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Sacrificial polymers for nanofluidic channels in biological applications

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Abstract
Chip based bio/chemical analysis relies on networks of fluidic channels that are connected to reaction chambers and sensors. For sensitive detection it is important to scale down the size of the channels so that they approach the relevant length scales of the molecules of interest. Here we have made sealed channels on the 100 nm scale using nanoimprinting to pattern the sacrificial polymer polynorbornene over areas of several square centimetres. We have combined channels of different cross sections and we have shown that the nanochannels can be made hydrophilic with DNA transported electrophoretically in these self-sealed channels.

1. Introduction

Micro- and nanofabrication technology is finding an increasing number of applications in biological and biophysical sciences [1–6]. Miniaturization has many potential benefits.

(1) Small amounts of samples are needed.
(2) Analysis can be performed in a parallel fashion in many separate devices defined on a single chip allowing high throughput.
(3) Well-defined and uniform matrices make it possible to study simple cases and to gain further knowledge of fundamental processes in separation science.
(4) Separation matrices are defined on the length scales relevant for single biomolecules, resulting in improved performance over traditional techniques.
(5) Maybe even more important than the above benefits is the fact that the analysis of a biological entity, such as a cell, can be performed on a single-cell basis and with all the steps required *integrated* on a single chip.

Integration makes it possible to handle small amounts of sample in a single cell. Long genomic DNA is too fragile to be handled macroscopically. In a microfabricated device genomic DNA can be extracted and treated chemically for labelling and identification without the risk of shearing associated with standard macroscopic laboratory practices. To make it possible to analyse the biomolecular contents of single cells we need to combine networks of fluidic channels with valves and pumps for sample transport. For detection we need to combine fluidic networks with optical or electrical detection schemes. Basing the microfluidic analysis system on silicon or quartz, advantages can be taken from technology and fabrication procedures already developed for the electronics and telecommunications industries. These include optical emission, optical detection and electrical detection schemes. Currently, the standard procedure to define microfluidic channels is first to make a trench in, for example, silicon, quartz or silicone and then to seal this trench in a second step. This procedure makes it difficult to create complex networks of nanofluidic channels to interface channels on different size scales with each other. Using sacrificial polymers, three-dimensional networks of channels can be defined [7].

Ultimately, we want to analyse single DNA molecules with respect to macromolecules bound to DNA. In order to

resolve individual binding sites, a greater than diffraction-limited optical detection scheme is necessary. To fully take advantage of sub-diffraction limited resolution the DNA molecules must be uniformly stretched so that any fluctuations in the projected length of the DNA are less than the size of the binding sites. Our goal is to localize transcription factors to a specific gene or, even better, to a specific binding site. The size of a typical gene is 1000 kbp and a typical binding site is 20 bp. The required resolution is therefore of the order of 10–100 nm. If DNA were rigid on the length scale of tens of microns it would be easy to align DNA molecules to this accuracy, but unfortunately DNA has a persistence length of about 50 nm [8] so that any structures must confine the molecule to at least 50 nm. In near-field microscopy it is paramount that the sample be as close as possible to the nanoscale light source. Typically, the distance should be on the order of or less than the required resolution. Therefore, in our device we need to keep the sample to within 10–100 nm from the light source by forcing the DNA into channels with an inner diameter of the same size scale. Our device analyses DNA on a single molecule basis. This allows us to obtain additional information on sample heterogeneity as compared to standard population based methods. With integration of the nanoscale DNA analysis devices to microfabricated cell separation devices [9, 10] the result of the DNA analysis can be correlated with the state of the cell, giving epigenetic information and information about the function of each gene [11]. To summarize, we need to create nanofluidic channels with a cross section on a scale less than 50 nm and we need to be able to integrate these channels with optical detection schemes and sample preparation schemes.

