The 2010-2011 Cornell NanoScale Facility Research Accomplishments
Abstract:

We have a wide range of projects: (1) studies of the ecological order that emanates from bacterial populations competing under starvation conditions, (2) the swimming collective dynamics of bacterial cells inside jagged, funnel-like geometries leads to the emergence of complex migratory patterns, (3) developing new approaches to study the evolutionary dynamics of drug resistance by studying collective response to drug-induced stress gradients, and (4) dynamics of cancer cells in 3-D structures.

Reports:

(1) Studies of the ecological order that emanates from bacterial populations competing under starvation conditions. Bacterial cells evolved under prolonged stress often have a growth advantage in stationary phase (GASP); we expect GASP cells to maintain a proliferative state and dominate wild-type cells during starvation, especially when nutrients are limited and the medium has been conditioned. However, when we compete GASP mutants against wild-type cells in a chain of microfluidic microhabitat patches (MHPs) with alternating nutrient-rich and nutrient-limited regions, we observe the reverse effect: wild-type cells achieve maximum relative density under nutrient-limited conditions, while GASP cells dominate nutrient-rich regions. We explain this surprising observation in terms of ideal free distributions, where we show that wild-type cells maximize their fitness at high cell density by redistributing themselves to sparsely populated MHPs. Figure 1 presents a highlight from this work.

(2) Swimming collective dynamics of bacterial cells inside jagged, funnel-like geometries. Complex collective phenomena may emerge from the seemingly simple behavior of the individual components of a biological system. For example, a self-propelled swimmer that runs in straight lines and reorients itself in tumbles will be redirected by and continue to swim along a microstructured wall, and will do so until it tumbles again. We reported on the emergence of spontaneously forming migrating bands of *Escherichia coli* (*E. coli*) bacteria inside a microchannel containing microstructured ratchets. We showed that a collection of bacteria is able to migrate against the funnel-shaped barriers by creating and maintaining a chemoattractant gradient. A transition between pure rectication and chemotaxis-driven collective motion is predicted from theoretical models, and is observed experimentally as the initial inoculation density is varied. Figure 2 presents a highlight from this work.

(3) Developing new approaches to study the evolutionary dynamics of drug resistance by studying collective response to drug-induced stress gradients. Emergence of resistance to antibiotics by bacteria is a growing problem, yet the variables that influence the rate of emergence of resistance are not well understood. In a microfluidic device designed to mimic naturally occurring bacterial niches, the time to development of resistance of *E. coli* to the antibiotic ciprofloxacin occurred within 10 hours. Resistance emerged with as few as 100 bacteria in the initial inoculation. Whole-genome sequencing of the resistant organisms revealed four functional single nucleotide polymorphisms attained fixation. Rapid emergence of antibiotic resistance in the heterogeneous conditions prevailing in the mammalian body may also apply to the emergence of drug resistance during cancer chemotherapy. Figure 3 presents a highlight from this work.

(4) Dynamics of cancer cells in 3-D structures. Metastasis is a three-dimensional invasion process where cells spread from their site of origin and colonize distant microenvironmental niches. It is critical to be able to assess quantitatively the metastatic potential of cancer cells. We have constructed a microfabricated chip with a three-dimensional topology consisting of lowlands and isolated square highlands (Tepuis), which stand hundreds of microns above the lowlands, in order to assess cancer cell metastatic potential as they invade the highlands. Figure 4 presents a highlight from this work.
Figure 1: (A) Two strains of *Escherichia coli* compete inside a microhabitat patch (MHP) system. Every other chamber has access to nutrient. A linear series of 85 such chambers is microfabricated in silicon. The nanoslits (yellow) allow nutrient to diffuse slowly from the nutrient reservoirs (purple) into only one of the chambers (blue). Cells retain their ability to swim and can move between MHPs using the inter-MHP channels (red). We performed the experiments in a device consisting of 42 such two-state systems placed on a linear array. (B) A computer-controlled microscope scanned the array of 85 MHPs and recorded a fluorescence micrograph of each MHP every 15 min.

Figure 2: (a) Fluorescence micrograph of individual cells (labeled green) inside the microchannel. Cell traps, where the rectification bias is reversed, have a higher concentration of cells. (b) False-color image showing the number of cells in each chamber as a function of time. Note that three spontaneously forming migrating bands of bacteria are observed.

Figure 3: (A) An overview of the entire microenvironment. (B) SEM image of the area of the array outlined by the box in (A). Each hexagon is etched to a depth of 10 µm; the interconnecting channels are 10 µm deep, 10 µm wide and 200 µm long. The insert shows a SEM image of the nanoslits at the microenvironment periphery. The nanoslits are etched down 100 nm and are 6 µm wide and 10 µm long. (C) Image of the expected Cipro concentration using the dye fluorescein as a marker. (D) The basic design of our microecology creates high stress using constriction of nutrient flow via nanoslits in the presence of an antibiotic gradient. We show here the apex of the device where the gradients are highest. Channels allow movement of motile bacteria.

Figure 4: Confocal images and the corresponding three-dimensional reconstructions showing the cell-chip system after reaching steady state. (A) A confocal z-slice of PC-3 cells cultured on the microchip. (B) The three-dimensional reconstruction shows PC-3 metastatic tumor cells attached to the sides and densely packed on the top. (C) A confocal z-slice of LNCaP tumorogenic cells cultured on an identical microchip. The focal plane is 25 µm below the top of the Tepui. (D) The three-dimensional reconstruction of LNCaP cells attached to the surface, showing that they barely reached the top.
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.

Summary of Research:

The implanted portion of our device consists of power and data secondary receiving coils; a small number of discrete components sealed into a titanium (Ti) can; and a custom-designed integrated circuit (IC) that contains clock and data recovery circuitry; current drivers for electrodes in a 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 (IrO\(_2\)) stimulating electrodes, which in turn give rise to the visual percepts mentioned above.

To date, the CNF-fabricated components of this system have been various proof-of-concept test structures and tools used in the research effort and an integrated combination of the external flexible circuit and a stimulating electrode array. Silicon (Si) wafers serve as carriers for these freestanding films during processing. The electrode leads are fabricated inside of ‘sandwiches’ of polyimide and amorphous silicon carbide (SiC), while the IrO\(_2\) 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 and solder attachment of discrete components onto an internal flexible circuit board which is hermetically sealed into an ultraminiature Ti can. The coils are soldered and glued to the integrated external flex-array which is in turn soldered to the hermetic feedthrough of the Ti can. Finally, the solder connections are overmolded for insulation and protection.

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 a radio frequency 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.

Scientific challenges still remain in realizing a chronically implantable retinal prosthesis. While our first generation device was primarily encapsulated in polymers for short term proof-of-concept implant studies, our second generation system focused on a device which would last many years in vivo. Our latest efforts are on developing a device with 200+ stimulation channels which is still small enough to be implanted in the ocular orbit and continue to function for many years in vivo. Thus, a major effort of this past year has been to develop a technological platform to build a robust, hermetically packaged, high-density subretinal visual prosthesis with a lifetime of > 10 years in biological saline that is scalable to hundreds of input/output (I/O) channels.
A key component of a high-density implantable prosthetic package assembly process is the reliable attachment of a flexible, microfabricated electrode array to the feedthroughs that carry signals into and out of the hermetic, sealed prosthetic package that contains the electronics. The hermetic Ti case is shown in Figure 1 where the feedthroughs are located on the upper surface rather than on the edge as in more traditional Ti can configuration. We used the CNF to laser-cut concept circuit outlines in polyimide film for evaluation by our surgical collaborators, and then using that approved form-factor we microfabricated a 200+ lead, flexible, microelectrode array with gold (Au) bumps. This was then bonded to the surface of an alumina feedthrough, shown in Figure 2. A portion of a bonded microelectrode array is shown in Figure 3.

To develop more advanced and higher fidelity retinal implant systems, we are pursuing 3D structures to improve electrode-cell coupling, and thus device performance. We have recently reported the results of our CNF efforts to develop a microfabricated polyimide-based subretinal penetrating electrode array, a SEM image of one 'post' is shown in Figure 4. This structure penetrates the retinal tissue so that the electrode is very close to the target cells. The structure was formed with SU-8 and has a high charge transfer material, sputtered iridium oxide film (SIROF), on the tip as the stimulating electrode, and a parylene-C coating electrically insulates the post metallization. These structures have been electrochemically evaluated and the performance has been found to compare well with traditional planar SIROF electrodes. These structures will form a key component of the next-generation retinal prosthesis.

References:


Figure 1: The side view of the ultraminature hermetic Ti case.

Figure 2: A portion of a 200+ conductor alumina feedthrough shown on a dime.

Figure 3: A close-up of a 200+ channel polyimide electrode array bonded to a ceramic substrate.

Figure 4: An SU-8 -based, SIROF tipped penetrating electrode.
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 [2].

Summary of Results:
Using µCCAs to Test the Toxicity of Nanoparticles:
We have used micro cell culture analogs (µCCAs) to test the toxicity of nanoparticles. Because of their small size and surface to volume ratio, nanoparticles possess unique properties, which can be utilized in medical applications such as diagnostics and drug delivery [3]. Particles might, for example, carry drugs or alter the absorption of drugs and nutrients that are administered orally. However, little is known of the particle’s fate within the body and tissues. We have recently developed a µCCA that combines cell culture models of the liver and the intestinal epithelium of the gastrointestinal tract in a physiologically realistic way (Figure 1) [4]. This model can be used to simulate the oral uptake of nanoparticles and other drugs.

We have used a µCCA that contained a model of the human intestinal epithelium and a model of the liver to simulate the oral uptake of 50 nm carboxylated polystyrene particles. To be able to compare the in vitro results with in vivo results, we also conducted a particle ingestion study with birds. In vitro, intestinal epithelial cells (Caco-2/HT-29/MTX) covered with mucous presents an effective barrier to 90.47% ± 2.85% of the particles over a period of 24 hours. The cell layer allows passage to single particles only. In addition, passing through the intestinal cell layer decreases the surface charge of the particles. At high doses — estimated in terms of possible daily human consumption — both Caco-2/HT-29/MTX and HepG2/C3A cell layers release aspartate transaminase (AST), indicating cellular stress and death. In vivo tests with birds suggest that the percentage of particles that transfer into the systemic circulation at the given concentration is too low to cause liver damage or inflammation, pointing to additional upstream or downstream mechanisms that were not present in the in vitro simulation.
Development of Microfluidic GI Tract Modules:

To obtain more detailed information from simulations with first pass metabolism μCCAs, we have developed a microfabricated gastrointestinal tract model that incorporates an on-chip membrane and integrated electrodes for transepithelial resistance measurements. The transepithelial resistance (TER) of the gastrointestinal epithelium is a measure for the intactness of its barrier function. To simulate the barrier function, gastrointestinal cells are cultured on membranes that allow access to either side (apical and basolateral) of the cell layer. Using GI-tract epithelial cells (Caco-2) and mucuous-producing cells (HT-29), co-cultured and grown to confluence on commercially available transwell membranes, we were able to simulate the GI-tract barrier and measure the transepithelial resistance (TER) [4,6]. From experiments with these static cell culture models of the intestinal epithelium, we know that nanoparticles alter the TER. To be able to measure the TER on chip, we have developed microfabricated electrodes and membranes that allow us to include the gastrointestinal tract model on a microfluidic chip that contains the systemic circulation.

In conjunction with the fabrication of flat, porous membranes, we have also developed 3D membranes that simulate the macrovilli characteristic of the GI tract (Figures 3 and 4). These structures increase the potential surface area available for metabolism and provide a more physiological microenvironment, which recent work using collagen-based villi has been shown to grow cell monolayers that closely mimic in vivo tissue morphology [7]. Synthetic villi up to 100 μm tall and between 25 and 100 μm wide have been achieved to date. The microfabricated 3D membranes have been shown to sustain long-term growth of the Caco-2 gastrointestinal cell line (Figure 4) and may be easily implemented in an on-chip GI tract module.

References:


Nanofluidics for Single Molecule Sorting

CNF Project # 762-99  
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Abstract:

Molecule separations are ubiquitous in molecular biology workflows and are necessary to isolate and enrich essential sub-populations for analysis. While many separation methods require input quantities of material at the nanogram-level or greater, identification and sorting at a single molecule level would access both the fundamental limit of sensitivity and material handling. We report the first demonstration of a fluorescence-activated single molecule sorter to perform high throughput selection of deoxyribonucleic acid (DNA) molecules based upon their fragment size. Using an M-shaped nanofluidic channel, we sorted 2.7 and 15.0 Kb DNA fragments, and achieved over 50 fold enrichment of the 15.0 Kb fragment from an input population of 16,000 molecules. Single molecule sorting can detect and collect rare sub-populations otherwise lost using conventional immunoprecipitation or gel electrophoresis methods and is reconfigurable to perform detection on many fluorescent markers simultaneously.

