Binding of DU145 Prostate Tumor Cancer Cells to Silicon Carbide

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Introduction:
Prostate cancer occurs in men worldwide. The risk factors vary among different ethnic groups. Prostate cancer has been detected in European and American men more so than in East and South Asian men [1]. DU145, one of the three “classical” cell lines of prostatic cancer, was isolated by K.R. Stone et al. from a wound in the brain of a patient with metastatic carcinoma of the prostate and a three year history of lymphocytic leukemia. The cell line is not detectably hormone sensitive and does not express prostate specific antigen (PSA) [2].

The long-term goal of this study is to explore an alternative technique to differentiate biological cells on silicon carbide substrates by their electrical properties using scanning tunneling microscopy (STM) and a probe technique. To this extent, the specific objective of this project was to examine the binding of metastatic DU145 prostate cancer cells to different types of silicon carbide (SiC): 3C-SiC grown on Si, 6H-SiC, and highly doped (HD) 6H-SiC to determine the most effective binding substrate.

SiC is biocompatible and is utilized in many biomedical applications such as stents, orthopedic implants, in drug delivery and tissue engineering [3]. For applications such as tissue engineering it is important to examine the binding potential and proliferative capabilities of cells as they develop into tissue on SiC substrates. The 3C, 6H and HD 6H poly types of silicon carbide were considered because of their electrical conductivity between that of metals and insulating materials [4]. Depending on their crystal structure and if the material is doped, then poly types have high thermal conductivity and high electron mobility that may be useful in a bio-electronic device.

The number 3 in 3C refers to the three-bilayer periodicity of the stacking structure and the letter C denotes the cubic symmetry of the crystal; similarly, the number 6 in 6H refers to the six-bilayer periodicity of the stacking structure and H denotes the hexagonal symmetry of the crystal.

Experimental Procedure:
Substrate Preparation. The substrates shown in Figure 1 were cleaned by ultrasonic bath using soap, water, acetone, and methanol sequentially for three minutes each. Cleaned substrates were placed in a dust free cloth lined container.

Tumor Cell. DU145 was purchased from American Type Culture Collection (ATCC). The cells were cultured in complete Roswell Park Memorial Institute (RPMI) 1640 media {5% fetal bovine serum, gentamycin (50mg/ml), penicillin/ streptomycin (5,000 units / 5,000 mg/ml)} in a humidified atmosphere of 5% CO2 and 95% air.

Binding of Cells to SiC Substrates. DU145 cells were harvested, either from a frozen sample by quick thaw or from trypsin-treated cultures. The cells were counted and adjusted to various concentrations. Substrates were placed into the wells of a 6-well culture plate; 2 ml of cells were added to each well. The plate was incubated overnight at 37°C in 5% CO2. An Olympus IX71 inverted microscope with an Olympus DP70 camera was used to confirm binding and sizes of the cells on the substrate. Optical microscope mages of the cells bound to 6H and HD 6H-SiC are shown in Figure 2.

Figure 1: Substrates from left to right: 3C-SiC grown on Si, 6H-SiC, and highly doped 6H-SiC.

Figure 2: DU145 cells at 5 × 10^4 cells/ml on [A] 6H-SiC, and [B] highly doped 6H-SiC.
Fixing of DU145 Tumor Cells to SiC Substrates. Substrates with DU145 cells were placed into individual wells of a six well plate; 2 ml of methanol was added to each well. After five minutes, the substrates were bathed in water. Five minutes later, the substrates were placed in an empty well and allowed to dry. Atomic force microscopy (AFM) was used to obtain three-dimensional high resolution images of the cells [5].

Experimental Design. Three different experiments (trials) were executed with various concentrations of DU145 cells to determine: 1) the optimal cell concentration for the examination of individual cells, and 2) the most effective substrate for binding cells.

Results and Conclusions:
A procedure for preparing samples that would allow for the discrimination of cells in a heterogeneous mixture of cells that could be used in STM and the probe technique was developed using the DU145 cell line. Luminal-like cells with a width and length of 44.091 µm and 6.452 µm, respectively, were observed on 6H-SiC and HD 6H-SiC while binding on 3C-SiC was undetermined because of its lack of transparency. The optimum concentration of cells was determined to be $5 \times 10^4$ cells/ml. Highly doped 6H-SiC was the better substrate for observing the cells’ morphologically.

Future Work:
We plan to apply STM and the probe technique to measure electrical properties (i.e. current-voltage curves) of the cells as an alternative method to differentiate biological materials.

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References:
Characterization of Polymer Films on Silicon Photonics Devices for Blood Analysis

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Introduction:

Silicon photonic devices employ nano- and microscale silicon features as optical guides to direct light in applications such as high-speed telecommunications and biosensing. The microring resonator, an archetypal silicon photonic device, consists of an optical waveguide fabricated in close proximity to a silicon ring (see Figure 1) [1]. Light at a resonant wavelength dependent on ring dimensions and effective refractive index couples into the ring; in biosensing applications, waveguides are modified using surface chemistry to facilitate selective binding of biomaterials from complex media such as blood. With any biological binding event, a shift in resonant wavelength is detected due to a change in the local refractive index at the waveguide surface. Surface protein fouling of these biosensors has been observed upon use in clinical samples, but growth of zwitterionic polymer films through atom-transfer radical polymerization (ATRP) minimizes non-specific binding events while enabling chemical immobilization of capture elements for biosensing [2].

Here, we developed a reliable protocol for the characterization of these polymer films using atomic force microscopy (AFM) to correlate topographical film quality and surface roughness with the ability of modified sensors to inhibit protein fouling. The reproducibility of the polymer film quality was essential for the potential use of these devices in clinical applications, particularly in antibody/antigen binding for performing serologic and phenotypic analysis of biologic samples.

Experimental Procedure:

Silicon microring resonators fabricated by Genalyte in arrays on chips on insulating oxide were piranha-cleaned using a 50:50 solution of 30% H₂O₂ and concentrated H₂SO₄. The chips were oven-dried for several hours prior to overnight solution functionalization with a silane initiator in toluene. Using carboxybetaine methacrylate (CBMA) as the monomer for ATRP, a batch polymerization scheme was employed to produce thin polymer films. Characterization of the polymer film was performed on several chips from different polymerization batches using AFM. Both 5 µm and 3 µm scans were obtained of the waveguide and cladding to measure the average height and large-scale topography of the features on substrates both with and without polymer. Small-scale scans were also obtained in regions near the waveguide at a size scale of 1 µm in order to compare surface roughness and morphology among the bare chips and various batches of polymerization. Polymer film thickness measurements were also deduced from bare silicon substrates used as control surfaces. This was done by scanning regions in which the polymer film had been scratched away down to the silicon surface. All of these measurements were obtained in tapping mode with an OTESPA tip in air or a FESP tip in water.

Results and Conclusions:

AFM was used to measure the polymer film thickness in a scratched region of an unpatterned silicon substrate (see Figure 2). While the numbers obtained via AFM were comparable to those determined using ellipsometry, the data was not within error. Therefore, it is necessary to further develop the protocol for determining film thickness using AFM before it can be used exclusively for this characterization on the microring resonator chips. Something besides ellipsometry is necessary to determine film thickness on the chips due to the complicated architecture and small sample size of the microrings.

Further characterization of the polymer film was performed using AFM in order to correlate topographical data with quantitative data regarding protein fouling. In Figures 3 and 4, images of the topography of the polymer film from two identical batches of polymerization are juxtaposed with graphs.
concerning the quantity of protein fouling. In Figure 3, the AFM image of the topography of the surface shows that it is smooth with some defects indicated by darker regions. This correlates with the graph that shows that the surface is not to non-fouling standards but does show a lower amount of protein fouling than a bare silicon substrate. In Figure 4, however, the graph shows unexpected results that could be due to a loss of polymer from the surface upon flowing plasma over the chips. This is supported by the AFM data showing that there are large features on the surface of the chip. AFM has been useful to relate the small-scale surface features to the quantitative protein fouling data and will be useful in the further optimization of the batch polymerization process.

**Future Work:**
The goal is to further optimize the procedure for the characterization of chips with polymer coating using AFM in order to use it more exclusively as a technique for determining polymer thickness on the microring chips for which ellipsometry is not possible. AFM will also be used in a characterization protocol to assist in the optimization of polymer film growth for minimizing protein fouling on the chips. Further, functionalization of the terminal ends of the polymer film will allow for specific immobilization of elements in complex media for detecting biological analytes.

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**References:**
Developing Nanoscale Electrodes for Sensitive Detection of Brain Cell Activities

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Abstract:
Vertical nanopillar electrode arrays have garnered much attention due to their applications in the study of the electrical behavior of neurons. Previous methods for measuring neuronal activity include the use of planar multielectrode arrays (MEAs); however, neuronal mobility and inefficient neuron-electrode interactions on flat substrate surfaces make it difficult to monitor the activity of specific neurons over extended periods of time. Vertical nanopillar arrays serve as neuron traps by effectively pinning the neurons and also offer a non-invasive measurement technique, thus allowing for long-term study. The aim of this work is to develop a novel electrode device composed of a patterned MEA fabricated on quartz and glass substrates using photolithography and subsequently electrodepositing vertically aligned Au nanopillars of varying diameters (200-600 nm) on the MEA. Using optical microscopy and scanning electron microscopy, it was noted that the specified quality and morphology of the devices were maintained throughout processing.

Introduction:
One of the main problems in measuring action potentials (AP) of neurons is neuronal mobility. Syntheses of many current nanostructures used to address this problem involve complex procedures and loading the neurons can be difficult and time consuming [1]. Other methods to control migration include the use of chemical modification or patterned deposition of proteins to promote neuron-electrode adhesion; however, it is difficult to control how many neurons are attached to each electrode [1]. Accurate AP measurements require efficient coupling of the measuring electrode and cell membrane. Intracellular methods such as patch clamping provide accurate measurements but are invasive and therefore reduce cell life, limiting recording times to only a few hours [2]. Extracellular techniques such as multielectrode arrays (MEAs) have lower signal quality due to the infrequent one-to-one neuron-electrode interactions [2].

Combining the extracellular and intracellular measurement technologies of multielectrode arrays and vertically aligned nanopillars, respectively, provides a non-invasive technique for accurate AP measurements. Vertical nanopillar arrays serve as low throughput intracellular recording electrodes, effectively pinning the neurons for long-term studies [2]. Previous work by Xie et al. has shown that neuritic protrusions wrap the nanopillars with a thin layer of membrane to improve interactions [1]. It is also proposed that vertical nanopillars serve as focal adhesion substrates, allowing for stronger anchorage of the cell matrix than on flat surfaces leading to preferential adhesion of neurons to the nanopillars [1].

In this work, MEA devices are designed and fabricated on glass and quartz substrates. These substrates allow for observation of the nanopillars and neurons on top of the device using optical microscopy. Vertically aligned gold nanopillars are electrodeposited on the surface of the MEA (Figure 1). Gold
is chosen because it is a good conductor, unreactive in most aqueous reagents and has good self-assembly chemistry for surface functionalization. This device will allow for both long-term and multiplexed measurements of the AP of individual neurons at the synapse.

**Experimental Procedure:**

Two masks with multiple square die, each of edge length 20 mm were designed (Figure 2) and used to pattern four-inch quartz and glass wafers with electrode (10 nm Ti/100 nm Pt) leads and pads using standard photolithography methods. The sizes of the inner contact pads were varied to facilitate variation in nanopillar diameter. The substrate’s surface was passivated with a 1.8 µm SiO2 layer deposited by low pressure chemical vapor deposition. A reactive ion etch was used to expose the outer contact pads of the device for wiring bonding during packaging. The wafer was diced into pieces. A simple electrical test was performed for each piece to confirm electrical connection using a digital multimeter. Electron-beam lithography was used to make holes of diameter 200-600 nm to facilitate pillar growth. A 24K pure gold solution (Gold Plating Services) maintained at 65°C was used for electroplating.

**Results and Discussion:**

Optical microscopy and SEM images show that the MEAs maintained the features of the original design (Figure 3 and 4) with clear lines and only small changes (1-2 µm) in feature size. These changes were expected since the device fabrication involved several processing steps. The electrical test confirmed all MEA devices were functioning as expected, indicating that the passivation layer had been successfully etched away from the surface of the outer contact pads.

Attempts to etch through the oxide following e-beam lithography were unsuccessful because the layer of e-beam resist used was too thin. The high etch rate of the resist led to significant reduction in oxide thickness, creating a surface that was not conducive to nanopillar growth. Nevertheless, dummy devices were used to confirm the gold plating conditions.

