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Direct Casting of Polymer Membranes into Microfluidic Devices

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ABSTRACT

Fully functional lab-on-a-chip devices for biological analyses require the capability for cell culture, separation, and purification, as well as analyses to be integrated on a single platform. To date, a great deal of research has been focused on analytical methods for the miniaturization of column-based separations. We have created a platform that provides the capability of including membrane separations as an intermediate stage in such

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devices. Our techniques adapt conventional silicon processing methods to the casting of membranes directly onto the silicon substrate through a wet inversion process. This process allows precise control of membrane thickness and pore size distribution based on the processing conditions. Using this methodology, we were able to fabricate devices that were found to be very robust with molecular weight cutoffs of approximately 350 Da as measured by solute flux in a dialysis mode of operation. These devices were also found to be suitable for cell culture, as evidenced by the high viability of fibroblasts grown within our device. On the basis of these results, a wide range of separations and coculture applications are possible.

Key Words: Polymer membranes; Microfluidic devices; Biological analyses; Cell culture; Molecular separation; Lab-on-a-chip.

INTRODUCTION

The recent explosion of microfluidic applications for chemical and biological analyses has had an enormous impact on the manner in which research is conducted. Through the use of manufacturing techniques adapted from the semiconductor industry, sample sizes and processing time have been greatly reduced, sometimes by orders of magnitude, compared with the traditional methods.[1,2] In addition to the benefit of reduced sample volumes, advances are progressing, so that analyses maybe performed on a single cell[3] or even a single molecule.[4]

Much of the research to date has addressed microfluidic transport and mixing, as well as molecular detection.[5–8] The development of separation and purification strategies located upstream from sensitive detectors and sensors have been of particular interest. Advancements in chromatographic and electrophoretic separations, as well as methods for packing microfluidic channels appear to have received the most attention.[1,9–11] The advances in these fields have enabled the use of highly sensitive detectors that are capable of quantifying extremely low solute concentrations without fear of contamination.

While these advances have been significant, the highly complex mixture of a bioreactor or a biological fluid sample may require intermediate separation steps prior to its introduction to column-type separations. Membrane separations may be an efficient method for sample size- and charge-based purification in the lab-on-a-chip environment. However, research relating to the incorporation of membranes into these systems has been relatively lacking. Several researchers have simply adhered small pieces of commercial membranes over microfluidic channels for separations.[12,13] In these cases,

the membrane pore sizes ranged from 0.1 μm with adsorbed protein as the separator, to as small as 8000 MWCO for performing dialysis. In other cases, membranes were incorporated into chip-based devices as cell culture substrates[14,15] where a larger 0.4 μm pore size was used to keep the cells in a specific location. Other researchers have etched small pores into the silicon substrate itself.[16,17] While this method provides precise control of pore size, the available surface area and pore density are limited by the material properties, and manufacturing may be very expensive and time consuming. Multiphase fluidic approaches, such as liquid membranes or simple extraction systems,[18,19] are interesting, as is the use of interfacial polymerization[20,21] to create polymer membranes within microchannels. In some cases, pore sizes smaller than 200 nm were achieved. However, a possible drawback to these methods is the limited surface area available for mass transfer.

In this paper, we present a method for incorporating polymeric membranes into silicon-based lab-on-a-chip devices that takes advantage of standard semiconductor processing equipment. The silicon substrate is coated with a thin nitride film[22] that serves as a support structure for the polymeric membrane and contains etched pores to permit fluid transport to and from both sides of the device. The use of a mechanical support enables the casting of thinner membranes across large areas with the potential for higher flux without compromising physical integrity. This method also allows membranes to be cast from any material of interest. The wet inversion process that is used requires no additional adhesives for attaching the membrane to the surface of the device. Additional reservoirs and fluidics may be attached, again without the need for adhesives, resulting in fully functional separation platforms. These devices have a broad range of applications, including the molecular separation of fermentation products or biological fluids. In addition, these devices are also attractive in cellular coculture applications where not only is cell separation necessary, but it may also be desirable to restrict the molecules that are allowed to communicate between cell types.