Summary of Research:

Nanofluidic channels were used to confine individual DNA molecules for fluorescence-activated sorting. The fluidic channels shown [Figure 1] were constructed in a fused silica substrate using a single layer of projection photolithography followed by reactive ion etch to construct channels with a cross-section measuring 250 nm by 500 nm. Fluid reservoirs access the channels by through-wafer ports and the final device was assembled with a direct wafer bond process. Nearly 20 sorting devices were constructed on a single, 100 mm substrate.

We have demonstrated the ability to identify single molecule fluorescence signatures in real-time and to actuate sorting on DNA fragments of different size. To perform this size-based analysis, a green intercalating dye (YOYO-1) stained a mixture of 2.7 and 15.0 Kb fragments. Focused laser spots on both the input and output nanofluidic tracks of the device were used to excite fluorescence and to identify molecules during transit through the device [Figure 2]. A field programmable gate array provided real-time evaluation of fluorescence bursts and actuated a high-voltage switch to direct the transport of molecules to either output. This method achieved 50 fold enrichment of the 15.0 Kb

Figure 1: Photomicrograph of a nanofluidic sorting device. An applied voltage drives molecules from the input (middle) microfluidic channel and through the M-shaped nanofluidic to either of the output (left or right) microfluidic channels. A focused laser beam (not shown) illuminates the nanofluidic at the input and each output to analyze the fluorescence emitted by each molecule passing through the device. Molecules are sorted by rapid switching of the applied voltage. Scale bar is 10 micrometers.
fragment within the sorted output, as verified in situ using the device’s M-shaped design, and demonstrated sorting at rates exceeding 1000 molecules/min. We have also applied this single molecule sorter to epigenetic analysis of methylated DNA to provide an alternative to conventional DNA immunoprecipitation based methods [1].

References:

An Array of Planar Apertures for Near-Field Fluorescence Correlation Spectroscopy

CNF Project # 762-99
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Abstract:

We have fabricated planarized apertures in a thin metallic film for near-field fluorescence correlation spectroscopy. This fabrication utilizes electron beam lithography to define the apertures, reactive ion etching to transfer the pillars in the fused silica substrate, and chemical mechanical polishing to planarize the 50 nm diameter apertures. The resulting device has 1.2 nm RMS roughness and an illumination area 14X smaller than a diffraction-limited area (0.11 vs. 0.0076 µm²) on both supported lipid bilayers and live cell membranes.

Summary of Research:

The organization and dynamics of cellular membranes is fundamentally important for a variety of cellular processes. The plasma membrane contains numerous receptors and signaling molecules utilized by the cell to sense the surrounding environment and transmit signals into the cellular interior. However, the nanoscale reorganization of membrane-bound molecules is largely not understood due to the experimental difficulties associated with dynamic nanoscale phenomenon [1].

Fluorescence microscopy provides high temporal and chromatic resolution, but is conventionally limited to the diffraction limit of ~ 200 nm resolution. Near-field excitation is one of the methods for improving on the diffraction barrier by using nanoscale metallic structures to concentrate the fluorescence excitation light at the dimensions of the fabrication [2,3]. To achieve confinement of excitation light to 60 nm diameter area of the membrane, we fabricated 50 nm diameter planarized glass-filled apertures in Al₉⁵Si₁⁵Cu₁% for transmission near-field illumination (Figure 1) [2].

Figure 1: Scanning electron micrograph of 50 ± 4 nm diameter planarized apertures for near-field optical microscopy (PANOMs). The four fused silica apertures are indicated by white arrows and are surrounded by the Al alloy film, which has noticeable metallic grain structure. (Reprinted with permission from Cell Press [2])
Fabrication of the planarized apertures utilized electron beam lithography to create 90 nm diameter pillars of NEB-31A3 on a 500 µm thick fused silica wafer. A reactive ion etch with CHF₃ and O₂ was performed to etch the fused silica and create nanoscale glass pillars. The glass pillars were etched with buffered oxide etch to yield pillars as small as 20 nm diameter. The wafer was then sputter coated with 200 nm of Al₉₅Cu₄Si₁ and planarized with chemical mechanical polishing, with a resulting RMS roughness of 1.2 nm.

Apertures were incorporated into a two-objective optical microscope (Figure 2) to examine membranes with fluorescence correlation spectroscopy (FCS). FCS analyzes the fluorescence intensity vs. time for individual fluorophores diffusing through the illumination spot. The characteristic dwell time for fluorophores in the illumination depends both on the diffusion rate of the fluorophores and the area of the illumination.

The small illumination area created by these apertures is shown by the fast dwell time for membrane-bound fluorophores via FCS (Figure 3). The half-time of diffusion for membrane-bound G₄₋ Bodipy in both POPC supported lipid bilayers and living RBL cells is 14x faster than in a calibrated far-field setup. Measuring the far-field illumination to be 190 nm diameter and the illumination area the near-field illumination was determined to be 60 nm diameter.

In future studies, we plan to utilize apertures of varying sizes for two-color cross-correlation analyses. This technique will permit us to examine microsecond dynamics of membrane-bound molecules and co-diffusion at 60 nm length scales.

Acknowledgements:

We appreciate collaboration with David A. Holowka and Barbara A. Baird. C.V. Kelly is supported by a NIH Kirschstein National Research Service Award postdoctoral fellowship (F32-GM092106). Additional research support came from the NIH (AI18306) and the Nanobiotechnology Center at Cornell University (NSF ECS-9876771). Device fabrication was performed in the Cornell NanoScale Science and Technology Facility (NSF ECS-9731293).

References:


Abstract:

Polydimethylsiloxane (PDMS) microfluidic devices for extraction and purification of genomic deoxyribonucleic acid (DNA) from small cell populations and single cells were developed. Hematopoietic stem cells trapped in a two-dimensional array of micropillars by size exclusion were chemically lysed releasing long strands of genomic DNA which were then immobilized within the microarray by hydrodynamic forces while cellular debris was washed away. The purified DNA was subsequently released from the microarray by enzymatic fragmentation under continuous fluidic flow conditions and collected for off-chip analysis by gel electrophoresis and fluorospectrometry. For a population of less than 100 cells, we have obtained a genomic DNA extraction efficiency of > 95%.

Summary of Research:

Various DNA extraction techniques have recently been implemented in microfluidic systems that provide better handling and manipulation of small sample and reagent volumes in engineered microstructures [1]. Microfluidic devices could perform the analysis automatically in an enclosed system thereby reducing the possibility of human error and cross contamination. These devices may also reduce the time and the cost of analysis by taking advantage of high reaction rates at the microscale and generally provide higher extraction efficiencies by utilizing features with high surface-to-volume ratios for improved DNA extraction, however they generally rely on DNA adsorption to silica or other biochemically functionalized surfaces.

The binding affinity is extremely sensitive to temperature, pH, and buffer composition which requires careful optimization to minimize DNA losses. Even after meticulous optimization, it is difficult to ensure that all the DNA fragments get adsorbed and the whole genome is represented in purified extracts obtained from a few cells and/or a single cell. Fundamentally different approaches to genomic DNA capture need to be explored to improve the extraction efficiency.

Here we explore a novel technique to extract and purify DNA by physically trapping long strands of genomic DNA in arrays of micropillars by hydrodynamic flow.

Figure 1: Photomicrographs of the fabricated PDMS microfluidic devices (Bottom) and of the array of micropillars for cell capture and DNA immobilization (Top).
MO-91 cells (hematopoietic stem cells infected with myeloid leukemia) were injected into the input of the microchannel and drawn under constant flow into an array of micropillars shown in Figure 1 in which they become trapped by size exclusion. The array with progressively decreasing spacing between microposts prevents channel clogging when larger numbers of cells and other debris are present in the growth medium.

The captured cells were lysed with a solution containing 1% sodium dodecyl sulfate (SDS) in Tris-EDTA buffer. Long strands of genomic DNA released from the cells become immobilized in the array of microposts by hydrodynamic flow. Additionally, the pressure driven flow is sufficiently slow as to prevent any shearing of the DNA.

After lysis, the genomic DNA is then rinsed and purified by flowing proteinase K and ribonucleases through the microchannels to remove histone proteins and RNA that may have become intertwined within the DNA. Thorough removal of cellular debris is important for the subsequent single-molecule studies as the labeled protein and lipid fragments can interfere with the analysis.

DNA capture by hydrodynamic forces allows separation of genomic DNA not only from proteins and lipids which are primary components of the cell lysate, but also from other nucleic acids such as mitochondrial DNA and RNA. This cannot be achieved with the alternative extraction methods. DNA strands extracted from four immobilized cells and from a single cell stained with PicoGreen fluorescent dye are shown in Figure 2. Purified DNA is released from the device by enzymatic digestion with restriction endonucleases (Bam HI and Hind III). The digestion process is captured in Figure 3.

All purified DNA is released from the microarray into the collection reservoir within two minutes. The digested DNA was collected into small elution volumes (~ 20 µL) for off-chip analysis with gel electrophoresis and fluorospectrometry (Nanodrop 3300). The cells captured in the array of micropillars were counted and the amount of the DNA was determined by assuming a quantity of 6.6 pg of DNA per cell. These fluorospectrometric measurements indicate that we are collecting and purifying and collecting > 95% of the genomic DNA released from the cells.

References:

Investigation of Functional Electrospun Bionanofibers in Microfluidic Channels

CNF Project # 802-99
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Abstract:

In this study, we investigated the incorporation of poly(vinyl alcohol) (PVA) nanofibers within poly(methyl methacrylate) (PMMA) microchannels in order to allow for sample preparation and concentration within microfluidic detection systems. Nanofibers were electrospun onto gold microelectrodes and integrated into polymer microfluidic systems using ultraviolet (UV)-assisted thermal bonding. We determined that nanofibers spun across microchannels maintain their morphology during fluid flow at linear velocities of 3.4 and 13.6 mm/s. In addition, we showed the ability of positively charged nanofibers to serve as bioseparators for negatively charged nanostructures using hexadimethrine bromide (polybrene) modified PVA fibers.

Summary of Research:

Sample purification and concentration are essential to the success of miniaturized detection assays that use small feature sizes and sample volumes in the nL-µL range [1]. Lab-on-a-chip devices aim to incorporate sample preparation and analyte detection within the same device, however this has proven difficult for many detection systems [2]. We aim to address this need for sample preparation within microfluidic systems by incorporating functionalized electrospun nanofibers within polymer microchannels.

Gold microelectrodes were fabricated on PMMA using standard microfabrication techniques. A 10 nm layer of chrome and a 200 nm layer of gold were evaporated onto the PMMA using a CHA evaporator. After evaporation, the PMMA was coated with positive photoresist (Shipley 1813) at 3000 rpm for 30 seconds. The PMMA was then exposed for 11 seconds using an ABM contact aligner and developed for one minute in MF 321. The PMMA was then etched for one minute in gold etchant and 15 seconds in chrome etchant.

We studied the effects of electrode spacing and width on nanofiber orientation using a basic five fingered electrode design with a large square electrode pad. Electrodes were fabricated with various gap sizes (0.1 mm to 10 mm), square sizes (50 µm to 500 µm), and electrode widths (1 mm to 2.5 mm). We determined that electrodes with a gap of 5 mm and width of 1 mm produced well aligned nanofiber mats between the electrode fingers (Figure 1).

The PMMA microchannels were formed using hot embossing with a copper template [3]. The copper masters were fabricated using negative photoresist (SU-8) photolithography and copper electroplating to produce raised copper channels on the copper plate. The microchannels were embossed onto PMMA using a Carver Laboratory Hot Press. Completed microfluidic devices were fabricated by bonding a piece of PMMA containing channels and a piece of PMMA patterned with a microelectrode and nanofibers using UV-assisted thermal bonding.

Nanofibers spun across microfluidic channels were subjected to fluid flow and analyzed to confirm that the fibers were not damaged during fluid flow. DI water was injected into the microchannels at 3.4 and
13.6 mm/s for 400 and 100 minutes respectively. The effluent was collected and analyzed using Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR). There are two FTIR peaks that indicate the presence of PVA in a solution: one for CH$_2$ (2930 cm$^{-1}$) and one for CH (2850 cm$^{-1}$). These peaks were not observed in the effluent samples, confirming that the fibers were retained within the channels during prolonged fluid flow. NMR analysis was also used to determine fiber stability during fluid flow. There are two NMR peaks characteristic of CH$_2$ in PVA, neither of which was observed in the effluent samples, once again confirming the stability of the fibers during fluid flow. We therefore concluded that the nanofibers are sufficiently stable for use in microfluidic devices for bioanalysis.

We fabricated positively and negatively charged nanofibers by incorporating hexadimethrine bromide (polybrene) and poly(methyl vinyl ether-alt-maleic anhydride) (poly(MVE/MA)) into a PVA spinning dope. Nanofiber composition was examined using FTIR and x-ray photoelectron spectroscopy (XPS) to confirm the incorporation of polybrene and poly(MVE/MA) within the fibers. Thermally stimulated current (TSC) measurements in pH 7 buffer solution confirmed the positive surface charge on fibers containing polybrene and negative surface charge on fibers containing poly(MVE/MA) [4].

Liposomes were used as a model analyte, and were modified with sulforhodamine B to allow for fluorescence imaging. Microchannels containing positively or negatively charged nanofibers were filled with HEPES-Sucrose-Saline (HSS) solution (pH 7) and a 1:1000 dilution of fluorescent liposomes at a flow rate of 1 µL/min. The liposome solution flowed through the channels for 30 minutes and was then washed out of the channels using a HSS solution at 1 µL/min for 60 minutes. The concentration of the liposomes within the channels was determined by measuring the fluorescence within the channels over time. As expected, channels containing positively charged nanofibers attracted and retained the liposomes, while channels containing negatively charged nanofibers did not retain a significant number of liposomes (Figure 3). The binding of liposomes to the positively charged fibers was quantifiable already during liposome flow and pre-wash buffer addition as peak fluorescence signals were significantly higher.

References:


**Figure 2:** Electrospun nanofibers spun as a thick filter within channels.  

**Figure 3:** The average pixel intensity of channels containing either positively or negatively charged nanofibers.

Charged nanofibers were spun across the microchannels in order to create a high density filter (Figure 2). It was hypothesized that positively charged polybrene-modified nanofibers would bind negatively charged nanovesicles, while negatively charged poly(MVE/MA)-modified nanofibers would repel the nanovesicles.
Ultra-Sensitive Biodetection Using Photonic Crystal Nanocavities

CNF Project # 810-99
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Abstract:

Nanocavities coupled to photonic crystal waveguides are investigated in this study for ultra-sensitive detection of nanometer sized particles and biomolecules. The nanocavity is formed by increasing the radius of an air hole adjacent to a photonic crystal waveguide structure. The device is fabricated on silicon-on-insulator (SOI) substrate using electron-beam lithography and reactive-ion-etching. The fabricated device is appropriate for multiplexed detection of virus-sized particles on a single platform.

Summary of Research:

In a recent work, we have developed a novel photonic crystal design where nanocavities are coupled to a two-dimensional photonic crystal waveguide for sensing applications [1,2]. This design allows light to be transmitted through the waveguide except at frequencies that correspond to the resonant mode of the nanocavity where a sharp transmission dip is observed. By tuning the nanocavity radius and placing the photonic crystal structures in series, multiple transmission dips can be obtained in the output spectrum, as each nanocavity possesses a distinct resonant wavelength. We have demonstrated very sensitive error-corrected detection of protein molecules with this new design [3]. This design also opens up opportunities for multiplexed biosensing of nanometer sized particles and biomolecules on a single platform, provided the nanocavity dimensions are appropriate.

We have designed a two-dimensional W1 photonic crystal waveguide structure by removing a single array of central air holes. A large nanocavity in the waveguide is formed by removing three holes from the first and second rows adjacent to the waveguide, and by creating a defect hole with double the radius of the surrounding air holes. The lattice constant ‘a’ is chosen to be ~ 400 nm for operation near wavelength of 1500 nm.

The photonic crystal waveguide device is fabricated on SOI substrate with a 450 nm thick Si device layer and a buried oxide layer (BOX) layer of 1 µm. The substrate is first cleaned using standard MOS cleaning procedure and a 130 nm thick hard oxide mask layer is then grown by wet thermal oxidation. For pattern writing, a 5.5% solution of 495k PMMA (polymethylmethacrylate) in anisole is spun on the SOI surface. The photonic crystal patterns are written using the JEOL JBX-9300FS electron-beam writing system. After developing the patterns, the exposed areas of the SOI substrate are dry etched in the Oxford 80 reactive ion etcher. The oxide hard mask is first etched using argon-assisted CHF$_3$ gas. Subsequently, the Si device layer is etched in the Plasma Therm SLR 770 machine using a gas mixture of CF$_4$ and BCl$_3$. After completion of the etching process, the samples are diced using the K&S 7100 dicing saw and the waveguide facets are polished in an Allied MultiPrep tool for coupling light from an external source.

Figure 1 shows the scanning electron microscope (SEM) view of the fabricated large nanocavity device coupled to ridge waveguides. The nanocavity has a radius of 225 nm. Computations show that the structure has a transmission dip at the resonance wavelength of 1511 nm (Figure 2).
The calculated cavity quality factor for the resonant mode is ~ 1000. The structure is theoretically found to be sensitive to the infiltration of a single nanoparticle of 50 nm diameter. A 0.6 nm red-shift in the resonance wavelength is observed when the 50 nm nanoparticle is present at the center of the nanocavity (Figure 1). Theoretical results thus suggest that the nanocavity coupled photonic crystal waveguide sensor can be used to detect single 50 nm sized viral particles.

References:


Electrochemical Detection of Neurotransmitter Using CMOS IC Biosensors

CNF Project # 848-00
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Abstract:

The signal transfer from presynaptic neuron to postsynaptic neuron is mediated by a neurotransmitter secretion process called exocytosis. Non-polarizable electrodes can be used to measure the oxidation current of neurotransmitter molecules released from single vesicles. This technique is called amperometry and provides the precise amount and time course of released molecules. However, amperometric recordings with conventional carbon fiber microelectrodes are very time consuming. By designing CMOS IC biosensors, we were able to incorporate a large number of electrodes and amplifiers in a small silicon chip (~ 5 mm^2), for automatic highly parallel amperometric recordings.

Summary of Research:

Exocytosis is the release of molecules from a secretory vesicle by fusion of the vesicle with the plasma membrane. This secretion is mediated by three key proteins: Synaptobrevin, SNAP-25, and Syntaxin. These proteins are called SNARE (Soluble NSF Attachment Protein RReceptor) protein. Some types of released molecules, such as serotonin, dopamine, epinephrine, and norepinephrine, are oxidizable by an electrode that is held at 700 mV and the electron transfer from the oxidation of a neurotransmitter molecule is measurable using a current to voltage amplifier.

An oxidation current spike recorded per each exocytotic event provides details of the vesicle-plasma membrane fusion kinetics. The total charge that is collected from a single vesicle release (quantal size) is closely related to the volume of the vesicle as the electroactive neurotransmitter concentration inside the vesicle is rather constant [1]. A so-called foot signal preceding the steep spike in the amperometric recording indicates the neurotransmitter leaking through a narrow fusion pore at the initial step of secretion by SNARE proteins. Previously, it has been discovered that quantal size is increased by the Parkinson’s drug L-Dopa and decreased by drugs such as reserpine [2] and the rate of release and the fusion mechanism are affected by manipulations that resemble BOTOX treatment [3].

To conclusively derive a result from a set of recordings, a large number of amperometric measurements must be performed and collected. This involves tedious positioning of microelectrodes under microscopic observation and many cells are unused as there are only so many experiments that can be conducted within cells’ survival time. Along with a highly skilled researcher, amperometry also requires a set of costly tools: a microscope, a manipulator, and an amplifier.

Complementary metal oxide semiconductor integrated circuit (CMOS IC) technology has been recently adapted to direct use for biological applications. As a large amount of amplifier circuitry can be integrated in an IC chip, incorporating biosensor electrodes on the IC chip can provide compact and low cost biosensors. As CMOS IC biosensor arrays including thousands of electrodes have a high number of electrodes per unit area, application of a cell suspension to the biosensor is sufficient to ensure successful recording on a large fraction of electrodes. Figure 1 illustrates the functional circuitry in the CMOS IC. By using an operational amplifier “A” the electrode is held at V_{ref} (700 mV). Oxidation charges entering through the electrode from the cell are accumulated at capacitor “C” which is then read out as the chip’s output.
Unfortunately, most vendors for CMOS IC fabrication do not offer non-polarizable metals in their fabrication. For an electrochemical measurement, a CMOS IC requires post-fabrication deposition of platinum onto the aluminum copper alloy pads as biosensing electrodes, followed by an insulation step to ensure the entire surface other than the platinum electrodes is insulated. Figure 2 illustrates the post-fabrication steps of platinization and insulation. The area of platinum that is exposed should be 10 µm × 10 µm as the chromaffin cells that we use for amperometric measurements may be up to 10 µm in diameter. The platinum that is exposed in Figure 3.1 will contact cells and measure the released molecules. In Figure 3.2, the complicated lines passing under the platinum electrodes represent the amplifying circuitry that is embedded in the IC.

References:
Revealing the Conformational Change of SNAP-25 during Exocytosis using Electrochemical Detectors and TIR-FRET Imaging

CNF Project # 848-00
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Abstract:

The direct relation of a conformational change in SNAP-25 to individual fusion events was investigated in bovine chromaffin cells over-expressing the SNARE Complex Reporter (SCORE). In the SCORE expressing cells we imaged the FRET change of SCORE localized to the plasma membrane by Total Internal Reflection - Fluorescence Resonance Energy Transfer (TIR-FRET) imaging and simultaneously recorded individual fusion events as amperometric spikes with a microfabricated electrochemical detector (ECD) array. Our study reveals the temporal correlation between the conformational change of SNAP-25 and membrane fusion.

Summary of Research:

The SNARE hypothesis postulates that the assembly of the Soluble N-ethylmaleimide-sensitive factor Attachment Protein Receptor (SNARE) complex is required to induce fusion pore opening and transmitter release. However, the direct relation between a conformational change in the SNARE complex and individual vesicle fusion pore openings, which is essential to understanding the molecular mechanism of exocytosis, has not been demonstrated.

To address this question, we performed experiments where a conformational change in the SNARE complex could be measured simultaneously with individual fusion events. SCORE was constructed by inserting as FRET donor Cyan Fluorescent Protein (CFP) and as FRET acceptor Venus (a variant of Yellow Fluorescent Protein) at the N-termini of the two SNARE motifs of SNAP-25, SN1 and SN2, respectively [1]. When Venus is excited, fluorescence resonance energy transfer (FRET) can occur if the SNAP-25 is in its folded state in the SNARE complex. In the SCORE expressing cells we imaged the FRET change of SCORE localized to the plasma membrane by TIR-FRET imaging and simultaneously recorded individual exocytic fusion events as amperometric spikes with a microfabricated electrochemical detector (ECD) array [2,3], (Figure 1).

The vesicle release location was determined with spatial resolution of ~ 300 nm based on the charge ratios of oxidation currents recorded by the four electrodes in the ECD array (2). And the accuracy of the position assignment...
was evaluated by the comparison of experimental recordings with simulated results, (Figure 2). We analyzed the fluorescence in a $2 \times 2$ pixel area $(330 \times 330 \, \mu m^2)$ surrounding the release site as determined from the amperometric recording. For single release events the signal-to-noise ratio is insufficient to obtain a clear FRET signal. To improve the signal-to-noise ratio we averaged the fluorescence traces after aligning them to the starting time points (set to $t = 0$) of their respective amperometric spikes. By averaging 339 events, a FRET change associated with vesicle fusion became clearly evident (Figure 3).

The time resolution of fluorescence imaging (219 ms/frame) is much lower than that of amperometric spikes. The fluorescence intensities obtained from individual frames were therefore converted to the same time resolution as the amperometric recording, assigning the same fluorescence value obtained from a particular frame to all the points corresponding to that frame. Using a simulated data set assuming that the FRET change occurs stepwise at $t = 0$ a FRET trace is obtained which shows a linear increase over the time of one frame that has its half time at the time of the spike, except for a small shift that results from the inter-frame interval of 19 ms between the 200 ms exposures (Figure 3 dashed line). In Figure 3, the measured data (solid line) shows a similar increase but at a time preceding the amperometric spike by about 100 ms. This result shows for the first time a conformational change in SNAP-25 that is directly related to fusion pore opening.

References:

Abstract:

We propose an integrated-optics-based bacteria sensor which would enable the detection of live bacteria in much shorter time periods than current day-long incubation based test. We demonstrate an optofluidic device with microring resonators which uses enzyme catalysis to identify the presence of bacteria and can therefore be used to quantify the number of viable bacteria in a fluid sample.

Summary of Research:

A number of different technologies exist for the detection and identification of pathogenic bacteria in diagnostic laboratories including biochemical and microbiological assays but these methods suffer from either long incubation times, large costs, or are highly labor intensive [1]. Therefore, there is a great need to develop a rapid, sensitive, and low cost detection technique that can test viability and enable follow-up analysis. We propose a device which can detect single bacteria without consuming expensive reagents or requiring high cost equipment. By combining nanophotonics with microbiological testing, the device can leverage high sensitivity photonic structures to enable massive parallelization of measurements and low cost.

The proposed device would use chromogenic agars, which change color due to enzymatic activity to detect bacteria. Instead of Petri® dishes, we propose depositing a thin layer of hydrogel on top of a microring resonator. The microring cavity allows for very sensitive optical absorbance measurements on surrounding materials [2]. The hydrogel would then serve as a growth media for the bacteria to grow on and the subsequent color change induced in the agar could be measured directly by the microring resonator.

The chromogenic assay relies on the cleavage of the tetrazolium salt XTT to generate a water soluble orange formazan product. The amount of the formazan dye, which is measured using optical absorbance, is correlated with the number of viable cells. To test the feasibility of the proposed bacteria/hydrogel/microring device, we perform measurements in solution and flow the absorbing liquid over top of the microring resonators. This allows us to determine the sensitivity of the microring resonators to metabolic activity of bacteria.

The absorption spectrum of this dye has a peak around 470 nm. Since there are no commercially available tunable wavelength lasers that operate at this wavelength, we developed a novel measurement method which allows us to use a fixed wavelength source to measure the resonance response of a microring. The measurement technique relies on the thermo-optic effect where the refractive index of materials change with temperature. Instead of sweeping the laser wavelength to measure the transfer function of the microring, we sweep the temperature of the ring which shifts the resonance through the operating wavelength allowing the transfer function to be recorded.

The nanophotonic and microfluidic components are fabricated using standard microlithography techniques. The material platform includes silicon nitride (Si$_3$N$_4$) as the waveguiding material on top of silicon dioxide (SiO$_2$). The fabricated microring is shown in Figure 1. The experimental process involved first growing $E$. coli in a liquid media containing XTT with samples extracted at roughly 8 hour time periods over the course of a 24 hour incubation period. The optical absorption of these samples were measured with a conventional laboratory spectrophotometer as well as with our microring resonator. Figure 2 shows an example of the resonance measurement to determine the microring attenuation coefficient. In Figure 3, we show the results of measurements on the four bacterial samples, plotted as
propagation loss in the ring as a function of the absorbance of the fluid measured. The ring propagation loss increases monotonically with absorbance of the fluid and the change in the microring performance is clearly visible over the tested range of absorbance values of 0 to 1 AU.

The use of XTT in chromogenic assays has been studied extensively and we can use results from literature [3] to determine the concentration of the bacteria as a function of absorbance. *E. coli* bacteria incubated for only 30 min with concentration $1 \times 10^8$ cells per ml leads to an absorbance of 0.6. At this bacteria concentration, the number of bacteria required to generate an absorbance of 0.6 within the 2 nL channel volume is 200 cells, which corresponds to a device sensitivity of 1 bacterium per 10 picoliter. The ability to manipulate picoliter volumes is within the capability of current microfluidic technology. Therefore, the demonstrated device can be used to perform fundamental single bacterium studies. The proposed platform of growing bacteria on thin layers of chromogenic hydrogels is promising. Further studies on the deposition of ~100 nm layer hydrogels and its change in absorbance due to bacteria metabolic activity need to be carried out.

We have demonstrated a novel measurement technique which allows single wavelength lasers to measure optical absorbance on an integrated optofluidic device. Using silicon nitride ring resonators with 10 µm radius and quality factors greater than 80,000, we measured the absorption due to the cleavage of XTT by the metabolic activity of *E. coli* bacteria. The device can measure absorption coefficients of 0.1 cm$^{-1}$ and can detect the absorbance due to a single bacterium in a 10 pL volume. These results demonstrate the suitability of microring resonators as inexpensive, compact and sensitive sensors for chromogenic assays and single cell studies.

**References:**


Abstract:

Tissue templates for reconstruction and regeneration in vivo have achieved clinical successes for homogeneous tissues in well vascularized regions. Outstanding challenges exist for applications in poorly vascularized regions and for spatially differentiated tissues. Here, we present a strategy to control the spatial patterns of cell and vascular ingrowth throughout the volume of a bioremodelable and resorbable matrix via well-defined micropores and networks of microchannels. Our presentation of this approach includes: a description of a lithographic technique to form deterministic microstructures within a matrix of native collagen; elucidation of multistep process by which microstructures drive rapid invasion and vascularization; and demonstration of long range guidance of invasion through the full thickness of patterned templates.

Summary of Research:

We have extended our methods [1] for the formation of microstructure within native type I collagen to allow for multi-level networks of interconnected microchannels (Figure 1). We exploited these microstructured tissue templates (MTTs) to investigate the geometry-dependent response of tissue to a bio-resorbable matrix [2-3]. For this purpose, we implanted MTTs with micro-pores of well-defined dimension and geometry subcutaneously in mice. Figure 2 illustrates the geometry-dependence of the density of cellular invasion into the templates. In particular, we find that at each time point, the density scales with the surface to volume ratio of the micro-features. Staining of the scaffolds at early times suggests that the cells involved in acute inflammation (neutrophils and macrophages) rapidly explore the interior volume associated with the pores. Figure 3 presents a comparison of the healing process with MTTs relative to the clinical standard (Integra®). We find significantly higher cell density (Figure 3a) and more mature blood vessels (Figures 3b-c) in MTTs relative to Integra®. We conclude that the inclusion of microstructure within simple biomaterials could provide a basis for increasing the rate and extend of engraftment in reconstructive surgeries.

Figure 1: Microstructured tissue template (MTT). a) Schematic diagram of fabrication of MTT. b) Schematic cross-sectional view of MTT. c) Fluorescence micrograph of MTT. Pore structure was filled with red-fluorescent microbeads.
Figure 2: Geometry-dependent invasion and immune cells. (a) Cell density within microstructure as a function of geometry (pore vs. slot) and size (pore diameter). (b) Cell density scales linearly ($R^2 > 0.85$) with surface to volume ratio of microstructure. (c-d) Immuno-histo-chemical staining on sequential histological sections of an MTT explant at day 3 indicates the preponderance of neutrophils (c – 7/4) and macrophages (d – F4/80) in microfeatures.

Figure 3: Invasion and vascularization in MTT and Integra®. (a) Cell density within periodic area (or equivalent in uniform materials) as a function of distance (z) from tissue after 14 day explants. (b-c) IHC showing endothelium (CD31) and perivascular cells ($\alpha$-SMA) in day 14 explants of MTT (b - 100 µm pores) and Integra (c - bottom).

References:


Effect of CXCL12 on Tumor Cell Migration using a 3D Microfluidic *in vitro* Model

CNF Project # 1209-04  
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Abstract:

Cancer metastasis is a complex and dynamic process that spreads cancer cells throughout a body, leading to most cancer death in humans. To study cancer metastasis *in vitro*, we have developed a hydrogel-based 3D microfluidic chemotactic device. The positive features for the devices were microfabricated using a standard photolithography. With a hypothesis that cancer cells may be chemotactic to chemokine gradients, various concentration gradients of CXCL12 were used to test if cancer motility and directionality are affected by the gradient. We concluded that CXCL12 gradients increase both chemokinesis based on speed and chemotaxis based on velocity towards a high concentration of the gradient of a malignant breast cancer cell line (MDA-MB-231).

Summary of Research:

**Device Design.** The microenvironment, including chemical and mechanical conditions, is known to be critical for cancer progression. To understand better how cancer interacts with its environment, systematic control over the microenvironment is important. We have developed a microfluidic gradient-generating device based on agarose (a hydrogel) to control chemical environments for cancer metastasis study. The master for the microfluidic devices was fabricated on a silicon wafer with a feature of three channels: the channel width is 400 µm, the ridge between the channels is 250 µm, and the thickness is 250 µm [1]. The microfluidic device (1 mm thickness) was made with 3% agarose in phosphate buffered saline (PBS) on the master wafer (Figure 1) and then assembled between a glass slide and a Plexiglas® top plate. A malignant breast cancer cell line (MDA-MB-231) at an initial concentration of $1 \times 10^6$ cells/ml was mixed with 0.15% collagen type I and injected into the center channel to form a 3D *in vitro* culture, mimicking natural 3D in vivo systems. Syringe pumps were connected at the side channels through a tubing to perfuse media and to establish a gradient of a chemokine. A time-course series of images at intervals of 5 minutes for 16 hours was taken under a bright-field microscope (Olympus IX 81 and Hamamatsu Orca-ER) in an environmentally-controlled chamber (37°C, 5% CO₂, and 90% humidity).

**Response to Gradients of a Chemokine.** The pattern of expression of chemokine receptors is correlated to cellular behaviors, and chemokines are suggested as a signal for metastasis of tumors [2]. Müller and colleagues reported that chemokine receptors, CXCR4 and CCR7, were highly expressed in human breast cancer lines, showing a chemotactic response of MDA-MB-231 cells to CXCL12 or CCL21 [3]. However, the currently prevailing *in vitro* system, the Boyden chamber, cannot provide information on a dynamic behavior of cells. We utilized our microfluidic devices to study chemotactic characteristics towards a...
chemokine gradient. MDA-MB-231 cells were chemotactic towards a high concentration of CXCL12, and their chemosensitivity peaks at 0.11 nM/µm (Figure 2). The migration velocity along the gradients follows receptor ligand binding kinetics with KD = 40 nM; in agreement with the value obtained using a biochemistry method [4]. We also found that the chemotactic response is partially due to an increase in chemokinesis (overall motility), which is not easily deducted from other chemotactic systems. With this detailed analysis of cell motility based on single cells, our system can provide valuable information about cancer behavior under a well-controlled microenvironment, leading to a better understanding of cancer metastasis.

References:


Silicon Nitride Cantilevers for Muscle Sarcomere Force Measurements

CNF Project # 1255-04  
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Summary of Research:

Silicon nitride cantilevers for sub-cellular force measurements were constructed at the CNF using photolithography and reactive ion etching as has previously been shown [1]. The stiffness of the cantilevers (22 pN/nm or 154 pN/nm) was adjusted by varying the dimensions of the devices and this gives us considerable flexibility in the types of experiments we can perform. The cantilevers are densely packed on 4-inch silicon wafers and this results in a very affordable, disposable force sensor for our work. Each cantilever pair costs approximately $1 to produce when all costs are considered.

The purpose of the study using these cantilevers (Figure 1) was to determine failure stresses and failure lengths of actively and passively stretched myofibrils. Myofibrils failed at average sarcomere lengths (about 6-7 µm) that vastly exceeded sarcomere lengths at which actin-myosin filament overlap ceases to exist (4 µm) and thus actin-myosin based cross-bridge forces are zero at failure (Figure 2). Surprisingly, however, actively stretched myofibrils had much greater failure stresses and failure energies than passively stretched myofibrils, thereby providing compelling evidence for strong force production independent of actin-myosin based cross-bridge forces.

Follow up experiments in which titin was deleted and cross-bridge formation was inhibited at high and low calcium concentrations point to titin as the regulator of this force, independent of calcium. The results of this study point to a mechanism of force production that reduces stretch-induced muscle damage at extreme length and limits injury and force loss within physiologically relevant ranges of sarcomere and muscle lengths.

References:

Figure 2: Mean failure stress (±1SD) versus mean failure sarcomere length (±1SD) for actively and passively stretched myofibrils. Failure stresses of actively stretched myofibrils (red diamond) were much greater than for passively stretched myofibrils (green square). Myofibrils stretched in a BDM and high calcium concentration solution (purple circle) failed at slightly higher stresses and sarcomere lengths than the passively stretched myofibrils. Titin deleted myofibrils (active-blue triangle and passive-yellow diamond) failed at very low stresses. Insert panel shows stresses of actively (red) and passively (green) stretched myofibrils as a function of sarcomere length. Insert data are for single active or passive lengthening tests on individual myofibrils pulled to failure. Values are means and ± 1 SD at discrete sarcomere lengths.

Figure 1: Image of a single cantilever pair.
Nanofountain Probes for the Delivery of Molecular Inks

CNF Project # 1296-04
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Primary Research Funding: This work was supported by the Nanoscale Science and Engineering Initiative of the National Science Foundation under NSF Award EEC-0647560. Horacio D. Espinosa acknowledges the support provided by the National Science Foundation through NIRT project No. CMS00304472
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Abstract:

Nanofountain probes (NFPs) are atomic force microscopy (AFM) probes designed for direct-write delivery of liquid molecular “inks” with sub-100 nm resolution. Liquid inks' stored in an on-chip reservoir are fed through integrated microchannels to apertured dispensing tips by capillary action. This allows continuous delivery either to a substrate for direct-write nanopatterning, or to a cell for in vitro injection. Recently a fourth generation of nanofountain probe with enhanced geometry was fabricated. Fluid flow models quantified how the probe dimensions affect directly the NFP patterning resolution. These results were implemented to the fabrication which will enable a sub-100 nm patterning resolution and reproducibility necessary for a viable large-scale nanomanufacturing.

Summary of Research:

The nanofountain probe (NFP [1-3]) functions as a highly miniaturized fountain pen that can be used to deliver a variety of materials with precision in the 50 nm to 1 µm range. As in a conventional fountain pen, the liquid material to be delivered (the “ink”) is contained in a reservoir and flows through a channel to an apertured dispensing tip (Figure 1). Past demonstrations of direct-write nanopatterning include proteins [4] and DNA [5] in buffer solution, gold nanoparticles in aqueous suspension [6], thiols [1,3], and drug-coated nanodiamonds [7]. Piezoelectric positional control of the NFP by an AFM enables ultra-precise prescription of pattern geometry. The accuracy of the NFP combined with the broad range of molecular delivery capabilities enable studies at a truly single cell level through two modes of delivery: direct write nanopatterning, and direct in vitro injection (Figure 2).

Figure 1: Schematic of the nanofountain probe. Liquid ink is stored in an on-chip reservoir and fed through enclosed microchannels to apertured writing tips by capillarity (inset, scale bar: 2 µm) [7].

Figure 2: Schematic of the two modes of delivery for the NFP. (left) For direct-writing nanopatterning, the tip is brought into contact with the substrate where an ink meniscus forms (right) for in vitro cellular injection, the tip is introduced to the cell membrane [7].
A fourth generation of NFP with enhanced features was fabricated jointly at Cornell NanoScale Science and Technology Facility, NY, and at the Center for Nanoscale Materials at Argonne National Laboratory, IL (Figure 3). A primary objective of this fourth generation fabrication was to incorporate the probe geometry optimized by fluid flow models [4]. A second aim is to better control the overall homogeneity of the probe geometry for a higher throughput yield in the nanopatterning. Fabrication issues typically encountered in previous generation limited greatly the control of the patterning [1]. The new generation addresses these problems through changes in the process flow.

Arrays of sharp silicon tips were created using silicon on insulator by wet anisotropic etching. Low stress silicon nitrides were consecutively deposited by low-pressure chemical vapor (LPCVD) to create the cantilevers with embedded microfluidics ending at the silicon probe. The apertured tip was then defined by etching selectively the outer nitride layer by inductively coupled etching (ICP) (Figure 4). The high-density ion plasma etching enabled a better control of the core shell-tip length definition, a critical feature for the patterning resolution. The inner microchannels were released by hydrofluoric acid-based wet etching. Channels were then sealed by depositing a silicon oxide by plasma enhanced chemical vapor deposition (PECVD). The PECVD parameters including gases and temperature were tuned to alleviate the stresses in the multilayer and prevent the cantilevers from bending after release of the chip by deep reactive ion etching (DRIE). The resulting sharper NFPs are expected to produce patterns with higher resolution and spatial control. They will be applied to building carbon nanotube-based NEMS by patterning catalyst for subsequent CNT growth and in vitro single cell nanoinjection.

References:
Single Protein Detection Using a Microfluidic System

CNF Project # 1393-05
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Abstract:

The understanding of protein interaction dynamics is important for signal transduction research, but current available techniques prove difficult in addressing this issue. Thus, using the microfluidic approach, we developed a digital protein analytical platform and methodology named “microfluidic system analyzing protein in single complex” (MAPS) that can measure the amount of target proteins and protein complexes at the digitally single molecule resolution.

Summary of Research:

We introduce MAPS, a novel digital protein interaction analysis platform and detection system using microchannels for single-molecule detection technique with solid stochastic algorism. It was designed to provide a rapid method to investigate the detailed protein-protein interactions for potential high throughput applications.

As shown in Figure 1, the microfluidic channels were fabricated on a 500- and a 170-µm thick ultraviolet laser (UV) grade fused silica wafer (Mark Optics, Santa Ana, CA) [1]. The detection channel has a width of 2 mm on a 500 mm thick fused silica wafer and CF$_4$ plasma was used to dry etch the channel to a depth of 500 nm. A protective surface coating was then spin-coated onto the wafer and injection ports were drilled by a sand-blast tool.

After the removal of any surface coating and a thorough piranha cleaning, a UV grade fused silica cover wafer of 170 mm thickness was carefully clinched to the substrate wafer using deionized (DI) water as an intermediate. Subsequently, these two wafers were permanently bonded by annealing at 1050°C in air for 5 h. The schematic diagram in Figure 2 shows the schematic diagram of MAPS.

A 375 nm UV laser was focused through 60X water immersion objective (NA 1.25) on the center of the microchannel. Photon bursts from target proteins were recorded by the avalanche photodiode (APD). EGFR proteins were labeled with the specific anti-EGFR antibodies, which were further recognized by QD605 conjugated secondary antibody. Their photon burst signals were obtained and smoothened by the FFT filter (Figure 3a). All events higher than the noise level obtained from QD605/EGFR sample were plotted in the 1D histogram with the threshold which was determined by QD605/ control IgG sample (Figure 3b). The events with higher photon burst than the threshold line are defined as EGFR. On the other hand, the events which have less photon counts than the threshold value line are defined as unbound QD605. After background subtraction, QD605/EGFR events were successfully obtained by MAPS.

References:

Figure 2: Protein detection system setup.

Figure 3: Detection of EGFR. (a) upper: photon burst of QD605/EGFR, lower: profile after FFT filtering. (b) Histogram of QD605/EGFR.
DNA Methylation Profiling in Nanochannels

CNF Project # 1483-06
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Abstract:

We report the profiling of the 5-methyl cytosine distribution within single genomic-sized DNA molecules at a gene-relevant resolution. This method linearizes and stretches DNA molecules by confinement to channels with a dimension of about 250 \times 200 \text{ nm}^2. The methylation state is detected using fluorescently labeled methyl-CpG binding domain proteins (MBD), with high signal contrast and low background. DNA barcodes consisting of methylated and non-methylated segments are generated, with both short and long concatemers demonstrating spatially resolved MBD binding. The resolution of the technique is better than 10 kbp, and single-molecule read-lengths exceeding 140 kbp have been achieved.

Summary of Research:

Epigenetic regulation is the inheritable modification of gene activity without influencing the underlying DNA sequence. DNA 5-cytosine methylation is one of the most widely studied mechanisms influencing epigenetic gene regulation, and is generally thought to suppress gene expression. A CpG pattern, in which cytosines on both strands carry this modification, can be maintained through DNA replication, and thus cell division. The methylation of CpG islands contributes to various biological processes such as parental genomic imprinting, x-chromosomal inactivation, cellular differentiation, aging, and cancer.

In our work, methylation patterns are detected through binding of a fluorophore-tagged probe to the interrogated dsDNA segment. The binding pattern along the DNA is detected by fluorescence microscopy. In order to achieve single-gene relevant resolution, DNA is stretched by confinement to a quasi one-dimensional nanochannel. Nanochannel stretching itself is an emergent technique [1] that has been used to map the length of DNA fragments, image the binding of GFP-fusion transfection factors, observe real-time ordered restriction mapping, and perform single-molecule melting temperature mapping. The particular physical structure of our device follows Reisner et al. [2], and is shown in Figure 1.

In order to provide a robust testing vehicle with minimal biological complexity, we used \lambda -phage DNA concatemers as a model system for genomic DNA (Figure 2). By concatenating fully CpG-methylated and non-methylated strands, we created a predictable barcode that enables us to judge both the detection efficacy and the mechanical properties of the probe-substrate complex. Fluorescently tagged methyl-CpG-binding domain (MBD) protein fragment serves as the methylation sensitive probe (Figure 3).

When the random methylation barcode after MBD-decoration was introduced we observe binding patterns as predicted by the random design of the DNA substrate [3]. Figure 3 shows a collection of observed fluorescence patterns from molecules incorporating MBD-labeled stretches. MBD-binding occurred in continuous stretches, and not as dots along a line. Each \lambda -monomer has about 3000 CpG sites which can be recognized by MBD. We observed barcode patterns that were consistent with the DNA used to construct the concatemers. However, we found that complexing of MBD to methylated DNA reduced the equilibrium length of the molecule to about one quarter of the MBD-free case.

Our technique is very distinct from the recently published single-chromatin analysis at the nanoscale (SCAN) [4]. These authors detect the methylation of DNA fragments in nanofluidic channels under flow and demonstrated coincidence of fluorescent MBD and DNA. We demonstrate that the binding location within the molecule can be determined since our molecules are efficiently stretched out, and can be observed over extended times.
References:


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Figure 1: Schematic of a device with two microchannel feeds (top and bottom) that are bridged by a nanochannel (inflowing arrows) containing an Alexa568-MBD labeled DNA concatemer. A shallow central shunt channel (outflowing arrows) allows the use of pressure-driven flow.

Figure 2: Schematic of possible outcomes of DNA concatemer formation with 5-cytosine methylated (5mC) and non-methylated segments.

Figure 3: Schematic of Alexa568-MBD to DNA concatemer. The entire molecule is stained using the greenstain YOYO-1, and Alexa568-MBD binds to methylated stretches.

Figure 4: (a) Fluorescence images of concatenated methylated and non-methylated λ-DNA labeled with Alexa568MBD (red) and YOYO-1 (green), stretched out in nanochannels. Within each panel, colors are split for clarity; (left) YOYO-1 only (DNA), (center) composite, (right) Alexa568 only (Alexa568-MBD). Schematic drawings in each panel illustrate the spatial position of the Alexa Fluor 568 MBD and the length of the λ-DNA. Panel (b) scale bar = 5 µm.
Three Dimensional Microscale Niches for Studies of Tumor Angiogenesis

CNF Project # 1540-07
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Abstract:

We are using microengineering approaches in combination with biomaterials and three dimensional (3D) cell culture to develop in vitro models of tumor angiogenesis. Lithographically defined microstructures were used to define microchannels in dense (1%) collagen scaffolds. Outer channels in a three-channel geometry were used to impose chemical gradients over a central endothelialized “blood vessel” channel, in order to study pro-angiogenic sprouting in response to chemical stimuli. We have observed directional sprouting in response to a gradient of vascular endothelial growth factor (VEGF), and are now exploring the dynamics of this sprouting process as well as response to additional tumor-secreted factors and anti-angiogenic therapeutics.

Summary of Research:

Growing tumors develop heterogeneity in terms of cell populations, physical parameters including matrix density and composition, and soluble factors such as metabolites and growth factors. In vitro culture systems are uniquely valuable for enabling precise control over these microenvironmental factors, all of which have been implicated in various aspects of cancer progression. We are developing 3D culture systems which allow improved control over these critical tumor components, with the specific goal of dissecting the role of different tumor resident cell types and resulting paracrine chemical signaling as these factors impact tumor angiogenesis.

We are developing microfluidic cell cultures in remodelable hydrogel scaffolds (Figures 1a and 1b). Type I collagen (1%) was chosen as an extracellular matrix material to allow for cellular engagement, as well as degradation and invasion as a result of cell released proteolytic factors. With this platform, we are studying sprouting by endothelial cells (HUVECs) in response to gradients of pro-angiogenic factors (schematic, Figure 2a). We are specifically interested in the dynamics of the individual and combined effects of vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8), tumor-secreted factors whose microenvironmental regulation we have previously illuminated [1]. We have initially performed experiments with 100 µm channels and 350 µm edge-to-edge spacing, with basal HUVEC growth media in the central endothelial and side sink channel, and VEGF supplemented (75 ng/mL) growth media in the side source channel. 40 kDa FITC-dextran was included with the VEGF-spiked media to act as a fluorescent tracer (fluorescence profile across the endothelial channel shown in Figure 2b after 48 hrs VEGF gradient culture). Sprouting was observed to emanate from the vessel corners as early as 24 hrs after gradient introduction, and sprouts were preferentially directed towards the VEGF source (Figure 2b, phase contrast image).

