Nanofiber Structures as Mimics for Cellular Membranes

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ABSTRACT

Dense arrays of vertically aligned carbon nanofibers are being used as membranes within fluidic structures. Size-dependent transport, perpendicular to the orientation of the fibers, can be controlled based on the wall-to-wall spacing of the individual fibers. The combination of size fractionation and chemical specificity can allow such membranes to selectively transport analytes. Further, addition of electrically addressable carbon nanofibers adds another dimension to controlling transport. However, integrating features across multiple length scales presents significant challenges. Here we address various approaches towards integrating nanoscale structures within fluidic devices. Ultimately, the biologically inspired design of VACNF structures will be useful for performing chemical separations and for mimicking the properties of natural membranes.

Keywords: carbon nanofibers, membrane mimics, cell mimics

1 INTRODUCTION

A fluid, lipid bilayer membrane envelopes natural cells. It serves as both a container and a controller of the chemical reactions inside the cell. Reagents are exchanged with the neighboring environment through the creation of chemical potential gradients, or actively transported using enzymatic systems. Membrane transport is molecule specific and is accomplished either passively, based on chemical potential gradients, or actively using energy transduction schemes. Creating semi-permeable barriers, or membranes, that function like their biological counterparts presents a significant challenge. These membranes must be able to selectively control the transport of molecular species, requiring engineering on the nanometer scale. The creation of even simple membrane structures would be enabling for many applications (e.g. chemical separations, drug delivery, sensing). We are pursuing the creation of membranes with nanoscale features by the directed growth of carbon nanofibers [1].

There are many material approaches to mimicking membranes [2]. One approach to the construction of synthetic membranes involves the classical techniques of forming lipid vesicles. Mechanical agitation, or sonication, of phospholipids forms discrete vesicles, or liposomes. Such liposomes are typically small, however other techniques can produce liposomes with diameters on the order of 100 microns [3]. Planar supported bilayers can also be constructed from phospholipids [4]. These artificial membranes, composed of naturally occurring membrane components, have been useful in understanding the physical and biological properties of cell membranes (e.g. permeability, molecular events in signal transduction). However, the design of discrete structures with specific permeability is not obvious with this approach. The seemingly beneficial structure that is both fluid and self-assembling also allows for free diffusion and reshaping of the membrane. Additionally, the long-term stability of such structures may preclude their use in synthetic applications.

Other membrane structures have been constructed from rigid polymeric films or metals containing nanopores [5]. Polyester, polycarbonate, or aluminum can be etched to create pore diameters as small as a few nanometers. Extremely small pores can also be created in glass by the repeated drawing and bundling of glass capillaries containing an etchable core. Tonucci and co-workers have

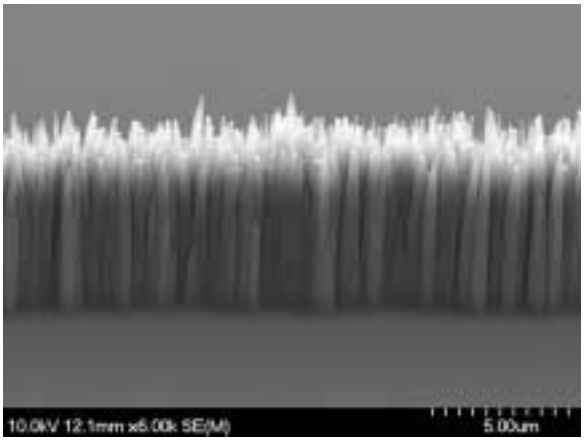

Figure 1: Example of vertically aligned carbon nanofiber structure.

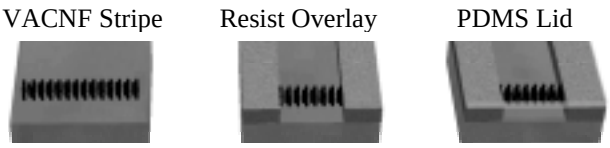

Figure 2: Fabrication overview using photoresist to create a sealed fluidic structure containing a VACNF barrier.

prepared nanochannel glass templates useful for creating porous membranes of various metals [6]. The pore diameters of these structures can be as small as a few tens of nanometers. Silicon substrates can also be used as nanoporous substrates. For example, block copolymer lithography has been used to prepare pores on the order of 20 nm at a pitch of 40 nm in silicon nitride-coated silicon wafers [7]. Nanopores have also been created in silicon by selective etching of carefully engineered oxide layers. Pores as small as 18 nm have been prepared for the construction of a silicon "biocapsule" useful for immunoisolation [8]. However, membranes based on these materials are typically thick (~100 μm) relative to biological membranes. This can considerably limit the rate at which material can transfer across a membrane potentially limiting chemical transfer rates compared to natural cell membranes.

An alternate approach to creating sieving structures is to create obstacles that are perpendicular to the direction of transport. For example, micromachined posts have been used as synthetic gel media in the electrophoretic separation of biomolecules [9]. These posts have been constructed, using electron beam lithography, with features as small as 100 nm and with a monolithic fluid enclosure [10]. In this approach to molecular sieving, the distance between the outer edges of the obstacles creates the "pore". The planned construction of these structures enables explicit definition of the separation capabilities, promising to be a superior alternative to the randomly arranged pores of polymer gels. In general, the limitations of conventional micromachining techniques prevent constructing such structures with molecular dimensions.

Template-based methods have been described that allow the ordering of nanoscale objects [5]. For example, arrays of nanowires and nanorods have been described [11]. However, these techniques only allow for a limited control of nanowire position and morphology on a larger scale. We recently have demonstrated that catalytically controlled growth provides a powerful method for directed self-assembly of vertically aligned carbon nanofibers (VACNFs) [12-14] into microscale and larger structures. This "bottom-up" approach to construction allows control over the physical features of VACNFs, and in combination with some "top-down" fabrication techniques (e.g. e-beam lithography) provides a powerful tool for the realization of complex microscale devices with functional nanoscale features. The ability to create fibers perpendicular to the substrate surface, with dimensions on the nanometer scale, provides the controlled synthesis and directed assembly required to realize membrane structures capable of controlling molecular transport.

2 EXPERIMENTAL

2.1 Carbon Nanofiber Growth

To prepare the membrane structures, VACNFs were grown on *n*-type (100)-oriented Si substrates. A 10-nanometer thick layer of Ni-Fe (1:1) alloy on a 10-nm thick Ti adhesion layer was deposited on the substrates. The Ni/Fe layer was used as a catalyst for growth of the VACNFs. The catalyst was patterned using contact photolithography to form catalyst stripes of defined dimensions. Acetylene (C_2H_2) as the carbon source and ammonia (NH_3) as an etchant, at gas flow rates of 65 and 80 sccm respectively, were used in the PECVD process. The dc plasma discharge was operated at 100 mA and the growth temperature was 710°C. The growth rate and time were selected to produce nanofibers of defined height, typically ~2.2 – 2.4 μm. An example of a resulting "forest" of nanofibers, randomly spaced within the catalyst stripe, is shown in Figure 1. Further details on the growth and application of VACNFs can be found elsewhere [12-14].

2.2 Device Construction

Several approaches to creating nanofiber containing fluidic structures were pursued. The first approach involved casting fluid channel structures in poly(dimethylsiloxane) (PDMS; Dow Sylgard 184) onto a silicon positive relief mold [1]. The mold was prepared using photolithography and reactive ion etching, 50 μm and 100 μm wide channels, 2 μm deep and 1.5 cm long were fabricated by pouring a 10:1 mixture (elastomer:curing agent) of PDMS onto the mold, followed by a 65° C cure for one hour. The resulting channel structure was peeled from the mold and overlaid on the VACNF containing silicon substrate with the PDMS channel oriented perpendicular to the VACNF stripe. The channels were constructed to be slightly shorter than the fiber height so that the VACNFs could presumably extend into the soft PDMS lid forming a floor to ceiling barrier.

The second approach involved construction of a VACNF barrier of defined dimensions and selective overlaying with photoresist to create a channel structure as pictorally described in Figure 2. VACNFs widths ranged from two microns to 100 microns and the fibers were grown to a height of 2 microns. This structure was then overlayed by spin coating a layer of photoresist (Shipley 1818). The resist layer was 1.7 μm microns thick so as to closely match the height of the fibers. The structure was soft baked and exposed using a channel pattern and a contact aligner. After developing in CD-26, the device was rinsed with water and the channel structure was then overlaid with a thin, flat layer of pre-casted PDMS. Fluid

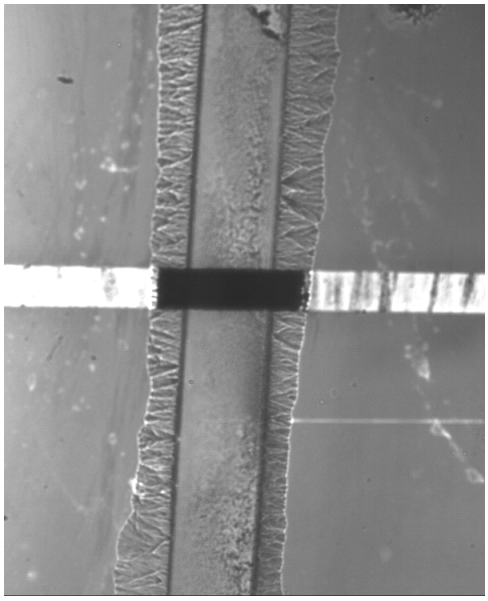

Figure 3: Electron micrograph of fibers grown across a channel previously etched in polysilica. The channel is 50 µm wide. The catalyst stripe stretches across the channel with excess nanofibers removed by scraping.

reservoirs were punched into the PDMS layer before overlaying.

The third approach involved construction of a VACNF barrier over a preformed channel constructed in a fused silica wafer. In this approach, a channel pattern was created using masking and etching with HF. Fluidic connection were created by drilling through the backed of the fused silica wafer to connect with the channel structure. After construction, the Ti adhesion layer and Ni/Fe catalyst layer were deposited and nanofibers grown. Fibers that were grown outside of the channel region were removed by mechanical scraping (see Figure 3). Finally, the channel structure was overlaid with a thin, flat layer of pre-casted PDMS to create the sealed structure

2.3 Electrode Interface

The VACNF structures were interfaced to electrodes as described in [15]. Briefly, the structures were prepared by a multistep process that involved overlaying the silicon substrate with tungsen. The electrode pattern was defined using a spin on resist (Shipley 1818) and exposing the pattern using a contact aligner. After development in CD-26, the structure was reactive ion etched (CF_4 + SF_6) to remove tungsten from everywhere except at the electrode sites. After construction, wire leads were glued to the contact pads and a flat piece of PDMS was used to seal the fluidic structure.

2.4 Experimental Characterization

The transport characteristics were assessed using fluorescently labeled latex beads [1]. Beads of various diameters, ranging from 100 nm to 1,000 nm (Polyscience,

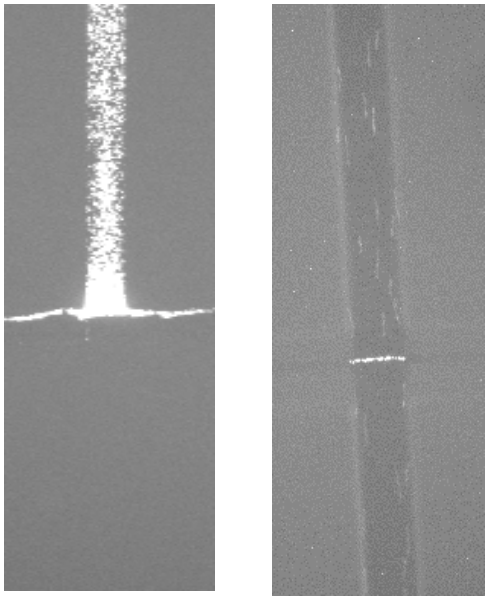

Figure 4: Fluorescent micrographs of fluorescently labeled latex beads traveling down fluidic channels intersected by VACNF barriers. On the left is a channel created by molding PDMS, on the right is a channel created in photoresist.

Inc.), were diluted to 0.15 % in an aqueous solution containing 1% sodium dodecyl sulfate (SDS) or in a Tris-Glycine SDS buffer. These solutions were introduced at one end of the open channels, and the transport of the beads was monitored using a Zeiss Axiovert 135 fluorescence microscope.

3 RESULTS AND DISCUSSION

Initial experiments were conducted to demonstrate the validity of the fiber membrane concept [1]. These initial experiments used a VACNF barrier and a simple overlay of a PDMS channel. This simple approach allows the use of PDMS, which is easy to mold in desired forms but cannot withstand the VACNF growth conditions. The difficulty associated with aligning a VACNF barrier precisely within a PDMS channel led to testing the membrane concept with VACNF barriers that extend well beyond the width of the channel. Although this leads to imperfect sealing at the region of the channel, it did allow demonstration of the concept.

These initial structures were tested with various sizes of fluorescently labeled latex beads (100, 200, 500, 750 and 1000 nm) [1]. Results showed that beads are trapped by the fiber barrier and build up regardless of the bead size. It was found that beads smaller than ~500 nm diffused relatively easily past the barrier compared to beads greater than 500 nm. These larger beads were effectively stopped as shown in the left of Figure 4. This is consistent with measurements of the interfiber spacing, ~230 nm +/-100 nm, as determined by electron microscopy. Also observed

in these experiments is leaking of the beads along the VACNF barrier due to ineffective sealing.

Presumably, with e-beam definition of the catalyst particles or altered density of a VACNF forest, size selection can be specified. To effectively test this assumption, improved device construction methods are required. Under investigation are two different techniques for placing VACNF barriers within the microchannel, without causing sealing problems or difficult alignment procedures. One approach involves "burying" unneeded VACNFs with photoresist while defining the channel structure with the same material. The second approach involves etching a channel in polysilica, overlaying with VACNFs and scraping away the unneeded VACNFs. The second approach results in a more rugged structure and is still under investigation.

These structures show greatly improved performance over the molded PDMS devices as seen in the images in Figure 4. On the left is an image of 0.5 μm beads flowing down a fluidic channel created using a molded PDMS channel. Flow, perpindicular to the channel, is clearly seen at the entrance to the VACNF barrier. On the left, is the resulting fluidic structure prepared using a photoresist defined channel. No leakage is observed.

The right hand image in Figure 4 also demonstrates the ability to integrate electrode structures within the device. VACNFs present in fluid reservoirs (not seen in the figure) are electrically connected to a power supply. Transport of the beads (seen as streaks due to the five second time lapse) in this image is by electrophoresis. One complication identified is control of the zeta potential in the channel due to the multiple surfaces. Often pumping would occur in the opposite direction due to electroosmotic effects. Ongoing are efforts to passivate the device surface as well as creating the channel structure out of a single material.

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