EXPERIMENTAL

Silicon Substrate Fabrication

The fabrication process is shown schematically in Fig. 1. Single-sided, polished 3-in. silicon wafers, <1 0 0> orientation, were cleaned with a standard metal-oxide semiconductor (MOS) cleaning solution and coated on both sides with approximately 2000 Å of silicon nitride in a Thermco Brute American XL-4000S-13 Model 4100 Series furnace system (Thermco Systems, Orange, CA). Next, the unpolished side of the wafers was first cleaned with

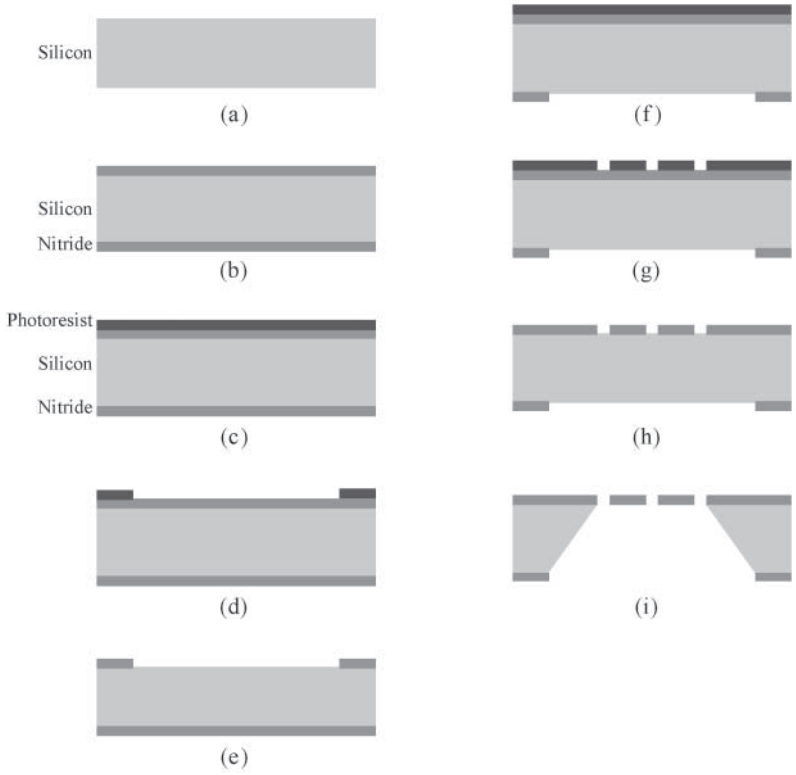

Figure 1. Schematic of silicon substrate fabrication. (a) Plain silicon wafer, (b) silicon wafer after growth of the silicon nitride layer, (c) silicon nitride coated wafer with photoresist spun across the backside, (d) wafer after exposure and development of photoresist creates backside pattern, (e) wafer after reactive ion etch transfers pattern into the silicon nitride layer, (f) photoresist spun across front side of the wafer, (g) exposed and developed photoresist on the front side of the wafer after mask alignment with the pattern on the back side, (h) reactive ion etch transfers front side pattern into the silicon nitride layer, (i) wet etch in KOH removes the interstitial silicon creating an etched silicon nitride film suspended across the through hole.

acetone and isopropyl alcohol, coated with Microprime P-20 (Shin-Etsu MicroSi, Inc., Phoenix, AZ), and spun dry. The wafers were then coated with a thin layer of photoresist (S1818, Shipley Co., Marlborough, MA) by spinning at 4000 RPM for 30 sec. The coated wafers were postbaked at 115°C for 1 min on a hot plate. An array of $\sim 1 \times 1\,\text{mm}^2$ was patterned into the photoresist by exposing the wafer to ultraviolet (UV) light through a chrome-on-glass mask using an EV620 Contact Aligner (Electronic