References:

Figure 1: (a) Schematic of a three-channel microfluidic device in 1% collagen with a central endothelialized vessel channel, and (b) photograph of an assembled device.

Figure 2: (a) Schematic of the operation of the three-channel device as a VEGF gradient generator, and (b) endothelial sprouts resulting from 48 hrs culture in a VEGF gradient, as imaged with a FITC-dextran tracer (intensity profile plotted).
Circulating Tumor Cell Release by use of Novel Immunocapture Chemistry in GEDI Microdevices

CNF Project # 1639-08
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Abstract:

We report the use of geometrically enhanced differential immunocapture microdevices (GEDI µdevices) to capture prostate cancer cells and present a framework for controllable non-mechanical, non-enzymatic methods of whole-cell and intact nuclei releases. We previously reported high capture yield, high purity circulating tumor cell (CTC) isolation using targeted anti-sera chemistry [1]. By altering the linker chemistry, we retain high capture rates while enabling substantial cancer cell elution. These data are the first demonstration of linker chemistry substitution for immunocapture and release of cancer cells. We also show one-step nuclei extraction from isolated cancer cells, which is critical for signaling, splice variant, copy number, and apoptosis assays [2]. The ability to control the temporal release of captured cells and nuclei will allow researchers to investigate many different routes of biochemical and genetic analyses to further understand prostate cancer progression.

Summary of Research:

Linear-shear flow cells were molded in PDMS and plasma bonded on glass. GEDI µdevices were fabricated in silicon using DRIE etching. GEDI µdevices have been previously characterized as having high capture efficiency and purity as compared to the literature [1]. All devices were functionalized with J591 monoclonal antibody (mAb) targeted to Prostate Specific Membrane Antigen (PSMA). Three unique J591 mAb linker chemistries were used: biotin, desthiobiotin (D-Biotin) and photocleavable biotin (PC-Biotin). Model prostate CTCs were captured on these µdevices to determine the ability to release whole cells and nuclei.

Once immobilized on the device, captured cells were exposed to either 100 µg/ml of exogenous biotin in PBS, or minimum five minutes of UV (365 nm) light to induce release. For nuclei extraction, model CTCs were immobilized on Biotin J591 surfaces and incubated with 2% Triton X-100 + 200 µg DAPI in PBS.

GEDI µdevices can be tuned through a combination of chemistry and fluid-mechanical forces to optimize targeted cell capture. To assess cell isolation and release as a function of these parameters, we employ µdevices with linear shear...
stress gradients along the centerline. The chemistries and shear stresses used correspond to those in GEDI µdevices. Results from these µdevices guided operational parameters for GEDI devices. D-Biotin, which reverses binding upon exposure to exogenous biotin due to differing desthiobiotin- and biotin-avidin binding affinities, and PC-Biotin, which is cleaved when exposed to UV light, are alternatives to standard, irreversible, biotin (Figure 1). Comparison of linker chemistries under flow showed that biotin and PC-Biotin captured model CTCs over a wide shear range; however, D-Biotin bonds were easily broken at low shear (Figure 2). Cells immobilized using PC-Biotin were released with short UV exposures (5-25 minutes) coupled with low-shear fluid agitation. These studies were replicated in GEDI chips (Figure 3). PC-Biotin functionalized devices had an average capture efficiency of 55%, comparing favorably with other immunocapture devices [3]. After UV pulsing, 30% of immobilized cells on PC-Biotin surfaces were released. Cell viability staining showed that all release methods minimally affect cell integrity. These experiments demonstrate that linker substitution works with little impact on cell viability. In contrast, removal via force [4] can induce apoptotic responses, and enzymatic digestion [5] can affect cell transmembrane proteins, altering native cellular responses to stimuli [6].

Additionally, devices were used to chemically isolate nuclei from model CTCs via membrane digestion (Figure 4) and release them with gentle fluid flow. Tuning of flow rate allowed for simple and accurate purification of nuclei without the use of mechanical homogenizers or centrifugation. This method could increase both purification yields and simplify sample preparation for a variety of genetic and biochemical clinical assays.

References:

A Laminar Flow Microfluidic Device for Quantitative Analysis of Electrochemical Activity of Bacteria

CNF Project # 1706-08
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Abstract:
A laminar-flow microfluidic bioelectrochemical system (BES) was fabricated for use as a quantitative analysis tool. The immediate effects of various chemicals on the electrochemical activity of Geobacter sulfurreducens were studied with this tool. Furthermore, a membrane-less microbial fuel cell was built based on this laminar-flow microfluidic BES.

Summary of Research:
Bioelectrochemical systems (BESs) harbor electro-chemically-active bacteria at electrodes as biocatalysts for redox reactions. BESs have been used for electric current generation from waste water, bioenergy production [1], and bioelectrochemical active bacteria research [2]. According to different reactor designs, the three main types of BESs are microbial fuel cells (MFCs), microbial electrolysis cells (MECs), and microbial three-electrode cells (M3Cs).

Figure 1: Photograph of the entire system, the inset is the photograph of the microfluidic device.
For the purpose of increasing the output power density of microbial fuel cell, BESs have been integrated with microfluidic systems [3]. However, in all the reported systems, thus far, the authors neglected the intrinsic flow properties of a microfluidic device. Laminar flow, which occurs in liquids flowing at a low Reynolds number (Re < 2100), is an important characteristic of microfluidic devices. This allows parallel flow of two separate streams without convective mixing. Here, we built a laminar flow microfluidic BES to eliminate the use of ion exchange membranes between the two electrodes. To build the two-electrode BES and create laminar flow in the microfluidic channel, two gold electrodes are aligned to a Y-shape channel (Figure 1). The Y-shaped channel (L*W*H = 2 cm * 0.5 mm * 0.1 mm) was fabricated with poly dimethylsiloxane (PDMS), employing standard soft lithography techniques. The gold electrode was made by depositing 2 nm gold on a borofloat wafer with 1 nm titanium as the adhesion layer.

We operate the laminar-flow microfluidic BES under an anaerobic environment, which provides the possibility to study obligate anaerobic electrochemically-active bacteria. *Geobacter sulfurreducens* was successfully grown in our laminar flow microfluidic BES (Figures 2 and 3). Due to the fast response and short hydraulic retention time, the laminar flow BES was further employed to study the immediate effect of different chemicals on the bioelectrochemical activity of *G. sulfurreducens*. Besides running the system as a potentiostatically-controlled two-electrode BES, we will also operate the system as membrane-less microbial fuel cell by exploiting laminar flow.

References:


Utilizing Micro-Patterned Protein Surfaces to Investigate the Properties of the Lysosomal Synapse

CNF Project # 1726-08
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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 techniques has not been explored, particularly in the area of molecular cell biology. Employing these technologies, we are now investigating the mechanisms of uptake of aggregated low-density lipoproteins (agLDL) by the macrophages by creating an extracellular hydrolytic acidic compartment [1]. An understanding of the mechanism of agLDL uptake that doesn’t require receptor mediated endocytosis may play an important role in foam cell formation and lead to new therapeutic approaches to prevent atherosclerosis. We use the micro-patterned protein arrays to investigate the properties of a novel cell-associated structure – the lysosomal synapse. These patterns are also used to investigate the formation of the lysosomal synapse with the fibrillar beta-amyloid plaques that are formed in brains of patients suffering from Alzheimer’s disease.

Summary of Research:

In the past, we established the use of micro-patterned protein surfaces as a tool for visualizing spatial distribution of signaling molecules, such as FcεRI bound immunoglobulin E (IgE) in Rat Basophilic Leukemia (RBL) mast cells [2]. We are now extending this technique to study the interactions of macrophages, which are a part of the immune system, with aggregated low density lipoproteins (agLDL). Among the various functions of macrophages in the human body, one of the medically important functions is their interaction with agLDL in the walls of blood vessels. This results in massive uptake of cholesterol and conversion of macrophages to foam cells, which is an early step in development of atherosclerosis [3]. The use of micro-patterned protein surfaces provides us a unique opportunity to investigate the properties of a novel cell-associated structure – the lysosomal synapse. We also look at the formation of lysosomal synapse with the beta-amyloid plaques that are often found in patients suffering from Alzheimer’s disease.

Previously, we prepared micro-patterned proteins on silicon surfaces. However, some fluorescence microscopy techniques, such as total internal reflection fluorescence microscopy (TIRFM) for live cell imaging, require micro-patterned proteins on glass surfaces (Figure 1). We use standard photolithography techniques and the polymer lift-off method to fabricate surfaces containing spatially defined fluorescently labeled streptavidin-LDL on glass cover slips.
In initial experiments J774 macrophages were incubated with micro-patterned protein arrays of agLDL for 30 minutes. Upon interaction of the macrophages with agLDL, regions of low pH were seen at contact sites (Figure 2). Since the agLDL is tightly bound to the surface this demonstrates that the acidic domains are extra-cellular.

In addition to the agLDL studies, we have used proteins micro-patterned on glass surfaces to image lysosomal synapses formed by the interaction of J774 macrophages with fibrillar beta-amyloid. The plaques that accumulate in the brain of Alzheimer’s disease patients are composed of fibrils of this beta-amyloid protein. Fibrillar Cy3 (red)-labeled beta-amyloid was conjugated to the micro-patterned surfaces. The lysosomes of the macrophages incubated on these surfaces were labeled with FITC-dextran. The green signal of FITC is strongest at neutral pH and is less intense at the more acidic pH that exists in the lysosome (Figure 3A). TIRFM movies of the macrophages interacting with the Cy3-fibrillar beta-amyloid patterns show that the green FITC signal increases in intensity as the lysosomal synapses form (Figure 3B), then eventually fades as the labeled dextran diffuses away from the lysosomal synapse into the media (Figure 3C).

In future work, we will investigate secretion of lysosome contents into the lysosomal synapse formed between macrophages and the patterned agLDL surface. We will also examine the signal transduction mechanisms involved and the SNARE proteins that participate in the plasma membrane fusion events.

References:


Figure 2 (left): J774 macrophages were incubated on a surface labeled with CypHer 5E and Alexa488. AgLDL attached in patterned features to the coverslip is exposed to an acidic environment underneath macrophages. The color intensity map represents the pH associated with those colors. (right) Histograms of the pH values of macrophage associated patterns (red trace) and patterns not in contact with the cells (blue trace).

Figure 3: Frames from a TIRF movie of macrophages with green-labeled lysosomes interacting with red-labeled fibrillar beta-amyloid conjugated to the patterned surfaces. Panel A arrows point to green-labeled lysosomes at the onset of imaging. In Panel B the lysosomes have moved and become brighter (indicating the formation of lysosomal synapses). In Panel C the green signal from lysosomes has faded or disappeared.
Microgeometries and Photonics for Studying Cell Mechanics and Cancer Metastasis

CNF Project # 1743-09
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Abstract:

We develop techniques involving microgeometries and photonics to study mechanical properties of metastatic cancer cells. Using confined microchannel environments with patterned geometric constraints, we quantify cell behavior during invasion into tight spaces. With nearfield photonic structures, we actively manipulate particles and cells via optical forces. Our techniques provide ways of probing single cells on-chip.

Summary of Research:

It has been demonstrated that in confined microchannel environments with cross-sectional areas comparable to the size of cells, cells can spontaneously migrate unidirectionally with high velocities and persistence [1]. To further investigate the effect of mechanical confinement on cell invasion and behavior, which may provide insights towards cancer metastasis, we introduce mechanically perturbative elements to elicit cell responsivity. Specifically, as shown in Figure 1, our device consists of confined PDMS microchannels with cross-sectional areas comparable to cell size, and a spatially tapered region in the channel reduces the cross-sectional area from $15 \mu m \times 10 \mu m$ to $4 \mu m \times 10 \mu m$. We analyzed the behavior of three different cell types—bovine aortic endothelial cells (BAECs), MCF-10A (human mammary epithelial cells), and MDA-MB-231 (highly metastatic breast carcinoma cells)—in the tapered junction (Figure 1 (a and c)) and characterized the relative probabilities of cell invasion vs. repolarization.

We found that MDA-MB-231 cells were the most invasive of the three. Furthermore, we analyzed the migration dynamics of cells as they exhibited physical spatial gradients. As shown in Figure 1 (b and d), we fitted the velocity profile of cells in the tapered junction to a dual-sigmoid function and characterized the cell transition dynamics with two time constants and a delay constant. There appears to be two acceleration phases separated by a transition lag [2].

Additionally, to actively probe cells, we have developed and fabricated optical waveguides with accessible evanescent fields, which can be used to induce localized optical forces [3]. Live and adherent cells can proliferate on these waveguides, and we can subsequently induce guided particle-cell collisions, as demonstrated in Figure 2.

References:

[3] “Optofluidic trapping and transport on solid core waveguides within a microfluidic device”; Schmidt, BS, Yang, AHJ, Erickson, D, Lipson, M; Optics Express, 15, 14322-14334 (2007).
Figure 1: Migration dynamics of repolarizing and permeating cells. A-B. Time-lapse image stack (A) juxtaposed on top of the data and sigmoid curve fit of the velocity profile on the same time interval (B) of a permeating MDA-MB-231 cell during transition in a spatially tapered junction, which connects a 15 µm × 10 µm channel to a 4 µm × 10 µm channel. C-D. Time-lapse image stack (C) juxtaposed on top of the data and sigmoid curve fit of the velocity profile on the same time interval (D) of a repolarizing MCF-10A cell during transition in the tapered junction.

Figure 2: Time-lapse image stack of a cell on an optical waveguide. An MDA-MB-231 cell adheres to a waveguide (2.8 µm wide) and 3 µm particles are optically propelled onto its surface. Each successive frame represents two seconds of time elapsed.
Micropatterned Hydrogel Substrates of Tunable Stiffness

CNF Project # 1859-10
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Abstract:

It is widely accepted that substrate topography and mechanical properties can influence cell behavior. Several groups have investigated the effect of topographically patterned polymeric substrates on cell behavior [1-3]. However, most of these studies used materials with a Young’s modulus that is significantly above physiological ranges. Likewise, multiple groups have investigated the effects of substrate stiffness on cell behavior, but less is known about how cells interpret simultaneous cues of substrate stiffness and topography similar to those encountered in vivo. Here we present the fabrication and characterization of polyacrylamide (PA) hydrogel substrates of tunable Young’s modulus (E) containing defined topographical features.

Summary of Research:

Topography was introduced using standard photolithography, and the regime of feature sizes that display good fidelity across a range of stiffness (E) was explored. Time-lapse microscopy was used to evaluate cell response to both substrate mechanics and topography. These substrates will further enable the study of cellular behaviors in response to 2D and 3D-like environments with a tunable stiffness.

Photolithography and etching were used to introduce an array of rectangular features into silicon wafers. Feature length was kept constant at 200 µm while height and width were varied. PA gels of varying E were cast on the molds, immersed in cell culture media containing 17.5 mM 40 kDa FITC-dextran, and visualized using confocal microscopy. Gel stiffness was modulated between 5 kPa and 30 kPa by varying the total concentration of acrylamide (T) and the percentage of it that is bis-acrylamide cross-linker (C). An increase in either parameter will result in a less compliant (stiffer) gel. Cell response was observed using time-lapse microscopy of bovine aortic endothelial cells (BAECs) on patterned PA gels coated with 0.1 mg/mL collagen.

Figure 1 shows two examples Si molds. Confocal imaging of the patterned features indicates that pattern transfer and feature shape retention is dependent on gel formulation.

Figure 1: SEM of Si molds, Length × Width × Height = 200 × 10 × 17 µm (top) and 200 × 25 × 35 µm (bottom). Scale bar = 200µm.
(Figure 2). The parameters T and C are known to control the pore size of PA gels [4] and hence gel swelling [5,6]. For a given T, pore size reaches a minimum when C = 5%. This supports our observation that gels with C close to 5% (10 kPa, T/C = 8.49%/4.46%) exhibit better feature shape retention than other formulations (5 kPa, T/C = 8.30%/2.28%; 30 kPa, T/C = 13.28%/2.28%, not shown). Therefore, pattern fidelity is not a function of gel stiffness but rather gel formulation. Figure 2 also suggests that for a given stiffness gel there exists a maximum feature aspect ratio (of height to width) for which feature shape is maintained. BAECs cultured on the patterned substrates exhibited a high degree of contact guidance when in contact with features, regardless of the E of the substrate (data not shown). Time-lapse microscopy revealed that depending on the feature height, cells either crawl over features (not shown) or up the sides of, and onto the feature (Figure 3).

These results illustrate that feature shape retention can be achieved in hydrogels of tunable stiffness when using a formulation that minimizes swelling. The patterned hydrogel substrates described here have promising potential as a tool to study cellular response to topography with consideration of the mechanical properties encountered in vivo.

References:

Abstract:

We have fabricated suspended carbon nanotube (CNT) devices specifically designed for biological sensing applications. We use six inch wafer processes to maximize yield. Our fabrication process produces suspended CNT with clean surfaces and minimal environmental noise. The electrode layout is compatible with a microfluidic system for delivering biological molecules to the sensor.

Summary of Research:

The two-terminal resistance of a carbon nanotube (CNT) device is sensitive to the binding of biomolecules onto the CNT surface. Several groups have demonstrated this effect using CNTs lying on substrates, however, there are compelling reasons to extend these experiments to suspended CNT devices. Suspended CNTs are further away from charge traps in insulating substrates which introduce noise and limit the sensitivity of CNT-based electronic sensors. Suspended CNT devices also allow measurements of the interactions between the biomolecules and CNTs without interference from the substrate.

An optical image of a partially completed device is shown in Figure 1. The substrate is Si/SiO$_2$ (1 µm oxide thickness). The device has undergone two processing steps. First, a pair of metal pads (Pt) were patterned. These metal pads are separated by a 1 µm gap. Second, a SiO$_2$ etch (reactive ion etch, 1 µm depth) was used to remove the SiO$_2$. The etched region is a 20 µm wide stripe. These processing steps are done on 6-inch wafers, allowing us to produce 30 chips in parallel.

The final processing step is growth of CNTs. We use alumina supported iron nanoparticles, patterned by photolithography, to seed the growth of CNTs. The growth of CNTs takes place in a chemical vapor deposition growth system. Figure 2 shows an SEM image of a completed device. Because the CNT was grown last, we expect the CNT surface to be pristine (no photoresist residue). On every chip, a fraction of devices have a CNT bridging the gap between electrodes, giving us two or three useful devices for our experiments.

Preliminary electrical data of our suspended CNT devices shows significant improvements in device sensitivity. We have observed higher transconductance than standard CNT devices and are currently evaluating the environmental noise in a dry environment. Our next experiments will involve measuring devices in a liquid environment.
Figure 1: Optical image of a device before the growth of CNTs. Scale bar 20 µm.

Figure 2: Scanning electron microscope image of a suspended CNT bridging the gap between the electrodes.
Zero Mode Waveguides for Single-Molecule Real-Time Detection

CNF Project # 1920-10, 917-00  
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Abstract:
This work reports fabrication of submicron zero mode waveguides in aluminum by lifting off a sacrificial material, selective to aluminum and the fused silica substrate. The sacrificial pillars have been made using JEOL 9300 electron beam lithography and ASML PAS550 DUV stepper.

Summary of Research:
Nanofluidic waveguides in aluminum have been reported for observing single molecule events in real time [1]. Sub-100 nm waveguides were fabricated in aluminum on a fused silica substrate using electron beam lithography [2]. The nanostructures in this report were fabricated by creating organic (or inorganic) pillars, followed by metal evaporation and lift-off, selective to aluminum and the substrate. The method was used to create structures from 60 nm to 200 nm, by using the JEOL 9300 electron beam lithography and 200 nm to 500 nm by using the ASML PAS 5500 DUV stepper at CNF, shown in Figures 1 and 2.

References:
Abstract:

To educate undergraduate students on mixing at the microscale, a laboratory experiment was designed where students directly measure diffusive mixing along the length of a simple microfluidic T-channel micromixer. The SU-8-on-silicon facilities at CNF were used to fabricate masters for the micromixers. Students cast PDMS micromixers from the masters and experimentally measured mixing of the fluorescent dye fluorescein using an epi-fluorescent microscope. Results were compared to theory.

Summary of Research:

Molecular transport in fluidic systems at micrometer scale can be significantly different from that at the macroscale [1]. At the macroscale, mixing of different reagents is often realized by the introduction of turbulence (e.g. small vortices introduced by pipetting up and down in an Eppendorf tube). However, at the micrometer scale, the fluid flow is predominantly laminar due to the small length scale, and mixing is a challenging task [2].

To educate undergraduate students on mixing at the microscale a laboratory experiment was designed where students directly measure diffusive mixing along the length of a simple microfluidic T-channel mixer.

Masters for the microfluidic mixer were fabricated for the students at CNF using the SU-8 photoresist on silicon photolithography process. Students used soft lithography to cast PDMS micromixers from the reusable masters. A syringe pump was used to control the flow rate of fluorescein fluorescent dye solution into one inlet of the micromixer and water into the other. Students used an epi-fluorescent microscope to take fluorescent images at different distances along the mixing channel from the point of contact of the two fluids and repeated the experiment at different flow rates (Figures 1 and 2).

The program ImageJ was used to plot fluorescent light intensity vs. distance along the width of the mixing channel to measure the fluorescein gradient. Students compared experimental results to theory.

References:

Figure 1: Fluorescent and brightfield image of the microfluidic T channel where fluorescein (left) and water (right) come into contact. The low Reynolds number of the system results in separate laminar flow streams in the mixing channel (bottom).

Figure 2: Fluorescent image of fluorescein gradient across the width of the mixing channel.
X-Ray Lab on a Chip: A Microfluidic Mixing System for Small-Angle X-Ray Solution Scattering

CNF Project # 1940-10
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Abstract:
Biological small angle x-ray scattering (BioSAXS) is an increasingly popular technique for obtaining low-resolution structural information on bio-macromolecules in solution. To further extend the method while conserving the sample, chip-based microfluidic devices for BioSAXS have recently been introduced. Building upon a chip design from the Technical University of Denmark, we have bonded x-ray window material at the Cornell NanoScale Facility and successfully tested our first microfluidic devices on two x-ray beamlines at the Cornell High Energy Synchrotron Source. In addition to examining known protein standards, we have investigated the solution behavior of glutaminase, a protein known to play a key role in metabolic profiles of cancer cells.

Summary of Research:
Small-angle x-ray solution scattering on biological samples (BioSAXS) is rapidly becoming popular among molecular biologists due to its ability to produce structural information from a wide range of solution conditions without the need for crystals [1]. X-ray scattering intensity at very small angles (less than 5 degrees) is collected on an area detector under very low-background conditions and integrated to yield a one-dimensional intensity profile. To further extend the power of the method, it is desirable to be able to mix various components in real time so that ranges of solution conditions with various additives and concentrations can be examined. Lab-on-a-chip microfluidic technology for BioSAXS promises researchers new versatility in exploring solution conditions with minimal sample consumption.

Figure 1: Microfluidic chip for biological small-angle x-ray solution scattering (BioSAXS) mounted on beamline F2 at the Cornell High Energy Synchrotron Source. White block (lower right) is the sandwich-style vacuum housing for the chip. Fiber optic boroscope image (LCD screen on left) shows the in vacuo view of the x-ray window on the chip. Computer controlled syringe pumps feed the chip through standard chromatography fittings (lower right). Magda Møller is shown operating the purge.
We have adapted the microfluidic BioSAXS chip design of Toft et al. for use on the Cornell High Energy Synchrotron Source (CHESS) beamlines F2 and G1 [2]. The CHESS design features a fully vacuum enclosed x-ray beam path in which the chips are sealed to vacuum via o-rings in an easy-access sandwich-like assembly (Figure 1). A novel vacuum-tight fiber optic boroscope configuration allows users to monitor the x-ray window region of the chip for bubble formation and possible window fouling due to radiation damage. The chip is connected to standard liquid chromatography fittings via mini face-seal o-rings (Figure 2). The whole unit is temperature regulated for biological work at 4°C using a commercial circulating water chiller system.

The poly(methyl methacrylate) (PMMA) chips used for our first experiments were micromachined at the Technical University of Denmark and fitted with x-ray windows at the Cornell NanoScale Facility. Input ports for three buffer components mix ahead of the fourth port where sample is introduced as a thin laminar stream. The x-ray sample chamber at bottom of the chip is a 1 mm wide by 2 mm deep flat-walled cell through which the 250 µm x 250 µm x-ray beam travels (Figure 3). Choice of thin x-ray window materials is critical to BioSAXS performance due to the extremely weak scattering signals produced by biological molecules in dilute solution. Current experiments suggest that polystyrene film (25 µm) windows rival natural mica for low background x-ray scatter.

