Microfabricated nanochannels: new tools for molecular motion control

MICRO Y NANOCANALES INTEGRADOS: NUEVAS HERRAMIENTAS PARA CONTROL DE MOVIMIENTO MOLECULAR

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Abstract—This paper presents a review of work on the fabrication and use of nanochannels in silicon and polymers for the control of molecular transport. The method of Sacrificial Layer Lithography is reviewed and demonstrated for silicon and polymers. A novel technique for the production of conical nanopores through a polymer membrane is also reviewed. Nanochannels and nanopores have many potential applications for drug delivery, immunoprotection of cell implants, blocking of globular proteins from biosensor surfaces, and diagnostic devices. All of these applications benefit from the more direct interactions of devices with biomolecules.

Keywords—Biomolecular separations, Nanofluidics, Silicon and polymer microfabrication, Therapeutic applications.

I. INTRODUCTION

There is a revolution occurring in biological research. Emphasis is rapidly shifting towards the view of biology in terms of a complex series of physical and chemical interactions, and interdisciplinary research between engineers, biologists, physicists, and clinicians is becoming the modus operandi [1]. One major component of this viewpoint is the new abilities to interact with biological molecules based on advanced technologies developed for rapid identification and precise interactions with proteins and nucleic acids. This paper presents a relatively new tool for direct interactions and control of biomolecules: fluidic nanochannels.

Fluid conduits with at least one minimum dimension from <1 nm to 100 nm (from here referred to as “nanochannels”) occur in abundance within natural settings, from nanotubes in zeolite crystals and nuclear membranes of biological cells to the larger openings in the silica frustules of the diatoms. Nanoporous inorganic structures result from the atomic arrangement of the components and most of what we perceive as their function is an artificial arrangement, but the organic nanoporous structures in general gain function from the localized change in the physical chemistry that accompanies nanometer-scale structures. This localized difference in the chemistry is what provides the control.

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over molecular motion through biological pores, and can provide both transport and catalytic control. Some researchers have suggested the use of nanochannels for direct physical models of ionic channels in cells [2]. By being able to mimic or harness these localized effects, we can gain amazing control over molecular motion, and by arranging nanochannels in massively parallel systems we can achieve this on a large scale.

Many researchers are attempting to exploit the unique features of nanochannels for molecular motion control. Some of the applications being explored include the sieving of DNA fragments for length analysis [3], immunoisolation of biological cells for xeno- and allotransplantation for treatment of molecular deficiency diseases [4], release of drug molecules in a highly controlled fashion [5], and direct analysis of DNA sequences through interrogation of nucleotides as they pass through a nanopore [6]. Most of these applications take advantage of the limited dimension of nanochannels to restrict or prevent the motion of otherwise globular molecules through the nanochannels. Recent research has also looked to imitate functions seen in biological nanopores, either through direct use of the nanopores or through design of synthetic pores to mimic natural pores. Some of the functions that can be obtained in this manner include ion selectivity, maintaining a chemical gradient across a membrane, adiabatic energy ratcheting, and size separation of molecules.

II. FABRICATION OF NANOCHANNELS AND NANOPORES

2.1 Silicon nanochannels and nanopores

The fabrication of nanochannels can be achieved through several different techniques, including track-etching of polymers and direct writing using e-beam lithography. The technique presented in this paper is known as Sacrificial Layer Lithography (SLL), in which a layer of a sacrificial material (i.e. one that gets dissolved after complete processing) is deposited during the fabrication of either monolithic or membrane structures. This technique avoids more expensive methods of nanopatterning by using a controlled deposition of a sacrificial layer to define the nanochannels. By creating a sandwich of two structural materials around this sacrificial layer, a precise spacing is defined. Similar to the use of sacrificial oxides to free up structural layers in silicon MEMS, the use of a sacrificial layer for nanochannels maintains a defined distance between the structural materials during the processing, and is then removed after the fabrication process is completed. When the sacrificial material is removed, a space of the same thickness or shape of the sacrificial material is left between the structural materials. There have been several geometrical configurations of nanochannels fabricated, including channels parallel to a membrane [7], channels normal to a membrane [8-9], and periodic nanochannels in series with microchannel [3]. Fig. 1 schematically demonstrates this process for silicon nanochannels through a membrane.

![Fig. 1. SLL process for silicon nanochannels: (a) growth of buried nitride layer and base polysilicon deposition; (b) hole definition in base polysilicon (1st thin film structural layer); (c) growth of thin sacrificial oxide on top of polysilicon and patterning of anchor points; (d) deposition of plug polysilicon (2nd thin film structural layer); (e) planarization (polishing) of plug layer; (f) deposition and patterning of protective nitride layer, KOH etch through wafer, and final release of structure in HF [9].](image)
Briefly, the fabrication process can be viewed as the surface machining of the nanochannels and then the bulk machining of the support wafer. To separate the two portions of the machining, a low stress silicon nitride (LSN or nitride), which functioned as an etch stop layer, was deposited using low pressure chemical vapor deposition (LPCVD). The base structural polysilicon layer (base layer) was deposited on top of the etch stop layer. The etching of holes in the base layer was what defined the shape of the pores. For this membrane structure shown in Fig. 1, the mask consisted of separated square holes (Fig. 2a), but other pore structures can easily be adapted to this protocol. As an example, Fig. 2b shows linear nanochannels fabricated using a linear (instead of square) mask pattern. These holes were defined with standard UV photolithography, using on silicon oxide etch mask patterned with photoresist and Cl-based reactive ion etching to etch the polysilicon layer. After the pores were defined and etched through the base layer, the pore sacrificial oxide was grown on the base layer. The sacrificial oxide thickness determines the pore size in the final membrane, so control of this step was critical to reproducible pore sizes in the membranes. Thermal oxidation of polysilicon allowed the control of the sacrificial layer thickness of less than 0.5 nm across the entire wafer.

Anchor points are defined in the sacrificial oxide layer to mechanically connect the base layer with the plug layer (necessary to maintain the pore spacing between layers for a membrane structure). For a flow-through configuration of nanochannels (e.g. for diagnostic applications), this step is not necessary, as the two layers will be connected. One method for defining anchor points is to use the same mask shifted from the pore holes by 1 μm diagonally (see the defined anchor points in Fig. 2a, noticeable as the flat regions beside the nanochannels), or a second mask can be used with features orthogonal to the etched features. The shifted mask approach produces anchors in one or two corners of each pore hole, which provides the desired connection between the structural layers while opening as much pore area as possible. After the anchor points were etched through the sacrificial oxide, the plug polysilicon layer was deposited (using LPCVD) to fill in the holes. To open the pores at the surface, the plug layer was planarized using chemical mechanical polishing (CMP) down to the base layer, leaving the final structure with the plug layer only in the pore hole openings.

A protective nitride layer was deposited on the wafer, completely covering both sides of the wafer. The backside etch windows were etched in the protective layer, exposing the silicon wafer in the desired areas, and the wafer was placed in a KOH bath to etch. After the silicon wafer was completely removed up to the membrane (as evidenced by the smooth buried etch stop layer), the protective, sacrificial, and etch stop layers were removed by etching in concentrated HF.

### 2.2 Polymer nanochannels

For many biomedical applications, silicon is not the ideal material, due to its high surface potential and its brittle mechanical nature, so polymers have been explored as materials for nanochannel applications. The use of a sacrificial layer in planar form to fabricate nanochannels has also been extended into polymer structures [10]. Fig. 3 shows a cross-sectional view of nanochannels fabricated in silicone rubber (polydimethyl siloxane, PDMS). The main requirement of the two or more materials used in the SLL process is an absolute difference in chemical properties, such as the absolute selectivity of concentrated HF in etching silica compared to silicon. Because of the rather unique chemical properties of silicone, the sacrificial layer used in this process can either have different etching properties (such as metals), similar to the silica-silicon pairing in the fabrication of silicon nanochannels, or...
different dissolution properties, since silicone (once cross-linked) is insoluble in nearly all solvents. The nanochannels shown in Fig. 3 were fabricated using a sacrificial polypropyl methacrylate (PPMA) layer, which was dissolved in acetone to leave the 150 nm channels within a monolithic piece of silicone. A more robust protocol that allows more massively parallel fabrication of nanochannels in polymers is currently being investigated.

For many biomolecular interaction applications, it is desirable to have pores through a membrane rather than the planar channels demonstrated above. While track-etch membranes can provide pores with sizes in this range, the pore density is limited to maintain accurate sizes and the resulting pores are symmetrical through the membrane. For the application of energy ratcheting as well as for mechanical strength considerations, asymmetrical pores are desirable. A novel nanofabrication method capable of economically producing well-defined, asymmetrical nanopores on thin polymer layers has been developed [11]. This approach is similar to the “lost cast” molding process used for producing large-scale metal pieces. A master consisting of high-density arrays (10^7/cm^2) of tapered nanotips is fabricated from a bundle of commercial silica imaging fibers (Fig. 4a). These image fibers are sharpened into conical tips by differential wet etching in a buffered oxide etchant (BOE). To fabricate nanoporous membranes, the master with nanotip array is immersed in a liquid resin (or a polymer solution) layer and onto an underlying support layer. The master is removed after the resin is cured (or the solvent is evaporated), leaving a female daughter mold (Fig. 4b). Adjusting the probe core angle or controlling the penetration depth of the tip array into the underlying layer can tune the sizes of the two ends of the pores. An Instron MicroTester was converted into the processing platform for accurate control of the master displacement (< 1 μm over 1 mm travel distance, higher resolution for smaller travel ranges). To transfer the nanoscale pattern from the daughter mold into a membrane, a sacrificial solution is cured into the features, providing a sacrificial template as the mold for the lost casting process (Fig. 4c). Pouring a thin layer of the final polymer solution onto the sacrificial template and allowing it to cure below the surface of the sacrificial template creates a polymer membrane with asymmetrical pores. The membrane can be integrated into a structure with or without the sacrificial template attached. The final release is the dissolution of the sacrificial template in the solvent or etchant that selectively removes only the sacrificial template (Fig. 4d). The example shown in Fig. 4 uses polyvinyl alcohol for the sacrificial template, which dissolves in water, as opposed to most biomedical polymers.

![Fig.3. Cross-sectional view of 150 nm channels (nanochannels going into picture) within PDMS substrate.](image3)

![Fig.4. Steps in the production of nanoporous polymer films with conical pores: (a) etched master of nanotips from differential etching of fiber optic array; (b) cast PDMS mold from the nanotip master; (c) PVA sacrificial cast of PDMS mold; (d) top view of cast PMMA membrane with assymetrical nanopores after dissolution of PVA sacrificial layer.](image4)
III. CONTROL OF MOLECULAR MOTION WITH NANOCHANNELS

Nanochannels have been investigated for several applications that can benefit from the direct physical interaction with biomolecules or from the extremely high surface to volume ratio within the nanochannels. Some applications include diffusion controlled drug delivery, electroosmotic driven drug delivery, sieving of biomolecules for sizing applications, and size-based exclusion of large biomolecules (especially for immunoisolation). The tight size distribution of nanochannels that can be fabricated using the SLL technique [9] allows for more deterministic engineering of biomedical devices for interacting with biomolecules.

The diffusion of molecules through nanochannels has been studied extensively for both drug delivery and size-based separation of different biomolecules. Because of the hindered diffusion regime that can be controllably designed with nanochannels, this tool holds the potential for more highly engineered diffusion membranes for drug delivery applications. A few basic rules can be derived from these experiments: (1) for nanochannels with dimensions much larger than the molecule diffusing through them, the diffusion coefficient approaches what would be expected for the same size opening of unhindered diffusion; (2) as the nanochannel dimensions approach those of the molecule diffusing through, diffusion slows much more than for a macroscopic opening, demonstrating a pure hindered diffusion; and (3) the ratio of nanochannel size to molecular size at which this transition occurs varies based on molecular size, from order of 10 for larger molecules such as IgG (presumably based on their interactions with the exposed surfaces) to order of 1 for smaller proteins such as albumin [12]. One underlying principle of these diffusion studies has been that, given a membrane with nanochannels of a specific size, the diffusion rate is predictable for a given molecule, and ratios between different molecules are more pronounced than for random network diffusion barriers. This becomes critical in the design of biomedical microdevices, where one hopes to completely exclude larger molecules while allowing as much diffusion of smaller molecules as possible. For immunoisolation, this means preventing the diffusion of immune molecules (such as IgG) while permitting the diffusion of viable cellular products of interest (e.g. insulin for diabetes treatment), nutrients (glucose and ions), and waste products [8]. For size-based protection of biosensors in vivo, this means the prevention of blood serum proteins (e.g. albumin) from diffusing into direct contact with the sensor, while allowing the full diffusion of the analyte molecule (e.g. glucose) [13].

Perhaps more interesting than a controlled design of diffusion parameters that one achieves with nanochannels is the change in the physical electrochemistry within the confined space of the liquid within them. The confined charges on the surface of the nanochannel provide a local space charge that produces excess counterions at the surface of the channel. This leads to increased ionic concentration within the nanochannels (due to Donnan equilibrium effects) and therefore an increase in the ability of the liquid within the channels to be moved under the influence of an electric field, known as electroosmosis. Due to these effects, much higher electroosmotic mobilities are observed for fluids within nanochannels than even in microfluidic channels [14-17]. This leads to higher flow rates through nanochannels for lower applied voltages. Using this possibility, one can easily envision methods to push fluid and drugs through these nanochannels with low voltages across a membrane for absolutely controlled drug delivery regiments. One potential disadvantage of this approach is the large shear stresses present in the fluid within the nanochannel, which could potentially disrupt the structure of molecules being delivered, thus voiding their potential usage. These physical regimes may also become useful for novel diagnostic approaches through different separation techniques for molecules based more on their mechanical properties.

IV. CONCLUSION

The fabrication and several applications of nanochannels made using Sacrificial Layer Lithography were reviewed. The basic technique of SLL can be applied to both silicon and polymers with proper selection of material pairings. The physical interaction of biomolecules with the surfaces of nanochannel structures can lead to highly engineered diffusion barriers for controlled delivery and selective blocking of molecules. The highly confined space charge within nanochannels leads to greatly increased electroosmotic effects, which has potential uses for drug delivery, diagnostic application, and basic biophysical research.

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