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SHORT COMMUNICATION

Embedding off-the-shelf filter in PDMS chip for microbe sampling

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Abstract Filtration for microfluidic sample-collection devices is desirable for sample selection, concentration, preprocessing, and manipulation, but microfabricating the required sub-micrometer structures is an elaborate process. This article presents a simple method to integrate filters in polydimethylsiloxane (PDMS) devices to sample microorganisms in aqueous environments. An off-the-shelf membrane filter with 0.22-μm pores was embedded in a PDMS layer and sequentially bound with other PDMS channel layers. No leakage was observed during filtration. This device was validated by concentrating a large amount of biomass, from 15×10^7 to 3×10^8 cells/ml of cyanobacterium *Synechocystis* in simulated sample water with consistent performance across devices. The major advantages of this method are low cost, simple design, straightforward fabrication, and robust performance, enabling wide-utility of chip-based devices for field-deployable operations in environmental microbiology.

1 Introduction

Advances in lab-on-a-chip technologies have generated interest in the environmental research community. These are especially useful in scenarios where portable devices and in situ instruments for bioanalysis are needed to perform experiments in real-time (Gardeniers and van den Berg 2004; Marle and Greenway 2005; Liu and Zhu 2005; Paul et al. 2007). The requirements for these applications are similar to those of point-of-care medical instruments. However, in contrast to common medical samples, environmental water samples have much lower cell concentrations. For instance, red blood cell (RBC) concentration in human blood is around 5×10^9 cells/ml (Furie 2003, p. 234), but bacterial concentrations in typical lake water are in the range of only 10^6–10^7 cells/ml (Kepner and Pratt 1994; Tranvik 1997). Hence, a typical first step in the analysis of microbes in aqueous environments is to concentrate cells by filtering off the water, preferably with a high-flow rate so as to accumulate a large amount of biomass within a short duration. However, high-flow rates are not amenable for microfluidic devices whose small channels or chambers restrict flow rates. Integrating filters in these devices further increase flow resistance unless the area of the filter is large.

Conventional microbe sampling uses physical filters to retain bacteria that are larger than the pores of the filters. Such methods effectively concentrate of bacteria and their selectivity is driven by the pore size of the filter. Lab-on-a-chip developers have adapted this approach to fabricate different types of physical filters in microfluidic devices. Some on-chip filters retain cells utilizing obstacles such as arrays of microscale columns, small gaps between microstructures, or beads columns that trap objects bigger than the gaps between obstacles (Broyles et al. 2003; Andersson et al. 2000; He et al. 1999; Zhu et al. 2004; Yang et al. 1999). Although these on-chip filters successfully trap large particles or mammalian cells, the dimensional requirements for the gaps to retain bacteria (conventionally achieved using filters with 0.22-μm pores) necessitates expensive equipments and elaborate skills. Furthermore, these devices had very small filter areas that restrain the flow rates. On-chip filters can also be made by irradiating a designated area with heavy ions to generate sub-micrometer pores (Metz et al. 2004). Although compatible with

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common chip fabrication methods and effective in retaining microbes, this method requires heavy-ion accelerators which are not easily accessible to general researchers.

One efficient, yet inexpensive, approach to build microfluidic chips filters is to integrate off-the-shelf filters with the chips. The advantage is that these commercial filters are well-characterized and inexpensive. Comparing with microfabricated obstacles, chips with commercial filters cost significantly lower, yet the filtering performance can be better because the quality of commercial filters is very consistent. This approach is usually applied on chips made of polydimethylsiloxane (PDMS), an inexpensive and biocompatible polymer (Ng et al. 2002). Because PDMS is liquid before curing, researchers demonstrated that uncured PDMS can be used as a glue to bind different layers of a microfluidic device (Wu et al. 2005; Noblitt et al. 2007). The drawback of this method is that an excess of liquid PDMS might clog the filter or microchannels (Aran et al. 2010). Aran et al. presented an approach to bind a porous membrane to glass or PDMS using a chemical crosslinking agent (Aran et al. 2010). However, this method is only applicable to the chips whose entire layout is occupied by the filter. It is difficult to use this method to fabricate a chip where the filter only covers a portion of the chip yet the other areas are designed for analysis and detection.

In this article, we present a cost-effective filter prototype where we embed an off-the-shelf filter within a microfluidic chip to retain micro-organisms. We apply well-established soft lithography-compatible methods to fabricate PDMS microchannels and sequentially integrate the filter into the chip. The produced chip has a large filter area that facilitates a large sampling of biomass. The fabrication process is compatible to integrating a hybrid chip that includes a sampling area and an analytical area. We characterize our prototype device by studying the rate of biomass accumulation and pressure across the filter as we sampled the microorganisms. Our results indicate consistent device performance with different concentrations of bacterial cells in the water.

2 Materials and methods

2.1 Chip fabrication

The key fabrication step was to embed an off-the-shelf filter in a PDMS layer. A hydrophilic polyvinylidene fluoride membrane filter (Durapore 0.22-μm pore, 25-mm diameter, Millipore, Billerica, MA) was integrated in the PDMS layer by anchoring the edges of the filter in the polymer. The fabrication procedure is shown schematically in Fig. 1a and b. Two acrylic circular plates of 17.7 mm in diameter and 0.8 mm thick were used as molds to keep the center part of the filter from uncured PDMS: the bottom acrylic plate was glued to a Petri-dish, and the top plate was placed so as to sandwich the filter (Fig. 1a). PDMS with a 1:10 cross-linker ratio was poured into the Petri dish until the content covered the top acrylic plate (Fig. 1b). Because PDMS does not mix with water (Chao et al. 2007), 20 μl of de-ionized water was dispensed on the center of the filter. The acrylic piece was then added, preventing PDMS to spread in the center and clog the filter. A clamp applied pressure on the top acrylic plate to secure the assembly during the 3-h curing process at 60°C. After being demolded from the acrylic pieces and petri dish, the product was a PDMS sheet with an embedded circular filter of 17.7-mm diameter and a total thickness of 1.5 mm.

The cured filter layer could be integrated with other PDMS microfluidic layers into a complete chip. In our experiment, the chip was composed of three layers. The top and bottom layers contained channel structures, while the middle layer was the filter layer (Fig. 1c). Sample water flowed through the filter via the inlet channel in the upper layer and left the chip via the channel in the lower layer. The top layer consisted of a circular chamber linked to a channel as the inlet. The chamber was 17 mm in diameter, and the channel was 27.94 mm long and 2.54 mm wide. The lower layer contained a 27.9-mm long channel. The fabrication for the top and bottom layers was based on the xurography process (Bartholomeusz et al. 2005). The three PDMS layers were bonded into a complete chip using standard plasma bonding (Fig. 1d): The conditions used were 500-mTorr chamber pressure, 6.8-W power, and a 15-s exposure time.

2.2 Retained biomass measurement

Biomass retained on the filter obstructs transmitted light. We measured the transmitted light intensity on a bright-field microscope (Nikon Eclipse 100) as an indication of retained biomass: low brightness indicated a large bacterial biomass. We selected to use photosynthetic Synechocystis for all tests because their dark green color can be easily identified on micrographs, therefore the change in transmitted light intensity was easily noticed. In our experimental setup, we used a 10× objective to observe the central region of the filter and recorded images every 2 min. To calibrate lighting conditions and camera settings, we adjusted the illumination and image contrast using pre-stained dark dots and the white background. The time-lapse micrographs were 8-bit grayscale images looking at the center of the filter, so the transmitted light intensity at each pixel was digitized into $2^8 = 256$ dimensionless gray levels (from 0, the darkest, to 255, the brightest). The mean transmitted light intensity within the field-of-view was calculated upon completion of the experiment.

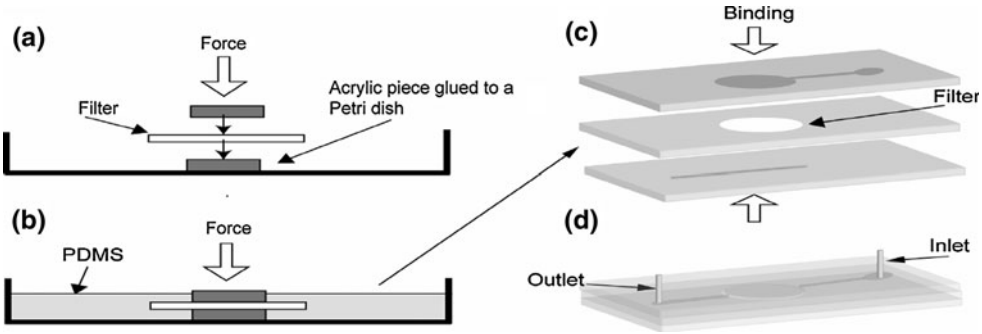

Fig. 1 Schematic of the insertion of the filter inside PDMS

2.3 Pressure measurement

The pressure drop between the filter was monitored during the course of the biomass measurement. The pressure downstream to the filter is approximately the same as the ambient pressure, because the outlet channel provided a low-resistance path to the ambient atmosphere. To measure the pressure upstream to the filter, we inserted a glass capillary having an inner diameter of 0.5 mm at the location where a linear air bubble had been intentionally trapped between the plugged end and the oil-filled open end which connects to the inlet channel of the chip (Fig. 2). The change in length of the air bubble was used to calculate the pressure during the experiment. The ambient temperature was set to be constant and assuming that air in the bubble is an ideal gas, the pressure in the filter chamber can be obtained by

$$\frac{P}{P_{\mathrm{ATM}}} = \frac{L_0}{L}, \tag{1}$$

where P_{ATM} is the pressure of one atmosphere, L_0 is the original length of the air bubble under one atmosphere, and L is the length of the bubble when pumping the bacteria medium. A digital camera with a close-up lens was used to monitor the air bubble inside the glass capillary. Images were taken every 2.5 min and then analyzed offline. The length of the air bubble was derived from the recorded images, and the pressure inside the chip was consequently computed using Eq. 1.

3 Experiments

Figure 2 illustrates the experimental setup before and during the filtration process. A peristaltic pump delivered a constant flow of 200 µl/min of bacterial medium. The flow rate was kept constant for all the experiments. The maximum flowrate that we could apply without creating leakage was 300 µl/min. Three concentrations (5×10^7, 7.5×10^7, and 10^8 cell/ml) of Synechocystis were used to characterize our device. Three milliliter of solution was filtered, accumulating the bacteria on the filter. The bacteria were not flushed out for this experiment.

4 Results and discussion

We performed various experiments to determine the biomass retention capacity of the filter and the effect of process variations on the filter. We collected liquids from the outlets of all chips at the end of experiments and used bright-field imaging to search for bacteria in these liquids. We did not observe any bacteria, indicating 100% retention. We observed no leakage in all experiments.

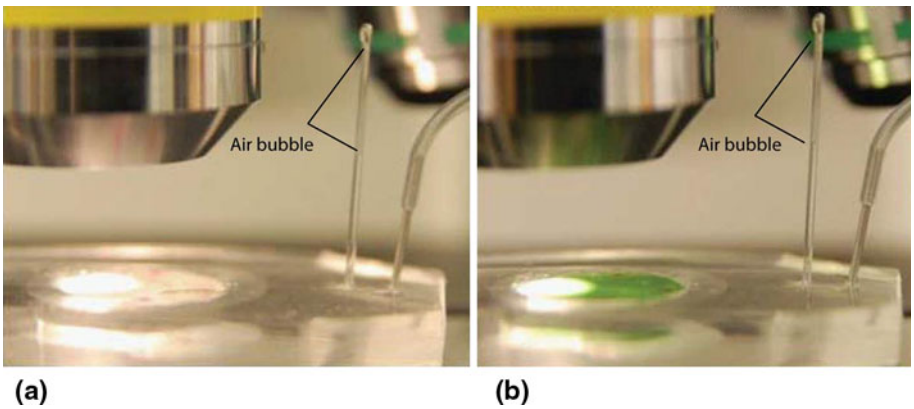

Fig. 2 Side view of the device connected to the pump under the microscope. **a** Before filtrarion, **b** during filtrarion

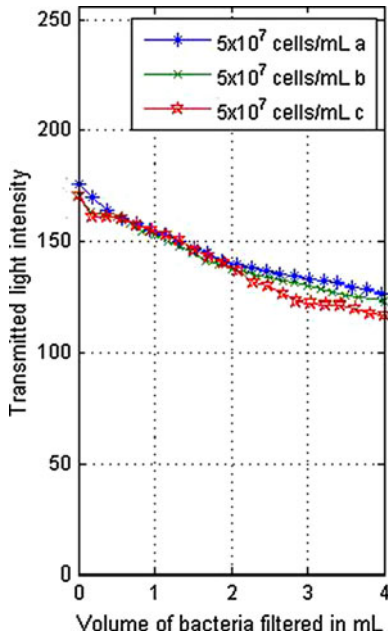

Fig. 3 Comparison of transmitted light intensity variation profiles for three filtering iterations of equal volumes of bacteria kept at constant concentration

Figure 3 depicts transmitted light intensity profiles of filtration experiments on three filter chips made in separated batches, each with 5×10^7 cell/ml concentration and same flow rate. The largest variation in transmitted light intensity observed between the three curves was 8% of the mean transmitted light intensity when 3 ml of medium had been filtered. Considering that this variation was contributed by chip-chip variation, pump fluctuation, cell concentration non-uniformity, the performance variation between chips is relatively small.

The biomass measurements show decreasing values corresponding to an increase in 8-bit transmitted light intensity (Fig. 4), where low transmitted light intensity indicates high biomass. The flow rate is kept constant, hence the biomass increased linearly with time. As expected, high cell concentrations yield fast biomass accumulation. No major variation of pressure has been observed during the experiment.

The filter was also tested using an environmental sample of wastewater containing many different types of microorganisms (Eikelboom 2000). The concentration of the sample was 3.2×10^8 cells/ml so we diluted it 10 times and filtered 1 ml, accumulating a total biomass of 3.2×10^7 cells on the filter. Pictures of the filter were taken before and after filtration using a bright-field microscope (Nikon Eclipse 100) using a 4× objective. The results are presented in Fig. 5. Before filtering the activated sludge, the filter was white and featureless at low magnification (Fig. 5a). After filtration, thick biomass was accumulated on the upstream side of the filter (Fig. 5b), while the downstream side of the filter remained featureless (Fig. 5c). Figure 5c appears gray because the transmitted light was block by the biomass on the upstream side of the filter.

We did not observe any biomass at the bottom of the filter which showed that 100% of the biomass was retained.

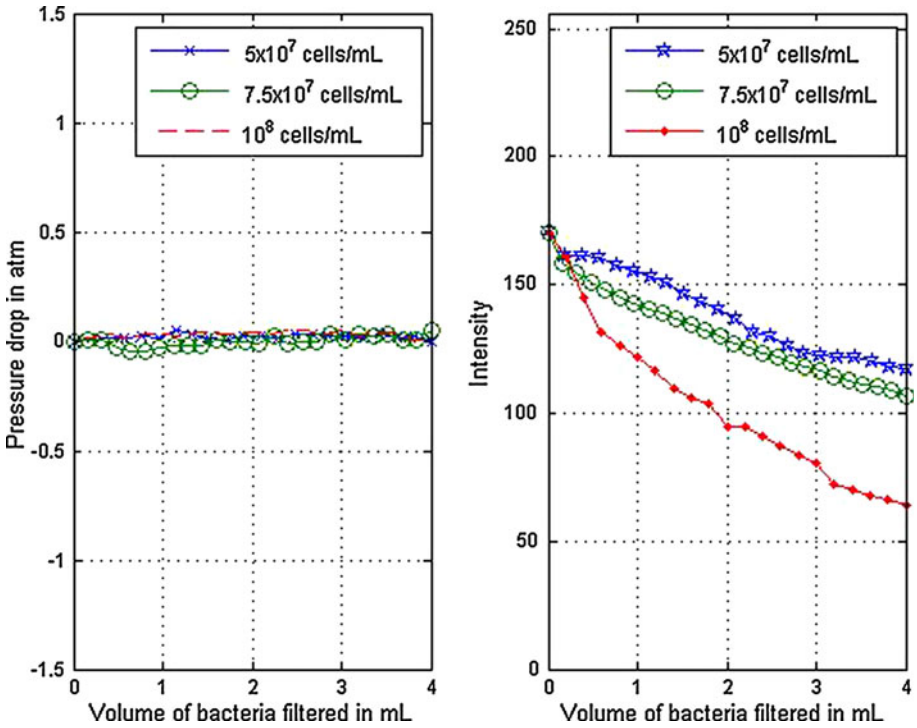

Fig. 4 Variation of the pressure drop and the transmitted light intensity over the volume of bacteria filtered

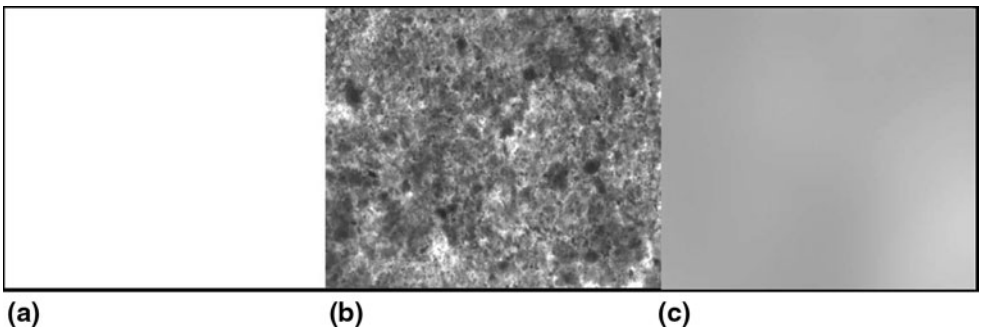

Fig. 5 Micrographs of the sampling filter: (**a**) Upstream side, before filtration; (**b**) Upstream side, after filtration; (**c**) Downstream side, after filtration

Similar to the previous experiment, we collected the liquid from the outlet of the chip at the end of experiment and used bright-field imaging to search for bacteria. We did not observe any bacteria, giving us confirmation of the 100% retention. We observed no leakage in this experiment, and the pressure stayed constant. This experiment showed that our method is reliable when dealing with water from the field that contains a great variety of microorganisms.

5 Conclusion

We have designed and fabricated a PDMS chip containing a commercially available 0.22-μm filter to sample microbes. The fabrication process we adopted is simple, fast, and cost-effective. The proposed filter can be seamlessly integrated with microfluidic channels that are fabricated by soft lithography. The performance of our device was determined to be robust with minimal process variations. The proposed method may be especially useful for small portable devices that collect and/or analyze biological samples at remote sites where the access to traditional instrumentation is not available.

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