A bank of three computerized syringe pumps controlled by a custom-designed Python graphical user interface controls fluid flow in the chip. X-ray scattering patterns are recorded on 2D CCD-based x-ray detectors and reduced to one-dimensional profiles with the open-source data processing software RAW, now maintained at CHESS [3].

Initial runs to test the efficiency and accuracy of the new system were done with bovine serum albumin (BSA), a widely used protein standard. Reproducible concentration series were obtained, and tests progressed to actual research examples. Glutaminase C is a key metabolic enzyme responsible for the conversion of glutamine to glutamate, which is up regulated in many cancer systems and believed to play a key role in the Warburg effect, whereby cancer cells undergo an altered metabolic profile. It has long been thought that glutaminase C forms a tetramer in the active state, though little is known about the true oligomeric state behavior in solution. It is therefore of importance to understand changes in the oligomeric state of the protein in solution as a function of concentration, additives such as phosphate, and known enzymatic inhibitors.

Shown in Figure 4 is our first example of a series of x-ray scattering profiles of glutaminase as phosphate concentration is scanned over a range of experimentally relevant values. While a complete analysis of the results is still in progress, early results suggest a more complex picture of behavior in which higher oligomers than the tetramer may play an important role in enzymatic activation.

References:


Abstract:

Recently, it has been shown that lymphatic drainage induced interstitial flow directs tumor cell migration by autocrine CCR7 signaling [1]. Inspired by this fact, we have designed and fabricated a microfluidic device specifically to investigate the effects of interstitial like flow on tumor cell migration in a three dimensional (3D) matrix and therefore analyze the intravasation through endothelium cell barrier using a collagen hydrogel based microfluidic technology. This device would be a chemically and mechanically tunable microenvironment. Therefore, the device would enable us to study the effect of flow on tumor cell’s invasiveness through an endothelium cell layer in a controllable manner.

Summary of Research:

Many advantages of this microfluidic device include, but not limited to, the study of spatial and temporal dynamics of the cells in 3D matrix environment as compared to the end-point analysis of macro-scale essays. With the small size of the device, the time factor of the experiments will decrease by five-fold, and cell cultures by 100-fold. Also, the device will be able to decouple mechanical and chemical tuning factors. This work would be an improvement over devices with similar intentions [2,3] from the perspective of having side-by-side channels of collagen matrix with no structures interrupting flow as well as a simple fluid introduction mechanism.

The silicon master was fabricated through a two-step photolithography and two step etching processes at the Cornell NanoScale Science and Technology Facility (see Figure 1). Initially, two layers of patterns, ridge and device, were prepared using L-Edit CAD software (Tanner Research Inc.) (see Figure 2, top picture). And the patterns were printed on two separate chrome-coated glass masks using an optical mask making tool (PG Mask Writer, PG3600). First, the ridge layer was fabricated, followed by the device layer fabrication.

For ridge layer fabrication, S-1813 was spin-coated on the silicon wafer at 3000 rpm for 45 seconds with an acceleration rate at 6000 rpm/s. The resulting film thickness was 1.4 µm. Spin-coated wafer was baked for 60 seconds on a 115°C hotplate and allowed to air-cool. The cooled-down wafer was exposed to broadband UV (365–405 nm) through the mask held in soft contact with a contact aligner (EV620, Electronic Visions Inc.) for 11 seconds in continuous exposure mode. The exposed wafer was developed for one minute (Microposit MF-321) using a double puddle process (HamaTech-Steag wafer processor, HamaTech AG). The wafer was etched for 12 cycles in a deep reactive ion etcher for one minute (The Oerlikon Versaline System, Oerlikon Systems) giving 5 µm deep ridge structures. After hot bath, the residual photoresist was stripped by oxygen plasma etching (GaSonic Aura 1000 Asher, Gasonics, Inc.).

Once ridge structures were formed, the wafer was primed (Shipley P20, Rohm and Haas Company) and then spin-coated at 2500 rpm with photoresist (Megaposit SPR220-7.0, Rohm and Haas Company) for 40 seconds for device layer fabrication. The thickness of the resulting film was 7.7-7.8 µm. Spin-coated wafer was baked for 90 s on a 115°C hotplate and allowed to air-cool. Cooled down wafer was exposed to broadband UV (365-405 nm) through the mask held in soft contact with a contact aligner (EV620, Electronic Visions Inc.) for 90 s in continuous exposure mode. Following a 90 min hold time, the wafer was post-baked at 115°C for 90 s and then developed for 90 s (AZ 726 MIF, AZ Electronic Materials) using a double-puddle process (HamaTech-Steag wafer processor, HamaTech
AG). The wafer was etched for 75 min. in a deep reactive ion etcher (The Oerlikon Versaline System, Oerlikon Systems) giving 251 µm deep devices. After hot bath, the residual photoresist was stripped by oxygen plasma etching (GaSomics Aura 1000 Asher, Gasonics, Inc.)

Once the silicon master was ready, a PDMS base and curing agent (10:1 ratio) mix was poured over the Si master and cured in 60°C for 1.5 hours. Once curing occurred, the PDMS mold was peeled from Si wafer and inlet/outlet channels were opened. An oxygen plasma (Harrick Plasma cleaner) was applied for about one minute to device surfaces. And the device was placed facing a clean glass slide (See Figure 2, bottom picture). The device and the facing glass slide were sandwiched inside a Plexiglas® manifold for better insulation and maintenance. After a 40 min. waiting time, the previously prepared collagen matrix was introduced in the mid-channels and confinement in the mid-section could be observed.

The next usage for this device will be to observe and set flow rates across the collagen mid-section, and proceed with the cell culture experiments with breast cancer cells.

References:


Fabrication of Flexible Microelectrodes for Chronic Implantation

CNF Project # 1960-10
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Abstract:

In this work we discuss the fabrication of flexible implantable probes for recording in vivo neuronal activity. We fabricated such probes bearing platinum electrodes, using polyimide as a substrate and SU-8 as an insulation layer. The fabrication process was simplified through the use of laser ablation to define the probe outline. The probes showed not only good mechanical flexibility, but also the required stiffness for implantation. Histology results and electrical recordings of neuronal activity lend support to the idea that the combination of polyimide and SU-8 represents a good choice of materials for the fabrication of implantable neuronal probes.

Summary of Research:

Conventional silicon-based probes with multiple recording sites make it possible to collect neural signals at the sub-millimeter scale from small volumes of tissue [1-3]. However, the rigid nature of these probes creates a tissue scar that, with time, impairs the quality of the recordings. Therefore, a great deal of research in neural engineering deals with the development of flexible probes to decrease the mismatch between the mechanical properties of the implanted probes and biological tissue. The central aim of our work is to fabricate probes with long-term implantation capacity, high quality signal recording, and facile packaging. In this report, we discuss the fabrication of flexible implantable probes and their in vivo evaluation in recording neural activity in the rat brain.

The fabrication of probes, as shown in the Figure 1, involved the deposition and patterning of alternating layers of polyimide, platinum (Pt) and SU-8. Some of the processes used are adapted from literature [4,5]. The platinum electrodes were defined by lift-off and sandwiched between the polyimide and SU-8. The CNF CO2 laser engraving/cutting tool (Universal Laser Systems VersaLaser) was used to define the probe outline in the polyimide layer. The SU-8 photoresist was used as an insulation layer and permitted the definition of the recording sites in the probe. The combination of polyimide with SU-8 improve the stiffness of the probes, making easier their manipulation during the implantation into the brain, as well as facilitating their interconnection to external electronics.

Figure 1: Process flow diagram for the fabrication of probes.
Figure 2: Layout of the polyimide probe. The insets show the cortical recordings: a) of an anesthetized rat; b) after bicuculline adding.
The final probes contained 12 metal electrodes with $20 \times 20 \, \mu m^2$ or $10 \times 20 \, \mu m^2$ recording areas placed at the end of the probe shank (Figure 2). The interconnect pads were soldered to an Omnetics connector using insulated metal wires.

For in vivo evaluation, the probe was placed on a stereotaxic holder in order to precisely control the implantation. A small craniotomy was performed in the right hemisphere above the somatosensory cortex and the dorsal hippocampus, centered relative to Bregma. Two miniature stainless steel screws were driven into the skull as ground and reference electrodes. The electrical recordings were performed in the somatosensory cortex. The recorded signals were amplified, band pass-filtered and acquired continuously at 32 kHz on the 64-channel Neuralynx system. The neurophysiological recordings were explored before and after the addition of bicuculline – a GABAA receptor antagonist. The recorded signals showed the typical pattern of slow neural activity that is recorded under anesthesia; Figure 2 (a) [6]. Increased network activity resulting from the action of bicuculline, deposited at the surface of the cortex, was also recorded; Figure 2 (b).

In addition, a chronically implanted brain was removed after 14 days for histological studies using Nissl coloration, and anti-GFAP immunostaining. Histology showed that the probe could be followed through the cortex and up to the CA1 region of the hippocampus (Figure 3). The anti-GFAP staining revealed the presence of only a small glial response (an immunological response of the brain tissue to a foreign body), which is an encouraging result for obtaining good quality long-term recording of the neural activity.

The fabricated probes presented in this work show good biocompatibility, good quality recordings of neural activity, and they provide good mechanical flexibility. These results support the idea that the combination of polyimide with SU-8 represents an advantageous choice of plastic materials for the fabrication of implantable neuronal interfaces.

References:


Moisture Responsive Artificial Micro-Pores 
in a PEGDA Membrane with Anisotropic Swelling Properties

CNF Project # 1961-10
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Abstract:
We wish to create a moisture responsive membrane with artificial micro-pores that open up when the membrane is exposed to moisture. These pores will then close back up when the membrane dries. We plan to accomplish this by controlling the swelling properties of a poly(ethylene glycol)-diacrylate (PEGDA) hydrogel and creating artificial pores using photolithography.

Summary of Research:
A large scale membrane of this sort has already been created by Professor Jin-tu Fan’s group at Hong Kong Polytechnic University. They essentially synthesized a PEGDA membrane, placed fibers aligned in one direction within this membrane, and then cross-linked the membrane by ultraviolet light. This created a membrane which expands anisotropically, expanding mainly in the direction perpendicular to the fibers within the membrane. They then cut holes about 1 mm radius in size in to the membrane and observed their increase in size as the membrane swelled when exposed to moisture. Figure 1 shows the unpublished work conducted by Jin-tu Fan’s group.

We wish to essentially miniaturize this experiment. We plan to accomplish this by selectively crosslinking areas of a PEGDA hydrogel with a photomask. Small holes, or pores on the micrometer scale, will be prevented from crosslinking by the photomask and then washed away. Using lithography, these pores will then be filled with various layers of different molecular weights of PEGDA as well as hydrophobic polymers. The overall swelling of the membrane will be controlled by aligned fibers similar to that of the work done at Hong Kong Polytechnic. We hope this will result in pores which increase in area, or open up, when the membrane is exposed to moisture and swells. These pores will then reduce in area, or close up, when the membrane dries and shrinks back to its original size.

Currently, only a few CAD files have been created for the photomask design. Preliminary research is still being conducted outside of the CNF facilities involving microporous membranes and moisture responsive materials. Characterization techniques are also being developed.
Figure 1: Membrane created by Jin-tu Fan’s group at Hong Kong Polytechnic University demonstrating anisotropic swelling properties and resulting pore size changes.
Abstract:

We report on the fabrication of a multichannel microfluidic device that geometrically confines cancer stem cells to divide in a row, permitting tracking of phenotype variations over generations of the cell lineage.

Summary of Research:

A device to facilitate the tracking of generational variations in a cell lineage of colon cancer inducing (CCI) cells was created, using a design inspired by work done to track lineages of yeast cells [1]. Cells are introduced at the inlet and aggregates are broken up or caught by an array of posts that acts as a filter. Individual cells encounter the trapping channel array, travel down one of the channels in the array before getting lodged in a constriction at the end of the channel. Upon becoming lodged in the constriction, the hydraulic resistance of the channel increases significantly, preventing additional cells from entering the channel. In this manner, only one cell is captured in each channel.

Due to the tight confines of the channel, the cells are constrained and division occurs longitudinally, like beads on a necklace. Typical trapping channel dimensions are 16 µm deep (with a square profile) and 960 µm long. Silicon master is created using the DRIE Bosch etcher and final devices cast in PDMS.

References:
