FABRICATION OF MASSIVELY-PARALLEL
REGULAR NANOFILTERS FOR HIGH-
THROUGHPUT BIOMOLECULE SEPARATION
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ABSTRACT
We have developed a top-down fabrication strategy for massively-parallel, regular vertical nanochannel membranes with a uniform, well-controlled gap size of ~50 nm and a depth up to ~40 μm, by using only standard semiconductor fabrication techniques [1]. The vertical nanofilter membranes are fabricated into an anisotropic nanofilter array, which enables to integrate nanofilters and micron-sized channels/pores seamlessly. In addition, we have demonstrated efficient continuous-flow separation of DNAs and proteins in a two-dimensional vertical nanochannel array device. These ultrahigh-aspect-ratio nanochannels have the advantage of large open volume, enabling for high-throughput applications.

KEYWORDS: Massively-parallel, Nanofilters, High-throughput, Separation

INTRODUCTION
Patterned regular sieving structures have achieved great successes as an alternative to conventional nanoporous gels for various applications such as biomolecule separation and manipulation. One critical bottleneck of these planar nanofilters, however, is that these nanochannels generally exhibit small open pore volume, and as a result, sample and fluid throughput are much lower compared with more traditional membrane materials such as gels. Although alternative fabrication strategies, such as colloidal nanoparticle templating and nanofabricated ultrathin membranes, have been successfully demonstrated to provide high-throughput biomolecule separation, these techniques still come short of providing enough flexibility to integrate nanofilters within microfluidic systems in an arbitrary or carefully-designed manner, which may be critical to provide advanced functionality. Recently we reported a robust top-down fabrication technique of producing massively-parallel, regular nanochannels with good control of pore size [1]. Our approach is to build high-aspect-ratio vertical nanochannels with a well-defined lateral width down to ~50 nm. In this paper, we have successfully integrated vertical nanochannels with microchannels seamlessly to realize advanced applications such as sample separation [2] and Pre-concentration [3]. Also, we have built a two-dimensional vertical nanochannel array system and demonstrated high-throughput biomolecule separation.

EXPERIMENTAL
The overall fabrication process is outlined in Figure 1. This method uses standard photolithography and anisotropic etching techniques to produce deep trenches.
with smooth sidewalls. Then the gap size is narrowed down to the desired value by thermal oxidation and channels are sealed by depositing non-conformal plasma-enhanced-chemical-vapor-deposition (PECVD) oxide. This process can be well controlled with uniform gap sizes down to 50 nm (Figure 2) and low defect density, and allows much flexibility and easy system integration. For example, nanochannels and microchannels can be seamlessly integrated to realize new functions such as sample preconcentration, as shown in Figure 3. Also, this method is straightforward to create massively-parallel nanochannels for large-scale membrane applications.

**RESULTS AND DISCUSSION**

Using this technique, we developed a two-dimensional anisotropic vertical nanofilter array for continuous-flow separation of DNA and proteins. The device consists of a two-dimensional periodic pillar array, produced by etching longitudinal channels and horizontal channels sequentially (Figure 4a). As shown in Figure 4b, the λ-DNA Hind III digest was continuously separated into four distinct streams within
one minute with decent electric fields applied in two orthogonal directions. Also, with a smaller gap size of ~40 nm, we were able to separate R-phycoerythrin (MW~240 kDa) and fluorescein isothiocyanate (FITC) (MW~389 Da), which demonstrates the potential of fractionating protein mixture in native conditions. An important feature of this device is the sample processing rate as high as ~1 μL·h⁻¹, and further improvement of sample throughput can be achieved by upscaling channel depths. We believe that this device could be a key to the efficient proteomic sample-preparation microsystems as well as purifying and separating bioparticles and nanoparticles.

Figure 4. (a) Schematic diagram of the two-dimensional anisotropic pillar array device including a sieving matrix and surrounding microfluidic channels. The top-view and tilted-view SEM images show structures of sieving matrix. (b,c) Fluorescence micrographs of continuous fractionation of DNAs and protein through the nanofilter array device. (b) Separation of λ-DNA Hind III digest. The gap size (width) of horizontal channels is 60 nm. $E_x=80$ V cm⁻¹ and $E_y=40$ V cm⁻¹. Band assignment: (1)23.13kbp; (2)9.4kbp; (3)6.58kbp; (4)4.36kbp. (c) Separation of the mixture of FITC (2) and R-phycoerythrin (1). The gap size is ~40 nm. $E_x=250$ V cm⁻¹ and $E_y=40$ V cm⁻¹. It is obtained by combining two fluorescence micrographs taken in the same run with two different filter sets since they have a different spectrum.

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REFERENCES

