Microfabricated Capped Channels for Biomolecular Motor-Based Transport

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***Abstract*—Kinesins are molecular motors that transport intracellular cargo along microtubules and provide a model system for force generation that can be exploited for biomotor powered nano- and micro-machines. To use this biological system for microscale transport, the most common approach is to reverse the biological geometry and move microtubules along surfaces functionalized with kinesin motors. The microtubules then become potential transport vehicles for sensors and lab-on-a-chip applications. A key requirement for extracting useful work from this system is confinement and control of microtubule movements over kinesin-coated surfaces. The open channel approaches used to date are limited because microtubules that lose contact with the kinesin motors rapidly diffuse away. As a step toward making stand-alone devices incorporating kinesin motors and microtubules, we have developed methods to fabricate capped channels that provide three-dimensional microtubule confinement. We first tested the activity of kinesin motors on a range of surfaces and found that motors were functional on a number of hydrophilic surfaces and nonfunctional on hydrophobic surfaces. In this work, SU-8 photoresist is used to fabricate open channels and a layer of bisbenzocyclobutene (BCB) or dry-film photoresist is used to encapsulate the channels. To allow sample introduction, we fabricate a hierarchical series of microfluidic channels. In this approach, macroscale ($\sim$ 250-μm) channels in glass or silicon substrates are used to hold fine-gauge stainless steel tubing and allow connection to various fluid sources and intermediate scale ($\sim$ 50-μm) channels fabricated in thick ($\sim$ 50-μm) dry-film photoresist are used to connect the macroscale channels to microscale (1–15-μm) SU-8 photoresist channels. This paper is the first demonstration of kinesin-based microtubule transport in enclosed microfluidic channels and provides an important step toward packaging these biomolecular motors into functional devices.**

***Index Terms*—Kinesin, microfabrication, microfluidics, microtubule, molecular motor.**

I. Introduction

ONE important area of nanotechnology is the scaling down of electromechanical devices or machines to molecular dimensions. In this paper, we employ biological nanomachines—kinesin molecular motors and their microtubule tracks to create microscale transport systems driven by biological components. Kinesins are a class of microtubule motor proteins that power the movement of membrane-bounded vesicles and organelles along microtubule tracks. The two motor domains of kinesin motors alternately bind to the tubulin subunits of the microtubule in a walking or waddling type of motion [1], hydrolyzing one adenosine triphosphate (ATP) molecule per 8-nm step [2], a distance corresponding to the spacing between tubulin subunits. Conventional kinesin motors take $\sim$ 100 steps per second and can generate peak forces of roughly 6 pN [3], [4]. Microtubules are polymers of the protein tubulin, which are arranged in a head-to-tail fashion to form cylindrical tubes measuring 24 nm in diameter [5] and up to tens of micrometers in length. The structural polarity of the microtubule lattice is recognized by molecular motors—kinesin steps toward the plus-end of the microtubule, which is normally found at the cell periphery.

The ability to express and purify kinesin motors using standard methods of biotechnology and combine them with microfabricated materials permits the design of hybrid devices, where biomolecular motors serve as force generating and transport modules in artificial environments. One concept of such a device is the biomolecular transporter, a nanoscale transport system designed for the controlled manipulation of molecules in an aqueous environment. The potential uses for such a system include alternatives to microfluidics for transporting materials at micro- or nano-scale, molecular assembly systems, nanoscale sensors, and devices for single molecule studies.

A prerequisite for obtaining useful work from the kinesin-microtubule system is controlling the direction of movement, and there have been a number of reports using surface chemistry and/or physical barriers to control the direction of microtubules moving over motor-functionalized surfaces. For example, Hiratsuka *et al.* [6] showed that microfabricated tracks can be used to confine microtubule movement and the direction of microtubule motion in tracks can be controlled by adding arrowhead patterns to the tracks. Hess [7] utilized channel-like tracks with 200-nm high walls undercut at their bottom to prevent microtubules from crawling up the sidewalls. In our previous research, Moorjani, used SU-8 photoresist microchannels on glass substrates to localize motility to the channel bottoms and direct microtubule movements [8]. However, these approaches all have limited effectiveness because of their open-top design—any microtubules that lose contact with the kinesin motors simply diffuse away. To package biomolecular motors into functional devices, techniques must be developed for achieving microtubule movements in enclosed microchannels. In this paper, we construct capped channels that provide three-dimensional (3-D) microtubule confinement, and we develop a hierarchical channel design to en-

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able sample introduction. The eventual goal of this microtubule transport work is to attach cargo to the microtubules and transport the cargo to desired locations in engineered devices.

II. Materials and Methods

A. Motility Assays

In this paper, kinesin motors were absorbed to surfaces and fluorescent microtubules observed moving over the motor-functionalized surfaces. Surfaces are first treated with 0.5-mg/mL casein to prevent motor denaturation, and then full-length *Drosophila* conventional kinesin (typically 30 μg/mL) is adsorbed to the surface [9]. Finally, rhodamine-labeled microtubules (32-nM rhodamine-labeled tubulin) are washed in along with 1-mM ATP and an antifade cocktail, and the sample surface is examined by fluorescence microscopy as previously discussed [8], [10].

III. Results and Discussion

A. Effects of Surface Chemistry on Molecular Motor Function

Because the kinesin motors need to be absorbed to the surface in order to propel microtubules, the interactions of kinesin motors with the surface are critical for motor function. Previously, Lipscomb *et al.* described a technology for patterning nonfouling (protein repelling) surfaces (pp4G, plasma-polymerized tetraglyme) on a fouling (protein absorbing) surface such as silicon or glass [11]. The nonfouling coatings are effective enough to block protein attachment and a final rinse removes any nonattached proteins. In a different study, Nicolau *et al.* employ high-resolution e-beam patterning exposure of the surface of poly[(*tert*–butyl–methacrylate)-*co*–(methyl methacrylate)], a common e-beam and deep-UV resist used in semiconductor microlithography, to induce sharp transitions in the surface hydrophobicity [12]. The differences in hydrophobicity resulted in the selective attachment of myosin motors to hydrophobic (unexposed) surfaces, allowing patterning of active motors.

Here, we report that the chemical properties of the surface can affect the interaction of kinesin motors with the surface, and, thus, determine the activity of the motors. Contact angle measurements were used to evaluate the wetting properties and surface-free energy, and motility assays were used to access motor function. We observed that motors are inactive on low energy, hydrophobic surfaces (for example, octadecyltrichlorosilane treated glass surfaces). As shown in Fig. 1(a), microtubules may bind to the surface but are immobile. However, on a range of high energy, hydrophilic surfaces (for example, oxygen plasma treated glass surfaces) motors are active and can move microtubules long distances [Fig. 1(b) and (c)]. The SU-8 photoresist that we use for microchannel fabrication provides a hydrophobic surface with negligible motility [8]. However, when SU-8 surfaces are treated with oxygen plasma, the surface energy changes from low to high, and the motility on this surface is comparable to the motility on glass. We found that, in general, surfaces with contact angles smaller than 60° support kinesin-driven motility, while surfaces with large contact angles deactivated motors and did not support motility (Table I).

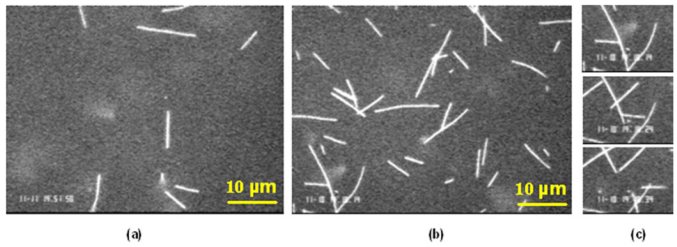

Fig. 1. (a) Octadecyltrichlorosilane (OTS)-coated surface (contact angle $\sim 95°$). Few microtubules bind to the surface, and those that do are immobile. (b) Oxygen plasma etched glass surface (contact angle $< 10°$). Microtubules bind and move on plasma etched glass surface. (c) Sequence of images at 10-s intervals from (b). (Color version available online at http://ieeexplore.ieee.org.)

TABLE I
Motility as a Function of Contact Angle for Various Surfaces

Surface	Contact Angle	Motility
Silicon (native oxide) with O_2 plasma treatment	<10°	√
Bare glass with O_2 plasma treatment	<10°	√
SU8 surface with O_2 plasma treatment	<10°	√
Bare glass with Acetone/IPA cleaning	~27°	√
Bare glass without cleaning	~58°	√
SU8 surface	~64°	None
HMDS vapor prime treated glass	~71°	None
OTS (Octadecyl Trichlorosilane) treated glass	~95°	None

Note: glass (ProSciTech, coverglass #1, 50mm round)

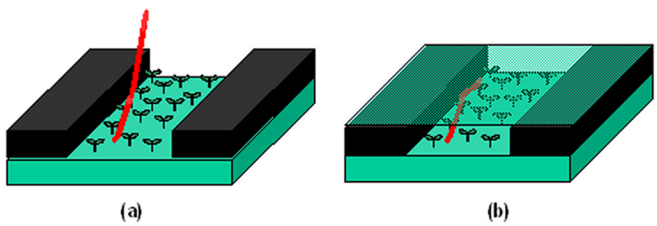

Fig. 2. (a) Open channel. (b) Capped channel structures. (Color version available online at http://ieeexplore.ieee.org.)

B. Capped Channel Fabrication

The open-channel approaches mentioned above have limited effectiveness because any microtubules that lose contact with the kinesin motors diffuse away. As an alternative, we are investigating capped channels that provide 3-D microtubule confinement as shown in Fig. 2. These enclosed channels are a prerequisite for building self-contained devices incorporating molecular motors.

There have been many micromachining processes used to fabricate capped microchannels, such as wafer bonding after bulk micromachining and sacrificial etching by surface micromachining [13]–[17]. However, those processes either are complex, expensive, or require high temperatures or electrical fields. For simple and low-cost fabrication, SU-8 photoresist has been used extensively to fabricate microchannels. There have been a number of methods reported for fabricating buried channel structures using SU-8 resist including the following:

1) SU-8 plus filling material to form channel spacers [18];
2) a metal mask used to protect regions of SU-8 from a second exposure forming the channel space [18];
3) a proton beam used to partially expose SU-8 to form a buried channel [19];

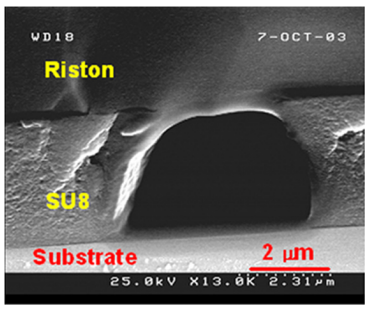

Fig. 3. Dry film photoresist Riston capped channel. (Color version available online at http://ieeexplore.ieee.org.)

4) an antireflection coating at the SU-8 bottom surface used to prevent reflected UV-light from inducing exposure [20];
5) laminated SU-8 and Riston film to form channel a space [21].

Because our microchannels are 1.5–5 μm deep and 3–15 μm wide, the first and the second approaches are not suitable for our application. In addition, it is difficult to remove the filling material or unexposed SU-8 from the buried microchannel, and any residual material could have undesired effects on microtubule motility. In the third approach, although a proton beam can achieve good dosage control, it is an expensive source for common use. The fourth method, the dosage-controlled UV exposure on SU-8 is difficult for thin (less than 5-μm) photoresist layers. In our paper, we have chosen an approach similar to the fifth method—we fabricate the SU-8 microchannel first and then put a dry-film photoresist (Riston) cap on top to form the microchannel space. As a second, similar approach, we use a transferred bisbenzocyclobutene (BCB) layer to encapsulate SU-8 channels.

C. Dry Film Photoresist Capped Channel

Our initial capping material was Riston (DuPont, Research Triangle Park, NC), a photoimagable dry-film suitable for simple and low-cost processing. The principal advantages lie in the layer thickness uniformity and the possibility of forming large and multilevel structures. On the other hand, the lithographic aspect ratio is approximately 1 and the sidewalls are not perpendicular to the substrate (typical wall angle is $\sim 10^{\circ}$ from perpendicular). Riston film is available with thickness between 20–150 μm, and is sandwiched between a polyolefin foil removed prior to lamination and a polyester protective foil. The process starts with lamination of the Riston layer at 50 °C and a speed of 1 cm/s. After the sample is cooled to room temperature, it is exposed with a standard UV source through a mask aligner. The polyester protective layer is removed before the developing by immersion in a sodium carbonate solution at 40 °C.

We used 40-μm-thick Riston to cap our SU-8 microchannels. Fig. 3 shows that intrusion of the Riston layer into the 5-μm-wide SU-8 channel is minimal for these lamination conditions. The combined SU-8/Riston technology is a simple and potentially useful approach for polymeric microchannel devices. However, the 40-μm thickness of the dry-film layer is not optimal for some applications. As an alternate approach, we investigated the use of a thin polymer layer to cap microtubule microchannels.

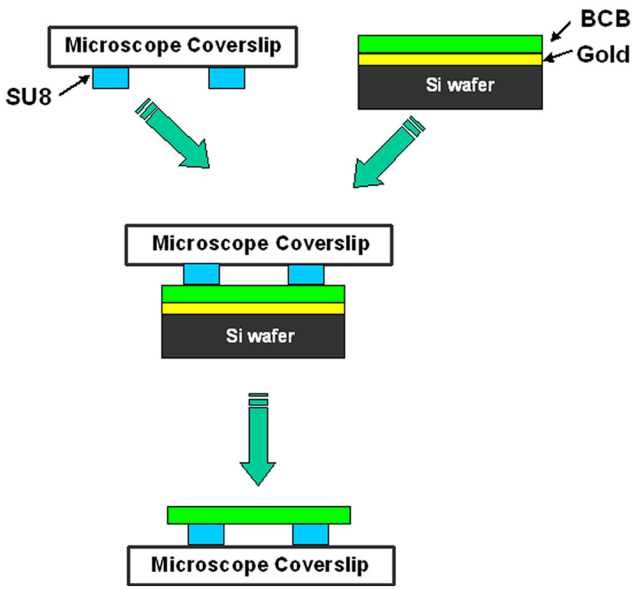

Fig. 4. BCB capped channel fabrication process. (Color version available online at http://ieeexplore.ieee.org.)

D. Benzocyclobutene (BCB) Capped Channel Fabrication

BCB is available from Dow Chemical in two types: dry-etch BCB (non photosensitive) and photo BCB (photosensitive). Thin-film coatings of 1–26 μm are achievable in a single spin-coat application using standard IC processing techniques (Dow Chemical, Midland, MI). BCB is useful for applications where a thin dielectric layer is required at the wafer level and is also suitable for low-temperature wafer adhesive bonding [22]. Since BCB bonds well with various materials and does not release significant amounts of byproducts during its curing process, void-free bonds can be achieved without the need for complex ventilation channels at the bond interface. Cured BCB coatings have excellent resistance to a variety of acids, alkalis, and solvents, and a high transparency across the visible spectrum, making it a good material for fluidic, optical, and packaging applications [23].

To minimize the thickness of the cap layer, we used 1-μm-thick BCB as the cap layer material. SU-8 channels fabricated on microscope coverslips were brought into contact with BCB-coated gold metallized silicon wafers (the BCB was prebaked on a 70 °C hot plate for 5 min). With moderate pressure and 2 h of curing at 200 °C, the BCB bonds with the SU-8 and, because gold adheres poorly to silicon wafers, the capped channel structure is easily peeled from the silicon wafer. The gold layer is then removed from the BCB by etching to complete the capped channels. The BCB capped channel fabrication process is shown in Fig. 4, and an SEM picture of a BCB capped channel is shown in Fig. 5.

Our design requires fluidic reservoirs for sample introduction, and the BCB capping approach described here offers a potentially useful approach for making open reservoirs. When channel or reservoir widths were 10 μm or greater, the thin BCB layer collapsed, providing an opening to the channels (Fig. 6).

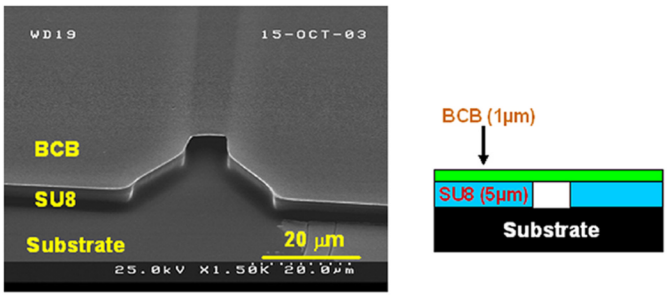

Fig. 5. SEM picture of BCB capped channel fabricated using technique shown in Fig. 6. (Color version available online at http://ieeexplore.ieee.org.)

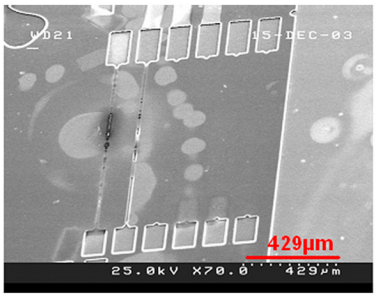

Fig. 6. Scanning electron microscope (SEM) picture of a set of dry-etch BCB capped channels. The channel widths of two left-side channels, two central channels, and two right-side channels are 10, 5, and 3 μm, respectively. BCB caps that on top of the reservoir areas and the 10-μm-wide channels both collapse. (Color version available online at http://ieeexplore.ieee.org.)

Positioning the microfluidic inlets in these open reservoirs enables facile sample introduction. In contrast to using fine-gauge drills to drill holes through capped channels, this method does not crack capped material and no fragments generated from the drilling process block the microchannels. However, in our fluorescence microscope investigations, we found that BCB has a strong autofluorescence in the range of the rhodamine fluorophore we use to label our microtubules, making it difficult to fluorescently image our microtubules in these channels.

E. Dry-Film Photoresist Hierarchical Channel Structure

The two channel capping approaches have their relative strengths, but for a useful microfluidic system, we also need mechanically robust connections for sample introduction and outflow, as well as a geometry that enables microscopic visualization of the fluorescent microtubules. A useful microfluidic system for biomolecular motor-based transport has four major requirements. First, biocompatibility with microtubules: the presence of oxygen will cause microtubule photobleaching and depolymerization. Any material with high oxygen diffusivity and high oxygen solubility such as polydimethylsiloxane (PDMS), which is a popular material for fabricating channels using micromolding techniques, should not be incorporated into microfluidic system [24]. Second, the visualization of the fluorescent microtubules: the thickness of capping material must be in the range of fluorescent microscope focal plane (less than 200 μm) while maintaining good physical strength and it must not autofluoresce. Third, the dimension of microchannel must be less than 15 μm wide and 5 μm deep, large channel

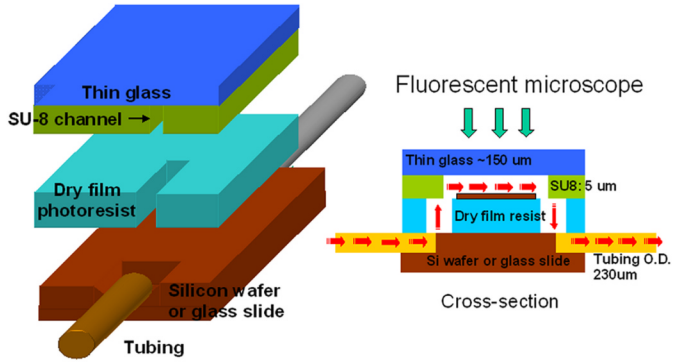

Fig. 7. Hierarchical channel structure and assembly. (Color version available online at http://ieeexplore.ieee.org.)

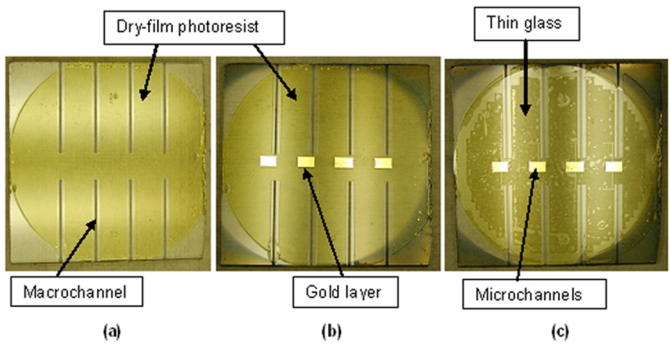

Fig. 8. Capped microchannel sample (size: 2 × 2in). (a) Glass substrate with hydrofluoric acid (HF)-etched macrochannels and laminated with dry-film photoresist on top. (b) Thin gold layer deposited in order to prevent illumination and autofluorescence of the dry-film photoresist. (c) Completed capped channel array. (Color version available online at http://ieeexplore.ieee.org.)

dimensions do not properly confine microtubule movements. Fourth, the process temperature must be as low as possible (at least lower than 200 °C) to enable future optical detector incorporation.

We chose to use the dry-film photoresist for our cap and further strengthen it with a backing of glass or silicon wafer, to deposit a layer of gold between the SU-8 layer and the capping material to block any autofluorescence, and to invert the sample to visualize the SU-8 channels through the coverslip on which they are patterned. With this inverted geometry (Fig. 7), we constructed a hierarchical series of microfluidic connections for sample entry and outflow.

In our approach, macroscale ($\sim$ 250-μm) channels in glass or silicon substrates are used to hold fine-gauge stainless steel tubing and allow connection to various fluid sources, and intermediate scale ($\sim$ 50-μm) channels fabricated in thick ($\sim$ 50-μm) dry-film photoresist are used to connect the macroscale channels to microscale (1–15-μm) SU-8 photoresist channels where kinesin-driven motility takes place. The macro- and intermediate-scale channels are fabricated on a glass or silicon support substrate, and the microscale channels are fabricated on a second thin glass substrate. The two substrates are then aligned and laminated, using the dry-film photoresist as an adhesive layer, to complete the channel structures. Fig. 7 shows the hierarchical channel structure and assembly, and Fig. 8 shows a completed channel array.

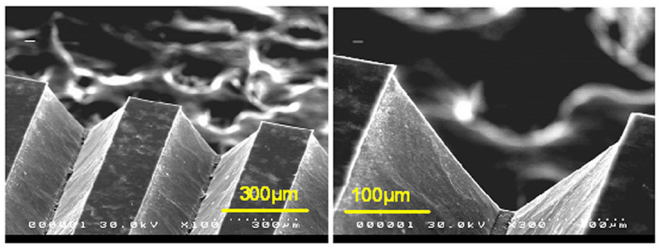

Fig. 9. SEM pictures of KOH anisotropic etched macroscale channel in silicon. (Color version available online at http://ieeexplore.ieee.org.)

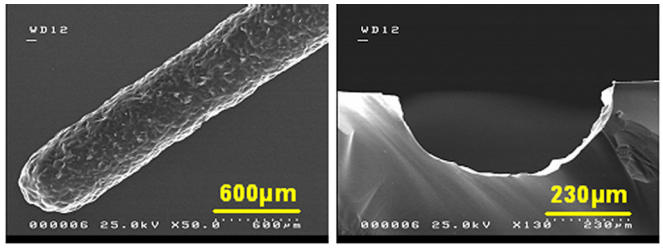

Fig. 10. SEM pictures of 49% HF deep glass etched sample. Channel width before 49% HF etching is 100 μm and after etching is 600 μm. These two images demonstrate the pinhole free surface and the cross section of a macroscale channel. (Color version available online at http://ieeexplore.ieee.org.)

F. Macroscale Channel Fabrication

In our dry-film photoresist hierarchical channel structure, macroscale channels in silicon wafer or glass substrate are used to hold 230-μm outer diameter size tubing, so the macroscale channels need to be about 250 μm wide and deep. Chemical wet etching can be used to fabricate deep macroscale channels in both silicon and glass substrates. Based on our results in Table I, both glass and silicon substrates can be used for the microtubule motility assay. The V-groove anisotropic etching of silicon provides easy alignment of fine-gauge tubing, but silicon is opaque. The transparency of glass enables a variety of observations to be performed. In our research, we have investigate both silicon and glass as our substrates.

G. Silicon Substrates

For silicon substrates, KOH solutions are widely used for V-groove anisotropic etching [13]. In our work, we used $\langle 100 \rangle$ n-type silicon wafers with 1-μm-thick thermal oxide as the etch masking material. Macroscale channels are aligned along a $[100]$ direction and etched at about 1 $\mu\mathrm{m}/\mathrm{min}$ at 70 °C in 4 M KOH. Six hours of etching results in V-grooves roughly 360 μm deep (Fig. 9).

H. Glass Substrates

Bulk micromachining of glass substrates is widely used for the fabrication of microfluidic structures such as fluid flow channels and thin diaphragms for pumps, active valves, and dispenser applications [13]. Glass has many advantages as a material for microfluidic applications including good mechanical properties, good optical properties, high chemical resistance, and high electrical insulation, and glass can be easily bonded to silicon substrates at temperatures lower than required for fusion bonding. For our glass macroscale channels. we used a combination organic/metal etch mask and etched the glass in concentrated (49%) hydrofluoric acid (HF). Etching for 35 min at room temperature results in channels approximately 250 μm deep (Fig. 10).

I. Microtubule Movements in Capped Channels

After fabricating our capped channels and microfluidic connections, we introduced protein solutions to achieve microtubule transport in the enclosed channels. Capillary forces can be used to introduce single solutions into the hierarchical channel structures, but for the sequential solution processing required for kinesin motor functionalization and microtubule introduction, solution exchange by capillary action is much too

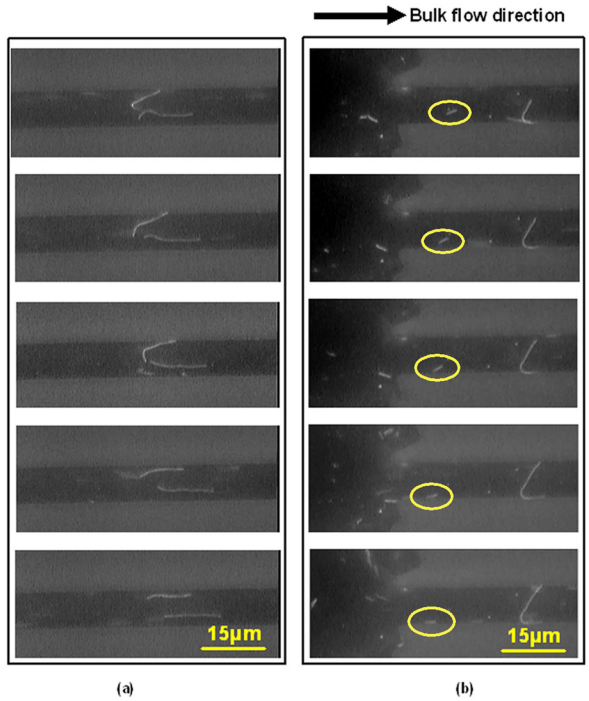

Fig. 11. Microtubules moving in capped microchannels (a) in the direction of bulk flow and (b) against bulk flow. Channel widths are 15 μm, images are 10 s apart. (Color version available online at http://ieeexplore.ieee.org.)

slow. To avoid this problem we optimized a single-step solution mix for obtaining motility in capped channels. Because the surface-volume ratio increases sharply in capped channels compared to open-channel design, we needed to significantly increase the concentration of casein and kinesin to achieve sufficient motor adsorption to enable long-distance microtubule transport. The concentration of enzymes and the oxygen scavenging (antifade) system was also increased. The final recipe of this cocktail is: casein (5 mg/mL), kinesin (75 μg/mL), microtubules (92 nM), paclitaxol (50 μM), ATP (5 mM), and antifade cocktail (0.1 M D-Glucose, 0.1 mg/mL Glucose Oxidase, 0.04 mg/mL Catalase and 0.35 M β-Mercaptoethanol). This cocktail was introduced into the microfluidic channels.

Fig. 11(a) shows microtubules moving in capped microchannels. The movement characteristics were comparable to those seen in open channel systems, and the overall transport performance was clearly improved by enclosing the channels. Moorjani *et al.* [8] reported that 7% of microtubules hitting SU-8 channel walls in an open-top system either diffused

away or stall at the surface. Additionally, microtubules moving in open channels microtubules periodically detach from the surface without hitting walls, meaning that in open-channel systems, an appreciable number of microtubules are nonfunctional from a device standpoint. To assess the functionality of our new design, 50 moving microtubules were tracked. It was found that all microtubules hitting walls moved along the walls (differential motility was not required due to 3-D confinement), microtubules that detached rebound to the surface, and no microtubules stalled when they hit walls. Fig. 11(b) shows that the kinesin-driven microtubules movement is independent of the bulk fluid flow in the channel. This phenomenon demonstrates that motor-based transport has a distinct advantage over traditional microfluidics-only microtubules, and those molecules bound to them are transported, whether the fluid flow is with or against the direction of movement. These transport characteristics suggest that molecular motor systems can offer a new approach for transport in hybrid biological/engineered devices, and the hierarchical channel structure is the key to providing a platform for integrating biomolecular nanomotors with microfabricated sensors and control devices.

IV. Conclusion

Because of their exquisite transport abilities in cells, there has been considerable interest in using the kinesin-microtubule system for microscale transport in microfabricated channels. Up to now, the many reports of localizing microtubule movements in microfabricated channels have all used open-top systems that are optimized for study under the fluorescence microscope. In this paper, we designed and constructed capped channels with microfluidic connections and achieved microtubule movements in these microscale enclosed channels. Enclosing these channels is a prerequisite for fabricating functional devices that incorporate biomolecular motors as transporters.

In choosing optimal materials for kinesin functionalization, we found that conventional kinesin adsorbed to a range of hydrophilic surfaces retains its functionality, but hydrophobic surfaces with contact angles greater than $\sim 60^{\circ}$ do not support motility, presumably because the kinesin motors denature on these surfaces. These measurements provide quantitative design constraints for choosing materials for biomotor investigations. We next devised two channel capping approaches, dry-film resist and a thin layer of BCB. Due to the lack of mechanical integrity and autofluorescence of the BCB, we chose the dry-film resist approach for our subsequent work. By etching grooves into our glass or silicon substrates and inserting tubing, we made microfluidic connections that were used to introduce kinesin motors and microtubules into the channels. The movement observed in these channels was comparable to that seen in normal flow cells or in open-top channels, demonstrating that the rate of ATP consumption in these enclosed environments does not limit motility. This result demonstrates a key technological advancement for devices based on motor protein driven microscale transport. There are various drawbacks for the open-channel approach concerning practical integration and packaging of molecular motors in functional devices. The open-channel approach requires handling of bulk fluid above the channels, which hinders packaging of the device and presents problems such as evaporation of fluid. The hierarchical capped channel structure presented here shows improved mechanical stability and efficient fluid handling when compared to an open-channel device. The capped channels also improved overall microtubules transports by preventing stalling at walls and diffusion of transiently detached filaments away from channel surface.

To increase the functionality of these enclosed channels, the next task will be to improve fluid exchange by shortening tubing connections and improving fluid pumping. One future application of these biomotor-based systems is to separate specific proteins or DNA/RNA strands from a complex mixture (i.e., a cell lysate) by binding them to microtubules and transporting them to a collection chamber for sequencing, analysis, or retrieval. To bind the analytes to the microtubules, the most obvious approaches are either to design analyte-filled channels that cross the microtubule channels, or to add a selective membrane that permits introduction of the analyte into the microtubule channel. These design issues are the topic of our current investigations.

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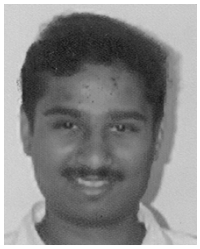

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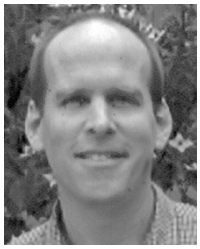

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