Visions Inc., Phoenix, AZ). The exposed photoresist was removed with Microposit MF-300 developer (Shipley Co., Marlborough, MA). The pattern in the photoresist was transferred to the silicon nitride layer by a reactive ion etch with CF_4 plasma using a PlasmaTherm 72 RIE (Plasma-Therm, Inc., St. Petersburg, FL). The remaining photoresist was stripped using a Branson/IPC P2000 Barrel Etcher (TePla America, Inc., Corona, CA). The process was repeated on the polished side by aligning a second mask containing arrays of $2-8\,\mu m$ holes with the previously patterned regions on the unpolished side. After the silicon nitride was patterned on both sides, the interstitial layer of silicon was removed by etching in KOH (23% v/v) solution at 90°C. The resultant substrate consisted of a silicon wafer with a 2000 Å porous silicon nitride layer suspended across 1 mm^2 holes etched completely through the wafer.

Membrane Fabrication

Membranes were cast directly onto silicon wafers from cellulose acetate solutions. Polymer solutions were prepared by dissolving cellulose acetate (39.8 wt% acetyl content, $M_n = 30{,}000$) in *N*,*N*-dimethylacetamide, both purchased from Sigma Aldrich (St. Louis, MO), yielding a 20–25% (w/v) solution. Casting was accomplished using an AC201 Photo Resist Spinner (Headway Research, Inc., Garland, TX) to spin a small volume of the polymer solution across a silicon wafer that had been previously cleaned with isopropyl alcohol and treated with Microprime P-20 primer. Spinning times and speeds generally varied from 15 to 30 sec and 1500 to 3000 RPM. After spinning, the coated wafers were immersed in a water bath for 10 min, removed, and allowed to air dry. The thickness of the cast membranes was measured by an MT12 high-accuracy length gauge (Heidenhain Corp., Schaumburg, IL).

Membranes used for transport tests were cast from 25% (w/v) solutions onto etched silicon substrates by spinning at 2000 RPM for 20 sec. The casting protocol was the same as described above, with the exception that the etched wafer was adhered to an unetched wafer with double-sided tape prior to spinning. This was done so that a seal could be maintained during the spinning process using a vacuum chuck.

Device Assembly

Membranes cast onto etched silicon wafers were incorporated into flow devices by attaching fluid reservoirs fabricated in polydimethylsiloxane (PDMS), (Dow Corning, Midland, MI) to the front and back sides of the wafer. Fluid reservoirs were molded from a glass coverslip glued to

the bottom of a 60-mm Petri dish. The two components of the PDMS were mixed in a 10 : 1 ratio according to the manufacturer's instructions. The mixture was then degassed for 15 min to remove any dissolved air. The degassed mixture was poured into the mold and degassed a second time. Next, the PDMS was cured at 60°C for 1 hr. After curing, the PDMS was removed from the mold, tubing ports were punched into the reservoir area, and silicone tubing was inserted. The PDMS was cleaned with a laboratory glass cleaner, rinsed with water and then isopropyl alcohol, and dried with N_2. Next, the PDMS was cleaned in O_2 plasma for 2 min using a Harrick PDC-001 Plasma Cleaner (Ossining, NY). Upon removal from the plasma cleaner, the reservoir was aligned and pressed onto the membrane-coated wafer, creating a fluid seal that is capable of being disassembled and reestablished. Assembled membrane filtration devices were connected to a Brinkmann IP4 peristaltic pump (Ismatech, Zurich, Switzerland) and a Waters 484 Tunable Absorbance Detector (Millipore, Billerica, MA) in series.

Membrane Characterization

The rejection characteristics of assembled membrane filtration devices were determined previously[23] by passing single- and multi-component solutions across the membrane in a tangential flow mode. Membrane fouling was determined by preparing two solutions: 5 mM caffeine in phosphate buffered saline (PBS) and 5 mM caffeine + 10% bovine serum albumin (BSA) in PBS. The caffeine solution was passed across the membrane using a recirculating tangential flow for 90 min. The device was rinsed and then the caffeine + BSA solution was passed for 90 min. After rinsing a second time, the single component caffeine solution was passed across the membrane again. In each case, the flux across the membrane was recorded by measuring the increase in UV absorbance with time. The flow rate in all cases was maintained at approximately 200 μL/min.

Light microscopy images were collected on an Olympus SZX12 stereomicroscope (Olympus America Inc., Melville, NY). Cellulose acetate membranes were also characterized by scanning electron microscopy (SEM) using a Leo 1550VP FESEM (Leo Electron Microscopy Inc., New York). Fracture surfaces were obtained by freezing the sample in liquid nitrogen prior to breaking. Membrane samples were sputter coated with gold for 1 minute prior to observation.

Cell Viability

Cellulose acetate membranes spun across unetched silicon wafers were used as substrates for the attachment and growth of human embryonic

lung (HEL) T.18 fibroblasts. Reservoirs for the cell culture media were cast in PDMS and attached to the membrane, as described earlier. Fibroblasts were plated at 1×10^5 cells/mL in Dulbecco's Modified Eagle's Medium (DMEM) + 10% fetal bovine serum (FBS) and incubated for 2 days at 37°C and 5% O_2. After incubation, the cells were stained with propidium iodide (PI) (Sigma-Aldrich, St. Louis, MO) and DAPI. PI stains the nuclei of dead cells while DAPI stains the nuclei of all cells, allowing the viability of the culture to be assessed. Widefield fluorescent images were collected on an Olympus BX-41 microscope with a 10X objective (N.A. 0.30) equipped with a MagnaFire S99806 CCD camera (Olympus America, Inc., Melville, NY).

RESULTS AND DISCUSSION

Membranes cast onto unetched silicon wafers were characterized for thickness, dimensional stability, and adhesion to the substrate. The dimensional stability and adhesion vary depending on the particular polymer used.[23] Cellulose acetate displays good dimensional stability and strong adhesion such that films did not shrink after casting and could not be peeled from the substrate without extensive tearing. On the basis of this result, cellulose acetate was the sole material used in these studies. The thickness of cast membranes could be controlled by the proper selection of spinning speed and time. Figure 2 shows the variation in film thickness with spinning time for a 20% (w/v) solution spun at 2000 RPM. As can be seen from the figure, a desired membrane thickness can be achieved by simply adjusting the time in a manner similar to photoresist processing. Variations in the concentration of the solution or the spinning speed will also have an effect but were not manipulated here.

Microscopic observation showed that cellulose acetate membranes cast by this method, from a 25% (w/v) solution and spun at 2000 RPM for 20 sec, were about 120-μm thick, and were well formed without tears or macroscopic holes. Furthermore, examination from the backside of the support substrate (Fig. 3a) showed that the polymer solution did not penetrate the silicon nitride layer during spinning and that the membrane was confined to the top surface of the wafer. This is a highly desirable morphology that allows for unrestricted bulk fluid transport from below the support substrate, through the silicon nitride layer and to the membrane. The SEM analysis (Fig. 3b) shows that the cellulose acetate membranes were asymmetric in nature with a thin skin on the top and bottom surfaces and the interior of the membrane consisting of large macropores that are several micrometers in diameter. These pores did not break through to either the upper or lower

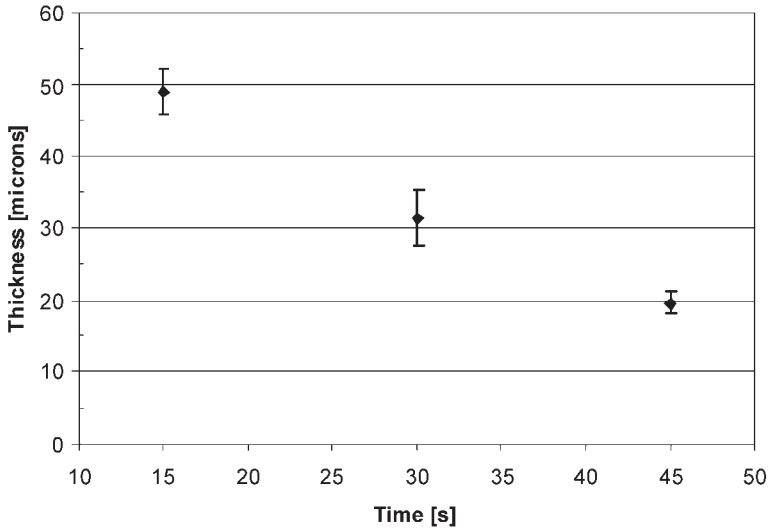

Figure 2. Variation in membrane thickness with spinning time. All membranes were cast from 20% (w/v) solutions in DMAc, spun at 2000 RPM, and precipitated in a water bath. Thickness measurements were taken after the film was completely dry.

surfaces of the membrane across the entire region. Therefore, filtration of small molecules occurs solely at the membrane surfaces and possibly in the thin layers just below the surface. The pores that penetrate through these thin layers to the surface are on the order of 30 nm or smaller as shown in Fig. 3c. In this morphology, the macroporous interior provides mechanical support to the membrane, but should not be involved in molecular separation. Therefore, this system should result in the desired separation of small molecules without compromising the integrity of the device by collapsing. On the basis of this mechanism, changes in processing parameters that affect membrane thickness may result in changes in permeability due to different path lengths. The MWCO should not be affected by changes in thickness, but could be sensitive to changes in other parameters, such as the solvent/nonsolvent system, which affect the actual pore structure of the membrane.

The rejection characteristics of assembled cellulose acetate membrane filtration devices, as previously determined[23] with the addition of sodium nitrate, are summarized in Table 1. For this determination, 5 mM solutions in de-ionized water were used as the feed stock. Sodium nitrate, which has the lowest formula weight, had the highest flux across the membrane at 4.49 E-3 mol/m^2sec. Molar fluxes decreased with increasing molecular weight, as

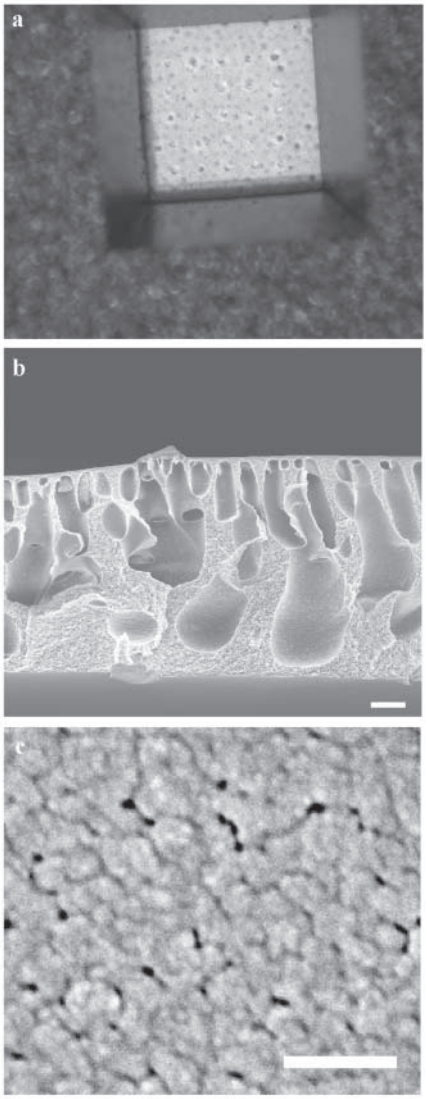

Figure 3. (a) Cellulose acetate membrane spun across an etched silicon wafer as viewed from below. The casting solution does not penetrate through the pores in the silicon nitride support during spinning, resulting in a well-formed membrane on the top surface. The silicon nitride support is $1 \times 1\,mm^2$ suspended across the top of the etched region. The macropores in the interior of the polymer membrane are visible through the thin surface layer, as shown in (b) Fracture surface of cellulose acetate membrane. Magnification bar is $20\,\mu m$. (c) Top surface of cellulose acetate membrane. Magnification bar is 500 nm.

Table 1. Molar flux of various solutes (all 5 mM) across a spin-cast cellulose acetate membrane.

Solute	Formula weight	Molar flux (mol/m^2sec)
Sodium nitrate	85	4.49E-3
Caffeine	194	1.16E-3
L-Tryptophan	204	2.14E-4
Methyl red	269	6.45E-6
Toluidine blue O	305	4.16E-5
Methyl orange	327	6.21E-6
Phenol red	354	0

expected, down to phenol red, which was undetectable. This result suggests that the flux of molecules above 350 Da is extremely low in these devices enabling the purification of very small molecules, such as amino acids, short peptides, and other signaling molecules. An equimolar mixture of four compounds—caffeine, L-tryptophan, toluidine blue O, and phenol red (0.25 mM in each component)—was also passed through the device as reported previously.[23] After circulation, the filtrate was collected and analyzed by measuring the UV/Vis absorbance over the entire wavelength range. Extinction coefficients for each component in the mixture were previously determined from single-component solutions at 272, 278, 435, and 630 nm. These coefficients were then used in a multicomponent analysis at each wavelength using Beer's law on the complete spectra (shown in Fig. 4). This method allowed the concentrations of each component in the mixture to be calculated, even though there is not a baseline separation between all of the components in the spectra. The results of this analysis are summarized in Table 2. This shows that even though the yield is low due to the short dialysis time, the filtrate is already significantly enriched in caffeine, which comprises 77% of the sample. The other components of the feed mixture are also present in the filtrate but are very dilute. This result is in agreement with the single-component data, which suggested vastly lower fluxes for these components. It is interesting to note that the amount of caffeine that passes across the membrane is substantially higher than that of L-tryptophan, even though the molecular weights of these compounds are similar. This indicates the presence of secondary interactions that result in higher rejections for certain molecules based on the physical and chemical characteristics. The specific interactions leading to this phenomenon are the subject of further investigation.

Realistic membrane separations often involve mixtures of molecules with widely separated molecular weights. In these cases, high molecular weight

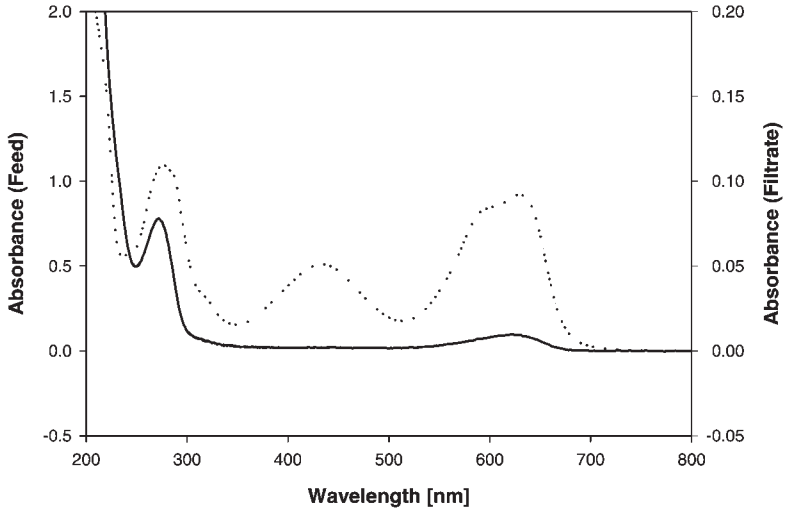

Figure 4. Absorbance spectra of multicomponent feed mixture (dotted line, scale on left) consisting of equimolar (0.25 mM) amounts of caffeine, L-tryptophan, phenol red, and toluidine blue O, and the membrane filtrate (solid line, scale on right) after circulation across a cellulose acetate membrane for 90 min. The peak at 435 nm is characteristic of phenol red and is absent in the filtrate. The toluidine blue O peak at 640 nm is greatly reduced. This result shows that the filtrate is enriched in caffeine and L-tryptophan compared to the feed mixture. From Ref.[23]

solutes, such as proteins, or highly absorbent molecules, may block the pores or bind to the membrane surface and greatly reduce the flux of smaller molecules that should pass through the membrane with ease. In order to assess the degree to which this occurs in our devices, membrane fouling was tested by using a mixture of caffeine (5 mM) and BSA (10 mg/mL) in PBS.

Table 2. Composition of multicomponent filtrate circulated through device for 90 min.

Component	Filtrate composition (%)
Caffeine	77
L-Tryptophan	10
Toluidine blue O	7
Phenol red	5

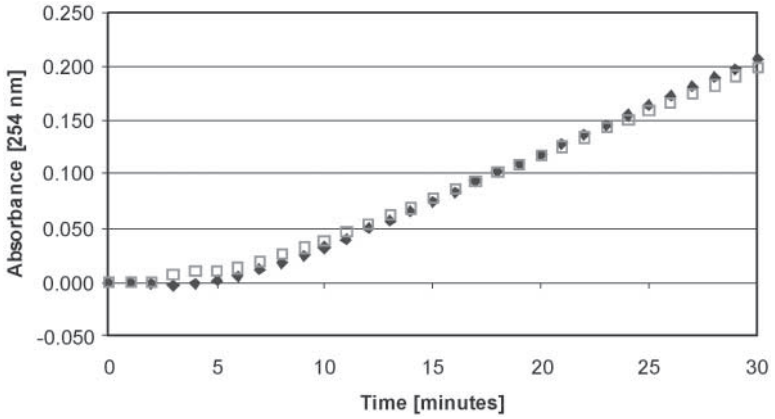

Figure 5. Increase in absorbance over time as a result of caffeine flux across a membrane in the presence of 1 mg/mL BSA (◆) and in the absence of BSA (□) after washing the membrane with buffer. The constant increase in absorbance and overlap of the two curves indicates that transmembrane flux is not significantly altered by protein fouling in this configuration.

This solution was circulated across the membrane as described above and the absorbance of the filtrate was monitored. Figure 5 shows the comparison of the absorbance increase over time for the caffeine/BSA mixture and for caffeine alone. It is clear from the figure that the flux of caffeine across the membrane is not reduced by the presence of BSA in solution. Furthermore, single-component solutions of BSA resulted in no increase in absorbance when circulated in the absence of caffeine (data not shown). This result indicates that these membranes have low fouling characteristics, at least for BSA, and are well suited for complex separations.

Finally, the viability of cells cultured on the membrane was assayed. This was done to ensure that the presence of the membrane, PDMS, or residual solvents was not toxic to cells. The HEL T.18 fibroblasts were cultured on cellulose acetate membranes for 2 days, as described above. After this time, the fibroblasts were stained with PI and DAPI to determine the viability of the cells. Fluorescent microscope images of cells cultured on membranes and coverslips are shown in Fig. 6. As can be seen from the figure, neither the membrane nor the coverslip shows positive staining for PI. Because PI only stains the nuclei of dead cells, this result indicates that the viability of fibroblasts cultured in these devices remains high and there are no problems associated with either toxins leaching from the materials of construction or of the cells being in contact with the membrane.

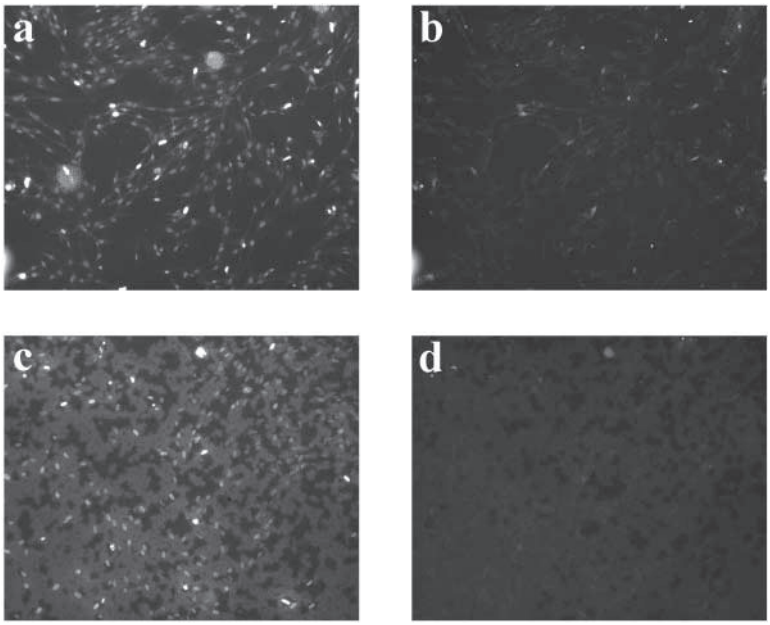

Figure 6. Fluorescent micrographs of HEL T.18 human embryonic lung fibroblasts cultured on coverslips (a and b) and cellulose acetate membranes (c and d) for 2 days. (a) and (c) show DAPI staining for quantification of total nuclei, while (b) and (d) show PI staining for dead nuclei. From (b) and (d), it is apparent that the presence of the cellulose acetate membrane does not result in cell death.

CONCLUSIONS

We have successfully combined aspects of membrane casting and semiconductor processing to produce microfluidic substrates with spun-on polymeric membranes. We have found that cellulose acetate possesses excellent material properties, and can result in membranes with nanoscopic pore sizes. Permeability tests showed that these membranes may be successfully integrated into closed devices and that membranes produced by this method may have molecular weight cutoffs as low as approximately 350 Da, as evidenced by the high rejection of phenol red. This level of performance exceeds that reported for other microchip-based devices. Further studies showed increases in flux across the membrane as the molecular size decreased, as expected. In some cases, differences in the rejection of two molecules appeared larger than what could be expected based on size alone. Some

factors that might cause this difference are molecular shape, charge effects, solvation, and hydrophobic interactions, among others. Further research is necessary to quantify these effects and clarify this issue. The performance of these devices was also characterized on mixtures of several components, including the higher molecular weight proteins. These tests confirmed the results of the single-component analyses and also showed that fouling did not occur to any appreciable extent. Finally, fibroblasts were successfully cultured within these devices without significant cell death.

The results of this study offer a very promising start to the integration of membrane separations into microfluidic devices. Complex separation strategies may use these methods to reduce the number of components in a mixture prior to its introduction to more specific onboard operations or sensitive detection elements. Not only can these devices be used as an intermediate stage in a more complex separation scheme, they may also be employed in a cell coculture environment. Current coculture applications generally use membranes with large pore sizes to physically isolate the two cell types. Our devices have the potential to perform the same task with smaller samples while also restricting, which molecules are allowed to communicate between the two cultures. The fabrication methods also allow the flexibility of tailoring the rejection characteristics of the membrane to the different molecular weight ranges. For example, changes in the concentrations and/or components of the solvent/nonsolvent system will affect the pore structure and may alter both the permeability and the MWCO of the membrane. By controlling the molecules that are allowed to influence a culture, it is possible to design experiments using these devices, which may help to identify the effects of various toxins, growth factors, or signaling molecules that trigger cell growth, immune response, cell death, or the progression of disease.

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