Nanofabrication of Membranes Based on Poly(Hydroxy Styrene) for Biological Applications

CNF Project # 386-90
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Abstract:

We have fabricated 3 µm thick polymeric membranes based on poly(hydroxy styrene), to contain pores ranging in size from 50-800 nm in diameter using electron beam lithography followed by a three step etch process. The membranes are used as a mimic of basal lamina in making in vitro models of the blood-brain-barrier.

Summary of Research:

Current in vitro models of the blood-brain-barrier involves the use of porous polymeric membranes that are 10-40 µm thick, with pores that are 400 nm, 1 µm, or 3 µm in diameter. These membranes are used to culture two different cell types on each side, and further study the electrical resistance and permeability of the cell layers on both sides on the membrane.

To better promote cell to cell contact through the pores, we have synthesized high molecular weight poly (hydroxy styrene) and spin-coated it on a silicon wafer to make a membrane that is approximately 3 µm thick. We then deposit a low-stress silicon dioxide layer using IPE 1000 plasma enhanced chemical vapor deposition system on top of the polymer layer to act as a mask. Leica VB6-HR electron beam lithography system is used to write a hexagonal pattern of octagons that range in size from 50 nm to 800 nm, spaced 2, 10, and 20 µm apart. The pattern area is approximately 0.5 × 0.5 cm². ZEP-520A is used as e-beam resist due to excellent etch resistance. We etch through the silicon dioxide layer using a CHF₃/O₂ recipe in the Oxford Plasmalab 80+ RIE system [1]. We then etch through the thick polymer layer using an oxygen clean recipe with reduced pressure in oxford plasmalab 100 RIE system. Finally, we remove the silicon dioxide mask using the first stated recipe in oxford plasmalab 80+ RIE system [1].

Pore sizes at the end of the process are characterized using the Zeiss Ultra scanning electron microscope.

References:

Figure 1: Cross-section view of a sample containing 600 nm pores. The top layer is the silicon dioxide mask, with the thick polymer layer underneath. The etch profile is anisotropic, but due to the thickness of the polymer layer (3 µm) the side-walls are sloped. The top of the profile grow by approximately 200-300 µm.

Figure 2: Top-down view of 600 nm pores. Illustrates the size growth on the top-side of the profile.

Figure 3: Cross-section of a line. Further illustrates the under-cut caused in Oxford Plasmalab 100 RIE system. The actual size in the mask is 1 µm, but after etching it grows to 2.7 µm.
Hydrodynamic Metamaterials: Nanofabricated Arrays
to Steer, Refract and Focus Streams of Biomaterials

CNF Project # 398-91
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Abstract:
We show that it is possible to direct particles entrained in a fluid along trajectories much like rays of light in classical optics. A nanostructured, asymmetric post array forms the core hydrodynamic element and is used as a building block to construct nanofluidic metamaterials and to demonstrate refractive, focusing and dispersive pathways for flowing beads and cells. The core element is based on the concept of deterministic lateral displacement where particles choose different paths through the asymmetric array based on their size: particles larger than a critical size are displaced laterally at each row by a post and move along the asymmetric axis at an angle to the flow, while smaller, sub-critical particles move with the flow. We create compound elements with complex particle handling modes by tiling the core element using multiple transformation operations: we show that particle trajectories can be bent at an interface between two elements and that particles can be focused into hydrodynamic jets using a single inlet port. Although particles propagate through these elements in a way that strongly resembles light rays propagating through optical elements, there are unique differences in the paths of our particles as compared to photons. The unusual aspects of these modular, nanofluidic metamaterials form a rich design toolkit for mixing, separating and analyzing cells and functional beads on-chip.

Summary of Research:
Generally the term metamaterial describes structured periodic features designed to achieve performance beyond that of conventional materials. Although typically defined in electromagnetics, we apply the broader definition to nanofluidics and design a nanostructured media that enables the motion of particles across non-mixing streamlines and along trajectories different from the bulk fluid flow. For example, two birefringent elements can be connected in series in order to change the angle of a particle’s trajectory through the metamedia. Alternatively, the $+\alpha$ and $-\alpha$ elements can be stacked to create a particle focusing element. If the $-\alpha$ element lies on top of the $+\alpha$ element, the new metamaterial element, denoted as $+F$, focuses particles—continuously creating a hydrodynamic particle jet. The $+F$ focusing element does not act as a lens, focusing particles to a single point, but instead focuses particles to a line. Particles above a critical radius will thus be actively focused to a line as they move down the channel, while those below a critical size will simply move unfocused. This characteristic is reminiscent of axicon optical elements used to create “non-diffracting” bessel beams over finite distances. Unlike light rays, which exit the focal line and continue on to project a ring for collimated incident light, particles in a focusing nanofluidic metamaterial element do not cross the centerline but continue forward entrained in the fluid flow. The $+F$ element is able to attain a rapid increase in local particle concentration along the centerline. The element demonstrates full removal of all bumping mode particles from the remaining flow and has potential applications for continuous flow concentration of rare biological species. Creating a hydrodynamic jet
usually requires carefully balancing flows across three input channels, but using the focusing element we are easily able to concentrate particles into a jet from only a single inlet reservoir. The concentration is remarkably compact and is completed in distance of only 5/2 channel widths for the angle shown of $\alpha = 1/5$.

This work was done in collaboration with Keith Morton, Jim Sturm and Steve Chou at Princeton University.

Figure 1: Complex nanofluidic metamaterial. A. Schematic of a complex metamaterial constructed by tiling several focusing, defocusing, and refractive elements. B. Tilted, cross-sectional SEM image showing the interface between four sub-elements. C. Collage of time exposure images showing particle motion through a series of different $+F$ and $-F$ elements, motion is from left to right using just a single inlet and single outlet port. D. A. Similar device with two separate inputs allowing two different-colored bead streams in the top and bottom halves of the device. Observe that particle cross-over between the two halves of the device is rare; particles only mix when dynamically hydrodynamically trapped along the center reflection axis.
A Population Ratchet for Self-Propelled Agents

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Abstract:
Randomly moving but self-propelled agents such as *Escherichia coli* bacteria are expected to fill a volume homogeneously. However we show in our experiments that asymmetric obstacles can result in an inhomogeneous distribution of swimming bacteria. When a population of bacteria is exposed to a microfabricated wall of funnel shaped openings, the random motion of bacteria through the openings is rectified in one direction. This ratchet-like effect leads to a buildup of a difference in cell number densities between two sides of the funnel wall. Similarly, we show that a series of such walls functions as a multi-stage pump and can guide motile bacteria from one reservoir to an other with high efficiency. The asymmetric obstacles can be arranged along arbitrary shapes and cause the bacteria to form well defined patterns. The ratchet effect may also have implications on the transport and distribution of motile microorganisms in irregular confined environments such as porous media, wet soil or biological tissue, or act as a selection pressure in evolution experiments.

Summary of Research:
Motility, of course, greatly enhances the chances of survival in a changing environment, thus it is an important part of the competition strategy of many different organisms. For that reason, the research on bacterial motility has been very active over the past three decades. Both the basic physical aspects (stochasticity, hydrodynamics) and the coupling to biological and biochemical processes (e.g. chemotaxis) have been extensively studied. On the other hand, very little has been done to put the gathered knowledge into use and gain control over the motion of microorganisms.

In our work, we demonstrate that properly shaped microstructures can interfere with swimming bacteria and guide, concentrate and arrange populations into arbitrary patterns.
Figure 1: Microstructures with funnel walls. (A) A schematic drawing of the interaction of bacteria with the funnel opening. Bacteria on the left side may (trace 1) or may not (trace 2) get through the gap depending on the angle of attack. On the right all bacteria colliding with the wall gets diverted away from the gap (traces 3 and 4). (B) Scanning electron micrograph of the device. (C) Distribution of incoming outgoing angles for bacteria colliding with a wall. Data is taken for 70 events.

Figure 2: Distribution of bacteria in a structure with a funnel wall. (A) Uniform distribution after injection. (B) Steady state distribution after 80 minutes. (C) $A(t) = \rho_1/\rho_2$, the ratio of densities in the left and right compartments vs. time. The circles are experimental data and the dashed line is a fit using hydrodynamic analysis.
Stress in a Nutrient Landscape Results in Stable Segregation of Selfish and Altruistic Populations

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Abstract:

Landscapes in ecology have a profound influence on the adaption and evolution of competing populations for resources. We are interested in how altruistic populations survive in the presence of selfish individuals in a non-stirred, closed and complex nutrient landscape. Well-stirred (landscape-free) but closed environments have a depressing future, known as the Tragedy of the Commons, when selfish individuals arise in a population. We have shown, using a non-stirred nanofabricated habitat landscape, that altruists and selfish bacteria can in fact coexist, that they can maintain phenotype diversity and avoid the Tragedy of the Commons. However, this avoidance of the tragedy comes at a cost: segregation of the two populations into highly complex spatially distinct regions at many length scales. This emergent spatial segregation of competing populations under stress greatly changes, we believe, our perception of the true sophistication of bacterial response to stress and competition, and has broad implications for the adaptive strategies of higher organisms under stress in complex environments. The Tragedy of the Commons is avoided at the cost of segregation.

Summary of Research:

What do we learn from this experiment? First, we think we should move away from the prisoners dilemma terminology of “defectors” and “cooperators” with the pejorative implications for the defectors, although our terminology of “selfish” and “altruists” is equally pejorative. In reality, probably both strains of altruists and selfish bacteria are necessary for the stable existence of the species in the presence of the complex and ever-changing nutrient landscape that is presented outside the confines of the microbiology laboratory. The facile change of the genome from the wild-type altruist genome JEK1036 to the rpoS mutant JEK1033 in a few days under stress with a 48 bp repeat of the adjoining sequence in the rpoS gene surely indicates that the GASP phenotype is programmed in, and is not a random event.

We have shown here that the growth advantage adapted strain phenotype does not result in a Tragedy of the Commons ecological disaster as long as the nutrient landscape is allowed to develop and is complex in topology, although admittedly ours is a very simple form of complexity.

The real test of the general importance of this result will be the extrapolation of these experiments to true 2 and 3-D nutrient landscapes and the use of eucaryotic cells in addition to bacteria. Ultimately, we believe that by learning the rules of engagement and observing the dynamics and steady-state solutions that competing communities develop as they cope with a rough fitness landscape that we will gain insight into a fundamentally analog systems problem which cannot be coded on conventional digital computers: how do agents improve fitness while competing for scarce resources? Do metapopulations follow the logic of Game Theory or do they find solutions that are illogical from a game theory perspective yet maximize strain fitness?
Figure 1: Schematic of a 2-level nutrient landscape device. The left-hand side habitat patch is a “black” one with no nanochannels open, the right-hand side one is a “white” patch with full openings. Nutrient and lysis molecules (due to the death of bacteria) are denoted by brown and green spheres respectively.
**Single Molecule Correlation Spectroscopy in Continuous Flow Mixers with Zero-Mode Waveguides**

**CNF Project # 398-91**  
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**Abstract:**  
This work illustrates how the use of a floor of zero-mode waveguides (ZMW) sustains sensitivity to diffusion measurements for fluorescence correlation spectroscopy (FCS) in high velocity flow channels, as occur in continuous flow microfluidic mixer (CFMM) designs. The basic idea is very simple: the floor of a CFMM is carpeted with an array of ZMWs which sample the local concentration of molecules at a particular region of the flow pattern but are shielded from the advection of the flow by the walls of the ZMW. Single molecules within a ZMW have a characteristic residence time given by their diffusion coefficient and the effective volume of the ZMW. Although above the entry of the ZMW the fluid is advecting, within the ZMW there is no advection and hence we expect the mean residence times in the ZMW waveguide, and hence the determination of the diffusion coefficient of the molecule, to be independent of the speed of the external flow.

**Summary of Research:**  
This result has important consequences. CFMM designs allow studies of biological reaction and mixing kinetics with low reagent consumption and microsecond time resolution. The flow velocity profile assigns reaction times to different distances from inlets. Hydrodynamic focusing achieves sub-microsecond time resolution and mixing times less than 10 µs, enabling protein folding kinetic measurements. We show that combining zero-mode waveguides with fluorescence correlation spectroscopy in a continuous flow mixer avoids the compression of the FCS signal due to fluid transport at channel velocities up to ~ 17 mm/s. Thus zero-mode waveguides make FCS suitable for direct kinetics measurements in rapid continuous flow.

This work was done in collaboration with Rob Ilic and Harold Craighead of Cornell University.
Figure 1: (a) and (b) A zero-mode waveguide method confines intensity fluctuations measurement to diffusers proximate to the metal substrate. (c) and (d) Repeated SEMs including those shown indicate typical scales $h = 163.3 \pm 8.8$ nm, $R = 209.3 \pm 4.4$ nm, and $r = 26.1 \pm 1.3$ nm.
Measurement of Stepwise Length Changes in Single Thick Filament as well as Interaction of Thick Filament with Thin Filament by Using Nanofabricated Cantilevers

CNF Project # 486-93
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Abstract:

We investigated the dynamics of isolated Mytilus anterior byssus retractor muscle thick filaments manipulated by nanofabricated silicon-nitride levers. Single thick filaments were suspended between the tips of two microbeams oriented perpendicular to the filament’s axis. One was a deflectable cantilever and the other a stationary beam (Figure 1). Axial stress was applied by translating the base of the deflectable nanolever away from the stationary beam. The tips of the flexible nanolevers and the stationary beam were imaged onto a photodiode array to track their positions. Filament shortening and lengthening traces, obtained immediately after a motor had imposed stress on the filament, demonstrated steps with a size ~ 2.7 nm and integer multiples thereof (Figure 2). Steps of this same size paradigm have been seen both during contraction of single sarcomeres [1] and during the active interaction between single isolated actin and myosin filaments [2], raising the question whether all of these phenomena might be related. Active thick filament shortening and thin filament shortening propagation on thick filament are considered as possible sources of length change in sarcomere.

Summary:

In researching the mechanism of muscle contraction, considerable attention has turned toward the elastic properties of the muscle filaments, themselves. Early studies [3] attributed almost all of the sarcomere’s elasticity to cross-bridges, rather than to filaments. However, recent investigations on isolated thick filaments from Mytilus and Limulus have shown that physiological stresses produce filament-length changes of up to 23% and 66% respectively [4]. It has also been found that actin filament can exist with in two states: short and long, which have a different structural and conformational state of globular actin [5]. All these data suggest that muscle contraction is not only due to simple sliding of actin and myosin filaments, but also due to shortening of the filaments themselves.

In a previous study, novel, nanofabricated cantilevers were employed to manipulate single myosin filaments. For the first time dynamic length-change measurements on isolated thick filaments were reported. It was also found that isolated thick filaments from the anterior byssus retractor muscle of the blue mussel Mytilus...
edulis changed length in steps. Steps were observed consistently and their size was indistinguishable from that found both during contraction and stretching of intact activated sarcomeres, and of isolated activated actin-myosin filament pairs [1,2].

Currently our research group is investigating if thick filament changes play a major role in muscle contraction. To answer the question, we are observing behavior of individual thick filament, attached to micro-fabricated levers, during activation. By tracking the position of the nanolevers, length change of the filament was detected.

Another possible partial source of stepwise muscle contraction may be the shortening of the actin filament. In order to answer this question, fluorescent labeled actin will be used (Figure 3). If actin filament shortening takes place during its interaction with myosin, detecting of the length change of the actin filament can be made by measuring distances between the fluorescent markers as functions of time. An assumption is that the length change will propagate from one end of the filament to the other. Following these studies we aim to work with single actin and single thick filaments. To control both filaments, the micro fabricated cantilevers will be used to bring one filament close to the other. Interaction between the filaments would be initiated by adding ATP and Ca.

After recent upgrades, a higher resolution than before (16-bit record, 32-bit control) was achieved and the system with the nanolevers can take pictures at a variable shutter speed of 4 to 227 frames/s, yielding a temporal resolution of 250 to 4.4 ms, considerably beneficial to the study of muscle and microfilament dynamics.

References:

Figure 3: Image of speckled actin filaments.
Non-Planar Nanofluidic Devices for Single Molecule Analysis Fabricated Using Nanoglassblowing

CNF Project # 551-95
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Primary Research Funding: The work was supported in part by the Nanobiotechnology Center (NBTC), an STC Program of the NSF under Agreement No. ECS-9876771. This research was performed in collaboration with Dr. Samuel Stavis who held a National Research Council Research Associateship Award at the NIST.

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Abstract:

“Nanoglassblowing” [1] was developed as a method to fabricated integrated micro- and nanofluidic fused silica devices with wide, shallow nanochannels and areas of gradual channel depth change. Using this method, channels were constructed with out-of-plane curvature of channel covers from over 10 µm to a few nanometers, nanochannel aspect ratios smaller than $2 \times 10^{-5}$:1 (depth:width), and nanochannels as shallow as 7 nm. These low aspect ratios and shallow channel depths are difficult to obtain using other fabrication techniques without collapsing the channel cover. The gradual changes in channel depth also eliminate abrupt free energy barriers at the transition from microfluidic to nanofluidic regions, facilitating loading of double-stranded deoxyribonucleic acid (DNA) molecules. The nanochannel depths and aspect ratios formed by nanoglassblowing allowed measurements of the radius of gyration, $R_g$, of single λ deoxyribonucleic acid (DNA) molecules confined to slit-like nanochannels with depths, $d$, ranging from 11 nm to 507 nm.

Summary of Research:

Nanofluidic devices are used for a variety of research applications, including biomolecular analysis, and interest in slit-like nanochannels in particular continues to grow. The utility of these structures remains limited, however, by planar device architectures as well as the need for high-resolution nanofabrication processes. A simple fabrication method termed “nanoglassblowing” is presented here that enables control over out-of-plane curvature of channel surfaces and improves attainable aspect ratios of shallow nano-channels in device regions without curvature. This method results in continuous nanoscale channel depth variation, which arises due to the outward deflection of a softened glass channel cover by increased air pressure during annealing of a bonded device. Because this method enables device bonding without the collapse of low aspect ratio,

![Figure 1: (a) Illustrated side-views of otherwise identical channels annealed without and with access holes demonstrate the effects of these holes on nanoglassblowing. The dashed lines 1 and 2 in (a) and (b) correspond to the locations of scanned height measurements 1 and 2 in (c). (b) White light interference patterns are visible in this photograph of adjacent channels with (32 ± 2) nm etch depths (scale bar: 1.25 mm). The left channel has no access hole, while the right channel has an access hole through which air could pass during bonding and annealing. (c) Out-of-plane curvature varies smoothly across the channel widths, as seen in these single representative scanned height measurements.](image)
slit-like nanochannels, contact photolithography can be used to pattern wide precursor trenches. This facilitates the fabrication of nanochannels that are both shallow and wide, a combination needed to prevent hydrodynamic or other nanochannel edge interactions that could hinder dynamic physical measurements, such as observation of long DNA molecules in constrained environments or continuous flow molecular analysis. The ability to subsequently fabricate variable nanochannel depths from this single layer of photolithography and etch depth obviates the need for multiple levels of aligned lithography. These fabrication benefits permit the seamless integration of device features across the centimeter to nanometer length scales relevant to miniaturized fluidic systems, including the critical micrometer-to-nanometer transition. When combined with the desirable optical and chemical properties of fused silica, the resulting devices are well suited to the manipulation and observation of single biomolecules.

To make devices using nanoglassblowing, 500 µm thick fused silica channel substrate wafers (7980 fused silica, Corning Inc., Canton, NY; Mark Optics, Santa Ana, CA) were patterned using contact lithography, wet etched at 22°C without agitation using 100:1 buffered oxide etch with surfactant (Ultra Etch, Air Products and Chemicals Inc., Allentown, PA), and fusion bonded to 170 µm thick fused silica cover wafers. Nanoglassblowing occurred while bonded devices were annealed at atmosphere using the following process: ramp from room temperature at 150°C/h to 1050°C, dwell 6 h at 1050°C, and cool to room temperature. The amount of out-of-plane curvature of the channel cover was observed to be influenced reproducibly by the presence of access holes during annealing, channel width, channel etch depth, and device geometry.

Figure 2(a) shows fluorescence micrographs of λ DNA molecules in a 3 mm wide loading channel of a device with 7 nm deep nanochannels. The out-of-plane deflection was sufficient to accommodate DNA molecules in their bulk radius of gyration. Figure 2(b) shows two DNA molecules loaded into the 7 nm deep, 300 µm wide slit-like nanochannel and confined by the dense matrix of surface roughness features. DNA molecules often remained stretched around these features upon removal of the applied electric field, which may be useful for applications requiring DNA elongation and observation, such as single molecule genomic mapping. Radius of gyration, $R_g$, measurements are plotted in Figure 2(c) versus channel depth.

Nanoglassblowing has the potential to extend the availability and utility of glass microfluidic and nanofluidic devices, by providing a simple means to fabricate and integrate non-planar device features, continuous changes in channel depth ranging from tens of micrometers to a few nanometers, and wide, shallow nanochannels.

Certain commercial equipment and materials are identified to foster understanding. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials and equipment identified are necessarily the best available for the purpose.

References:


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Figure 2: (a) λ DNA molecules in their bulk conformation in a deep, curved loading region (scale bar: 10 µm). (b) λ DNA molecules electrophoresing through a 7 nm deep and 300 µm wide slit-like nanochannel (scale bar: 10 µm). (a) and (b) show unprocessed image data. (c) $R_g$ is plotted against nanochannel depth, d. Each data point combines the results from ten λ DNA molecules and is corrected for the effects of diffraction limited optical resolution and image pixelation. Error bars represent the addition of one standard deviation of the mean and estimates of error from optical resolution, image pixelation, digital filtering, and noise thresholding. Uncertainty values for the nanochannel depths are smaller than the data symbols. Brochard’s predicted theoretical scaling is shown as a dashed line overlaid on the experimental data.
BIO

Nanochannels Fabricated in Polydimethylsiloxane using Sacrificial Electrospun Polyethylene Oxide Nanofibers

CNF Project # 599-96
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Abstract:
We have used electrospun polyethylene oxide (PEO) nanofibers as sacrificial templates to form nanofluidic channels in polydimethylsiloxane (PDMS). By depositing fibers on silicon templates incorporating larger structures, we demonstrate that these nanochannels can be integrated easily with microfluidics. We use fluorescence microscopy to image channels filled with dye solution. The utility of the hybrid micro- and nanofluidic PDMS structures for single molecule observation and manipulation was demonstrated by introducing single molecules of λ DNA into the channels. This nanofabrication technique allows the simple construction of integrated micro- and nanofluidic PDMS structures without lithographic nanofabrication techniques.

Summary of Research:
Using sacrificial electrospun polyethylene oxide (PEO) fibers, we have formed nanofluidic channels in polydimethylsiloxane (PDMS). Electrospinning is the process of forming nanofibers from a polymer solution using an electrically forced fluid jet [1]. These fibers can be used as lithographic masks [2-4] or sacrificial structures [5] to form nanoscale features in other materials. In this work, electrospun fibers were deposited onto a silicon chip, and PDMS was poured on top and allowed to cure. The PEO fibers were removed from the cured PDMS by soaking the material in water, leaving nanochannels (process outline in Figure 1).

We demonstrated that these nanochannels can be easily integrated with standard microfluidics by depositing fibers on patterned silicon chips. Imaging the channel cross-sections using a scanning electron microscope revealed the channels to have sub-micron diameters (Figure 2). To ensure that the channels were open, we filled them with fluorescent dye and imaged the filled
channels. Figure 3 shows images of filled devices consisting of random and aligned channels.

We also introduced single molecules of λ DNA into the channels, demonstrating the utility of these integrated micro- and nanofluidic structures for single molecule observation and manipulation. The paths of several isolated molecules of DNA are shown in Figure 4. Using this nanofabrication technique, it is possible to fabricate hybrid micro- and nanofluidic PDMS structures without using expensive and time-consuming conventional nanofabrication techniques. These fluidic structures could be used for several purposes, ranging from separating and analyzing biomolecules [6] to forming materials with artificial vascular structure [7].

Of the materials used to form microfluidic structures, PDMS remains one of the most popular due to its versatility and ease of use. Similarly, PEO is one of the most popular materials to electrospin because it is easy to work with, water soluble, and non-toxic. A fabrication process that combines these two materials is advantageous because the materials systems involved are well characterized and commonly used. Moreover, it should be straightforward to scale up this fabrication process to allow high throughput formation of micro- and nanoscale devices, rendering several applications previously confined to the research lab potentially commercially viable.

References:

**Nanomechanical Resonators for Prion Protein Detection**

**CNF Project # 599-96**

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**Abstract:**

In this study, arrays of nanomechanical resonators were employed to detect small molecules using specifically bound nanoparticle mass labels to amplify the frequency shift associated with chemical binding. As a model we used established antibody-based binding of prion protein (PrP). The detection limit of the system was improved by the use of antibodies and nanoparticles as mass labels, both specific for the presence of PrP.

**Figure 1:** Schematic of secondary mass labeling and our experimental setup. The resonators were operated in the fundamental mode of vibration and the total active surface area of the resonator was 56 µm² as shown by the broken lines.

**Summary of Research:**

The resonators are fabricated from a 200 nm thick layer of low-stress silicon nitride deposited on a 1.5 µm thick layer of silicon dioxide. Devices were patterned using optical photolithography and released from the substrate by removing the underlying sacrificial oxide with hydrofluoric acid. A schematic of the working principle and device dimensions are shown in Figure 1. They were loaded into a small vacuum chamber mounted on a motorized stepper stage and the resonant frequencies were measured using optical techniques [1]. A 405 nm diode laser was modulated in intensity and used to periodically heat and excite the resonators. Thermal expansion mismatch between the silicon nitride and oxide is ultimately responsible for sensor actuation. Resonant frequencies were determined interferometrically by measuring the reflectance variation from an incident HeNe laser (632.8 nm) focused at the free end of the resonator.

Computer control of the stage and spectrum analyzer allowed easy measurement of large arrays of resonators in a short amount of time. For each concentration of PrP, two arrays of resonators (24 devices) per chip were chosen randomly for use. The average values and errors were calculated based on frequency measurements from 24 resonators.

The primary antibodies were coated on the surface using amino-propyltriethoxysilane and gluteraldehyde [2]. The resonators were exposed to PrP alone and subsequently with secondary antibodies and nanoparticles for mass labeling. For the detection of PrP alone, the resonant frequency of each device was measured before and after exposing the resonators to PrP. The frequency shifts from the control (no PrP) were then subtracted from that of the sample (with PrP). It was observed that PrP could not be detected at concentrations of 20 µg/mL and below (data not shown). Therefore, mass labeling was required in order to detect PrP at these concentrations. Because both the secondary antibodies and nanoparticles specifically bind to PrP, mass labeling corresponds to the presence of PrP on the device surface. The frequency shifts due to secondary antibody binding for different concentrations of PrP (20 ng/mL, 200 ng/mL, 2 µg/mL and 20 µg/mL)
are shown in Figure 2a. This sandwich assay improved the detection limit for PrP to 2 µg/mL.

In order to further amplify the frequency shift and improve the detection limit, streptavidin conjugated nanoparticles were then attached to biotinylated secondary antibodies present on the resonator surfaces. The frequency shifts due to the presence of nanoparticle mass labels for different concentrations of PrP (from 200 pg/mL to 20 µg/mL) are shown in Figure 2b. The results indicate that the nanoparticles improved the detection limit to 2 ng/mL of PrP.

Figure 3 shows an SEM image of a resonator functionalized with nanoparticles for a PrP concentration of 2 µg/mL.

References:
Patterned Positively Charged Polymer Brushes to Guide Neuron Growth

CNF Project # 640-97

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Abstract:

Quaternized poly(dimethylamino ethyl methacrylate) brushes were directly synthesized by surface-initiated atom transfer radical polymerization on silicon or glass surfaces. These positively charged brushes were patterned by photolithography. Silicon regions uncovered by brushes were backfilled with poly(ethylene glycol). Such surfaces were used to guide rat hippocampus neurons to grow.

Summary:

The ability to control cell morphology is very important in tissue engineering, cell-based assays and many other similar areas. Poly(ethylene glycol) (PEG) is famous for its ability to resist protein and cell attachment to substrates [1]. PEG or polymers containing PEG side chains are widely used to generate arrays of cells [2] or to assist in reducing non-specific adsorption in protein patterning [3]. Here, we utilized PEG between positively charged brushes to restrict neurons to grow in the brush regions. Quaternized poly(dimethylamino ethyl methacrylate) brushes were usually synthesized by quaternizing poly(dimethylamino ethyl methacrylate) brushes with methyl iodide, which is highly toxic [4]. Here, we synthesized such positively charged polymer brushes by direct polymerization of its quaternized monomer. Such brushes were shown to be antibacterial [5], which can be a big advantage for its usage as a cell culture substrate.

We found that the ratio of neuron population to glia cell population is much higher on such brush modified surfaces than on polyllysine covered surfaces. The viability of rat hippocampus neurons on such surfaces is very good. Using photolithography, we are able to pattern the polymer brushes as shown in Figure 1. The uncovered regions between brush regions were backfilled with PEG monolayer. As shown in Figure 2, neurons grown on such patterned surfaces followed the brush region very well. Such surfaces are very useful for controlling cell morphology.

References:

Figure 1: Optical image of patterned quaternized poly(dimethylamino ethyl methacrylate) brushes on silicon surface. The lines in the image are 2.5 µm wide.

Figure 2: Living hippocampal neurons labeled with calcein AM ester. The neurons were cultured for 7-14 days on the polymer brush / PEG patterned surface.
Direct Patterning of Polymer Brushes by Electron Beam Lithography

CNF Project # 640-97

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Abstract:

We have studied the possibility of patterning polymer brushes directly using electron beam lithography. Conventionally patterned polymer brushes are fabricated by patterning the initiator layer, followed by the surface initiated polymerization of the desired monomer. However, contamination or loss of activity of the initiator is always a concern. Also, growth of patterned brushes in this way can lead to lower resolution features when the brush height is comparable in length to the pattern width, due to chain relaxation into the voided regions during growth. We have synthesized poly(methyl methacrylate) (PMMA), poly(2-hydroxyethyl methacrylate) (PHEMA), and PMMA-block-PHEMA brushes via atom transfer radical polymerization (ATRP) and successfully patterned them using a 0.5-1 nA beam current with doses ranging from 10 to 1500 µC/cm². We have shown that feature sizes close to 20 nm (for PMMA brushes) and close to 50 nm (for PHEMA brushes) can be achieved using e-beam lithography. The sensitivity curves of these brushes have also been studied.

Summary:

Patterned polymer brushes have attracted great interest because of the need for systems in the fields of molecular-scale electronics, magnetic storage, biosensing and other areas of nanotechnology [1,2]. We have studied the possibility of patterning polymer brushes directly using electron beam lithography. Conventionally, patterning of polymer brushes is done by first patterning an initiator layer on the substrate, followed by surface initiated polymerization of a suitable monomer [3,4]. However, it would be favorable to make this a single step process as this would reduce the possibility of surface contamination. In addition, direct patterning of brushes could help prevent pattern collapse which may occur during polymerization of patterned initiator sites due to chain relaxation into the voided regions [5].

To investigate the feasibility of patterning polymer brushes in a single step, brushes of poly(methyl methacrylate) (PMMA) and poly(2-hydroxyethyl methacrylate) (PHEMA), known electron beam resists [6], were prepared via atom transfer radical polymerization (ATRP) and direct patterning was carried out using electron beam lithography. The brushes were patterned using 0.5 nA to 1 nA beam currents, with doses ranging from 10 to 1500 µC/cm². Development was done using 1:3 ratio of methyl isobutyl ketone and isopropyl alcohol mixture for 90 seconds. Figure 1 shows an scanning electron microscopy (SEM) image of a patterned PMMA brush.
AFM images of the e-beam patterned PMMA and PHEMA brushes are shown in Figure 2.

“Direct” patterning of PHEMA brushes by e-beam lithography was carried out after pre-baking at 160°C for 5 minutes. A 0.5 nA beam current was used with doses ranging from 10 to 120 µC/cm². The patterned brushes were developed in 0.9 N TMAH solution for 60 seconds.

We also investigated the sensitivity of these polymer brushes. Figure 3 shows the sensitivity curves for a 45.3 nm thick PMMA brush and a 50 nm thick PHEMA brush. Line edge roughness can be improved if the development conditions are optimized. Typical development conditions used for spun coat samples are not adequate enough as chain lengths are much shorter in the brushes than the high molecular weight polymers used in spun coat samples, which can lead to chain relaxation into the patterned areas. This phenomenon is noticeable in the patterned PHEMA sample in Figure 2b.

Currently, we are looking to explore the use of other monomers for brushes to pattern directly. We will study their electron beam resist behavior and also incorporate them into a study of patterning other block copolymer brushes other than PMMA-b-PHEMA. We are also very interested in the patterning of block copolymer brushes in order to combine top-down lithography with bottom-up self assembly of the block copolymers. By studying the patterning of various block copolymer brushes with differing molecular weight ratios, and by working with various monomers, we expect the convergence of the top-down and bottom-up methods to result in smaller periodic structures than what is currently achievable with current lithographic techniques. Lastly, we believe it to be possible to create “nano-channels” with the direct patterning method. Using diblock copolymer brushes where the top block crosslinks to electron beam exposure while the bottom block depolymerizes, nano-channels of various sizes can be achieved.

Acknowledgements:
The National Science Foundation (Grant DMR-0518785) is gratefully acknowledged for funding the project. Additionally, we are grateful to the Cornell NanoScale Science & Technology Facility (CNF) and the Cornell Center for Materials Research (CCMR) for use of their facilities.

References:
Fabrication of Patterned Fluorinated and PEGylated Monolayer Surfaces for Fundamental Marine Fouling Studies

CNF Project # 640-97

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Abstract:

Various designs for coatings that resist the attachment of marine organisms are based on the concept of “ambiguous” surfaces able to present both hydrophobic and hydrophilic functionalities as surface domains. Information is needed on the scale of the domains that the settling marine organisms are able to distinguish. To further this pursuit, silicon wafers were chemically modified to produce a pattern of squares containing alternating fluorinated and polyethylene glycol (PEG)ylated stripes of different widths on either a uniform fluorinated or PEGylated background. Each 1 cm × 1 cm square contained stripes with widths of 500, 200, 100, 50, 20, 5, or 2 µm as well as an unpatterned square with chemistry opposite that of the background. The integrity of the patterned monolayers was checked using protein adsorption and subsequent fluorescence microscopy and fundamental biofouling studies were carried out using the green alga Ulva.

Summary:

The fouling of ship hulls and other man-made marine structures is a significant problem that causes high operational and maintenance costs to industry [1]. Furthermore, these problems are being further exacerbated by the ever-rising cost of energy. In the closely related biomedical field, success in preventing fouling has been achieved using hydrophilic materials such as poly(ethylene glycol) (PEG) [2]. It is anticipated that PEG’s ability to resist nonspecific binding is through the formation of a hydration layer capable of hindering the nonspecific adsorption of proteins [3] and the adhesion of cells and microorganisms [4].

The green seaweed Ulva is a prominent fouling alga found throughout a wide range of marine environments. Distribution is chiefly through dispersion of microscopic zoospores, capable of settling on a solid surface [5]. Generally, a preference for Ulva to

Figure 1: Schematic diagram of the process used for creating alternating stripes of FOTS and PEG on silicon substrates using photolithography.

Figure 2. Diagram showing layout of chemically patterned Si wafer.
settle on hydrophobic versus hydrophilic surfaces has been established [6]. However, using this knowledge, fabrication of alternating PEGylated monolayers (PEG-SAM) and fluorinated monolayers on silicon presents an opportunity to probe the length scale at which the settling spores can discern the difference in hydrophobic and hydrophilic surface domains.

Alternating stripes of fluoroocetyltrichlorosilane (FOTS) and PEG-SAM were produced on silicon substrates using photolithography. The overall process is depicted in Figure 1. Silicon wafers were first cleaned by immersion in piranha solution. Vapor deposition of FOTS was then performed using an applied microstructures vapor deposition system MVD-100 (1). The wafers were coated with Shipley S1818 positive-tone photoresist (2) and subsequently patterned using contact photolithography. (3). The patterns were developed using a Hematech-Steg wafer processor (HMP900) (4), and subsequently subjected to oxygen plasma using a Harrick Plasma Cleaner (5). The patterns were then chemically backfilled using a PEG silane (6), and finally the photoresist was stripped (7) leaving the alternating patterns of PEG-SAM and FOTS.

Figure 2 demonstrates the layout of the eight patterned 1 cm$^2$ areas on the silicon wafer. 0 refers to a uniformly covered square of either PEG-SAM or FOTS opposite to the background. The number denotes square with stripes of FOTS and PEG-SAM of widths 2, 5, 20, 50, 100, 200 and 500 µm. The background is either pure PEG-SAM or pure FOTS.

The chemically patterned wafer surfaces were incubated with fluorescein-tagged BSA protein (BSA-FITC) in PBS buffer solution and then imaged using fluorescence microscopy. As depicted in Figure 3, protein adsorption was generally found to be significantly lower on the PEG-SAM regions of the patterns demonstrating the fidelity of the patterning process. Additional work found consistent behavior for settlement testing using Ulva zoospores above some threshold size (between 5 and 20 µm) at which the zoospores were no longer able to differentiate the surface domains [7]. Future work will be focused on exploring different shape features and also trying to further narrow down the domain size threshold at which the zoospores are no longer to differentiate the surface’s chemical properties. This includes, but is not limited to, checkerboard patterns as depicted in Figure 4.

References:

Retinal Implant Project

CNF Project # 657-97

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Abstract:

The purpose of the Retinal Implant Project is to restore useful vision to patients who are blind with degenerative retinal diseases. The primary illnesses we hope to treat are retinitis pigmentosa (a primary cause of inherited blindness) and age-related macular degeneration (the leading cause of blindness in the developed world). Both these diseases cause the eventual destruction of the photoreceptor cells – rods and cones – in the retina, leaving intact the ganglion cells which transmit electrical impulses (and hence visual information) to the brain. The ganglion cells may be stimulated, however, with biphasic current pulses from a microfabricated electrode array. Blind surgical volunteers have consistently described visual percepts that resulted from such stimuli, and this has led our team to develop a wireless, implantable retinal prosthesis a prototype which is shown in Figure 1 assembled on a model eyeball and in close-up in Figure 2.

Summary of Research:

The implanted portion of our device consists of power and data secondary receiving coils, a small number of discrete components, and a custom designed integrated circuit (IC) which consists of a delay locked loop for clock and data recovery, current drivers for each of the 15 electrodes in the stimulating electrode array, and a programmable function generator capable of stimulating with a wide range of pulse widths and amplitudes. The current outputs drive high-charge capacity iridium oxide stimulating electrodes, which in turn give rise to the visual percepts mentioned above.

The CNF-fabricated components of this system have been various proof-of-concept test structures and tools used in the research effort, the flexible circuit on
which the remaining components of our prosthesis are assembled, the stimulating electrode arrays themselves and most recently an integrated combination of the external flexible circuit and the electrode array as shown in Figure 3. Si wafers serve as carriers for these freestanding films during processing. The electrode leads are fabricated inside of a ‘sandwich’ of polyimide or parylene-C, while the IrOx electrodes themselves are fabricated by reactive sputtering.

Assembly of the intraocular components of the prosthesis is accomplished by flip chip stud bumping of the IC onto the host flex circuit, and epoxy die attach of the discrete components and coils. An external patient interface unit, under development by our team, will consist of a video camera for capturing images in the patient’s environment, a digital signal processor, and an RF transmitter and coil to relay power and data to the implanted device. The patients will also be offered the ability to adjust the electrical stimulation parameters to optimize their perception, in much the same manner as modern hearing aids and cochlear implants.

Challenges still remain in realizing a chronically implantable retinal prosthesis. Early prototypes were made by flip-chip stud bumping a microfabricated electrode array to the external flexible circuit. Difficulty in making this connection led us to pursue an integrated approach. As shown in Figure 3, the external flexible circuit and electrode array are now being microfabricated as one piece and include a removable ‘test tail’ for testing purposes. Integration of these two parts has necessitated microfabrication process developments such as the capability to create bonding pads on both sides of the polyimide-based devices as well as including multiple metal layers in the polyimide ‘sandwich.’ The flexible circuit is currently fabricated with two metal layers to allow conductor cross-overs. The flexible circuit also has a series of bonding pads which, because of size constraints, require that adjacent pads alternate between the top and bottom surface to minimize the overall width while still maintaining sufficient pad separation for solder bonding.

A second effort has been to develop the capability of increasing the number of stimulation electrodes which can be utilized in future generations of our retinal implant.

We anticipate that the 15 stimulating electrodes of our present design must be increased by at least an order of magnitude and likely more. An increase in the density of the metal interconnects must be realized without greatly increasing the conductor resistance to achieve this goal. Though Figure 4 only shows the cross-section of a two conductor system, through a series of test fabrication runs we have demonstrated the capability to include up to four metal layers in our flexible circuit/electrode array system.

The first generation of our retinal implant has now been successfully implanted in an animal and has continued to function for over 2 months and counting. While this has not yet demonstrated efficacy, it does represent a very important milestone in the development effort. The implant is operational and surviving the in vivo environment for an extended period of time.

References:


Microfabricated Mixers for Experiments with Proteins

CNF Project # 692-98
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Abstract:
In past years, this project has focused on the development of microfluidic mixers for use with a variety of experimental probes of macromolecular folding or conformational change. This year, we report the successful application of an optically transparent microfluidic mixer to study sub-millisecond scale conformational changes of fluorescently labeled proteins, using multiphoton microscopy.

Summary of Research:
Past reports have described the fabrication of a microfluidic mixer, shown in Figure 1, which decouples hydrodynamic focusing from diffusive mixing. The use of multiple inlet ports allows rapid hydrodynamic focusing of an inlet solution. Once the dimensions of this macromolecule-containing solution are reduced to the micron scale, contact is made with a second solution, which contains the small ions that trigger a conformational change. The diffusion of these small ions into the protein-containing solution enables rapid and uniform mixing of the two solutions [1, 2] and eliminates mixing artifacts. The mixers were fabricated from optically transparent Zeonor, following the procedures outlined in Ref. [3].

These improvements have enabled a measurement of the time scale for the conformational dynamics of the protein calmodulin: a ubiquitous calcium-sensing protein present in eukaryotic cells, as illustrated in Figure 2. In conjunction with more standard experimental techniques (e.g. stopped-flow mixers), we have identified two separate kinetic transitions. The rapid event, detected by the microfluidic mixer, occurs in less than 0.5 ms and corresponds to the binding of Ca ions to the C terminus of the protein. A slower event, occurring in tens of ms, corresponds to Ca ion binding to the N terminal domain of Calmodulin [4].

Devices that can sense microsecond scale dynamics are important for quantifying the time scales of biological conformational changes.

References:
Figure 1: A photograph illustrating the six-port mixing device fabricated from Zeonor.

Figure 2: Rapid conformational changes from (fluorescently labeled) apo-calmodulin (structure shown schematically at the lower left, based on 1QX5.pdb) to Ca2+-calmodulin (lower right, 1CLL.pdb) are studied with a microfluidic mixer in conjunction with multiphoton microscopy. The microfluidic device enables measurements of ultra fast chemical kinetics with ~40 microsecond time resolution by using sheath flow (from diagonal channels) to prevent premixing of the reactants (from orthogonal channels).
Microfluidic Cell Culture Analog Devices
to Mimic Animal Exposures to Toxins and Drugs

CNF Project # 731-98
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Abstract:
Our group has developed microfluidic in vitro devices that mimic the response of humans or animals to drugs, toxins, or nanoparticles. Each device, or cell culture analog (CCA), contains an array of pseudo tissues that are interconnected by microfluidic channels [1]. The recirculation of blood surrogate through the microchannels allows us to study tissue-tissue interactions, such as the breakdown of a parent compound in the liver, and subsequent transport and reaction in the lung. We combine these in vitro device experiments with physiologically-based pharmacokinetic model simulations to predict toxin and drug dynamics in humans.

Summary of Results:
Development of Estrogen-Responsive µCCA System:
To visualize the susceptibility of human breast cancer (MCF-7) cells and endometrial carcinoma Ishikawa cells to estrogen and estrogenic compounds present in the environment, we infected both cell lines with estrogen response elements–E4 promoter-GFP reporter gene constructs that caused the expression of GFP when estrogen or estrogenic compounds bind to the cells’ estrogen receptors [2]. When challenged with 17-beta-estradiol in µCCA devices, both cell lines expressed GFP in a dose-responsive manner. Both cell lines also responded with dose-responsive GFP expression when exposed to the cancer drug metabolites diethylstilbestrol, 2-hydroxyestradiol, and the environmental toxins bisphenol A, genistein, and o-p′-DDT. In contrast, we observed tissue-specific responses with the drugs

Figure 1: µCCA chip in plexiglass housing.

Figure 2: Response of Ishikawa-GFP cells to estrogen antagonists ICI 182,780 (ICI), tamoxifen (Tam) and raloxifene (Ral). Antiestrogens were tested with or without co-administration of 1 nM E2. The data are reported as fluorescence units (FU) per pixel of cells.
Raloxifene and Tamoxifen. These drugs were effective antiestrogens in breast cancer cells, but acted as partial estrogen receptor agonists in Ishikawa-GFP cells. Raloxifene and Tamoxifen did not synergistically effects the cells [3].

Development of GI Tract Modules: Using GI-tract epithelial cells (caco-2) and mucous producing cells (HT-29), co-cultured and grown to confluency on transwell membranes, we were able to simulate the GI-tract barrier and part of the digestion process. Operated in connection with multi-organ µCCA chips, compounds of interest pass through the apical chamber of the GI module, are absorbed and digested through the epithelial layer, and then circulate into the µCCA chip. Using this system, we were able to digest acetaminophen and simulate its metabolites’ toxic effects on liver and lung cells [4]. We were also able to observe the cells’ detoxifying response – a decrease in glutathione, a molecule that binds toxic acetaminophen metabolites (Figure 3).

Toxicity of Nanoparticles: We have also simulated the digestion of nanoparticles and have found that carboxylated nanoparticles, 50 and 200 nm in size, lower the transmembrane resistance of GI-tract epithelial layers by decreasing the tightness of junctions between the cells. As a result nanoparticles are able to pass from the apical to the basolateral side of the GI-tract module and increase the ability of iron to pass through the GI-tract barrier. We are now measuring the effects nanoparticles have on other organ analogs by using added-on GI-tract modules upstream of µCCA devices in which digested nanoparticles are re-circulates to other organ chambers. Preliminary results suggest that a concentration of 2.5 × 10¹⁰ of carboxylated 200 nm sized particles alter the morphology of liver cells. We will conduct further experiments and simulations of PBPK models to establish concentrations at which nanoparticles affect liver and kidney cells.

3-D µCCA Devices: To realistically mimic the effects of anti-cancer drugs on liver and colon cells, we developed µCCA devices with 3-D organ analog constructs. For this purpose, liver and colon cells were embedded in Matrigel, placed into µCCA chips, and their survival rate in the devices was measured over a period of several days. Over a period of 72 hours, cell viability dropped from 100%, measured immediately after device assembly, to about 80(+) %. After 72 hours of operation the cell viability dropped significantly due to nutrient depletion of the recirculated medium [5]. Using the 3-D µCCA devices, we tested the cytotoxic effects of Tegafur, a cancer drug that metabolizes in the liver to 5-FU. The metabolite 5-FU acts as a chemotherapeutic agent for colon cancer. Operating the devices without liver cells, we found that Tegafur itself is somewhat toxic to colon cancer cells (HCT-116). Adding liver cells (HepG2/C3A) to the system caused Tegafur to be converted to 5-FU by cytochrome P450 enzymes. The drug now exerted measurably higher toxic effects on HCT-116 cells (figure 3) [6]. This level of toxicity on HCT-116 cells were neither observed in 96-well plate experiments (without liver cells), nor in µCCA experiments in which liver cells were absent. The results we were able to observe in vitro have previously only been seen in vivo in animal experiments or studies involving humans. They confirm that µCCA devices are able to reproduce part of the liver metabolism and its consequences on HCT-116 cells.

References:

Figure 3: HepG2/C3A and L2 viability (A) and glutathione levels (B) after 6 hours of 30 mM APAP digestion.
A Bilayer Resist Method for Creating Silica Microfluidics

CNF Project # 762-99
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Abstract:
In support of a growing collection of lab-on-a-chip applications utilizing inexpensively formed microfluidics [1-2], we have demonstrated a new method for creating silica microfluidic networks. Unlike some existing bilayer resist processes involving hydrogen silsesquioxane (HSQ) [3-4], this process utilizes a single photolithographic step. The resulting silica microfluidics offer several advantageous material properties over polydimethylsiloxane (PDMS).

Summary of Research:
We formed microfluidics using various thicknesses of HSQ, which were spun and exposed to oxygen plasma to cross-link a 10 nm thin barrier layer. This barrier was robust against photoresist solvents, allowing a bilayer stack to be formed without altering the underlying HSQ bulk. Photoresist was then spun, patterned with optical lithography, and used as a mask layer. A wet-chemical etch was used to transfer the pattern into the barrier layer, followed by development to isotropically dissolve the HSQ bulk. Microfluidic networks formed with this developer-based transfer are self-terminated on the underlying substrate without inducing surface damage. Cross-sectional electron micrographs of these channels revealed a sponge-like film composition, which was compacted into a dense silica film during a subsequent high-temperature anneal (Figure 1).
This annealed film had excellent chemical solvent resistance. The resulting microfluidic channels have widths 1.5-3.1 µm and heights of 80-520 nm, respectively (Figure 2).
The microfluidic networks were sealed and used to directly observed flow of fluorophore-labeled deoxyribonucleic-acid (DNA) using fluorescence videomicroscopy. Future applications of this fabrication method may include integration with other components such as MEMS/NEMS or nanowire sensors.

References:
Figure 1: (LEFT) Cross-sectional electron micrograph of HSQ film prior to annealing, exhibiting porous structure. (RIGHT) HSQ film structure following a high temperature anneal. The previously porous structure is collapsed into a dense, amorphous film.

Figure 2: Electron micrograph of a microfluidic channel constriction formed in HSQ with cross sectional dimensions 1.5 (w) by 0.08 (h) micrometers.
Nanofluidic Channels for Biological Manipulation and Analysis

CNF Project # 762-99

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Abstract:
Nanoscale fluid filled channels can be used as tools for manipulating and observing fluorescently labeled deoxyribonucleic acid (DNA) molecules. A DNA molecule with a radius of gyration larger than the nanochannel width can be forced into a channel using externally applied electric fields. Once inside, it is subject to confinement induced forces which cause it to elongate in the direction of the nanochannel axis. Here, we report on the dynamics of DNA molecules which initially enter the channel with a looped front end. Such folded molecules are observed to spontaneously unfold over a period of time ranging from seconds to minutes and depending on the length of the initially folded portion.

Summary of Research:
Nanofluidic channels have shown great promise as tools for the analysis of genomic length DNA molecules. Assays for fragment length analysis, real time enzymatic degradation, and localization of hybridized probes have all been demonstrated. Additionally, detailed studies of the conformation and dynamics of single molecules in nanochannels have corroborated models for self-excluding polymers in confined environments. Recently, molecular dynamics and Monte Carlo simulations have been applied to the study of confined polymers and overlapping (but unconnected) polymer chains. While it is experimentally difficult to position two DNA molecules in a nanochannel so that they partially overlap, it is relatively straightforward to insert a single molecule into a nanochannel such that the front end is folded over on itself.

We have achieved this using the strategy depicted in Figure 1, an overview of the experimental procedure. Left: A long DNA molecule sits in a microchannel, next to the entrance of a nanochannel. Middle Left: The electric field pulls the DNA into the nanochannel. Because molecule’s entrance was initiated at some point along the backbone (not by one of the two ends), it enters the channel in a looped conformation. Middle Right: The electric field is turned off and the DNA strand is allowed to sit inside the nanochannel, in a high energy looped state. It gradually unfolds, thereby reducing its conformational free energy. Right: Molecule has completely unfolded and remains in the channel, extended in its equilibrium conformation. Long DNA molecules (T4 bacteriophage) are electrophoretically driven through a microchannel towards an array of nanochannels. Just before entering the channel, the field is turned off and the molecules are allowed to sit near the entrance, where thermal agitation causes them to experience a number of different conformations. When a molecule happens to be in an appropriate conformation and position relative to the channel entrance (as depicted in Figure 1A), the field is turned on and the molecule is driven into the channel, often with a folded front end. After the entire DNA molecule has entered the channel, the field is switched off and the dynamics of the molecule are observed. Here we pay particular attention to the spontaneous unfolding of the looped end, a process that we theorize to be entropically induced.

All unfolding events are observed with fluorescence microscopy. Videos are taken using a cooled CCD camera, recorded to disk and analyzed using a custom program written in MATLAB. A time trace graph, showing a single DNA molecule in a nanochannel as it unfolds over time is shown in Figure 2, a time trace of a fluorescently labeled
DNA molecule confined to a nanofluidic channel. Each column of pixels in the time trace image represents the fluorescence intensity along the axis of the channel in one movie frame. Many of these vertical lines are placed side by side to produce the image showing the position, length, and contour of the DNA molecule in the channel over the course of the entire movie clip. The molecule in this time trace initially has a looped front end, with the folded segment of the molecule being more intense than the unfolded part. Over the course of 35 seconds this molecule is observed to spontaneously unfold. White dots show the end points of the folded segment of the molecule as identified by an automated image analysis routine. The part of the molecule that is looped over on itself is roughly twice as bright as the unlooped portion of the molecule.

A simple model, based on the balance of entropic and frictional forces, is depicted in Figure 3, an unfolding model. The points ×1, ×2, and ×3 are the endpoints of the folded and unfolded portions of the molecule. The separation force is localized at the point ×2, and results from the difference in free energy between a conformation in which the blue segment overlaps with the looped end and a conformation in which the blue segment slides left, no longer overlapping with the looped end. The resulting forces, Fs and -Fs, are equal in magnitude and opposite in direction. They act on the upper and lower segments of the molecule in a localized manner as depicted. The upper and lower segments are accelerated until two frictional forces resist the motion. The hydrodynamic frictional forces act on the entire upper and lower segments. The forces are proportional to the length and velocity of each segment. The entropic force causing separation is thought to be localized to the interface between the looped and unlooped segments, and is constant in magnitude throughout the unfolding process. Hydrodynamic friction depends on both the length and velocity of a moving segment of DNA. The equations resulting from a balance of forces were solved numerically, resulting in the plot seen in Figure 4, the fit of a numerical solution of the unfolding model to data. Plotted on the bottom is the length of the folded part, for a given frame, and plotted on the top is the end to end length the whole molecule. By fitting the model, it is possible to extract the parameter $F_s/\rho_{hd}$, which is the ratio of the unfolding force to the hydrodynamic friction factor per unit length of DNA. Using a previously measured value for $\rho_{hd}$ [1], the unfolding force is determined to be on the order of 10 fN. The residual between the generated points and the data points was minimized by an algorithm which adjusted the model parameters, allowing for an estimate of the unfolding force magnitude.

The nanochannel devices were patterned on a mirror-polished fused silica wafer with a thickness of 500 mm (MarkOptics, Santa Ana, CA) using a combination of electron beam and optical lithography. Both micro- and nanochannels were etched simultaneously using a reactive ion etch process. Access holes were created by alumina powder blasting from the backside of the wafer. Finally a 170 mm fused silica cover wafer (MarkOptics) was touch-bonded and annealed at 1050°C to the device wafer, enclosing the channels. Nanoports (Upchurch Scientific, Oak Harbor, WA) were sealed to the access holes forming buffer reservoirs.

References:

**SELEX-on-a-Chip: Microchip Integration of the Sol-Gel Derived Affinity Column for Monitoring RNA-Protein Interaction**

**CNF Project # 762-99**

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**Abstract:**

A microfluidic chip that integrates a sol-gel derived affinity column with a microheater is developed. This microchip includes five electric heaters covered with patterned PMMA for individual heat elution from five sol-gels with target molecules. This study is the first to demonstrate the detection of protein-aptamer interactions on the sol-gel microfluidic system and further describes possible compatibility with Systematic Evolution of Ligands by Exponential Enrichment (SELEX).

**Summary:**

Systematic Evolution of Ligands by Exponential Enrichment (SELEX) has been widely used for identification and characterization of interactions between RNA aptamers and proteins or proteins and peptides [1]. Aptamers, nucleic acid species that have been selected through repeated rounds of SELEX, offer utility for biotechnological and therapeutic applications as they offer molecular recognition properties that rival that of the commonly used antibodies. To obtain a high-quality RNA aptamer requires typically 8-12 rounds of SELEX, where each round includes both positive and negative selection. This can require a few to several weeks of effort. Therefore, multiplexing and more efficient SELEX technologies could significantly reduce the cost of generating these reagents.

The synthesized silicate glasses with nanoporous sol-gels, have been investigated for their ability to entrap proteins and yet maintain their activity over months. Protein immobilization in sol-gels should allow the construction of miniaturized devices that hold protein without requiring affinity capture tags and enabling the entrapment of various proteins in their native state [2]. Furthermore, interest in scaled-down analytical processes, combined with advances in microfluidics, is motivating various chip-based methods in which analyses can be carried out more rapidly and at lower cost via small-scale systems than with current laboratory bench-scale methods [3].

Based on these techniques, we report a novel method to develop a SELEX chip (Figure 1) that will allow high-throughput selection and characterization of RNA aptamers. This microchip utilizes porous sol-gel networks to immobilize proteins, micro-channels to deliver RNAs to the sol-gel droplets, and a heat source to selectively elute the target RNAs that bind the protein after the RNAs selection.

We have developed a microfluidic chip that integrates the sol-gel derived affinity column of a SELEX system. These chips were fabricated on a glass-slide using photolithographic processes and with
polydimethylsiloxane (PDMS) as a main microfluidic structure. The fabricated chip includes electric heaters covered with patterned poly (methyl methacrylate) (PMMA) for individual heat elution and sol-gel droplets containing the target molecules and immobilized on the PMMA surface. These electrodes work as a localized heat source. We confirmed precise heat elution by monitoring fluorescent signal loss so that we were able to elute SYBR-I Green labeled dsDNA from the sol-gel spot. For further confirming SELEX compatibility, in Figure 2, a known aptamer targeting TBP proteins was delivered to TBP spots, eluted from spots and collected for reverse transcription polymerase chain reaction.

This product was analyzed by gel electrophoresis and confirmed to be the correct aptamer. This study shows the potential of mass-produced, low-cost microfluidic systems capable of performing multiplexed in vitro selection with improved SELEX cycle efficiency.

References:


Patterning Cell Arrays Using a Versatile Polymer Template

CNF Project # 762-99 and 1540-07

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Abstract:

Cell-cell interactions play a critical role in tissue homeostasis, and dysregulation of this interplay may contribute to cancer initiation and progression. The goal of this study is to create tailored, cell-patterned surfaces to study the angiogenic capability of tumor cells in the absence and presence of cell-cell interactions. To this end, we fabricated a versatile polymer template on a glass cover slip that enables the patterning of individual cells and clusters of multiple cells in a well-defined manner. Our polymer template offers several advantages over the widely popular polydimethylsiloxane (PDMS) used in micro-patterning.

Summary of Research:

Alterations in cell signaling contribute to tumor vascularization, a hallmark of cancer [1]. By comparing the angiogenic behavior of individual cancer cells and cell clusters containing varying number of cancer cells, one may be able to identify signaling pathways leading towards enhanced tumor vascularization. Knowledge of these pathways is important in designing more efficacious anti-cancer treatments, as well as identifying biomarkers for cancer diagnosis and patient prognosis. Cell arrays have previously been patterned by micro-contact printing (µCP) using PDMS stamps, to study cell-cell interactions, to measure cellular forces and to introduce time-variant stimuli to cells [2].

In this work, we describe a versatile polymer (Parylene C) template to create tailored surfaces for patterning cell arrays of individual and clustered cells, for studying the angiogenic behavior of tumor cells as a function of cell-cell interactions. Parylene is chemically inert, pinhole free and resist swelling in aqueous medium. Our parylene templates overcome the problem of sagging and swelling associated with the PDMS in µCP, thus leading to more reproducible feature patterns. In addition, we can fabricate parylene templates with features, ranging from nanometers to micrometers. We have reported the use of parylene templates to pattern cells [3], DNA [4] and lipid bilayers [5].

Briefly, the fabrication process is shown in Figure 1. Using standard photolithography techniques and oxygen plasma etching, we fabricated parylene templates with micrometer-sized features on glass cover slips (Figure...
2). The parylene film served as a template for coating defined areas of fibronectin onto the underlying cover slip, and afterwards this template was peeled off. Laying down the fibronectin was necessary for cell adhesion in later steps. Figure 3 shows fibronectin coated cover slips, as visualized by immunostaining. The cover slip surface was treated with a blocking agent (e.g. bovine serum albumin, polyethylene-glycol) to prevent non-specific cell adhesion. Figure 3 also shows the cell array patterned using this method. We quantified the number of adherent cells based on fluorescent DAPI-staining of the nuclei. The surface area of the features determined the number of cells adhered and thus, able to interact with each other.

We are currently using these parylene template cover slips to pattern oral squamous cancer cells and study their ability to up-regulate angiogenic factor secretion as a function of cell-cell interactions.

References:
Novel Biosensors Based on Organic Electrochemical Transistors and Their Integration with Microfluidic Channels for Multianalyte Sensing

CNF Project # 775-99
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Abstract:
Organic electrochemical transistors (OECTs) were integrated with a surface-directed microfluidic system to yield multianalyte sensor arrays. The surface-directed microfluidic system was able to distribute the analyte solution from one reservoir to four wells over the OECTs, and do so by spontaneous flow and without any external pressure. Only small quantities of analyte and enzyme solution (less than 5 microliters) were required for detection. These integrated devices are easily fabricated and show good specificity, hence they may be useful for lab-on-a-chip technologies.

Summary of Research:
Organic thin film transistors (OTFTs) have received considerable interest in recent years because of their potential for low cost, large-area, flexible electronics. One of their potential applications is as a transducer in chemical and biological sensing. Among OTFTs, organic electrochemical transistors (OECTs) are particularly promising for biological sensors because they operate in aqueous media. These devices also operate at a low voltage and can provide effective detection of biological analytes in a very small sample volume. OECTs consist of a conducting polymer channel, an electrolyte, and metal layers for source, drain and gate electrodes. In these devices, the migration of a small quantity of ions from the electrolyte into the conducting polymer channel (which is the result of the application of a gate voltage) results in a large modulation of the drain current due to dedoping. This electrochemical process is reversible and therefore, OECTs can be effectively switched on and off in aqueous electrolyte media, making them valuable devices for ion-to-electron transduction.

A novel microfluidic technique to direct fluid flow involves patterning of surface energy. This surface-patterned microfluidic channel consists of hydrophilic microfluidic pathways and hydrophobic ‘virtual’ walls. When a drop of aqueous solution is placed on an appropriately patterned surface, the aqueous solution is confined by the hydrophobic ‘virtual’ wall and allowed to flow ‘spontaneously’ only along the hydrophilic pathways. The capillary action generated by the hydrophilic surface is the driving force that causes flow in this system and therefore no external pressures are required to direct the flow.

In this project, we demonstrated OECT biosensor arrays in which the flow of analyte solution can be guided via a surface-directed microfluidic system. The OECT sensors are preloaded with a solution of an enzyme (e.g. glucose oxidase) and give specific responses to particular analytes (e.g. glucose). Because most analyte solutions of interest to biosensing are aqueous, the integration of OECT sensors with surface-patterned microfluidic system described above can easily yield simple and effective devices for multianalyte sensing. Also, direct and precise alignment of the microfluidic channel with the OECT arrays can be achieved via contact alignment tools during the fabrication.

Figure 1 shows the optimized process for the fabrication of the OECT biosensor arrays with microfluidic channels. Devices were composed of a top reservoir and bottom wells which were connected via microchannels. In order to make the surface-directed microfluidic channel, (tridecafluoro-1,1,2,2-tetrahydrooctyl)trichlorosilane (FOTS) was deposited on a glass slide substrate with Pt contacts deposited on it. FOTS treatment was carried out with the MVD 100 and resulted in a hydrophobic surface with a water contact angle of 100°. The poly(3,4-ethylenedioxythiophene) doped with poly(styrene...
sulfonate) (PEDOT:PSS) channel was patterned using a parylene lift-off technique. After the formation of the PEDOT:PSS channel, standard photolithography and oxygen plasma etching were carried out to form a hydrophilic microfluidic channel within the FOTS hydrophobic area. The size of reservoir and the width of microfluidic channel were 1.2 mm and 500 µm, respectively. The spontaneous flow of water in the surface-patterned channel is shown in Figure 2. After placing a drop of water on the top reservoir, the water began to flow spontaneously along the hydrophilic channel and reached each well at the same time.

The integration of OECTs with microfluidic channels makes it possible to distribute an analyte from a single drop to four channels. To evaluate the detection capability of the OECT biosensor array, a 10 mM glucose solution in PBS was placed on the reservoir. After the glucose solution filled each well via spontaneous flow, the enzyme glucose oxidase (GOx) was added to one of the wells. Figure 3 shows the relative change of drain current (I_{ds}) as function of gate voltage (V_{g}) for two different OECTs. The drain voltage was fixed at -0.2 V during the measurement. On the well in which the GOx was added, I_{ds} decreases by almost 90% at 0.5 V of V_{g}, as compared to a 20% decrease of I_{ds} in the well with PBS. This result demonstrates the potential of these microfluidic-based biosensor arrays for multianalyte sensing devices.

To optimize the response of OECTs, a thorough understanding of the device physics is necessary. For this purpose we studied the response of OECTs having different ratios of gate/channel area with metallic (e.g., Pt) and PEDOT:PSS gate electrodes (Figure 4). We have developed a micro-fabrication process that enables the fabrication of OECTs with high throughput and good reproducibility of electrical characteristics. The fabrication process was carried out on 4 inches glass wafers and consists of three main steps that combine photolithography, metal and polymer deposition, etching, unconventional patterning and surface treatment. The first step (lift-off) leads to the definition of the metallic electrodes on the glass surface. In the second step, the transistor channels and the PEDOT:PSS electrodes are defined through a parylene process. Finally, a selective surface treatment with a FOTS layer creates hydrophobic regions on the substrate so that electrolyte solution is confined in the OECT channel. These devices will provide valuable information on the effect of device geometry and gate material on electrical characteristics and sensor response of OECTs. Besides that, they will be used in scanning probe microscopy studies that are expected to shed light onto the mechanism of ion transport in OECTs.
PMMA Lab-on-a-Chip for RNA Detection

CNF Project # 802-99
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Abstract:

We describe investigations toward a disposable polymer based chip for the isolation, amplification, and electrochemical detection of eukaryotic mRNA. We introduce a method to fabricate a copper master for rapid prototyping of poly(methyl methacrylate) (PMMA) substrates, as well as a novel means to realize gold interdigitated ultramicroelectrode arrays (IDUA) directly on the PMMA surface without the use of a metal adhesion layer. Finally, we demonstrate the capability of this lab-on-a-chip system to isolate the mRNA of Cryptosporidium parvum and to detect the amplicon from a single C. parvum oocyst.

Summary of Research:

We have been working on efforts to develop a lab-on-a-chip for the detection of specific eukaryotic mRNA. Our first objective was the microfabrication of a sturdy and easily producible hot embossing master for imprinting microchannels into PMMA substrates. This was accomplished by electroplating copper channel molds onto a copper plate patterned with a KMPR negative photoresist (Figure 1). The channel molds had a height of approximately 35 µm high with a variation of ±1 µm. The copper material has a linear coefficient of expansion closer to that of PMMA than silicon which is traditionally used for hot embossed prototypes, and therefore released the substrate much easier.

Microchannels were then made from the copper master which were used for the mRNA isolation of Cryptosporidium parvum. With oligo (dT)25 beads, and an incorporated sawtooth micromixer [1], the device was able to isolate enough hsp70 heat shock mRNA from as few as five C. parvum oocysts to result in a successful nucleic sequenced based amplification off-chip.

The next objective was to incorporate a working IDUA into the microchannel for the detection of the hsp70 amplicon. In order to avoid galvanic reactions, a secondary metal substrate was avoided as the adhesion layer. Instead, the PMMA surface was UV-modified for carboxyl formation, and then conjugated with...
cystamine to provide a thiolated surface. A gold electrode was then patterned on the PMMA using the thiol-gold interaction as an adhesion mechanism. The PMMA containing the IDUA was then UV-bonded [2] to the hot embossed PMMA (Figure 2).

For the amplification detection, superparamagnetic beads tagged with a capture probe were mixed in the channel along with the amplicon from a NASBA reaction. Added to this were liposomes containing potassium ferro/ferricyanide which were tagged with a reporter probe [3]. The bead and liposome formed a sandwich assay with the available amplicon and were then captured by a magnet over the IDUA.

Following a washing step, a detergent was then pumped into the channel to lyse the liposomes and release the potassium ferro/ferricyanide. A 400 mV potential was applied across the electrode and the resulting current was proportional to the bound liposomes.

This device was able to detect the amplicon resulting from a single C. parvum oocyst. Our lab is currently working on developing on-chip NASBA amplification for the final component of the lab-on-a-chip.

References:


Platinum Electrochemical Detectors to Study Exocytosis and Stimulus-Secretion Coupling

CNF Project # 848-00
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Abstract:

Neurons release neurotransmitters in a process called exocytosis, wherein packets of transmitter molecules are released from the cells upon stimulation. Adrenal chromaffin cells and mast cells, among others, also undergo exocytosis. Release of adrenaline from chromaffin cells or serotonin from mast cells can be measured using the electrochemical technique of amperometry, historically employing a carbon fiber electrode [1]. To gain information about the exocytotic mechanism, we have developed various planar amperometric electrode arrays on glass coverslips employing platinum as the electrode material. We have successfully measured electrochemical events using these electrodes, and have developed devices that include stimulatory molecules patterned onto the coverslips to study stimulus-secretion coupling in mast cells. Because the electrodes are fabricated on glass, the devices can also be combined with fluorescence imaging techniques, yielding more information about the exocytotic process.

Summary of Research:

Several important cell types, including neurons, chromaffin cells of the adrenal gland, and mast cells (responsible for such things as allergic immune response), release transmitter molecules via exocytosis—the release of membrane bound packets or “vesicles” of transmitter molecules. Exocytosis can be observed by amperometry, an electrochemical detection method. In amperometry, transmitter molecules released during an exocytotic event are oxidized by a nearby electrode, generating a measurable current signal. Historically, carbon fiber electrodes have been employed for this purpose [1].

We have fabricated a variety of planar electrodes and electrode arrays that take advantage of this electrochemical detection technique. Electrodes are fabricated of platinum (Pt) on glass coverslips, with fused silica (SiO₂) or photoresist insulation. The advantage of fabricating the electrodes on glass is the ability to see through the coverslips to the cells above, and allows additional information about cell function to be recorded via brightfield or fluorescence microscopy simultaneously with the electrochemical measurements.

Previously, we demonstrated that amperometric...
signals from chromaffin cells can be measured using Pt electrodes patterned on a glass coverslip while simultaneously observing fluorescence from the cell between the electrodes. And, that the Pt electrodes behave similarly to carbon fibers [2].

Recently, we have made several advances in our electrode design and functionality. We have redesigned our original four-electrode arrays such that over three times as many experiments can be done with the same coverslip (Figures 1 and 2). The four-electrode arrays were created for simultaneous amperometric measurement from four electrodes placed about a cell, which allows us to determine the location of an exocytotic event on the surface of the cell [2]. The new four-electrode array design allows a much higher experimental throughput, with less manipulation of the coverslips.

Furthermore, we have developed electrode designs that incorporate stimulatory molecules onto the coverslip surface. We demonstrated direct measurements of single vesicle exocytosis of rat peritoneal mast (RPM) cells stimulated by Poly-D-Lysine (PDL). Planar platinum electrodes were insulated with fused silica and a circular region (~5 µm diameter) in front of the individual electrodes was selectively coated with PDL using dry lift-off of Parylene-C. RPM cells were placed on top of the electrode/PDL regions using a glass pipette and standard patch-clamp techniques (Figure 3). Exocytosis was monitored amperometrically as current spikes corresponding to oxidation of serotonin, which is secreted by RPM cells (Figure 4). This method provides direct measurement of single cell exocytosis stimulated by a localized stimulus patterned with micrometer precision.

Taken together, these devices make up a unique tool set to study the exocytotic mechanism on a cellular and sub-cellular level.

**References:**


Microfabricated Components for DNA Extraction

CNF Project # 854-00
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Abstract:
BioArray Solutions (BAS) is developing BeadChip™ array based automatic DNA assay platform. DNA extraction is an important step in the assay process. It is costly to integrate commercially available kits for DNA extraction in BAS’s assay platform. We are developing microfabricated components that can be used for DNA extraction in BAS’s assay platform.

Summary:
DNA extraction components were fabricated on silicon. The fabricated structures are based on proprietary designs to facilitate DNA retention from samples. These DNA extraction components will be integrated with the BeadChip™ platform previously developed for DNA detection based on color-coded microparticles (“bead”) assembled into a silicon chip [1].

References:
Nanofabrication of Quartz Cylinders

CNF Project # 868-00

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Abstract:

Optical tweezers help scientists study and manipulate biological structures at the single molecule level. Recently, a variety of methods have been proposed for torque generation with optical tweezers via the transfer of spin angular momentum. Performing rotation experiments with these modified tweezers necessitates specialized, anisotropic trapping particles. We have nanofabricated a large quantity of crystalline quartz trapping particles in order to facilitate such optical rotation experiments.

Summary:

Our design consists of a circular cylinder made from crystalline quartz, such that the extraordinary susceptibility, \( \chi_e \), of the crystal lies in a plane parallel to the cylinder’s radial direction. Quartz cylinders were nanofabricated from a single-crystal quartz wafer using standard photolithographic techniques. Resist was spun onto the quartz surface, patterned, and developed. The wafer was etched, and the cylindrical posts were removed from the surface.

We calibrated the optical properties of the quartz cylinders, including the maximum force and torque that can be exerted on a cylinder, and assessed heterogeneity using a single beam optical trap. A 100×, NA 1.3 objective is mounted in an inverted microscope. The quartz cylinder is rotated in the trap by controlling the direction of polarization of incident light. The laser’s polarization angle is controlled with a response time of a few microseconds using acousto-optic modulators. External torque exerted on the particle is measured by monitoring the angular momentum change of transmitted light using detectors that measure the difference between right and left circular polarizations. Cylinders fabricated in the same batch have a narrow distribution of optical properties.

Upon calibration, the cylinders were used in our angular optical trap to study torsional response of deoxyribonucleic acid (DNA). We directly measured DNA torsional modulus, determined basic relations regarding the dependence of torque on applied force and observed abrupt plectoneme formation.

References:


Figure 1: Array of 0.5 µm diameter circular posts etched into a single-crystal quartz wafer.

Figure 2: Quartz cylinder after removal from the wafer surface.
A Three-Dimensional Hydrodynamic Focusing Manifold

CNF Project # 884-00

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Abstract:
A manifold was developed to focus a fluid stream into the central volume of a square microchannel. The manifold was designed for operation in rectangular microchannels and can be readily constructed with photolithography. A set of channel junctions of cleverly designed geometry was employed to confine an input stream on all sides by sheath fluid and position the input stream at the center of the microchannel. The fluid distributions were observed downstream of the manifold with confocal microscopy and were observed to match the simulations.

Research Summary:
Fluid focusing manifolds have been developed to operate within microchannels for several applications including flow cytometry, diffusion mixing, and microfiber synthesis. Hydrodynamic focusing is currently the more commonly employed method of fluid-focusing within microchannels and is achieved by applying a pressure differential across the inputs and output(s) of a manifold junction. Hydrodynamic focusing within microchannels operated at low Reynold’s Number leverages the property of laminar flow, allowing the impinging of fluid streams into one another to be choreographed by informed manifold design.

Here, we report the design and characterization of a simple hydrodynamic focusing manifold for which the central design requirement was that the input stream be maneuvered into the geometric center of the channel. A secondary design consideration was fabrication simplicity. In particular, our manifold was designed for construction from two patterned surfaces of polydimethylsiloxane (PDMS) manifold housing. In addition, the number of fluid inputs was minimized in order to simplify packaging and run-time operation.

Manifolds satisfying the design considerations were simulated using ANSYS Computational Fluid Dynamics Modeling Software. Several variations were defined as a 3D FLOTRAN-142 element. The feature height for both layers was chosen to be 125 µm, and the width of the input and output channel was chosen to be 125 µm so that the input and output channel have square cross-section. The simulation is a two-species transport solution obtained by loading the central input with a species of molecular weight 480 (fluorescein) and by loading the sheath inputs with a species of molecular weight 330 in water (rhodamine). The geometry was solved with 60-global iterations of the preconjugated residual method over the manifold volume, which was meshed with an element edge length of 5 µm.

The manifold of this geometry was fabricated in the Cornell NanoScale Facility using standard soft lithography techniques and was observed to function as predicted using food coloring indicator dye and an optical microscope.

Confocal microscopy was used to experimentally image the cross-section of the output of the manifold. Fluorescein dye was pumped into the input for focusing, and rhodamine dye was pumped into the sheath fluid inputs (Figure 1). The experimental result is shown side-by-side with the simulated concentration distribution of fluorescein (Figure 2). The manifold is shown to operate as predicted and could become useful in a microfabricated flow cytometer, a diffusion micromixer, or a microfiber gellation/polymerization system.
Figure 1: Schematic of confocal microscopy imaging experiment. Sheath fluid labeled with rhodamine and sample fluid labeled with fluorescein are pumped into the flow-cell, and the output is imaged with a confocal microscope to obtain the concentration distributions across a cross-section of the channel.

Figure 2: 3D Fluid Focusing. A fluorescein-labeled stream is cylindrically focused by rhodamine-labeled sheath fluid. The channel dimension is 125 µm height by 125 µm width. Left: Simulation based on the Finite Element Method. Right: Experimental measurement with confocal microscopy.
Measuring the Mechanical Response of Single-Cell *E. coli* to Chemotactic Agents using Microfabricated Silicon Nitride Cantilevers

CNF Project # 884-00
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Abstract:

A method for measuring the mechanical response of single *Escherichia coli* cells to chemotactic agents using a microfabricated gold-coated silicon nitride cantilever. Changes in motion of the cell immobilized near the free end of the cantilever due to changes in the chemical environment are sensed by a shift in the resonance frequency (1st flexural mode) of the cantilever. The thermally excited resonance frequency of the cantilever is measured using a PicoPlus liquid cell setup by focusing the laser on the back of the cantilever. The cantilever with the immobilized cell is immersed in leucine solution, an *E. coli* chemotactic attractant at low concentrations, and a 0.97% increase in frequency is observed.

Summary of Research:

Microfabricated gold-coated silicon nitride cantilevers are used to immobilize and measure the mechanical response of single *Escherichia coli* cells to chemotactic agents. The fabrication process of the gold-coated silicon nitride cantilevers are summarized in Figure 1. Briefly, thermal oxide was grown on a silicon substrate, followed by formation of a silicon nitride film by low pressure chemical vapor deposition (LPCVD). Cantilever arrays are patterned in the silicon nitride film and a thin layer of gold with 10-micron square openings at the free tip is defined using standard photolithography and an e-beam evaporation and liftoff procedure. Release of the cantilevers is conducted via a through-wafer backside KOH etch, followed by a buffered oxide etch and critical point drying. Scanning electron microscopy (SEM) verification of the device dimensions (Figure 2) and finite element analysis were performed to estimate the resonant frequency of the cantilever.

Figure 1: Process flow for fabrication of gold-coated cantilevers with a silicon nitride opening

Figure 2: 45º-tilt scanning electron microscope (SEM) image of a 400 µm-length gold-coated cantilever with a SiN square pad.
The gold is passivated with PEG-thiol, and a single cell is immobilized on the exposed nitride pad via the electrostatic interactions of poly-l-lysine hydrobromide to the silicon nitride (Figure 3) on top of the cantilever. The functionalized cantilever is dipped in a concentrated culture of GFP-modified *E. coli* cells for cell capture, and washed extensively to remove background cell adhesion, and verified fluorescently (Figure 4).

The reflected laser beam bounces off the back of the cantilever and is monitored in a PicoPlus AFM liquid cell setup. The free thermal noise vibrations of the cantilever are recorded using Thermal-K® software, both in air and in buffer solution. In buffer solution, an increase in the resonant frequency was not observed with the adhesion of bacterial cells at the free end. In low concentration leucine, an *E. coli* chemo-attractant, the resonant frequency of a 400 µm-long cantilever is seen to increase by 50 Hz (0.97%) indicating an increase in tensile stress on the cantilever.

We hypothesize that this change in tensile stress to be the result of stimulated flagellar motion. Since cantilevers exhibiting a large bending modulus and a small length scale are known to be able to retain a larger amount of surface stress and hence higher sensitivity, we are currently working on performing measurements with smaller size cantilevers in order to achieve higher sensitivity in our measurements. We are also trying to quantify the response of the bacterial cell to different concentrations of chemotactic reagents.

**References:**


Bacterial Colonization and Communication in a Microfabricated Fluidic Channel

CNF Project # 935-01
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Abstract:

We are studying biological aspects of the bacterial plant pathogen Xylella fastidiosa. This bacterium is confined to live in the water (sap)-conducting xylem vessels of plants. The mechanisms of infection and plant colonization of this pathogen are being studied in microfluidic chambers used as “artificial xylem vessels.” Using this approach, we were able to understand X. fastidiosa movement against the sap flow, and explain the role of two types of pili in this bacterium. We are currently studying the phenomenon of autoaggregation, in which cells rapidly attract each other and form large masses in microfluidic channels.

Summary of Research:

The plant pathogenic bacterium, Xylella fastidiosa, causes important losses in grapevines and other high value crops. The bacteria exclusively inhabit the water conducting xylem vessels of the plant hosts where it is believed that X. fastidiosa causes disease by developing bacterial aggregates and biofilms that block xylem sap flow. Because temporal and spatial activities of the bacteria can not be observed microscopically within the plant, microfluidic devices have been developed to mimic features of plant xylem.

Using such devices, we discovered that X. fastidiosa migrates upstream via hair-like structures (type IV pili) [1,2] that are repeatedly extended and retracted from the bacterial cell in a movement known as twitching. Such movement explains, in part, the spread of bacteria in the host plant against the flow of sap [1]. In addition to type IV pili, X. fastidiosa possess shorter type I pili positioned at the same cell pole. Another aspect of the disease caused by these bacteria is that after some period of time (months), disease symptoms seemingly appear ‘overnight’ as thought all the bacteria accumulated at the ends of the xylem vessels and formed a plug interfering with water flow. Earlier observation in the microfluidic chambers indicated that an “autoaggregation” of individual cells occurred after several days. This phenomenon was further explored this past year.
Autoaggregation occurs as widely dispersed *X. fastidiosa* cells come together to form compact cell masses, over a period of hours (2-8h) following 7 to 11 days of growth in microfluidic chambers (Figure 1) [3]. Studies involving the use of mutants defective in polar-positioned shorter type I, and longer type IV, or both types of pili, revealed the importance and role of pili in the autoaggregation process (Figures 2-4). Cells need the long type IV pili in order to form spherical and compact cell masses (Figure 2). Formation of such large masses of cells, albeit in a shorter time frame, possibly explains in part the sudden appearance of disease symptoms in plants. It is possible that widely dispersed *X. fastidiosa* cells in xylem vessels reach a critical population density, aggregate into large cell masses and plug the vessels thus leading to disease symptom expression.

Through the use of microfluidic devices we are gaining information on the biology and the infection process of a xylem-inhabiting bacterial plant pathogen, which may lead to the development of novel control methods.

References:


Microfluidic Chip to Measure Response of Single Cells to Extracellular Stimuli

CNF Project # 990-01
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Abstract:

We have developed a microfluidic chip that is capable of measuring volume change in single cells in real-time. The sensor chip was constructed using photosensitive polymer (SU-8) on a Pyrex® glass substrate. Using MDCK cells, we have measured the response of a single cell to osmotic challenges. The results show that the impedance based sensing provides the required sensitivity and resolution to detect volume change down to a single cell. The microfluidic chip allows rapid and convenient change of solutions, enabling detailed studies of various drugs and chemicals that may play important role in cell physiology at the single cell level.

Summary:

The transport of ions and other solutes across a cell membrane reflects cellular metabolism, neurotransmitter activity, as well as interaction with extracellular environment [1,2]. Since transport activities introduce the changes in intracellular and extracellular osmolarity, it causes the cells to change their volume. Thus, real time measurements of cell volume provide direct access to studying transport activities and cell physiology. The microfluidic lab chip enables handling and analysis of single cells on chip in a convenient manner, and provides continuous delivery of various solutions with precise control. It is an ideal platform for studying cellular physiology at the single cell level [3].

We have developed a lab-chip that can measure the volume changes of a single cell in real-time. We demonstrate that the impedance based sensing method can be utilized to measure the activities of single cells with high sensitivity and resolution. The chip allows rapid change of solutions enabling high throughput screening of cell response to extracellular stimuli. Moreover, the morphological change of cells can be simultaneously monitored using an optical microscope.

The sensor chip exploits the fact that cell membranes are electrical insulators at low frequencies. As in a coulter counter where the cells passing through an orifice change the impedance of the aperture, a captured single cell in a defined sensing zone of the chip causes the resistance of the sensing zone to change [4,5]. In this work, a narrow sensing region was created along the fluidic pathway, within which a chevron like feature was designed to capture suspended cells in the solution. Once captured, various solutions can be readily perfused through the channel, and the resulting

Figure 1: SEM image of single cell sensor. The narrow rectangular region with two sensing electrodes defines sensing region. The enlarged micrograph shows the details of V-shape feature in the sensing region for localizing cells.
changes in cell volume can be followed via impedance measurements in real-time. The single cell sensor chip was constructed using photosensitive polymer (SU-8) on a Pyrex glass substrate. A standard optical lithography technique was used to fabricate the sensor chip. Using the lift-off technique, 200 nm thick thin film platinum electrodes were deposited on the glass wafer by e-beam. The fluidic channel was then constructed using SU-8 photoresist following the standard processing protocol. A programmed temperature ramp-up hard-baking process was applied after lithography; this process also helped to heal the micro cracks in the SU-8 structure.

The single cell sensor was tested by measuring the impedance change due to the capture of individual single-cell. The cell capturing events in the microfluidic channel were simultaneously monitored using an optical microscope. A dilute cell suspension of MDCK cells in isotonic solution was then perfused through the channel inlet at a flow rate of 1.02 µL/min giving a linear velocity of approximately 1 mm/s between the chevron. A stepwise increase was observed whenever a cell was captured.

The typical sensor output of a single cell capturing event was 8 to 14 mV, depending on the original size of individual cells. The cell volume change in response to changes in extracellular solution was recorded as a function of time. When a single cell was arrested in the sensing zone, the solution was switched to isotonic solution (without cells) to establish a baseline representing the resting cell volume. The channel was then perfused with hypotonic solution to cause cell swelling. The volume change of the single cell in response to hypotonic challenge was measured in real-time using impedance measurement, and the change in cell volume was simultaneously recorded using optical microscopy. Results show that both maximum swelling and the time course of the swelling are different in different cells.

References:
Patterned Surfaces to Investigate Spatially Regulated Mechanisms in Immune Cell Signaling

CNF Project # 996-01
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Abstract:
Micro- and nano-fabricated surfaces have been widely used for applications in cell and tissue engineering. However, the full potential of these technologies has not been explored, particularly in the area of molecular cell biology. By using these technologies we are investigating fundamental mechanisms in immune cell signaling, specifically IgE receptor (FcεRI) signaling involved in allergic responses on mast cells. We are interested in learning the spatial regulation mechanisms for intracellular signaling events and the role of the actin cytoskeleton and early signaling components in these processes.

Summary:
We have been using patterned surfaces as a tool for visualizing spatial distribution of signaling molecule upon IgE receptor activation on the cell surface [1,2]. We have used standard photolithography techniques and the polymer lift-off method to fabricate surfaces containing patterned lipid bilayers or immobilized protein carriers with haptenst that serve as antigens [3]. Immobilized antigens/hapten bind and cross-link FcεRI-bound IgE on the surface of mast cells, thus activating signaling events in these cells (Figure 1). By spatially clustering receptors on the surface of mast cells we are able to control and observe the local environment in which signaling molecules undergo a series of biochemical events. We are studying the dynamics of the actin cytoskeleton and other signaling components including Syk and Protein Kinase C (PKC) following FcεRI mediated activation. We have found that F-actin and other actin binding proteins commonly associated with focal adhesions such as vinculin, talin and paxillin are recruited to the clustered receptor sites (Figure 2) and that this local recruitment may be mediated by interactions with Lyn kinase. Biochemical data confirms that paxillin plays a role in IgE receptor signaling and that activation of vinculin and paxillin is specific to IgE clustering and not due to mechanical tension. In addition, we have found that Syk but not PKCβ is locally recruited towards the activated IgE receptor and PKCβ shows an oscillatory translocation to the plasma membrane.

References:
Figure 1: Cartoon representation of the interaction between receptors on the cell surface and the patterned lipid bilayers.

Figure 2: Visualization of the redistribution of signaling components upon stimulation with fluorescence microscopy.
Low Noise Biomimetic Differential Microphones
Inspired by the Ears of the Parasitoid Fly, *Ormia Ochracea*

CNF Project # 1116-03
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Abstract:

A prototype of a miniature differential microphone having a low noise floor was successfully fabricated and tested. The input sound pressure-referred noise floor of a differential microphone increases as the distance between the two pressure sensing locations decreases, which causes the sensitivity to decrease. By combining novel diaphragm design inspired by the coupled directionally sensitive ears of the fly *Ormia ochracea* and the use of low-noise optical sensing that has been integrated into the microphone package, both the diaphragm thermal noise and the electronic noise of the microphone are minimized. The measured sound pressure input-referred noise floor of this miniature differential microphone is less than 36 dBA.

Research Summary:

The purpose of this project is to fabricate a miniature differential microphone that is able to accurately detect acoustic pressure gradients with minimal influence of microphone noise. The microphone is intended to be used in hearing aids. The sensitivity and noise of directional microphones are strongly dependent on their size, which must be kept to a minimum in hearing aid applications. By combining a novel biomimetic microphone diaphragm and low noise optical sensing, a miniature differential microphone with minimal thermal and electronic noise has been successfully fabricated and tested.

The differential microphone developed in this project is inspired by the mechanically coupled ears of the
fly *Ormia ochracea*. The microphone diaphragm is designed to behave like a rigid plate that rotates about a highly compliant central hinge due to an applied moment resulting from an incident sound wave. The mass, stiffness and passive damping is minimized in the diaphragm design in order to achieve adequate sensitivity to sound and low thermal/mechanical noise. This differential microphone responds well to minute sound pressure gradients even though the dimensions are only 1 mm by 2 mm.

A high-sensitivity, low-noise optical sensing scheme instead of the commonly employed capacitive sensing scheme is incorporated in order to transduce the motion of the diaphragm into an electronic signal. This avoids the instability due to the bias voltage required in capacitive sensing when used on highly compliant diaphragms and allows us to have a very compliant diaphragm to achieve good sensitivity to pressure gradient. The thermal noise is also minimized by keeping sources of fluid damping by the air to a minimum.

The combination of a low-noise optical detection scheme and a highly compliant and responsive diaphragm has resulted in a system having low noise relative to the desired pressure gradient being sensed over much of the audible frequency range. The measured response of the microphone matches very well with predictions.

**Acknowledgements:**

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**References:**

Microfluidic Materials

CNF Project # 1119-03

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Abstract:

Materials often act as hosts for physical, chemical, and biological processes: e.g., as supports for catalysts, matrices for drug delivery, and scaffolds for tissue engineering. In these contexts, the rate of the processes is prone to limitation by the rate of mass transfer, as the material hinders convection such that the delivery and extraction of reagents must occur by molecular diffusion. The incorporation of fluidic paths within such materials can ensure adequate rates of mass transfer by allowing fresh streams of fluid to flow through the convective paths, such that solutes in the fluid diffuse into the bulk of the materials. In several projects that involve work at the CNF, we are developing lithographic techniques to embed microfluidic structure in diffusively permeable materials such as hydrogels that are appropriate for biomedical as well as physical applications. These applications include: tissue engineering, active wound dressings, and mimicry of the transpiration process in vascular plants to manipulate water at negative pressures.

Summary:

Here we summarize progress on two projects:

1) Transport in biomaterials. We have introduced a new approach to engineer mass transfer within biologically compatible hydrogels based on the formation of microfluidic structures directly within the gel: the microfluidic network acts as a vascular system for the material to provide efficient, convective mass transfer down to a scale on which diffusion through the material is fast. We have pursued this theme in collaborations with Prof. Lawrence Bonassar (BME, Cornell), Prof. Suzanne Schwartz, M.D. (Surgery, Weill Cornell), and Prof. Claudia Fischbach (BME, Cornell).

Progress: i) Fabrication. We have developed methods to fabricate functional microfluidic structures within biocompatible hydrogels (Figure 1A - Cabodi et al., 2005; Stroock and Cabodi, 2006; [2]): prior to this work, microfluidic systems had been formed in diffusively impermeable materials such that chemical or biological function was limited to the fluid-filled channels: the bulk of the host material acted only as a passive, mechanical support. ii) Engineering principles. We have established principles for the design and operation of these microfluidic constructs to accommodate both engineering and biological constraints. In the contexts of active wound dressings [1] and scaffolds for three-dimensional culture (Cabodi et al., 2005; Choi et al., 2007), we have developed formal models of the relevant transport phenomena and validated these concepts with quantitative experiments. iii) Microfluidic tissue scaffolds. We have demonstrated the biological relevance of

![Figure 1: Mass transfer in a microfluidic scaffold seeded with chondrocytes. (A-B) Fluorescence images showing top (A) and cross-sectional (B) views of scaffold. Grayscale indicates degree of conversion of a metabolic dye delivered via microchannels. (C) Quantitative fits using kinetic rate as fit parameter of distributions of metabolite at three densities of cells. (Choi et al., 2007).](image-url)
microfluidic scaffolds for tissue engineering [3]. In particular, we have shown viability of a variety of primary and immortalized cells cultured in these scaffolds and microscale control of distributions of both reactive (e.g., metabolites) and non-reactive (e.g., growth factors) solutes (Figure 1C). These developments open important new paths toward the engineering of tissues of unprecedented dimension (e.g., by allowing convective delivery of metabolites to the core of a thick section of tissue) and complexity (e.g., by allowing for the maintenance of spatially heterogeneous distributions of growth factors).

2) Transport and thermodynamics of water at negative pressures. Vascular plants use liquid water at large negative pressures as a central component of the transpiration process: in the extraction of water from sub-saturated soil (via reverse osmosis) and in its movement vertically up to tens of meters against gravity (to maintain hydration during gas exchange with the atmosphere, provide evaporative cooling, and regulate the exchange of water with fruit and other tissues). Human technologies have never exploited water in this regime. To gain access to negative pressures in liquid water, we have mapped the process of transpiration onto engineering concepts and replicated it in a microfluidic system, a “synthetic tree” (Figure 2A).

**Progress:**

i) Microfabrication in hydrogels. We have developed methods to fabricate microfluidic structures directly within hydrogel-based membranes (Figure 4A [4]). This technique endows the membrane with a “vascular system” that provides intimate coupling between efficient, convective mass transfer over macroscopic distances and local permeation flow through the membrane. These techniques open new avenues to engineer transport processes in membranes for a variety of applications, such as separations by pervaporation, ultrafiltration, and water management in low temperature fuel cells. ii) Manipulating water at negative pressure. Based on these conceptual and experimental tools, we have developed experimental techniques to control the thermodynamics and dynamics of volumes of liquid water at large negative pressures (< -200 atms) via osmotic coupling to sub-saturated vapors [4]. Our “synthetic tree” represents a new experimental platform with which to access and control liquid water at negative pressures. Unlike previous methods of generating negative pressure in the laboratory, our method allows for net flow within the metastable liquid at steady state; this capability opens the opportunity to exploit liquids under tension in processes that require flow and to study the dynamic properties of this state for the first time. Highlights of our achievements with these synthetic trees include the first demonstration of evaporative heat transfer from liquids under tension and of continuous extraction of pure, liquid water from sub-saturated vapors (Figure 2B). These advances have dramatic implications for the design of heat transfer technologies (e.g., wick-based heat pipes) and for the management of water in environmental, civil, and agricultural contexts.

**References:**


Ultrasonic Microprobes with Integrated Sensors for Biomedical Applications

CNF Project # 1122-03
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Abstract:

We report on a microfabricated ultrasonic silicon horn actuator integrated with microprobes for biomedical applications. By incorporating microprobes at the narrow tip of the silicon horn, we have successfully demonstrated reduction of penetration force on biological tissues, thus serving as an efficient surgical tool. Sensors are microfabricated on the microprobes for tissue metrology. There are two main devices we have developed; 1) Microprobes with platinum electrodes along the length for in vitro cardiac action potential recording from canine left ventricle tissue, and 2) Piezoresistive strain gauges on the microprobes for mechanical and spatial characterization of seminiferous tubules in the testis to extract sperm.

Summary of Research:

Ultrasonically Actuated Silicon Microprobes for Cardiac MAP Recording: Silicon-based microprobes have been reported for three-dimensional electrical activity recording in neural tissues. The ventricular wall of the heart is known to have high tensile strength and so the microprobes will encounter a large force during insertion in cardiac tissues. Microfabricated silicon ultrasonic surgical tools have been reported before, which, by actuating the surgical tools at their ultrasonic resonance can reduce the cutting force on biological samples [1]. In this work, we integrate an ultrasonic horn actuator with the microprobes that are also able to reduce the penetration force significantly by ultrasonic actuation. For electrical potential recording, the microprobes have Pt/Cr electrode arrays along the length of the probes. Electrical recordings were obtained from isolated perfused sections of canine left and right ventricle. Figure 2a is a multichannel recording from the isolated perfused canine left ventricle, paced at a 700 ms cycle length by the surface stimulator and Figure 2b shows extracellular recording of VF. The activation time is identified as the moment of the maximum negative time derivative of the intrinsic deflection in
the cardiac signals. Taking the activation time in the uppermost channel (closest to the epicardial stimulator in this experiment) as baseline, the activation time delay progressively increases as the location of the electrodes moves downward, indicating the spreading of activation from the epicardial pacing site across the thickness of the ventricular wall. The mean time delay between the third and fourth, and the fourth and fifth channel from the epicardial surface is $\Delta t \approx 4$ ms. The conduction velocity can be estimated to be $\approx 0.5$ m/s, which is typical for ventricular myocardium.

**Silicon Ultrasonic Surgical Microprobes with Integrated MEMS Sensor:** We report on a microfabricated silicon probe integrated with ultrasonic actuator and polysilicon strain gauges for Micro-dissection TEsticular Sperm Extraction (TESE) surgery [2]. Tubules with low spermatogenesis have thinner walls and smaller diameter, as compared to healthy tubules. Our hypothesis is that by monitoring the force experienced by a microfabricated force probe inserted with 100-µm-scale incision, the surgeon can map the diameter and stiffness of tubule walls encountered during the incision.

The fabrication process of our microprobe is similar to the ultrasonic microprobes reported earlier [1], however in this work polysilicon strain gauges were integrated for force measurement, with differential force measurement across probes. Four polysilicon resistors are connected to form a Wheatstone bridge arrangement with two sense resistors for force measurement on each of the two microprobes (Figure 3). In this work, the Si microprobes are 5 mm long, 100 µm long wide and 140 µm thick projecting outwards at the tip of the horn. The tip of the microprobe has 2 sharp blade-like edges—a V-shaped edge on the top formed by the LPCVD nitride film and one at the bottom formed by silicon due to the backside-only KOH etch (Figure 4). The two sense polysilicon resistors are positioned on the microprobe near its clamped edge, so their resistance changes with the compressive/tensile strain experienced when the blade-like edges of microprobes pierce the tubule walls.

When the tubules are encountered by the blades on the microprobe, the strain experienced by the polysilicon resistors is due to a combination of pure axial compression and the bending of the cantilever. Depending on whether the tubules are encountered by the top (-0.977 V/N) or bottom (0.777 V/N) blade, the bending moment causes a compressive or tensile strain respectively. The microprobes are inserted into the rat testis tissue to a depth of 2 mm at 100 µm/s. Once inside the tissue, the blades on the microprobe puncture the tubules and the puncture artifact in the force signal is observed to be a negative spike (compression) for the top nitride blade and a positive spike (tension) for the bottom silicon blade (Figure 4). The average time between 2 consecutive puncture artifacts multiplied by the speed (100 µm/s) can be taken as the mean diameter of the tubules, since the tubules are densely packed wall-to-wall in the tissue. From the FFT of the positive and negative spike data, the effective tubule diameter was calculated to be 41.16 ± 1.58 µm (Figure 4). This is close to the expected tubule diameter of rat testis. At present we are integrating sample collecting channels in the probes that can be used to extract sperm from the ruptured vessels.

**References:**


![Figure 3](image_url)  
*Figure 3, left: Optical photograph of the fabricated device with integrated MEMS sensor.*

![Figure 4](image_url)  
*Figure 4: a) Detection of tubules (sensed force signal vs. time) in rat testis tissue, b) Normalized FFT of the time-domain force signal, with frequency modified to diameter of tubules sensed (x-axis).*
Fabrication of Thin-Walled and High-Aspect-Ratio Nanofluidic Channels

CNF Project # 1176-03
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Abstract:

Nanofluidics promise to solve the sample preparation problem in various spectroscopy experiments where the thickness of a sample is usually constrained by the penetration depth of the optical probe. We designed and successfully fabricated a nanofluidic cell which was used in a 2D-IR spectroscopy experiment to study the nature of the OH bond in water [1,2]. The fabricated structure employs a system of access holes and channels used for sample delivery and active thickness control.

Summary of Research:

The building blocks of the nanofluidic cell are two free standing membranes of low stress silicon nitride (SiN) back-etched on separate wafers using KOH. A silicon oxide (SiO₂) spacer deposited on one wafer is used to define a gap between the SiN membranes when the matching pieces of the structure are bonded together. The etching of the SiO₂ layer to create the sample gap is done with 6:1 buffered oxide etch (BOE). In our first prototype, the thicknesses of the SiN windows were 800 nm, and the cell was filled from side channels then sealed, trapping the liquid inside. Such a passive approach resulted in a poorly defined sample thickness due to the flexibility of the thin SiN membranes.

Therefore, for the new version, input and output access holes were etched into the surface of the structure as shown in Figure 1, and then connected to an external pumping system. The active control of the gap thickness was realized using the transmitted intensity of an IR beam through the cell as feedback signal. In addition, a 70 µm deep KOH etched channels were used to connect the narrow gap to the access holes therefore restricting the high flow resistance region to the sample area. Lastly, a hydrophilic surface is created by high temperature deposition (LPCVD) of ~ 10 nm silicon oxide [3] in order to enhance the filling of the cell. An assembled cell filled with water is shown in Figure 2. The variation in sample thickness is most apparent from the interference rings.

Figure 1: Schematic of nanofluidic device for nonlinear spectroscopy on thin sample liquids.

Figure 2: Nanofluidic cell filled with water. Edge thickness is ~ 1500 nm, center thickness is ~ 400 nm, window size 1 × 1 mm.
References:


A Novel Microprobe for Objective and Simplified Monitoring of Free Tissue Transfer

CNF Project # 1195-04
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Abstract:
We are developing an implantable device to monitor the concentration of oxygen and certain metabolic products in free tissue transfers. Prototypes of the device have been fabricated through a two-step lithography process. The micron-sized Pt electrodes aim to achieve fast diffusion from the surrounding tissue to the electrodes, and thus, guarantee rapid responses to changes in tissue oxygen concentration.

Summary:
For decades, reconstructive surgeons have attempted to patch or rebuild tissue deficiencies arising from congenital malformation, traumatic injury, or oncologic resection. The success of these surgical reconstructions relies on transferring well-vascularized tissue into the defect, especially when transferring tissue to compromised recipient beds that harbor infection or radiation injury. Autologous tissue transplantation using microsurgical techniques is currently the standard of care in many major reconstructive procedures. Aside from the technical difficulty that accompanies these complex and delicate surgeries, a major problem that remains is post-operative monitoring of the transferred tissue. Currently, post-operative monitoring is performed with few objective parameters and relies principally upon the experience and clinical acumen of those doing the monitoring. Therefore, a device that could measure indicators of transplanted tissue viability, such as oxygen partial pressure or lactate buildup, would be of significant clinical value.

The long range goals of this project are: 1) to fabricate a novel probe that can assess real time values of pO₂ and lactate concentration in tissue transplants; 2) to test the probe in vivo using flap models of both arterial and venous insufficiency; and 3) to apply and test this device in clinical trials involving human free tissue transfer.

Our initial prototypes have been fabricated on a four-inch silicon wafer. The process begins by using plasma enhanced chemical vapor deposition (PECVD) to deposit 500 nm insulation layer of silicon dioxide. Then, a 30 nm adhesive layer of Ti is deposited by vacuum evaporation. This is followed by deposition of 130 nm Pt layer, which forms the active electrode material. A lift-off process is
used to define the planar geometry of the electrodes. We employ a three-electrode configuration, which we expect will improve the stability of the system output during the measurement. The last step uses PECVD to form a 400 nm silicon nitride capping layer. After the last deposition, the sensitive areas of the electrodes, including bonding pads, are opened by exposure to \( \text{CHF}_3/O_2 \) plasma. The surface is then dip coated to form a Nafion membrane on top of the electrodes. Characterization of the probe by cyclic voltametry is in progress.

**References:**

Mimicry of Biological Adhesion Through Fabrication of Fibrillar Surfaces

CNF Project # 1225-04
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Abstract:

The extraordinary climbing ability of geckos is because of the fine structure of their toe pads. It contains a layer of fine fibrils terminated by spatula-like features. This project aims to fabricate artificial mimics of these structures to make dry adhesives. We have made adhesive surfaces using the polymer poly(dimethylsiloxane) (PDMS). Our samples have thin pillars (fibrils) standing on the PDMS base and are topped by a thin continuous film of PDMS. Great normal adhesion on this sample has been reported. We further investigate the frictional properties on these samples and have found this architecture can provide a significant static friction without altering its sliding frictional resistance. Our fabrication procedures include making a master mold by etching a Si wafer.

Sample Fabrication:

Samples with thin pillars/fibrils are made by molding PDMS (Sylgard 184, Dow Corning) in silicon molds. Silicon molds are made using standard photolithography and deep ion etch techniques. The depth of the holes is estimated by the duration of etch, and that decides the height of the pillars in the samples.

After the holes of desired depth and with desired pattern are made on the Si wafer, a self assembled monolayer (SAM) of the molecule n-hexadecyltrichlorosilane is deposited on the wafer to reduce its surface energy. The polymer is poured in the Si mold and sandwiched between a glass slide (having SAM on it) and the mold. It is then cured in an oven at 80°C for 2 hours and then kept in dry ice for around 6 hours. In dry ice, the PDMS shrinks more than silicon, making it easier to remove it from the mold.

The final step of affixing a thin terminal plate of PDMS on the pillars is accomplished by spin-coating a SAM-coated Si wafer with PDMS, and then placing the samples on this wafer with pillars in contact with liquid polymer. The assembly is then cured at 80°C for an hour in the oven. Glass coverslips are attached to the back of the samples while they are still on the wafer using O₂ plasma to activate adhesion between the two. Once the samples have the additional backing of a cover-slip, they are carefully pulled off the wafer. Figure 1 shows a typical sample.

Shear Experiment:

The shear experiments were conducted on a custom apparatus built on an inverted optical microscope on which the sample is placed. A spherical glass indenter with a 2 mm radius is placed on the fibrillar surface. The
surface of the indenter is treated with a monolayer to reduce the shear force between the indenter and the PDMS sample. The fixed normal force, \( P \), is applied by means of a mechanical balance. The actual value of normal force corresponding to this contact area is obtained by performing an independent indentation experiment as described in [2,4].

The shear is applied by translating the glass slide at a constant rate of \( \dot{u} = 30 \, \mu \text{m/s} \). The shear force is measured by a load cell in line with the balance arm. Deformation of the contact region is recorded by means of an optical microscope. Typical experimental force and displacement data for control and a fibrillar surface are shown in Figure 2. A set of images corresponding to different points on the force displacement curve (Figure 2b) is shown in Figure 3; the direction of motion of the substrate is indicated by the arrow.

Two main characteristics of the shear response of our fibrillar samples compared to the unstructured sample are: 1) the strong enhancement of the static friction force, and (2) dynamic friction force remains almost unchanged with changes in geometry.

References:


Fabrication of Inorganic-Organic Interfaces

CNF Project # 1239-04
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Abstract:

As feature sizes continue to decrease in semiconductor devices, a molecular level understanding of interfaces between dissimilar materials is becoming more critical in device manufacture. In this project, we are examining the growth of inorganic films by atomic layer deposition (ALD) on top of thin, surface-bound organic layers. Substrates on which the films are grown include well-characterized, flat SiO₂, as well as a porous low dielectric material. We find the kinetics of the ALD process are sensitive to the chemical functionality and microstructure of the interfacial organic layer.

Summary of Research:

Interfaces have always strongly affected the performance of solid state devices. From a simple diode to complex interconnects, inorganic-inorganic interfaces have defined semiconductor technology to date. As semiconductor manufacturing moves to future technology nodes, devices will shrink to dimensions approaching the molecular regime. At these small length scales, designs are envisioned which incorporate the use of organic molecules, both as active [1] and passive [2] components.

Organics are attractive because their structure and chemical reactivity can be customized using the tools of synthetic chemistry. Furthermore, they can be designed such that they form self-assembling structures which are organized on a molecular level. This project focuses on two stages of the formation of an organic-inorganic structure. The first is the growth and characterization of the organic layer, the second is the subsequent deposition of an inorganic film. We have mainly used “wet chemical” techniques to deposit the organic layer, while atomic layer deposition (ALD) in ultra-high vacuum has been used to deposit the inorganic layer. The main application envisioned for the work described here is the modification of substrates prior to ALD.

We begin by depositing or growing a layer of organic molecules on wet chemical prepared SiO₂ with a high surface concentration of -OH groups. In the past year, we have focused on branched molecules, with either -NH₂ or -OH termination. To obtain a branched -NH₂ functionalized film, first a monolayer of (3-aminopropyl) dimethylethoxysilane was attached to the SiO₂ surface. The surface was then treated with an acrylate to convert amine termination to a branched ester termination. Finally, the samples were immersed in a diamine solution to re-obtain -NH₂ terminal functionalities, in twice the concentration of the first monolayer. To obtain a branched -OH terminated organic layer, we have used a “single pot” synthesis technique which involves exposing an activated SiO₂ surface to glycidol [3], Figure 1.
The thickness of the resulting polyglycidol film, and thus its degree of branching, is determined simply by the length of time the substrate is exposed to the monomer. Once grown, the organic films are characterized using a variety of thin film analysis techniques, including ellipsometry, contact angle, atomic force microscopy (AFM), and x-ray photoelectron spectroscopy (XPS).

We next examine the reactions of the gas-phase precursors Ti[N(CH₃)₂]₄ (TDMAT) and Ta[N(CH₃)₂]₅ (PDMAT) with these organic layers [4,5]. In-situ XPS has proven especially useful in characterizing chemisorption of the organometallics. For both the -NH₂ and -OH terminated branched layers, increasing the degree of branching increases in the uptake of PDMAT. Furthermore, angle-resolved XPS shows that PDMAT penetrates on average 10-20 Å into the polyglycidol film, for films in the range of 35-80 Å. This is in contrast with previous chemisorption experiments on unreactive, -CH₃ terminated organic layers. In this case, the organometallic deposited on the SAM/SiO₂ interface [6]. Another key result from the chemisorption experiments is that the number of dimethylamido ligands lost by PDMAT on chemisorption increases with the thickness of the polyglycidol layer. On SiO₂, chemisorbed PDMAT loses on average one of its original five -N(CH₃)₂ ligands; on 30 Å polyglycidol it loses 3 ligands, while on 85 Å polyglycidol it loses 4 ligands (Figure 2).

These experiments are used to help understand the growth of TiN and TaN layers by ALD using the above mentioned precursors in combination with NH₃. A custom-designed UHV chamber in which the precursor is delivered to the substrate by a collimated molecular beam is used to carry out ALD. One advantage to using a collimated beam is that terraced films can be grown without a vacuum break, simplifying the examination of the film at varying stages of growth. We found that the thicker polyglycidol films, which caused PDMAT to lose more ligands on chemisorption, led to much lower ALD growth rates of TiN. ALD growth on thinner polyglycidol, however, was similar to growth on the branched -NH₂ terminated layers (Figure 3).

In addition to growth on planar, nonporous SiO₂, we have also studied the growth of organic films, and subsequent ALD deposition, on a porous low-κ material. We found that the infiltration of organic materials into the pore network of the low-κ can be controlled by changing the structure of the organic precursor. Furthermore, the kinetics of ALD TiN growth are similar on polyglycidol grown on SiO₂ and low-κ material.

References:

Figure 2: N(1s) and Ta(4p 3/2) XPS features of PDMAT adsorbed on 30 Å polyglycidol films. Fitting the features to a double peak allows for the calculation of the N/Ta ratio, indicating how many dimethylamido ligands were lost on chemisorption. The proposed chemisorbed species is shown on the right.

Figure 3: Growth vs. number of ALD cycles for a number of substrates. Note that for thin polyglycidol films, the growth rate is close to that for a branched -NH₂ terminated surface (see text). For thick polyglycidol, growth characteristics are similar to growth on an unreactive, 30 carbon -CH₃ terminated monolayer.
Polymeric Microfluidic Devices for Dielectrophoretic Manipulation

CNF Project # 1360-05
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Abstract:

This report discusses the development of a high-throughput separation technique aimed at separating cells based on phenotypic differences in membrane composition using dielectrophoresis. Successful separation of polystyrene microparticles with 20% variation in DEP mobility has been demonstrated. New device designs increase the dynamic range of possible separations by further modulating microchannel geometry.

Introduction:

This project involves the development of a dielectrophoresis-based, high-throughput microfluidic technique for identifying and separating cell populations with phenotypic differences in membrane composition. We focus on Mycobacterium in particular as the membrane composition of M. tuberculosis has been implicated in both pathogenicity and drug resistance of the organism. The gram positive bacterial membrane is composed of a cell membrane, peptidoglycan, and associated extracellular lipids. These outer membrane lipids include covalently and non-covalently attached moieties that we postulate contribute, in a detectable way, to the cellular “electrical phenotype” [3].

Mycobacterial membrane properties influence the cell’s effective electrical permittivity and conductivity and therefore change a cell’s electrical response as a function of membrane composition [4]; we take advantage of this change in electrical character using dielectrophoresis. We have designed a device that uses dielectrophoresis (DEP) — particle motion in response to a non-uniform electric field — induced using insulating constrictions in the depth of a microfluidic channel. An electrical potential is applied at either end of the channel by electrodes placed in open fluid reservoirs. This potential induces linear electrophoresis of particles and electroosmotic fluid flow. The constriction in the channel depth changes the current path, leading to a region of higher electric field intensity. The constriction region changes the electrical current path leading to non-uniform electric fields and dielectrophoresis.

Research Summary:

The dielectrophoretic force is particle dependent, but also depends on the magnitude of electric field gradients. Using geometric design of the channel constriction we modulate electric field gradients without resorting to microfabricated electrodes. This modulation is achieved by a curved constriction in channel depth (Figure 1).
We have developed a fabrication technique for hot embossing Zeonor 1020R polymeric devices from a silicon “stamp.” After the devices are embossed, a chemical bonding procedure is used to enclose the channel with a second Zeonor lid. These devices have a 250:1 width-to-depth aspect ratio, and must maintain high fidelity in order to operate [1,2]. The silicon master “stamp” was created using a two-step Bosch etch process to define microfluidic channels 50 µm deep and 2500 µm wide. Channel constrictions are 40 µm tall, leaving a channel gap of 10 µm. We have characterized our fabrication technique using mechanical profilometry after hot-embossing and confocal microscopy after enclosure.

After fabrication, fluorescent polystyrene microparticles are used to characterize device performance. A mixture of 1.7 µm and 2 µm diameter spheres are suspended in DI water and introduced via reservoirs affixed to the Zeonor substrate. Particle motion was observed using a X-Cite 120 fluorescence source and a Nikon TE2000U inverted microscope. Images and movies were recorded with a Q-Imaging Retiga EXi FAST camera and Phylum software. Image analysis was carried out using MATLAB (MathWorks).

Successful separation of the two microsphere populations was achieved and characterized using time-lapse images (containing approximately 200 frames of video) (Figure 2).

Most recently, we have demonstrated successful separation of 1 µm and 2 µm polystyrene beads using a curved constriction in channel depth placed in a curved microfluidic channel (Figures 3 and 4). The added curvature of the microchannel leads to predictable variation in the bulk electric field and additional modulation of electric field gradients.

References:
Ultra-Precise Cultured Cell Patterning Using Molecular Vapor Deposition of Self Assembled Monolayers and Lift-Off Technique

CNF Project # 1370-05
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Abstract:
Patterned growth of cultured cells is a technique gaining importance in a variety of different applications and fields, such as cell-based sensors, neurobiology and tissue engineering. In all applications, the fundamental idea is to form a pattern of alternating surfaces which are either permit (cytophilic) or inhibit (cytophobic) cell growth. The most frequently used technique to generate such patterns is microcontact printing (µCP). Microcontact printing does have some drawbacks, in spite of the fact that this technique is very simple and cost-effective. The major drawbacks that we have observed are the inability, using this technique, to create printed patterns precisely aligned to pre-existing structures on the substrate, and the inability to visualize the printed pattern prior to cell culturing. We developed a new cell patterning technique that is simple, effective and which eliminates the primary problems exhibited by its predecessors [1].

Introduction to Research:
The fabrication process for the cell-culturing substrate is illustrated in Figure 1 and is as follows. First, 100 nm of silicon oxide was deposited on a 3 inch silicon wafer at 130°C, and positive photoresist Shipley 1818 was patterned by photolithography. Then, a stack of two self-assembled monolayers was created by MVD. Heptadecafluoro-1,1,2,2-tetrahydrodecyltrichlorosilane (FDTS) was used as the organo-silane functional monolayer. The resulting FDTS film was annealed after deposition at 100°C for 30 minutes. FDTS SAM can be deposited directly on a silicon wafer.

In the subsequent MVD coating, a vapor deposited oxide layer is initially deposited, followed by a SAM. The silicon oxide functions as an adhesion layer and was created by the hydrolysis of silicon-chloride and water within the MVD reactor just prior the introduction of the FDTS SAM. The thickness of the adhesion oxide can be controlled to create sufficient optical contrast so that the pattern is visible under the microscope.

Figure 1: The patterning of GT1-7 cells using molecular vapor deposition of self-assembled monolayers and lift-off technique.
We chose to deposit a 10 nm thick oxide adhesion film on the top of a silicon wafer. Following MVD, the photoresist was stripped with acetone for 5 minutes, and the wafers were cleaned in ethanol by an ultrasonic cleaner. The wafer was then immersed in a 9 mM solution of trimethoxysilyl propyldiethylenetriamine (DETA) (Gelest, Inc., PA) for 1 hour, which has been shown to be favorable for cell growth. This step created a cytophilic surface wherever the MVD-deposited silicon oxide/cytophobic SAM bilayer was not already present. Then, the substrate was rinsed with ethanol and DI water, and dried with a stream of dry nitrogen to remove any excess solvent.

The prepared substrates were put into six-well cell culture plates (BD Biosciences, CA) and immortalized mouse hypothalamic neurons (GT1-7) were cultured in DMEM medium (Gibco, NY) with 10% fetal calf serum (FBS, Gibco, NY) at 37°C in an incubator with 8% CO2. After 48 hours of incubation, the GT1-7 cells were visualized with an E800 microscope (Nikon Inc, NY). The scale bars for all relevant figures were calibrated with a stage micrometer (OB-M 1/100, Olympus Inc, NY), and scale bars were added with the SPOT imaging software (Diagnostic Instruments, MI).

Figure 2 illustrates the cell patterning results we have obtained so far, and the pre-existing SAM patterns are included for comparison. In the upper panel of Figure 2, the first number on the left depicts the size of the square island suitable for cell growth (in this case, 20 µm, which we have determined is an appropriate size for anchoring a single GT1-7 cell). The second number depicts the width, in microns, of the pathway between two islands. For example, the width “0” denotes that there is no pathway between the islands, and that the islands are isolated.

On the left-hand side on Figure 2, one can see that eight out of sixteen available sites are populated by neurons. The right hand side of the same figure shows a well-developed neuronal network, with cell bodies occupying most of the 20 µm islands, with a network of neurites growing neatly along 5 µm-wide, cytophilic pathways.

One can see that we have obtained a faithful replica of the SAM pattern in the cultured neuron pattern.

**Summary:**

We have developed a simple and effective method for cell patterning, with single-cell resolution, utilizing molecular vapor deposition of a seed oxide/SAM bilayer. The patterns can be easily aligned with previously existing patterns and structures since the patterning alignment depends on photolithographic mask exposure rather than imprecise alignment during stamping. The primary benefit of this technique is that the SAM pattern is visible prior to cell culturing, which is beneficial for targeting sources of error in individual cell patterning steps. Furthermore, the hydrophobic surface is highly uniform and stable because of the silicon oxide seed layer which is a benefit for extended cell culturing times. Finally, this technique avoids the disadvantages of more traditional cell patterning techniques, such as deformation of the PDMS stamp fabricated using conventional molding process.

**References:**

A Microfabricated Cell Culture System for Modeling the Blood-Brain Barrier

CNF Project # 1372-05
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Abstract:

The blood-brain barrier is a complex polarized tissue formed by the interaction of several cell types including, neurons, astrocytes, and microglia, with vascular endothelial cells. It has been demonstrated that these interactions are necessary to induce blood-brain barrier phenotypes in endothelial cells. However, no study has yet to produce an in vitro model that accurately represents the permeability and transport properties of brain microvasculature, as measured by trans-endothelial resistance. We have developed a novel hydrogel-based method for modeling the blood-brain barrier and a microfabricated electrode array for making trans-endothelial measurements.

Summary of Research:

Our culture system consists of neural cells entrapped within an alginate matrix, functionalized by the covalent attachment of proteins and peptides. Neural cells attach within the hydrogel and display process outgrowth over time in culture. Endothelial cells can be subsequently cultured and allowed to attach to the alginate hydrogel surface (Figure 1a), thus producing a stratified culture for measurement of trans-endothelial electrical resistance (TEER), permeability to test compounds, and neural cell/endothelial cell contact [1]. Methods were developed for the efficient culture of primary brain capillary endothelial cells from rat and mouse. Expression of endothelial cell markers, including CD-31, von Willibrand factor, and tight junction proteins were assayed using immunohistochemistry for primary endothelial cell cultures, endothelial cell lines, and co-cultures of endothelial cells and glia.

Hydrogel co-cultures were constructed above microfabricated gold electrode arrays (3 mm x 3 mm x 310 nm) to permit measurement of TEER by tissue impedance spectroscopy. Electrodes were patterned on Pyrex® wafers by photolithography and lift off. The resulting devices contained 2 x 2 arrays of Ti/Au electrodes (10/300 nm) with connecting leads insulated with a triple stack of SiO₂, Si₃N₄, and SiO₂ (200 nm, 200 nm, 600 nm). Electrode impedance was characterized and ranged between 10 and 100 Ω for frequencies...
ranging between 10 kHz and 100 Hz. These impedance ranges allowed for sensitive measurements to be made of the electrical properties (resistance and capacitance) of the cell culture system.

Confocal fluorescence imaging was used to confirm the presence of an intact endothelial cell monolayer, the formation of tight junctions between endothelial cells, and to describe the direct interactions between co-cultured cells. Methods are being developed to permit transport studies of test compounds for comparison to other in vitro models and values measured in vivo.

References:
Planer Patch-Clamp Arrays: Neuronal-Electronic Interfaces for Examining Network Development in Neuronal Ensembles

CNF Project # 1377-05
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Abstract:
Recent advances in the experimental sciences and coupled data analyses have paved the way for investigating systems of increasingly complexity. One system of significant interest is neuronal networks: large composites of neurons interconnected via synaptic junctions. These networks not only form the physiological basis of many biological organisms’ central and peripheral nervous systems, but, functionally, are responsible for manifest higher order cognitive properties, including memory and logic [1]. Nevertheless, a thorough understanding of the manner through which these properties emerge as an undifferentiated collection of neurons comes to operate as a biological circuit has not yet been realized. And while the materialization of these functions implicates both the networks in question and the inputs they receive, understanding the spatiotemporal development of a model network’s connectivity in response to a series of applied inputs is an important first step toward decoding this emergence. To this end, we have fabricated planer arrays of patch-clamp electrodes in order that we might probe the afore-mentioned development on a one-electrode-to-one-neuron basis [2-5].

Summary of Research:
During the past year, we have completed the construction of a 10 × 10 planer array of patch-clamp electrodes at 100 µm pitch as a proof-of-principle. This test neuronal-electronic interface, meant to reproduce the geometry of many patch-clamp micropipettes protruding from a cell-culture glass coverslip, is based upon the previous work performed by Pantoja et al. [4] and Lehnert et al. [5]. Essentially, one hundred apertures or micronozzles, respectively, were generated in a silicon wafer using a series of gas etches.

Briefly, a reactive ion etch was used to define a silicon dioxide etch mask for a subsequent front side deep reactive ion etch. After these steps, the silicon wafer possessed a 10 × 10 array of 2 µm in diameter, 50 µm deep trenches. Using a backside-aligned photolithographic step, another reactive ion etch, and a second deep reactive ion etch, a 10 × 10 array of 25 µm in diameter trenches were etched to meet the front-side set, yielding 100 apertures within
the wafer. At this point, the wafer was either considered finished or micronozzles were made using a final front-side reactive ion etch and deep reactive ion etch. After electrically and electrochemically passivating the wafer with a series of MOS furnaces, it was ultimately diced for final packaging.

In the packaged state, as shown in scheme 1, the above-described interface is coupled to a PDMS-based microfluidic system and an electrochemically passivated platinum multi-electrode array. The microfluidic module is composed of two flow and two control layers stacked in an alternating fashion: 1) incoming flow, 2) lower-level control, 3) outgoing flow, and 4) upper-level control layers [6]. In the lower- and upper-level flow layers, the solution channels are 100 µm wide and 15 µm high except the central region where the 100 flow channels are individually directed toward the 10 x 10 array of electrode termini.

Here, the channel widths gradually reduced down to 30 µm. Each incoming flow channel in the lower level is aligned to a corresponding outgoing flow channel in the upper flow layer and vertical connecting channels are drilled with a laser prototyping instrument. Once the fluidic channels in both layers are filled with an intracellular solution, each circular metal pad in the electrode array is electrically connected to its corresponding top patch hole. Meanwhile, the lower- and upper-level control layers, with channel width and height 100 µm and 20 µm, respectively, contain integrated hydraulic valves enabling the electrical isolation of individual flow channels in the lower (incoming) - and upper (outgoing)-level flow layers, respectively.

By pressurizing the control channels (~10 psi) and thereby activating their hydraulic valves, the electrical resistance between neighboring electrodes can be increased to above 50 gigaohms. For the high fidelity of electrophysiological recording, a high seal resistance between cellular plasma membrane and patch hole substrate is desired. In our completed device structure, when the flow channels are employed to apply negative pressure to aid in membrane sealing, the seal resistances we measure are typically > 500 MOhm for 90% of 100 electrode-channel-patch-hole assembly.

Thus far, initial experiments have shown the viability of the implemented multi-electrode array, our microfluidic design, and the chip’s interface as a site for culturing cells. At present, we are in the process of testing our chip with patterned arrays of rat hippocampal neurons.

References:

Figure 2: Recordings from a rat hippocampal neurons. A & B: Voltage clamp traces obtained using a glass pipette (A) or our planar device (B). C & D: Current clamp traces obtained using a glass pipette (C) or our planar device (D). E & F Action potentials generated by injecting current with a glass pipette (E) or our planar device (F).
Abstract:

Detection of target protein direct from cell lysate without any prior cleaning procedures has been demonstrated by fused silica nanofluidic channel devices and two-dimensional (2D) photon burst analysis technique. Individual molecule events are scatter plotted in terms of their photon counts and burst width in 2D photon burst analysis. Detection of target protein is achieved by comparing the 2D photon burst analysis of the target sample with that of the control sample. This single molecule technique offers the advantages of high sensitivity, minute sample consumption and reduced processing time over conventional immunological ensemble measurements, such as western blot, immuno-precipitation, etc.

Summary of Research:

The nanofluidic device is fabricated on a 500 μm-thick UV grade fused silica wafer using standard photolithographic and etching techniques. A schematic layout of nanofluidic channels is shown in Figure 1. The detection channel has a width of 2 μm and CF₄ plasma is used to dry etch the channel to the depth of 500 nm. A protective surface coating is then spin-coated onto the wafer and injection ports are drilled with high-speed sand-blast tool. After removal of surface coating and a thorough pirahna cleaning, a cover UV grade fused silica wafer of 170 μm is carefully clinched to the substrate wafer using de-ionized water as intermediate. Permanent bonding is achieved by annealing the wafer at 1050°C in air for 5 hours. The target protein, Hemagglutinin (HA) epitope tagged MAX (HA-MAX), in cell lysate was conjugated to polyclonal rabbit anti-HA antibodies and hybridized to the secondary rabbit antibodies with quantum dots (QDs). Sample solution was then loaded into nanochannel and electrokentically driven through the channel at various potentials. QDs were excited by an external laser source while they are passing through the detection volume. Due to the small dimension of the detection volume in nanofluidic channels, photon burst counts strictly from single molecules can be obtained. In addition, because a few quantum dots were hybridized to targeted HA-MAX proteins, they were distinguished from the unbound individual QDs by comparing their two-dimensional photon burst diagrams (photon burst width vs. photon burst counts). From this diagram, the existence of HA-MAX proteins can be detected. The typical photon-burst peaks obtained from these samples from cells with and without HA-MAX are shown in Figure 2a and b respectively. The burst counts for HA-MAX protein is higher than that of the sample without HA-MAX protein.
Figure 1: A schematic layout of nanofluidic channels (scale bar inset 2 µm).

Figure 2: Photon-burst peaks from (a) QD525, (b) QD525 with HA-MAX from cell lysate. On left: the cartoons of QD525 and HA-MAX protein with a few QD525.
Abstract:

A nanofluidic trapping device was developed for highly improved surface enhanced Raman spectroscopy (SERS). This device has been used to detect various molecules and proteins, including β-amyloid (Aβ) peptide, one of the biomarkers for Alzheimer’s disease. It can increase the location concentration of the sample and provide a consistent and reliable detection spot.

Summary of Research:

SERS has been investigated for decades for its capability to provide an enhanced signature Raman signal of various analytes on the order of $10^6$ to $10^{12}$ by confining the analytes into “SERS-active substrates” [1-3]. However, SERS signals are not uniform over the sample and only visible at certain “hot spots” [4-7], limiting the reproducibility and sensitivity of SERS detection techniques. In this project, we designed a novel nanofluidic device that can overcome these problems and provide a convenient and efficient platform for ultra-sensitive characteristic molecule detection.

Figure 1 through Figure 3 show the schematic diagrams of this device. Figure 1 shows the side view of a nanofluidic trapping device (modified from Figure 1 (a) in [8]). Figure 2 shows the top view of a nanofluidic trapping device (modified from Figure 1(b) in [8]). Figure 3 shows the magnified view at the nanochannel entrance in a nanofluidic trapping device (Figure 1 (c) in [8]).

The device consists of a 2 µm-depth microchannel and 40 nm-depth nanochannel. The dimension of the nanochannel is selected based on the size of SERS-active substrate we used in followed experiment, which are 60 nm gold nanoparticles. Since the size of gold nanoparticle is larger than the depth of the nanochannel, they will be trapped at the entrance to nanochannel and construct a “hot spot” for detection. The device was fabricated by conventional top-down techniques on a UV grade 500 µm-thick fused silica wafer (Mark Optics, Inc., Santa, Ana, CA). With a standard photoresist as
masking material, contact photolithograph and CF₄ reactive ion etching were used to create the nanochannel on the fused silica wafer. Then the microchannel was aligned with the nanochannel and patterned by photolithography. Concentrated (49%) hydrofluoric acid was then used to etch the microchannel to a depth of 2 µm using electron-beam-evaporated Cr/Au as masking materials. The sample inlet holes were drilled through the wafer by a high-speed sand-blasting tool. After a complete cleaning process, a fused silica cover wafer is carefully bonded to the substrate wafer to create an enclosed fluidic channel and annealed at 1050°C for 5 hours. Plastic reservoirs are attached around the inlet holes with epoxy afterwards.

In the followed SERS experiment, 60 nm-Au-nanoparticle suspension is used as the SERS-active substrate. The interested analyte solution was blended with Au colloids at a volume ratio of 1:10 and dispensed into one reservoir. The mixture was then drawn into the device by capillary force within minutes. Au nanoparticles are efficiently trapped and form a highly dense “hot area” around the entrance to nanochannel. This “hot area” is consistently located at the nanochannel entrance and provides a reliable point of detection. Compared to the conventional SERS detection technique, the signal is increased by a factor of 10². Moreover, capillary force maintains a continuous flow of the sample through the interstices of Au nanoparticles, the concentration of Au nanoparticles-analytes at the nanochannel entrance increases over time because more nanoparticles-analytes are trapped. The local concentration of nanoparticles-analytes can be increased by ~ 10⁵ within ~30 minutes, which further increases the detection sensitivity of SERS [8]. Furthermore, this device can enhance the SERS intensity of a complex sample and is powerful at multi-analyte detection with high sensitivity and accuracy.

With these advantages, this device has many practical applications, such as disease diagnosis and detecting dangerous or toxic molecules in aqueous solution. An important application lies in the detection of Aβ, a biomarker of Alzheimer’s disease (AD) [9]. This device can be used to detect the SERS spectrum of Aβ in different conformational states (monomer and oligomer form), which provides important information for the understanding and early diagnosis of AD. With the multi-analyte detection capability, this device can also potentially identify Aβ from insulin and albumin, confounder proteins in cerebral spinal fluid.

References:
Sample Preparation Device for Biological Agent Detection

CNF Project # 1442-06

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Abstract:

We designed, modeled, and tested a fluid mixer for biological sample preparation based on electrode-induced vortices at metalized posts in a microfluidic channel. The device exploits the principle of induced charge electroosmosis (ICEO) to pump fluids over electrically polarizable surfaces such as metal coatings. The main fabrication challenge was patterning the metal coatings only on vertical sidewalls of the device. This was accomplished by ion milling at the Cornell NanoScale Science and Technology Facility. The resulting devices mix two co-flowing streams faster than diffusion-based mixers, and the process also includes through-wafer contacts for driving the device using low voltages (~ 10 V).

Summary of Research:

Mixing chemical or biological samples with reagents for the detection of specific agents is one of the most time-consuming operations on microfluidic platforms. This is, in part, due to the slow rate of diffusive transport in liquid systems, but it is also due to the relatively small emphasis placed on steps of the chemical analysis system not directly involved in detection. As a result, inefficient methods are often used to perform the mixing operations.

In response, we have used a scientific approach to develop designs for microfluidic sample preparation based on a novel electrokinetic phenomenon called induced-charge electroosmosis (ICEO) [1,2]. ICEO creates microvortices within a fluidic channel by applying alternating current (AC) electric fields. The microvortices are driven by electrostatic forces acting on the ionic charge induced by the field near polarizable (metal) materials. The ICEO approach requires no moving parts and allows for on-chip integration. By enabling mixing to be turned on or off within a channel containing fixed volumes of liquids, these electronically controlled mixers prevent sample dilution, which is a common problem with other sample preparation strategies.

AC fields are desired for pumping aqueous fluids because DC fields generate gas bubbles via electrolysis, even at small voltages (1.2V or greater). The ICEO principle enables AC fields to produce continuous flow rather than back-and-forth pumping. Briefly, this is because the charges in a metal-coated post will separate in the presence of an applied electric field, inducing a nonuniform surface charge on the post which attracts counter-ions in the surrounding electrolyte. Positive counter-ions are attracted to the negative surface charge on one side of the post, while negative counter ions are attracted to the positive charge on the other side of the post (Figure 1). The same applied electric field acts on these counter-ions, which drag the surrounding fluid (electroosmosis). When the AC field switches direction, both the surface polarization and the counter-ion flow direction change sign such that the direction of the fluid motion remains unchanged.

Figure 1: The arrangement of surface charges and counterions around a metalized post in an applied electric field.
A combination of experiments, modeling, and microfabrication was used to develop the sample preparation devices. A partnership between Sandia National Laboratories, the University of Louisville, and Cornell University was formed to provide expertise over a diverse set of disciplines. Early experiments used a polymer chip that contained an array of gold-coated posts within a microfluidic channel. An electric field was applied to the channel and particle image velocimetry was used to measure the flow velocities. These data were used to validate a three-dimensional computer model based on the finite volume method.

The electric field, fluid flow, and mass transport in a multispecies liquid were calculated using high-performance computers. The calculations were used to rapidly prototype a wide range of designs (Figure 2) involving a new fabrication process capable of producing embedded electrodes. Figure 3 shows the steps to produce posts with metalized sidewalls via ion milling; electrical contacts are made on the back side of the silicon-on-insulator wafer.

The calculations include simulations of the fluid loading process as well as the mixing process. The calculations show the ICEO flow that occurs when the electric field is applied and how it mixes the liquids. The level of mixing is quantified using a metric that goes from zero (no mixing) to one (perfect mixing). A comparison of the performance of the various designs shows that the “rectangle” and “octagon” designs were best. The octagon design makes use of two sets of electrodes such that the electric field they generate can be continuously rotated. This rotates the flow field along with it so as to sweep out “dead” regions that often exist in stationary flows. The model’s animation feature shows how the mixing process proceeds in the octagon design. Experiments using fluorescent dye flowing at 1-2 microliters per minute replicated the computer model (Figure 4) and mixed the streams significantly faster than diffusion alone [3].

Acknowledgments:

We thank Rob Ilic at the CNF for tips on sidewall metallization pertaining to this project.

References:

**Microfabricated Implantable Flow Sensor for Medical Applications**

CNF Project # 1453-06  
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**Abstract:**

A radio frequency (RF) wireless capacitive flow sensor was developed. The sensor has a pressure sensitive capacitor with an integrated inductor. The resonant frequency of the sensor changes as the capacitance changes with liquid pressure that is related to fluid flow. The sensor uses a low pressure chemical vapor deposition (LPCVD) silicon nitride membrane and the residual stress of the membrane has been measured as 139 MPa. Two pressure sensors are integrated together and capable of measuring flow rates of as small as 5 ml/hour.

**Summary of Research:**

Gas and liquid pressure and flow are required to be measured and controlled in many applications, such as biomedical systems, environmental monitoring and industrial control. Capacitive pressure and flow sensors have been developed and the detailed capacitive pressure sensors have been reviewed. Most capacitive sensors use silicon, glass, or silicon nitride membranes as sensing plates [1]. In some applications like implanted medical devices, poison gases and high temperature places, the flow is not easily recorded. One possible way to remotely sense the capacitance (and the corresponding pressure or flow) is to integrate an inductor with the capacitive sensor in a closed loop. The state of the capacitor can then be detected by mutual inductance with the LC circuit that includes the sensor. A newly designed RF wireless capacitive flow and pressure sensor was fabricated and tested that is sensitive enough to remotely measure flow rates that have applications in medical diagnostics.

The design has a parallel plate capacitor and a thin film spiral inductor as a resonant circuit. The capacitor is sensing pressure change, which in the case of a liquid, is directly related to the flow rate. A more sensitive device can use two sensors in the path of the liquid to allow for a pressure difference measurement, which is also related to the flow rate. The inductor is fabricated with the capacitor on a silicon wafer using MEMS techniques. An ultra low stress silicon nitride membrane was used as the sensing element in the capacitor. The deflection of a square thin membrane is described by in equation (1) [2]:

\[ P = \frac{C_1 t \sigma_0}{a^2} \delta + \frac{C_2 f(v) t E}{a^4 (1 - \nu)} \delta^3 \]

where \( P \) is the pressure, \( \delta \) is the deflection of membrane under pressure, \( t \) is the thickness of membrane, \( a \) is the half-width of the membrane, \( E \) is Young’s Modulus, \( \nu \) is Poisson’s ratio, \( \sigma_0 \) is the initial or residual stress, \( C_1, C_2 \) are dimensionless constants, \( f(v) \) is function that is geometry and model dependant. The sensor design was optimized using equation (1). Silicon nitride with a thickness, \( t = 500 \text{ nm} \) was used and the residual stress was measured for a 1.2 mm square membrane using a Michelson interferometer with the membrane as one of the mirrors. A fit of the deflection vs. applied pressure data (using equation (1)), yielded \( \sigma_0 = 139 \text{ MPa} \).

The starting silicon wafers for the sensor were purchased with LPCVD silicon nitride deposited on both sides. The sensor device was fabricated with a process that included 5 photolithography levels including a backside level to pattern an anisotropic etch of the silicon wafer. The process also included PECVD depositions of silicon.
nitride and silicon oxide, two chemical mechanical planarization steps, reactive ion etching, and wet chemical etching. Figure 1 shows examples of a single and dual sensor. Measurements with sensors shown in Figure 1 yielded a pressure response in the range of 30-110 (mm H₂O). When integrated into the appropriate fluidic package, the design allows for flow rates in the 5-200 ml/hour range.

References:


Fabrication of Scaffolds for Three-Dimensional Culture of Human Endothelial Cells

CNF Project # 1465-06
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Abstract:
Guided by the natural design of the microvascular network of the human brain, we have designed and fabricated cylindrical polydimethylsiloxane (PDMS) channel networks that serve as scaffolds for the in vitro growth of artificial microvascular networks using human umbilical vein endothelial cells (HUVEC). The reconstructed networks allow us to observe how the endothelium changes when treated with brain tissue factors and to determine whether these changes promote the for metastasis necessary passage of breast cancer cells through it. Varying endothelial responses towards tissue factors from different organs may hold the key to understanding tissue-specific metastasis occurring in the majority of patients suffering from breast cancer.

Summary of Results:
In 40-50% of the rising number of patients yearly diagnosed with malignant breast cancer, the tumor metastasizes and preferentially spreads to the lung, brain or bone [1-4]. During metastasis, breast cancer cells travel within the circulation and eventually adhere to the endothelium where they pass into the new host organ. The site of adherence is not, as one might expect, determined by the pattern of blood flow within the human body or by mechanical arrest in microvessels [3,4]. Rather, it is likely that the molecular makeup of the microvasculature in the brain, lung and bone promote cancer cells adhesion. It is also possible that these organs secrete tissue factors that weaken the endothelial barrier, allowing breast cancer cells to invade. To investigate these hypotheses, we are developing a device that realistically mimics the vasculature of the host organ and allows us to observe interactions between breast cancer cells and the microvasculature in vitro. We aim to identify adhesion promoting molecules and tissue factors that weaken the endothelial barrier and thereby further metastasis.

We use HUVEC grown within PDMS scaffolds with square, semicircular, and round cross sectional profiles to construct the artificial microvasculature. We then observe reactions of the endothelium to tissue factors as well as interactions of injected cancer cells with the modified endothelium. Of particular importance is the existence of a well-established endothelial barrier at the beginning of the experiment.

This barrier forms when HUVECs grow and their membranes come in contact with each other. Transmembrane proteins such as VE-cadherine bind to each other and form tight junctions comparable with those seen in in vivo endothelial barriers. We visualize these via immuno-staining of VE-cadherine. Only when the barrier is intact, the resulting pattern outlines the cells’ membranes (Figure 1A) [5]. The extent of the cells’ body can be confirmed via immuno-staining of fibrous actin (Figure 1B).

Using VE-cadherine staining, we have found that HUVECs are capable of growing to confluency and can form tight junctions on flat PDMS surfaces and PDMS surfaces that contain semicircular channels (Figure 2). A cross sectional view of the collected and deconvoluted fluorescent antibody signals in the semicircular channel shows that VE-cadherine is present not only at the bottom of the channels, but along the channel sidewalls as well (Figure 3). The image confirms that tight junctions can develop along the sidewalls of semicircular channels.
leading to the assumption that we will be able to grow complete endothelial networks within closed round channels. Cells grown in 50 µm square channels have not developed tight junctions. We assume that they develop them slower or not at all due to the arrangement of their cytoskeleton within the limited geometry of the channels (Figure 4).

The dimensions, shape, and fluid flow rates of the artificial microvasculature are replicated according to data observed in vivo. PDMS channel networks provide the scaffolds in which HUVECs grow. They are fabricated by casting the material on negative plastic replica of silicon originals that consist of 50-150 µm wide channels with semicircular cross sectional profiles. Semicircular profiles form when silicon is etched isotropically with xenon difluoride (6). We constructed round PDMS scaffolds from two mirrored PDMS casts that we aligned and bonded to each other. Square channels with feature sizes ranging from 50 µm to 200 µm were fabricated using PDMS casting of SU-8 imaged networks. All devices contain 1-10 µm connecting channels between two main channels. These provide a diffusion path from one channel to the other and are fabricated in cured PDMS using two-photon laser lithography (7). Using these connecting channels we will be able to manipulate the endothelium from the “tissue-side” with chemo-attractants that we deliver at precise locations via diffusion from a neighboring micro-channel. In response to effective chemo-attractants we expect to observe an interaction between cancer cells and artificial endothelium either due to the changed molecular composition on the surface of the endothelial cells or due to the weakening of their tight junctions.

References:
Abstract:

A nanowire was lithographically fabricated using 50 nm doped polysilicon, attached to small gold terminals separated from each other by 90-150 nm gap. Semiconducting properties and other electrical characteristics of the devices were demonstrated with under varying voltages. Robust bio-molecular detection model were demonstrated on these nano-device using electrochemical impedance spectroscopy (EIS) using pure *Staphylococcus aureus enterotoxin* B (SEB) protein molecules. The lowest detection limit of these molecules was observed in the range of 1-35 fM. These results will lead to the development of a portable detection kit which integrated with digital microelectronics for any biological detection in real time.

Summary of Research:

The development of rapid and ultra-sensitive detection technologies is a long standing goal for researchers in the bio-detection fields. Nanowire based devices [1-5] have shown great promise in label-free and ultra sensitive detection of biological agents. However, critical application problems in using this technology have not been addressed, particularly the difficulties of device sensing surface modification for various targets and lower detection specificity in real biological samples. A novel molecular signal transduction system overcomes such problems. With this system, various complicated bio-molecular interactions are “translated” into simple signal molecules with universal sequences. These signal molecules are captured on the sensing surface of nano-devices through sequence specific recognition and generate detectable electronic signals. Using this system, nano-devices become universal for detecting almost any bio-agents.

In this work, nano-devices were fabricated at the Cornell NanoScale Facility (CNF), Cornell University, Ithaca NY. Four-inch p-type silicon (500 µm)/silicon dioxide (200 nm) wafers were first processed in a polysilicon furnace to deposit 50 nm of a p+ type polysilicon film and later annealed. Desired nanowire structures were obtained by exposing on a Leica VB6 e-beam. The patterned wafers were then subjected to specific chlorine etches to obtain the desired shape and size for the nanowires. The nanowires ends were then connected with patterned Ti/Au (10 nm / 35 nm) layer leaving 100 nm gap between two electrodes. The outline of fabrication processes and scanning electron microscope (SEM) images of nanowire were depicted in Figure 1. Electrochemical impedance spectroscopy and source meter were used to characterize these nano-device
The sensing surface of the nano-device was modified using a self-assembled monolayer on the polyamide surface. These surfaces serve as bonding sites for capture probes, peptide nucleic acid (PNA) or antibodies, to capture specific bio-targets. Nyquist EIS of pure biological protein sample from 1nM to 1fM were used to demonstrate detection on this fabricated nano-device (Figure 4). A clear change in impedance was observed in nyquist semicircle with change in protein conc. demonstrating the device sensitivity to sense any change on its surface due to these protein samples.

We gratefully acknowledge the assistance of Michael Skvarla and Alan Bleier from Cornell NanoScale Facility (CNF). The fabrication work was performed in part at CNF, a member of the National Nanotechnology Infrastructure Network, which is supported by the National Science Foundation (Grant ECS-0335765). This work was supported in part by the USDA grant CSREES 3447917058, NASA grant NNG06GB45G and Hatch grant IDA 00709-STH.

References:
Nanoscale Optofluidic Devices for Biomolecular Analysis

CNF Project # 1472-06
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Abstract:

The recent interest in the development of nucleic acid biosensors and high-throughput screening techniques has been in part driven by the need to rapidly diagnose emerging viral threats. Currently the key challenges are to develop label free biosensors with low mass sensitivity and high specificity without sacrificing the extreme parallelism of the microarray format. In this project, we will present our work towards the development of nanoscale optofluidic sensor arrays (NOSA), which represent a potential solution to this problem.

Summary of Research:

Figure 1 shows a 3D illustration of our sensor design. A central defect in the 1D photonic crystal [1] gives rise to a defect state in the photonic bandgap. By varying this defect cavity spacing we can tune the resonant wavelength of this defect state across the bandgap of the side resonator. Analogous to ring resonators [2], light corresponding to the resonant wavelength couples evanescently into the side resonator and is sustained within it. This results in a dip in the output spectrum of the waveguide at the resonant wavelength. Since the resonant structures lie to the side of the waveguide the bandgap does not interfere with the light transmission outside of that which lies in the resonant peak. Thus our unique design allows multiplexing along a single waveguide by simple placement of a large number of side resonators along the waveguide, each of which is fabricated to have a slightly different resonant wavelength.

An SEM image of a typical nanoscale optofluidic sensor array (NOSA) [3] device is shown in Figure 2. These resonators are extremely sensitive to refractive index changes in the innermost holes. We use a two stage fluidic architecture to first immobilize the necessary DNA capture probes onto the sensing sites and then subsequently flow the sample liquid over the NOSA to

Figure 1: 3D illustration of a sensing element of the nanoscale optofluidic sensor array (NOSA).

Figure 2: SEM image of an array of sensing elements indicating multiplexing capabilities of this architecture.
Figure 3: Experimental data showing redshift in one of the resonators when a calcium chloride solution of higher refractive index is flown through a channel targeting it.

Figure 4: Successful detection of Serotype-3 of the Dengue virus using the NOSA platform.

perform the target detection. Figure 3 shows the working principle of our NOSA architecture. The plot shows the spectrum of a sensor having 5 side resonators with water in the channels targeting them. When we flow a higher refractive index solution of calcium chloride through one of the channels, we observe a redshift in the resonant peak of the corresponding resonator. It is important to note that the other resonances are not affected.

In this manner we can perform a large number of detections in parallel using this sensing architecture. With the Q-factor of these devices being between 2000 and 3000 and given the operational range of a standard 1550 nm laser, we estimate that we could have at least 50 such resonators on a single bus waveguide, thus allowing the possibility of performing 50 detections in parallel on a single waveguide. The Q-factor of such resonators can be significantly improved so these devices could be used to perform more than a hundred parallel detections.

The Si devices were hydroxylated, aminated, and then subsequently treated with dendrimers to increase the capture efficiency of DNA capture probes. Figure 4 shows preliminary results from an experiment where we successfully detected one of the serotypes of the Dengue virus using the NOSA. As can be seen, the resonance corresponding to the resonator which was functionalized with Serotype-3 capture probes shows the largest redshift. We see some minor shift in the resonance for Serotype-1 due to cross reactivities between the different serotypes.

References:
Microfluidic Networks for Studying Thrombosis

CNF Project # 1476-06
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Abstract:

The flux of platelet agonists into flowing blood is a critical event in thrombosis and hemostasis. However, few in vitro methods exist for examining and controlling the role of platelet agonists on clot formation and stability under hemodynamic conditions. In this project, we fabricated a membrane-based method for introducing a solute into flowing blood at a defined flux [1]. The device consisted of a track-etched polycarbonate membrane reversibly sealed between two microfluidic channels; one channel contained blood flowing at a physiologically relevant shear rate, and the other channel contained the agonist(s). We expect this device to be a useful tool in unraveling the role of platelet agonists on clot formation and stability.

Summary of Research:

During thrombus formation on the exposed subendothelial matrix, platelets pause or roll on von Willebrand factor, and then arrest and activate on collagen [2]. Adhered platelets become activated through outside-in signaling via glycoprotein receptors [3]. Activated platelets recruit and aggregate other platelets by producing soluble agonists and releasing them into the flowing blood. The cellular and molecular events of platelet adhesion to surface-bound proteins and subsequent platelet activation have been extensively studied in animal [4] and in vitro models [5]. In vitro models have been critical in defining the shear dependent bonding dynamics between platelet receptors and their surface bound ligands. Parameters such as on and off rates and adhesion efficiency have been measured by manipulating the composition and density of surface-bound ligands. However, there are no in vitro models for manipulating agonist flux into flowing blood.

In this project, we fabricated a membrane-based microfluidic device for controlling the flux of platelet agonists into flowing whole blood. The device consists of two channels separated by a membrane (Figure 1). One channel contains blood flowing at a physiologically relevant mean wall shear rate (250-2000 sec⁻¹). The second channel, containing the agonist(s), is oriented perpendicular to the first channel. At the intersection of the two channels, the flux of agonists into flowing blood is controlled by the concentration of the agonist and the transmembrane pressure. Channels were fabricated using standard soft lithography techniques using KMPR photoresist on silicon wafers as a mold master. A track...
etched polycarbonate membrane (pore diameter = 1 µm) was reversibly sealed between two PDMS layers using vacuum assisted sealing. Luciferase was used as a model solute to examine the roles of wall shear rate, the relative flow rates of the two channels, and protein fouling on flux. Analytical and numerical models were developed to describe the fluid flow and mass transport in the device.

As a proof-of-concept, the platelet agonist ADP was introduced at three different molar fluxes into flowing human whole blood (250 sec⁻¹). Post hoc electron (Figure 2) and confocal microscopy reveal that platelet activation and aggregation depend on the magnitude of ADP flux. Numerical simulations suggest that this flux dependent aggregation is a function of the boundary layer thickness formed downstream from the introduction of platelet agonists. This is the first demonstration of user-controlled ADP wall flux into flowing blood to trigger platelet deposition.

References:
Nanofluidic Electric DNA Detector

CNF Project # 1483-06
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Abstract:
Nanofluidic channels enable the linearization of deoxyribonucleic acid (DNA) for optical microscopy, and enable analysis of long DNA molecules. We are developing a device structure that combines this elongation, optical detection, and electrical detection.

Summary of Research:
Nanochannels with a cross-section of 100 nm have recently been investigated as a means for linearizing DNA for optical analysis [1,2]. As such, they have been shown to stretch DNA considerably (70% of contour length). They possess the remarkable property that molecules are at equilibrium and free to diffuse through phase space, since the molecule is not fixed or tethered.

However, the information content of images is dependent on probes, and the resolution of microscopy is limited by the diffraction limit and the number of photons. Thus an alternative, label-free detection method would be desirable. We are investigating the detection through detection of a transverse current by attaching two electrodes laterally and co-planar to the nanochannel, such as has recently reported by Liang and Chou [3].

Our devices are fabricated on fused silica using electron-beam lithography (JEOL JBX-9300FS), reactive ion etching and metal lift-off. An example of a 100 nm wide channel interfaced with an 80 nm wide electrode is shown in Figure 1. The closed fluidic system is formed at a later stage by bonding to a second wafer.

References:

Figure 1: SEM of a horizontal nanochannel with an attached vertical nanoelectrode pair.
Fabrication of a Micromirror with Sidewall Electrodes

CNF Project # 1502-06
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Abstract:
We designed and fabricated a micromirror with sidewall electrodes in the Cornell NanoScale Science & Technology Facility (CNF) for endoscopic application. The micromirror can perform 2-dimensional scanning. The static and dynamic performances of the micromirror are improved over that of a micromirror without sidewall electrodes. In addition, undesired spring-softening effect that is commonly found in electrostatic actuation is significantly reduced. Based on the fabrication process of a single micromirror, the 2 × 2 micromirror array with sidewall electrodes and 4 × 4 micromirror array with sidewall electrodes were fabricated in CNF as well.

Summary of Research:

Micromirrors are important micro-electric-mechanical system (MEMS) devices adopted in many application fields, such as barcode readers, digital light processors (DLP), optical switches, optical coherence tomography (OCT) and confocal microscopes. The performance and limitation of these devices are highly depended on the micromirror. Our goal is to design and fabricate a micromirror for an endoscope used in OCT system. The application of a biaxial micromirror used in OCT system to acquire 3-D images has already been demonstrated [1]. One of the major challenges is to design and build the actuators.

The micromirror actuating mechanisms such as electrostatic, thermal, piezoelectric, electromagnetic, electrowetting of liquid metals or mixed actuators are the subjects of intense research. Among these actuators, electrostatic actuator is widely used in different micromirror designs [2-4]. Electrostatic actuators are easy to fabricate by traditional integrated circuit (IC) process. Based on the electrostatic force, \( F = \frac{1}{2} \varepsilon_0 V^2/d^2 \), (\( \varepsilon_0 \) - dielectric constant of vacuum, \( d \) - the distance between electrode and mirror plate), if the same drive voltage is used, an increase in \( d \) will decrease the electrostatic force rapidly. Meanwhile, increasing the effect area of electrodes is effective to raise the electrostatic force. As a result, a micromirror with sidewall electrodes is proposed in Pu et al [3], however, its fabrication process is so complicated. Therefore, we created a model for sidewall electrodes and designed a novel micromirror with sidewall electrodes [5].

The micromirror with sidewall electrodes consists of a rigid mirror plate, spring hinges, gimbal frame, supporting frames, and actuating electrodes. The micromirror plate is suspended by the double-gimbal structure, which consists of two pairs of torsion bars for 2-degree-of-freedom (DOF) scans: \( \alpha \)-scan and \( \beta \)-scan, rotating about the x-axis and y-axis, respectively. The micromirror plate is electrically grounded and the quadrant electrodes (sidewall and bottom electrodes) offer \( V_1, V_2, V_3 \), and \( V_4 \) voltages. The parameters of the micromirror are listed: micromirror plate: 1 mm × 1 mm, torsion bar width: 2 \( \mu \)m, the gap between micromirror plate and bottom electrodes: 290 \( \mu \)m. Figure 1 presents a scanning electron micrograph (SEM) image of the as-fabricated micromirror with sidewall electrodes.

The fabrication process is divided into three steps; (i) micromirror plate fabrication, (ii) sidewall electrode fabrication, and (iii) assembling of micromirror plate and sidewall electrodes. The micromirror plate was built on the silicon-on-insulator (SOI) wafer. First, we fabricate the micromirror plate feature on the device layer and then pattern the back side of the micromirror on the handle layer. Reactive ion etching (RIE) and deep RIE (DRIE) were adopted during the process. After obtaining all the micromirror features on SOI wafer, we used wet etching to release the micromirror plate. Figure
shows the 2 µm width serpentine bar. Subsequently, Cr/Au thin film was evaporated as a reflecting surface.

Second, sidewall electrodes were built on another SOI wafer. The structure of sidewall electrodes was obtained by DRIE. Because the height of sidewall is 250 µm, we adopted a special mask-shadow mask, to pattern all the features of sidewall electrodes. Figure 3 presents the assembling of a shadow mask and the sidewall electrode wafer. Al layer (1 µm) was sputtered on the sidewall surface by CVC sputter deposition system. Finally, the shadow mask was removed by lift-off and the sidewall wafer was cut into single dies.

The last step is to assemble the micromirror plate and actuating electrodes. Flip-chip method was adopted. The micromirror plate was stuck on the sidewall electrode by SU-8 and HTG system III-HR contact aligner (HTG) was used to align these two. Afterwards, the micromirror device was put into 90° oven for 12 hours.

Figure 4 shows the characteristics of micromirror with sidewall electrodes. The bias voltage is 55V [4]. The differential method is adopted [2, 4]. The scanning angle reaches 2.87° when the drive voltage is 15V at α-scan, and 3° when the drive voltage is 25V at β-scan. The resonant frequency of the micromirror is 50.5Hz at α-scan, and 146.5Hz at β-scan, respectively.

In addition, 2 x 2 micromirror array with sidewall electrodes and 4 x 4 micromirror array with sidewall electrodes were fabricated as well. The fabrication process is similar with the single micromirror structure.

References:

Figure 1: SEM image of the micromirror with sidewall electrodes.
Figure 2: SEM of 2 µm width serpentine torsion bar.
Figure 3: Assembling of a shadow mask and the sidewall electrode wafer.
Figure 4: Static performance characteristics of micromirror with sidewall electrodes.
Abstract:
Precise placement of individual biological cells is desirable to obtain accurate quantitative data for a variety of experiments. In this project, we intend to manipulate cells by tagging them with paramagnetic beads and then electromagnetically maneuvering the beads. For this purpose, we are trying to develop a microelectromagnetic device, consisting of a two-dimensional array of current-carrying wires, which can be used to create localized magnetic field patterns that can be configured to manipulate beads with microscopic precision. Thus far, the results indicate that the magnetic fields produced by current loops are capable of trapping and manipulating beads when a substantial amount of current is applied.

Summary of Research:
Neuronal function and cellular release mechanisms are of great importance in understanding human health and certain neurological diseases including Parkinson’s disease. Various types of devices have therefore been developed so far to study such release mechanisms. Although these compact on-chip systems allow for fast, repeatable biological experiments at low cost with only a small amount of biological sample, one of their main difficulties is positioning and manipulating the individual sample cells with high precision at the microscopic scale. The techniques currently in use for single-cell isolation and manipulation include optical tweezers and dielectrophoresis. However, these techniques are far from ideal; one has a tendency to damage the cell surface, while the other introduces excessive signal noise.

In this project, we are trying to develop a technique based on a microelectromagnet to manipulate biological cells attached to paramagnetic beads with high precision. The magnetic manipulation scheme was chosen because of the biocompatibility of magnetic fields. Previously, electromagnetic traps, rings and guides have been designed to manipulate neutral atoms, magnetic nanoparticles, etc. The proposed device shall consist of a microfluidic channel and an array of lithographically defined platinum wires on a silicon substrate (Figure 1). Passing sufficient amounts of current through these wires will create localized magnetic field patterns that can be configured to precisely manipulate beads at the microscopic scale. The device can theoretically control almost any kind of...
magnetic particle, including biological cells attached to paramagnetic beads. In future, such a device can be integrated with on-board electronics, such as electrodes and amplifiers, which would allow for simultaneous positioning of cells as well as real-time measurements of cellular activity. In addition, it can also be used to assemble artificial tissues and investigate intercellular communications.

The magnetic field generated by the device is directly proportional to the current and inversely proportional to the distance, whereas the magnetic force generated by the field is directly proportional to the gradient of the field. As shown in Figure 3, the magnetic field profile above a current-carrying loop varies with height. Therefore, in the context of cell manipulation, the height of the insulation layer above the loop is critical. Based on our experiments with a variety of insulation heights, the optimum height has been determined to be $0.64r$ (where $r$ is the radius of the loop) for circular current loops and $\geq 0.5p$ (where $p$ is the wire pitch) for square-shaped loops (Figure 2).

Since circular current loops can only be constructed with a single metal layer, only a small amount of current can be used before power dissipation, due to internal resistance, leads to overheating and device failure. In addition, a single metal layer also limits the field gradient that can be obtained because of the closed ring trap geometry (Figure 4). Therefore, we are currently experimenting with square-shaped current loops fabricated using two stacked metal layers. Using MATLAB simulations and theoretical analysis, it is evident that using two metal layers instead of one offers a great deal of improvement in device operation and functionality.

References:
Fabrication of Elastomer Pillar Arrays with Modulated Stiffness for Cellular Force Measurements

CNF Project # 1545-07
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Abstract:
The mechanical properties of a cell’s environment can alter behavior such as migration and spreading, and control the differentiation path of stem cells. Here we describe a technique for fabricating substrates whose rigidity can be controlled locally without altering the contact area for cell spreading. The substrates consist of elastomeric pillar arrays in which the top surface is uniform but the pillar height is changed across a sharp step. Preliminary results demonstrate the effects on cell migration and morphology at the step boundary.

Summary:
Cells generate traction forces in the nN range during adhesion and migration [1]. In order to quantify the mechanical interaction of cells with their environment, it is important to control the mechanical properties of the cell’s environment, and measure the forces exerted by the cell. To this end, we use a high-density microfabricated array of elastomeric pillars, which provide direct measurement of the traction forces. The forces are deduced from the measurement of the deflection of these pillars. Previous studies demonstrated the importance of the rigidity of the substrate in numerous cellular processes (ranging from migration to adhesion and also cell differentiation) [2-4].

In this work, we want to study the effect of a boundary between two different rigidity substrates on cell behavior. In order to properly study the influence of a sudden change in rigidity, pillars with different stiffness but the same contact surface area need to be designed on a single substrate. To achieve this goal, we sought to fabricate polydimethylsiloxane (PDMS) pillar arrays in which the pillar diameter and top surface remains constant, but the pillar height, and therefore the stiffness, changes abruptly (Figure 1j). Mold for these arrays consisted of etched silicon substrates, as shown in Figure 2.

Figure 1 shows the process used to fabricate these molds.

Figure 1: Schematic drawing of the fabrication of PDMS posts. The substrate consists of hexagonal arrays of vertical PDMS posts whose deflections are directly proportional to the force exerted by a migrating cell. The diameter and spacing of the posts is kept constant but the height is changed from the base such that the top contact area of the posts lies in one plane. The height change will generate controlled step increase in substrate stiffness without changing the surface area presented to the cells.
The fabrication process starts with thermal growth of 950 nm oxide. The desired pattern was replicated in positive photoresist. After developing, it was treated with a long post exposure bake in order to smooth the sidewalls then descummed in O₂ plasma. The oxide layer was etched, using the resist as a mask, in fluorine based system. Silicon was then etched to desired depth in chlorine-based inductively coupled plasma reactive ion etcher (ICP-RIE) system using oxide masking. After removal of the oxide mask, oxide is deposited conformally on the whole wafer. The surface of wafer is then planarized by CMP process.

Photoresist was spun on the wafer and then half of the patterned area was exposed. After developing and etching silicon in Bosch process, the desired height step is obtained. The rest of the oxide was removed in BOE (Figure 2). The wafer was piranha cleaned, plasma cleaned and silanized with tridecafluoro-trichlorosilane in vapor phase. PDMS was poured over silicon substrate, cured at 70°C for 12 h, and then peeled off in ethanol. The scheme of the PDMS template fabrication is shown in Figure 3.

These PDMS substrates have the advantage that their stiffness can be easily modified by changing their geometry and can precisely detect the cellular activities. Surface rigidity modifies cell migration, adhesion and by likely force exerted by cells on the substrate.

Observation was made with mesenchymal stem cells plated on elastomer pillars with different heights and therefore different rigidity (Figure 4). A marked difference in phenotype can be observed in either side: they look more round on the soft part and more spread out on the stiff part. This behavior could lead to different differentiation paths in the two areas.

References:
Myosin Dependent Migration of Fibroblasts

CNF Project # 1545-07
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Abstract:

Cell polarization induced by topographical cues can be used to understand what are the means that cells are using to sense surface topography. Micro-grooved induced cell alignment is maybe related to the fiber response. Furthermore, this behavior is also a way to understand how cells are able to generate persistent directed migration and what are the necessary protein components needed. This last question would also give important insights about how cells are polarizing and choosing their migration direction. The preliminary experiments were done on polydimethylsiloxane (PDMS), but it cannot be aid for certain that the response was due to rigidity or the topology of the substrate. So 1 µm lines at pitch of 3 µm and various heights were fabricated at CNF on 170 µm thick fused silica wafers.

Preliminary Results:

Grooved Surfaces Will Cause Cell Orientation but Myosin Depletion Weakens Orientation: The goal of our preliminary experiments was to study the behavior of different adherent fibroblast cells on micron-sized square groove substrates. Characterization of different mutant cell lines on the structured surfaces has already enabled our group to define critical aspects of the alignment response. Engineered substrates were obtained by soft lithography, i.e. in pouring an elastomeric compound PDMS upon a photolithography-obtained textured surface and by curing the PDMS; we obtain a perfect PDMS mold with the features imprinted. The features of structured substrates used for our assay were square grooves of 1 µm width and 1.5 µm height separated from each other every 1 µm. Textured surfaces were coated with fibronectin full-length.

When control fibroblast cells (NIH 3T3 or mouse embryonic fibroblast) are loaded onto fibronectin coated micro-grooved surfaces, cells are showing after 30 minutes to 1 hour a strong alignment with the axis of the grooves as seen in Figure 1a. Moreover, we observed a typical bipolar shape, which correlate with a bipolar protrusive activity oriented in the direction of the

![Figure 1: DIC micrographs of adherent fibroblast cells 30 min after spreading onto grooved PDMS surfaces coated with fibronectin (scale bar: 30 µm). (a) Control cells (NIH 3T3 cells) are showing typical bipolar shape and bipolar protrusive activity behavior leading to cell orientation with the axis of the grooves. (b) Myosin II-A knockdown cells: Myosin II-A depletion weakens the cell orientation with the grooves. Also the protrusive activity of the leading edge is no longer bipolar and is showing more off-axis components than in the control cells. (c) MARCKS -/- cells: Cells which are lacking MARCKS protein, spread onto those micron-grooved surfaces without a broader leading edge as seen in the control cells and send long processes out on the 1 µm beams.](image)
grooves. This orientation of the protrusive activity leads to the overall cell orientation with the axis of the groove. At earlier times during cell spreading, we noticed two different behaviors. The first spreading behavior is showing cells, which are directly developing a polarized shape with a bipolar lamellipodia extending in an oriented manner with the axis of grooves. The second way of cell spreading is showing a more isotropic lamellipodial development with no preferential direction. Of course, when this first phase of spreading is ending, cells are polarizing in a similar manner than the other aligned cells. This indicates a possible role of the cell contractile machinery in the polarization phenomenon.

To check the role of the acto-myosin contractility in the alignment process, we studied the behavior of the same fibroblast cells in which we depleted the molecular motor myosin II-A by SiRNA technique. The results are particularly striking as it could be seen in Figure 1b. Myosin II-A depletion obviously weakens the cell orientation with the grooves. Also cell bodies are less stretched than in the control cells. Furthermore, the protrusive activity of the leading edge is no longer bipolar and strongly oriented. Indeed, we observed that the myosin II-A knock-down cells were showing more off-axis protrusive activity than in the control cells. From those results, it seems that the acto-myosin contractility has an important role in the cell alignment and in the persistent directionality of the protrusive activity.

**Marcks Deletion Disrupts Lamellipodial Structure of the Protrusive Leading Edge:** Another interesting results from our preliminary experiments is the effect of MARCKS protein on cell morphologies when plated onto micro-grooved substrates. As in Figure 1c, fibroblast cells which are lacking MARCKS protein, spread onto those micron-grooved surfaces without a broader leading edge as seen in the control cells and send long processes out on the 1 µm beams. The complete loss of a smooth and continuous lamellipodia due to the MARCKS deletion affects dramatically the ability of cells to polarize and move.

Figure 1 shows DIC micrographs of adherent fibroblast cells 30 min after spreading onto grooved PDMS surfaces coated with fibronectin 1 µm, double arrows are showing grooves direction axis, scale bar: 30 µm. For a better cell visualization, cell contours have been outlined with a black dashed line.

To get substrates that are not rigid 170 µm thick fused silica wafers were used. Pattern was transferred using photolithography. The wafer was coated with ARC (anti reflective coating) and resist (SPR 700). Glass was not a good choice as etching was glass made it rough. The wafers were etched using a CHF₃/O₂ gases. Figure 2 shows the resist profile before etching. The etched samples look very smooth and clean as compared to pure glass etched surfaces.

We are still in the process of using the actual substrates for plating cells, but they promise to unfold new frontiers defining the basic cell biology.

**References:**


Neural Recording Electrode

CNF Project # 1609-07
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Abstract:
The neurotrophic electrode is a FDA approved cortical electrode used in long term human recordings, which is currently built by hand. Improvements to the electrode can be made by using standard microelectronic fabrication techniques for manufacturing. Here, we have investigated new electrode structures based on the neurotrophic electrode design concept utilizing polyimide thin films.

Summary of Research:
Neural interfaces have successfully been implanted in human subjects for communication applications and potentially for motor restoration and speech. An electrode recording system that has been available for long term human implantation since 1996 is the neurotrophic electrode (NE) (Figure 1). Its unique design consists of a hollow glass cone containing gold recording wires that allow recording from axons grown into the glass cone under the influence of proprietary trophic factors [1]. The gold recording wires are coiled for strain relief between the glass tip and connected electronics mounted on the skull.

Recording from this reconstituted neuropil has produced APs that display robust signal-to-noise ratios over long time periods (four years in humans until death) [2]. The FDA-approved (and biologically compatible) recording system uses transcutaneous FM transmission of the amplified system and thus no wires. It is powered by air gap induction coils, obviating the need for batteries. This system has been implanted in six locked-in humans in efforts to provide them with control of a switch or a computer cursor, thus restoring synthetic speech, Internet access, environmental control, and other applications.

Improvements to the NE system would substantially increase neural signal throughput and usability. Most notably, an increase in the number of electrode sites at various locations across the cortex and individual axon isolation are critical requirements. The current NE has an internal cone diameter of 50 µm and can contain anywhere between 10-50 individual neurites. Reducing the diameter into the 20-25 µm range would effectively limit the number of neurites grown into the device which is desirable. In addition, the NE is manufactured by hand by drawing glass pipettes into cone structures with a glass puller. Increased controllability and manufacturing reliability can be achieved with standard microelectronic fabrication processes.
Initial feasibility studies were performed at the Cornell NanoScale Science and Technology Facility (CNF). The electrodes were designed with dimensions of 0.5 x 3 cm. Three electrodes were designed to branch from a 4 cm microribbon cable; the terminating end was designed to fit into a standard 12 channel zero insertion force female connector. The branch design would be appropriate for recording from multiple, separate, distant cortex areas while reducing the number of connectors and tethered wiring. This application is especially appropriate in our focus of developing a speech prosthesis where we would ideally like to implant electrodes in different articulatory premotor and motor areas. The electrode consists of top and bottom polyimide layers with an inner Ti/Au/Ti metallization layer. The cone structure is created by rolling or folding large surface area flaps at the tip of each electrode shaft into a cone and then cementing with ethyl-2-cyanoacrylate cement. Each electrode tip has four recording areas of 20 x 20 µm² on the inside portion of the cone. Three variations of the tip layout were investigated to determine the best way to create the cone structure. Two tips were designed to be 5× larger than the NE for ease of rolling the polyimide flaps to create the cone structure and one tip was designed to be approximately the same size as the NE. Tabs were included on one tip variation to act as mechanical hold during the cementing process to create the cone.

Pictures of the electrodes are shown along with microscopy photos of the recording site areas (Figures 2 and 3). An electrode tip with tabs is shown in Figure 2 during the rolling process. Alternative, the flaps could also be simply held together and cemented (Figure 3). This creates a flat edge similar to a fin that can be used to hold the electrode tip during cementing and also during surgical implantation. Metallization and reactive ion etch (RIE) vias for the recording sites can be seen in Figure 4.

References:


Figure 2, left top: A tab can be seen in the closest electrode tip, this tab acted as a mechanical hold during the cone formation process.

Figure 3, left bottom: Photo of electrode tip with cone structure.

Figure 4, above: Microscopy photos of the recording areas at the electrode tips.
Modeling Microvascular Structure In Vitro

CNF Project # 1613-07
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Abstract:

The goal of the project was to create robust microfluidic channels with a layer of collagen and endothelial cells to simulate microvascular environments in vitro. These channels would be of use in angiogenesis and transmigration assays as well as studies of endothelial behavior. The final design was a two channel poly(dimethylsiloxane) (PDMS) structure with an upper and lower channel separated by a membrane of polyethylene teraphthalate (PET), and a collagen cell-attachment surface. The channels were shown to support an endothelial cell population, and it was possible to create a diffusion-limited concentration gradient between the upper and lower channel.

Summary of Research:

In vivo microvasculature is a complicated branching plexus of small vessels composed of endothelial cells, support cells such as pericytes, and extracellular matrix all engaged in chemical signaling, experiencing flow, and remodeling. The scale of these structures is on the scale of tens to hundreds of microns [1]. In order to explore phenomena that occur in this environment, we designed a microfluidic channel system which could foster an endothelial cell layer on an extracellular matrix material, allow flow adjacent to the endothelial layer, and create concentration gradients perpendicular to the endothelial cell layer. A microfluidic model was selected due to precedent in creating concentration gradients in microfluidic channels [2], and collagen type I was selected as cell attachment material as collagen is the principle component of the extracellular matrix [3].

The completed device is composed of two PDMS components with channels facing each other, sandwiching a membrane of PET coated with type I collagen. As seen in Figure 1, the upper and lower PDMS channels are symmetrical, with a region of overlap in a straight section roughly 1 mm long. The channels are 800 µm in width and 160 µm in depth, ending in reservoirs. The PDMS channels were created through casting from a silicon master, which was created in the Cornell NanoScale Science and Technology Facility (CNF) using stereolithography, primarily plasma etching using a Unaxis SLR 770. The two PDMS channels were then placed together with a PET membrane from a BD Falcon Cell Culture Insert [BD Biosciences, Franklin Lakes, NJ] in between. This membrane is approximately 15 µm in thickness with 0.4 µm pores across its surface and is plasma treated. Onto this membrane was deposited a 40 µm thick layer of type I collagen. This collagen was extracted from rat tails using techniques developed
by George Davis and Jian Tan, and gelled upon the PET membranes by neutralizing with 10X PBS and 1N NaOH [Sigma Aldrich, St. Louis, MO].

Two functional tests of the device were performed, the first being a mass transfer experiment to verify that a diffusive concentration gradient was being formed across the membrane without significant convection. The second test was seeding of human umbilical vein endothelial cells (HUVEC) [Lonza, Allendale, NJ] into the device to determine its biocompatibility. For the mass transfer experiment, 10−4 M fluorescein was flowed in the lower channel while PBS was flowed through the upper channel, at flow rates between 2.5 and 20 µL/min, as applied by a PHD 2000 syringe pump [Harvard Apparatus, Holliston, MA]. The outflow from the upper channel was collected, and the concentration of fluorescein there measured. As any fluorescein in the upper channel was the result of mass transfer, we can anticipate that the concentration would decrease in a logarithmic pattern if the mass transfer is dominated by diffusion. This logarithmic pattern was observed, as demonstrated in Figure 2.

In the second test, 1.5 million cells/mL HUVECs were seeded in the upper channel of the device while cell culture media was flowed through the lower channel. The device was maintained in an incubator at 37°C and 5% CO₂ while maintaining flow in the lower channel for 12 hours. No flow was provided in the upper channel, primarily to allow cell attachment, but this also implied that the only nutrients were available by mass transfer through the membrane. At 12 hours, the upper channel was stained with calcein AM. Significant cell populations were observed, as shown in Figure 3.

The device presented here demonstrates the capacity to sustain endothelial cells in a biomimetic environment of collagen type I, and it demonstrates the capacity to create diffusive concentration gradients perpendicular to the endothelial layer, both requirements for a microvascular model.

References:


Device Fabrication for the Observation of Axonal Growth and Axonal Organelle Transport of Rat Hippocampal Neurons

CNF Project # 1624-07
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Abstract:
This work is towards developing a microfluidic platform to observe the axonal growth and axonal organelle transport of rat hippocampal neurons. The undertaking proceeds along two lines of inquiry: (1) controlled growth, and (2) spatio-temporal delivery of soluble chemicals. Presently, the patterning of poly-l-lysine on glass substrates to promote attachment of neurons and guidance of axon growth is explored. A photolithographic technique is employed, by which positive photoresist is exposed in the image of a desired pattern. The exposed pattern is then immersed in a hybrid solution of fluorescently labeled and unlabeled poly-l-lysine. The patterns have a minimum dimension of 2 µm.

Introduction:

*In vivo* observations of neuron growth and response to stimulus are extremely difficult. *In vitro* methods have been developed to facilitate study of such effects, with varying degrees of success. Cell culturing and observation chambers originally developed by Campenot [1] feature neurons placed on a collagen coated Petri dish, segmented by Teflon® dividers sealed with silicone grease. Axon growth is coaxed along pathways separated by etches in the collagen layer. Directed growth as well as compartmentalization is difficult in such devices. More recently, work by Wang, et al [2] accomplishes directed nerve growth in micro-wells and integrates microfluidic control via laminar flow techniques to generate extracellular chemical gradients. Shear due to the flow, however, potentially hinders the response of nerve cells to chemical gradients.

Summary of Research:

We implement a lithographic patterning procedure as described by Oliva, et al [3] to introduce a growth pathway for neuronal growth. Briefly, a mask is used to illuminate positive photoresist (Shipley 1818). The illuminated photoresist is then developed and removed, the substrate cleaned briefly in oxygen plasma, followed by incubation in a FITC-poly-l-lysine/poly-l-lysine solution (1:10 w/w, 1mg/1mL in .1 M Borate Buffer, pH = 9; two hour duration). Photoresist in the unpatterned areas is removed via acetone sonication (15 minutes) following the poly-l-lysine soak.

The time of the poly-l-lysine soak is significant. Long incubation times, for example, will increase the thickness of the poly-l-lysine film, resulting in decreased feature fidelity. This occurs due to extraneous poly-l-lysine films at the boundary of the pattern and the substrate.

Results:

Micrographs depict the outcome from the procedure. We observe strong adhesion of poly-l-lysine to the glass substrate, indicated by the contrast in Figure 1. All results are for an incubation time of 2 hours. Hippocampal rat neurons have been cultured on these poly-l-lysine features, as shown in Figure 2. The culturing and imaging was performed by Dr. Cheng Fang and Dr. Gary Banker, of Oregon Health and Sciences University. Future work will incorporate a superstructure for delivery of soluble factors, as well as a re-design of the poly-l-lysine features for guided growth.
References:

