed by quick release, they allowed clusters of enriched cells to pass into the outlet channel. The size of the cells or particles that could be handled in such device was limited only by the height of the actuation chamber. Large height to width ratios for the inlet and outlet channels could be easily achieved.

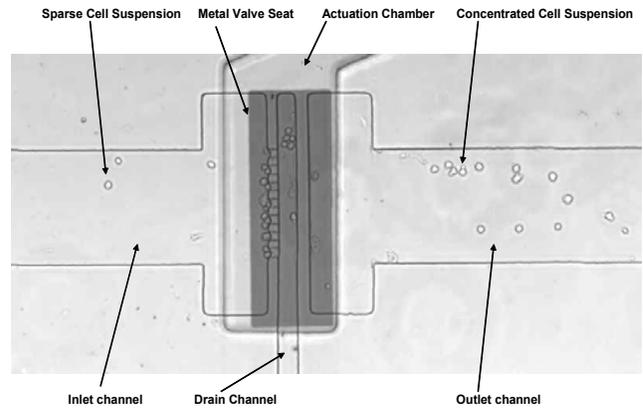


Figure 3: Microscopy image of the microfluidic device. Cells from a sparse suspension are trapped at the first leaky barrier. Brief lifting of the two barriers allows the transport of the cells into the outlet channel where they are concentrated. Scale bar $50\mu\text{m}$.

Through the operation of this new microfluidic device, the density of a sparse cell suspension was increased in the output channel by several orders of magnitude. Cell suspensions with concentrations ranging from 1×10^3 to 1×10^7 cells/mL were enriched to 1×10^7 cells/mL. The concentration increase was more dramatic, up to three orders of magnitude, in the case of sparse cell suspensions (Fig.4). With increasing concentration of the cell suspension, an increasing number of cells were trapped in the $30 \mu\text{m}$ space between the first and second microstructured barriers and wasted into the drain channel, limiting the yield of enrichment for cell suspensions above 1×10^7 cells/mL. For low concentration suspensions, the limiting factor for enrichment was the time required for draining the suspension liquid and trapping the maximum number of cells at the first barrier.

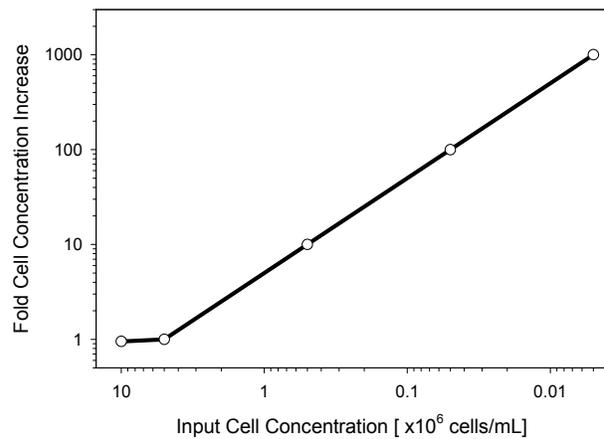


Figure 4: Several orders of magnitude cell concentration increase in the microfluidic device. The concentration increase is more dramatic for cell suspensions containing fewer cells.

One potentially important application of the presented method for cell concentration would be for lab-on-a-chip devices for blood analysis. While emerging new capabilities for the analysis of blood cells using microfluidic devices are opening new perspectives for clinical diagnostic, drug discovery and patient-tailored therapy in inflammation, infectious diseases, or cancer, the ability to provide self sufficient point of care analyzers to the bedside would be of critical importance [4]. In this context, gentle and accurate handling of complex cell suspensions like blood is poised to play significant roles in the development of fully automated in point-of-care devices.

4 CONCLUSION

Our work demonstrates the use of a microstructured membrane for the handling of mammalian cells and large particles on the chip. The new sample preparation capabilities would expand the range of cell handling and analysis that can be implemented on the chip and become part of emerging point-of-care technologies.

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