There are many examples of self-sealed channels that have been made on the micron scale with sacrificial materials such as polynorbornene (PNB) [7], polycarbonate [12, 13] or polysilicon [3]. The basic idea is simple. The sacrificial material is patterned on a surface using standard photolithographical techniques. It is then covered using a capping layer. This layer is typically silicon oxide, silicon nitride [3] or polyimide. The sacrificial material is then removed either chemically or by thermodecomposition. Each sacrificial material has its benefits and drawbacks. Polycarbonate typically has a low glass-transition temperature, making it suitable for low-temperature applications, but unsuitable for high-temperature oxide deposition. Polysilicon can stand high-temperature processes, which enables the deposition of high quality oxides. On the other hand, the process is complicated and the polysilicon is decomposed using a liquid etchant that must be removed from the channels for subsequent processes. PNB can be tailored to have a wide range of decomposition temperatures. Using PNB with a high decomposition temperature makes it compatible with moderately high-temperature oxide deposition. The decomposition takes place at an elevated temperature of 425 °C, leaving a minimum of residues. In this paper, we make channels as big as 40 μm and as small as 100 nm. There are alternative ways of making sealed channels. For example, using non-uniform deposition on trenches, the cross section of trenches can be decreased and eventually nanofluidic channels can be defined [14]. The advantage of this approach is that the trench profile can be manipulated and reduced to at least 10 nm. On the other hand, it is difficult to create a very thin capping layer which is necessary for near-field optical applications.

2. Fabrication

This section describes the fabrication of nanofluidic channels using a sacrificial polymer. The first part describes the polymer we used and how we modified the process as described in the literature to fit our equipment. The second part describes further development of the technique to combine it with nanoimprinting lithography.

Our goal was to create sealed channels with dimensions ranging from 1 μm to less than 100 nm. For the large structures we used standard photolithography procedures and for the small structures it was necessary to use alternative techniques. Standard nanofabrication technologies, such as electron beam lithography and focused ion beam milling, are capable of precise high-resolution lithography, but the throughput is very low. Instead we have chosen to use nanoimprint lithography (NIL). In NIL [15] a resist pattern is formed by pressing a mould into a thermoplastic under elevated temperature and pressure. The result is a replica of the mould in the thermoplastic. The patterned thermoplastic can now be used as an etch mask for subsequent pattern transfer into the underlying substrate or it can be used together with liftoff to transfer a metal pattern. NIL is capable of creating features with resolution down to 10 nm over large areas (100 mm wafers and larger) [16]. The whole process takes about 1 min per wafer.

2.1. Materials and general processes

The specific polymer used in this study is poly(butylnorbornene) (PNB). 10 wt% of the polymer is functionalized with triethoxysilyl (TES) side groups, to provide adequate adhesion of the polymer with the Si or oxide [7, 17]. The PNB, dissolved in 1, 3, 5-trimethylbenzene, was obtained from the electronics materials group of BFGoodrich Performance Materials[7]. The polymer has a molecular weight of 180 kDa. To asses the possible range of feature sizes feasible using this type of PNB we carried out a series of dilutions and spun the solutions with different spin times and different spin speeds. The resulting thickness of the PNB is measured using a DekTak IIa profilometer and a Gaertner Scientific L3W16 multi-wavelength ellipsometer. The result is presented in figure 1. From the spin curve it seems feasible to make structures ranging in depth from 3 μm down to 20 nm. With slower spin speeds we were able to make 10 μm thick PNB films. Films with thicknesses greater than 50 nm had the required uniformity to make continuous channels over square centimetre areas.

To make channels using PNB we modified the steps used by Bhusari [7] to make it compatible with our process equipment.

The PNB was diluted to a suitable concentration for the desired thickness and spun onto silicon wafers with 200 nm thermal oxide or fused silica wafers with a 500 rpm spread for 10 s followed by 4500 rpm for 40 s with a ramp of 40 s. The solvent was then removed by heating the wafer on a hotplate at 110 °C for 4 min. An additional solvent removal step took place over 3 h at 220 °C in a N_2

[7] The family of norbornene derivatives used in this application is Unity(TM) Sacrificial Polymers, marketed by Promerus LLC (formerly BFGoodrich Performance Materials) Brecksville, OH, USA.

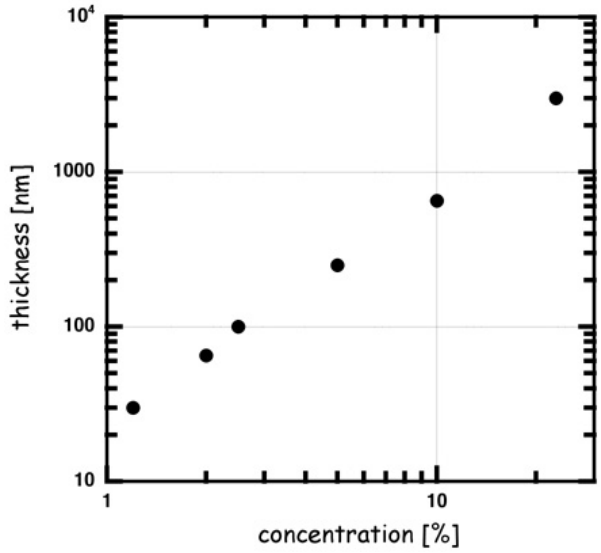

Figure 1. Spin curve for PNB diluted in 1, 3, 5-trimethylbenzene. The parameters are the following: 500 rpm spread during 10 s and 4500 rpm during 40 s with ramps of 100 rpm s^{-1} and finally a solvent flash on a hotplate at 110 °C for 4 min.

purged furnace. It is important to keep the oxygen content <100 ppm to avoid residue formation during the subsequent decomposition step. After the final solvent removal the PNB was no longer soluble in standard solvents and photoresist could be applied without affecting the PNB. We found that the PNB is not soluble in acetone, isopropanol, water, ethanol or photoresist (AZ5200 series photoresist with solvent: 1-methoxy-2-propanol acetate). The PNB was however readily soluble in HMDS (hexamethyldisilazane), which is commonly used as an adhesion promoter for photoresist. Treatment with either gas phase or liquid phase HMDS completely destroyed the PNB thin film. No HMDS adhesion promoter was needed for PNB on silicon or fused silica because of the TES functionalization of the norbornene.

For patterning of the PNB, reactive ion etching (RIE) was performed in a parallel plate chamber to transfer the resist pattern to the PNB. The following parameters were used: 4 sccm O_2, 1.5 sccm CHF_3 at a pressure of 10 mTorr. The total power was 50 W. With a plate area of 250 cm^2, that corresponds to a power density of 0.2 W cm^{-2}. The etching takes place at room temperature. With these settings the etch rate was typically found to be the following: for SiO_2 20 nm s^{-1}, for photoresist 50 nm s^{-1} and for PNB 65 nm s^{-1}, i.e. a relative etch rate of about 1:2:3 for oxide, resist and PNB. These parameters can be compared with the RIE conditions used in the Bhusari paper [7]: gas flow rates: 40 sccm Ar, 15 sccm O_2, and 5 sccm CHF_3; chamber pressure: 300 mTorr; power: 300 W with 12 inch plate, giving an area of 730 cm^2 and a power density of 0.4 W cm^{-2}. The etching was carried out at room temperature. The typical etch rate of PNB under these conditions was reported to be 250 nm min^{-1}. After patterning the PNB using RIE the photoresist was removed using acetone.

The capping layer was deposited using PECVD oxide deposition at a temperature that is 20 °C less than the second solvent removal step to prevent any residual solvent vapours from escaping from the PNB during the deposition process. Due to its low operation temperature, sputtering deposition would be a good alternative to PECVD oxide deposition, but we did not have a sputtering machine available at the time of the experiments. We used the following parameters for our PECVD deposition: N_2O at 475 sccm, SiH_4 at 200 sccm with pressure 700 mTorr and power density 0.1 W cm^{-2} for a time of 135 s to make a 100 nm thick SiO_2 layer.

We then transferred the sample to a quartz furnace with a nitrogen flow to keep the oxygen concentration below 5 ppm. The temperature was increased gradually from 100 to 440 °C at a rate of 1 °C min^{-1}. The decomposition time at 440 °C was about 3 h. After decomposition the temperature was decreased at a rate of 1 °C min^{-1} until a temperature of 350 °C was reached. At this temperature we introduced oxygen at a flow rate of 100 sccm for half an hour to make the channels hydrophilic. Finally the temperature was reduced to 100 °C at a rate of 1°C min^{-1} and the sample removed from the furnace. The whole process took place in a computer controlled Tystar quartz furnace with a total duration of about 15 h.

2.2. Fabrication of self-sealed channels on the micron scale

A 100 nm thick PNB was prepared by spinning a 2.5% solution of PNB in 1, 3, 5-methylbenzene at 4500 rpm for 40 s with a ramp of 40 s. The solvent was then removed by heating the wafer on a hotplate and in a Thermco furnace. The resulting thickness of the PNB was 100 nm, as verified by a DekTak IIa profilometer. A 0.6 μm thick photoresist, AZ5206 (Clariant Corporation, Somerville, NJ, USA) layer was spun on the wafer at 4000 rpm for 40s with a subsequent solvent removal bake on a hotplate at 100 °C for 4 min. Since HMDS cannot be used, the baking step had to be long to ensure adequate adhesion of the photoresist. We used a contact aligner in hard contact mode to transfer a pattern of 1 μm lines. The aligner works with mainly two wavelengths: 365 and 405 nm. The power density is set to 2.5 mW cm^{-2}. The exposure time was 25 s and the total dose was consequently 62 mJ cm^{-2}. The pattern was developed in AZ312MIF diluted in water with 1.2 volume units of water for each volume unit of developer. This provides higher contrast than the standard high-sensitivity 1:1 dilution protocol. The development time was 25 s. The PNB was then patterned by RIE as described above. A SiO_2 capping layer was deposited by PECVD as described above.

Channels 1 μm wide and 100 nm deep were successfully made using standard photolithography and our modified process protocol (see figure 2). With thicker PNB films we have reproduced the results reported in the literature on channels made with a cross section of 1 × 1 μm^2 [7].

2.3. Fabrication of fine self-sealed channels on the 100 nm scale

To make nanoscale channels over square centimetre areas nanoimprinting lithography [15, 16] was used. We used a mould that consists of an array of lines, 100 nm wide and 100 nm apart, over a 3 inch wafer. The complete process for creating nanochannels using nanoimprinting and PNB is described in figure 3.

The glass transition temperature (≈350 °C) of PNB is too high and too close to the decomposition temperature for direct imprinting. Instead we used a two-step process where Cr lines

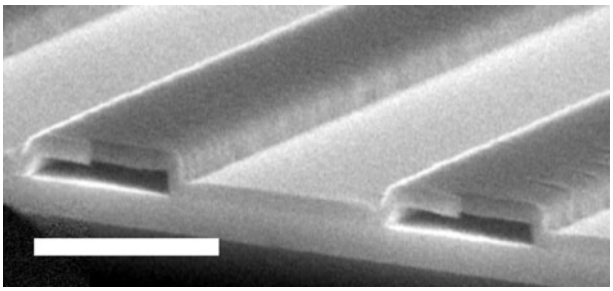

Figure 2. Shallow channels were made using UV lithography. 2.5% PNB was spun at 4500 rpm to achieve a 100 nm thickness. The scale bar corresponds to 1 μm.

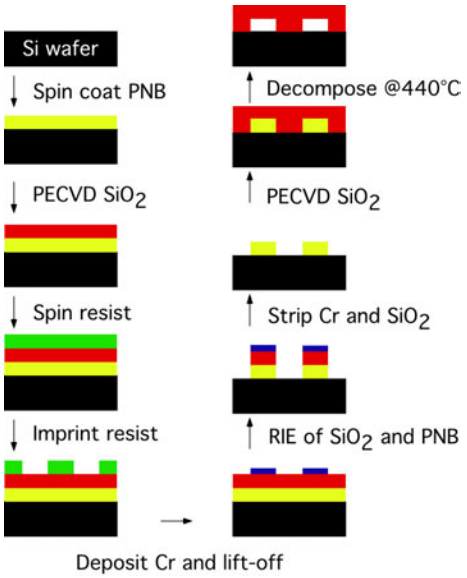

Figure 3. The process steps used for making nanochannels based on nanoimprinting of PNB.

(This figure is in colour only in the electronic version)

were first defined on top of the PNB using imprinting and lift-off. The imprinting resist that was used had to be specially formulated to be compatible with the rest of the process with respect to adhesive properties. In the second step the Cr lines were used as a hard mask for etching the PNB. Even though baked PNB is difficult to dissolve in organic solvents, we were forced to introduce a liquid barrier in the process to make it possible to soak the device during an extended time for the lift-off process of the chrome. A 50 nm thick PECVD deposited SiO_2 film on top of the PNB served this purpose. The hard mask was defined on top using lift-off in a 50 nm thin film of chrome. The oxide and the PNB is etched by RIE. After removing the chrome, the result is an array of 100 nm wide lines. The last steps are to coat a layer of SiO_2 on the PNB with PECVD and then decompose the PNB at 440 °C. We found that the higher temperature increases the decomposition speed without affecting the end result to any determinental degree.

The result is an array of sealed channels over several square centimetres. As can be clearly seen in figure 4 the channels have cross sections of 100 nm × 100 nm. Even smaller channels on the scale of 20 nm were made due to the isotropic nature of the PECVD deposition of the capping

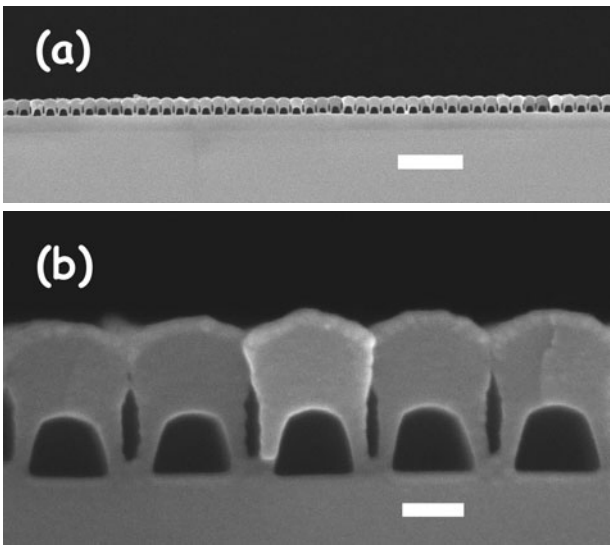

Figure 4. (a) Using nanoimprinting lithography nanoscale channels are uniformly defined over large areas. The scale bar corresponds to 0.5 μm. (b) Detail of the channels resulting from nanoimprinting of PNB. The scale bar corresponds to 100 nm.

SiO_2 layer. This approach to making nanochannels was independently pursued by Cao *et al* [14]. The benefit of the method based on a sacrificial material is that the capping layer can be defined at arbitrary thickness. Using the sealing technique based on isotropic deposition results in a thick cap [14]. A thin cap is important for near-field optical applications where a small aperture or light source is defined on top of the channels.

For efficient transport of liquid, channels need to be large, on the micron scale. On the other hand, for efficient stretching of DNA and to ensure high resolution and signal to noise ratio in integrated near-field applications the channels need to be very small, on the scale of the persistance length of the DNA and on the scale of the desired resolution, i.e. on the 10–100 nm scale. It is therefore important to integrate large micron scale channels with small nanoscale channels. Relying on the fact that the RIE is not perfectly anisotropic, we were able to create wide large channels connected to narrow shallow channels (figure 5). By fine tuning the degree of anisotropy the geometry of the cross section can be controlled.

3. Microfluidic and nanofluidics

The ultimate test of the utility of these nanochannels is their ability to be wet by water and for DNA molecules to move through them. We have conducted a very preliminary experiment that shows both the hydrophilic nature of the channels and the ability to image DNA molecules in the nanochannels. For ease of imaging, we chose for the initial experiments the relatively long λ phage molecule of length 48 502 basepairs (contour length 16.5 μm). The λ bacteriophage DNA was purchased from New England Biolabs. The DNA was stained with the dimeric cyanine dye TOTO1 (Molecular Probes, Eugene, OR, USA) and diluted to a concentration of 0.5 μg ml^{-1} in 0.5 × TBE buffer with 0.1 M dithiothreitol (DTT) to reduce photo-bleaching.

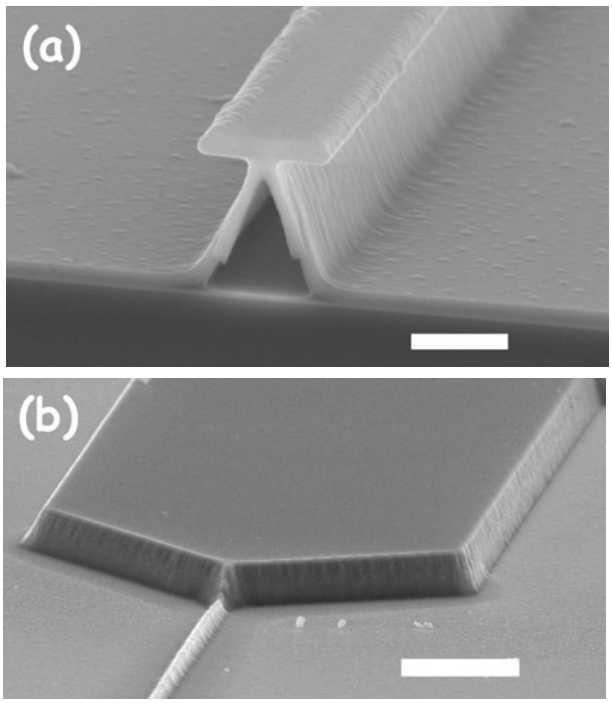

Figure 5. (a) This shows a PNB channel that was originally 2 μm square. Since our RIE process for etching PNB was not perfectly anisotropic, as the PNB was etched with an oxide mask, the side etching resulted in an undercut and a triangular profile. By taking advantage of this deviation from anisotropy of the etch, a wide channel can be made triangular and effectively narrowed. Here the etch was continued until the top of the two sides of the channel meet. The scale bar corresponds to 2 μm. In (b) the etch was continued until the height of the channel was narrowed down considerably to $\approx$0.5 μm. Note that the narrowed channel connects with a broad one on the top of (b). This broad channel is used to input DNA to the narrow channel. The scale bar corresponds to 5 μm.

Openings were etched in the channels before decomposition of the PNB. After decomposition reservoirs were attached at the entrances of the channels using optical UV curable adhesive (NOA68, Norland Products, Cranbury, NJ, USA). The DNA was introduced into the channels electrophoretically and imaged in an epifluoresence microscope with a SIT camera (Hamamatsu, Bridgewater, NJ, USA). Figure 6 shows that the channels wet and that the DNA moves in readily and is elongated into the channels.

4. Discussion and conclusions

We have shown that structures with a thickness of several tens of microns down to 100 nm can readily be made. To make even thinner structures, a sacrificial material with lower molecular weight must probably be used. The size of the channels can be decreased by decreasing the molecular weight of the polymer. The PNB that we used has a molecular weight of 180 kDa. Assuming a density of 1000 kg m^{-3} and spherical molecules this corresponds to a diameter of each molecule of $\approx$8 nm. Using a molecule with a molecular weight of 60 kDa would decrease the diameter by a factor of $\sqrt{3}$ to $\approx$5 nm. For direct nanoimprinting into the sacrificial polymer rather than etching it with a mask we need a sacrificial polymer that is thermoplastic with a low glass transition temperature. There are also applications

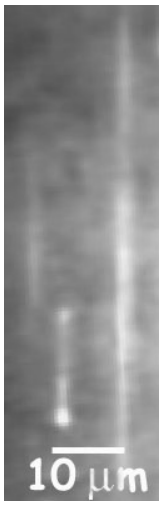

Figure 6. Several λ DNA molecules in 100 nm nanochannels. The variety of apparent lengths corresponds to different configurations of a polymer chain of finite persistence length in a channel of length less than the total polymer length. A fully stretched λ DNA is approximately 16 μm long and the right-hand side molecule is a λ concatamer. The scale bar corresponds to 10 μm.

where the polymer must be applied directly over a metal film, and in those cases a lower melting point polymer would be useful to maintain metal film integrity. There are many possibilities. Polycarbonate is a known possible candidate [12].

Acknowledgments

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