**Conclusions and Future Work:**

Patterned MEAs were successfully designed and fabricated using standard photolithography techniques. However, additional work needs to be done in optimizing the technique used in preparing the MEA surface for nanopillar growth. Once these challenges are overcome, nanopillar arrays of varying diameters (200-600 µm) will be electrodeposited on the MEA. In the future, we also hope to vary nanopillar shape and composition to determine how these properties affect action potential measurements in neurons. These nanopillar electrode devices have the potential to serve as high-sensitivity probes for detecting membrane potentials at the synapse level which will help in understanding the long term behavior of neuronal circuits and thus provide insight into the mechanics of the brain.

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**References:**


Delivery of Immunosuppressive DNA Drug for Treatment of Autoimmune Diseases

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Abstract:
The focus of this project was drug delivery methods for delivering various deoxyribonucleic acid (DNA) fragments to treat autoimmune disease. A cationic liposome was successfully used to transfect the DNA drugs into the mouse fibroblast cells, significantly reducing the immune response to large B-DNA molecules. The cationic liposomes have some limitations when utilized in other cell types, so other nanoparticle drug delivery methods involving silica nanoparticles were explored. Our experiments have shown that silica nanoparticles are ineffective carriers for these types of DNA drugs.

Introduction:
In autoimmune diseases, large DNA fragments such as the body’s own “self” DNA can stimulate an immune response, attacking your own cells. Small DNA fragments, however, do not elicit such a response, so these small fragments can be administered to a patient to occupy the receptors, stopping cells from responding to self-DNA fragments. When the naked DNA fragments were added to cells, there was a small immune suppression effect. In order to increase this effect, a more efficient drug delivery method had to be developed. Three different drug delivery methods were tested: cationic liposomes, silica nanoparticles coated in positively charged PEI, and streptavidin-coated silica nanoparticles bound to biotin-labeled DNA fragments. Experiments involving the two types of silica nanoparticles showed that both failed to suppress the immune response. This demonstrated that silica nanoparticles are an ineffective method of delivering the DNA drugs to cytosolic receptors.

Experimental Procedure:
We first transfected different DNA fragments into cells using a cationic liposome. Single-strand and double-strand versions of three types of DNA fragment were combined with lipofectamine, a cationic liposome, and transfected into cells in a 24-well plate. In each well, 10 µg of the DNA drug were added. After waiting one hour, 10 µg of B-DNA/lipofectamine were added into all of the wells. After 24 hours, the supernatant was collected from each well and tested to see the concentration of two cytokines (IL-6 and IFN-β). A greater amount of these cytokines indicates a strong immune response, while a lesser amount means that the DNA drug suppressed the immune response. To measure the cytokine concentrations, a process called enzyme-linked immunosorbent assay (ELISA) was used. This process uses antibodies to bind to the cytokines, creating a color assay that can then be read by a plate reader. The absorbance levels were compared to a standard curve, revealing the concentrations of the two types of cytokines.

After that experiment was finished, we employed a similar technique to test the silica nanoparticle coated with PEI. First, we made the DNA drug/nanoparticle complex. A silica/PEI solution was prepared with 0.05% PEI and 10 mg of silica nanoparticles. This was mixed for one hour and centrifuged at 150,000G for 30 minutes. The pellet was re-suspended in 5 mL of water. Three tubes were then prepared: one with double-stranded CpG, one with single-stranded CpG, and one control with only silica and PEI. In each, 1 mg of silica nanoparticles and 80 µg of DNA were mixed with water for a total volume of 1 mL. The three tubes were then placed in a rotating machine for one hour, centrifuged at 150,000G for 30 minutes, and re-suspended in 1 mL of water. Once the silica/PEI/CpG complex was finished, they were transfected into cells and the cytokine levels were measured in the same manner described above.

The next experiment involved streptavidin-coated silica nanoparticles bound to biotin-labeled CpG. The silica/streptavidin nanoparticles were first washed in a wash buffer, mixing 400 µl of the silica/streptavidin nanoparticle with 1 mL of wash buffer and centrifuging at 5,000 rpm for five minutes, discarding the supernatant, and re-suspending the pellet in 400 µl of wash buffer. Three tubes were then prepared: one with double-stranded CpG, one with single-stranded CpG, and one control with only silica/streptavidin nanoparticles. In the two tubes containing DNA, 80 µg of either single or double-stranded CpG were mixed with 2.5 µg of silica/streptavidin nanoparticles for a final volume of 200 µl. The silica/streptavidin/CpG-biotin complexes were finished, they were transfected into cells and the cytokine levels were measured using ELISA, as described previously.
When these silica-based delivery methods failed to suppress the immune response, one last experiment was conducted to confirm a hypothesis on why they were failing. Instead of binding the DNA drug to the silica/PEI nanoparticle, transfecting that into the cells, and then adding the B-DNA (to aggravate the immune system), the B-DNA was bound to the silica/PEI nanoparticle and then transfected into the cells. To make the silica/PEI/B-DNA complex, the same silica/PEI procedure described above was used, only the B-DNA was substituted for CpG.

Results and Conclusions:
The only drug delivery method that effectively suppressed the immune response was the cationic liposome, as can be seen in Figure 1. Double-stranded CpG showed 62% suppression in IFN-β and 74% suppression in IL-6. The two silica nanoparticle delivery methods showed no suppression. Figure 2 shows that PEI actually created an even greater cytokine output than the controls. In Figure 3, it can be seen that cells treated with the silica/streptavidin/CpG nanoparticles had the same cytokine production as the controls.

From this data, it was hypothesized that the silica nanoparticles were getting stuck in the endosomes of cells when taken up by endocytosis, so the DNA drugs weren’t able to access the receptors in the cytosol. This was tested by transfecting silica/PEI/B-DNA nanoparticles into cells, and the cytokine outputs seen in Figure 4 support this hypothesis, as even the B-DNA wasn’t able to get to the cytosolic receptors and elicit an immune response. From this data, it can be concluded that silica nanoparticles are ineffective carriers of DNA drugs when targeting receptors in the cytosol.

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Cisplatin-Based Metal Organic Framework Nanoparticles for Targeted Drug Delivery and Tumor Imaging

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Abstract:
Nanoscale metal organic framework (NMOF) particles are nanoparticles composed of an amorphous framework constructed from an organic linker ligand and a metal ion. These nanoparticles are excellent candidates for use in drug delivery due to their biodegradability, tunable size and high loading capacity. In this study, we synthesized novel NMOFs based on the anti-cancer drug cisplatin with high payload (83%). A targeted near-infrared (NIR) molecular probe was also incorporated into this nanoparticle to achieve NIR imaging capability and targeted drug particle delivery. The anticancer effect of the nano-construct was demonstrated on cancer cells and the targeted delivery of this nano-theranostic agent was evaluated both in vitro and in vivo.

Introduction:
Metal organic frameworks are materials composed of metal ions coordinated with organic linker ligands. When shrunk down to the nanoscale, the potential for drug delivery applications arise [1]. The drug can be directly loaded into the porous in the framework or alternatively the framework can be constructed with the drug acting as the organic linker. By building the framework from the drug itself, one can increase the payload of the particle while decreasing the overall unwanted material. However, not all drugs can be used in the framework directly in this fashion. For example, a stringent requirement is that the metal ions should form the framework with the desired drug.

In this project, we constructed MOF nanoparticles based on the anti-cancer drug cisplatin with a simple nanoprecipitation method. Different nanoparticle sizes were obtained when using different precipitation procedures. To achieve the controlled drug delivery and imaging capability, we coated the amorphous prodrug nanoparticle with polymer and silica shells, at the same time, an integrin targeted imaging probe LS301 was incorporated into the nano-construct to make it capable for targeted tumor imaging (Figure 1).

Experimental Procedure:
A disuccinatocisplatin (DSCP) salt was first synthesized [1]. Methanol was added to the mixture of DSCP prodrug NPs with terbium salt to form the NMOF construct through the nanoprecipitation process [2]. By modifying method to precipitate the prodrug NPs, we were able to obtain particles with different sizes. Polyvinylpyrrolidone (PVP) and silica shells were coated onto this NMOF construct subsequently to achieve the controlled drug release. At the same time, NIR imaging probe cyrate-cyclic GRD (LS301) [3] was also incorporated into the nano-construct to make it capable for targeted tumor imaging (Figure 1).

Results and Conclusions:
The particles were characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), and dynamic light scattering (DLS) to determine the morphology and size. Figure 2a shows a SEM image of NP1-uncoated, and
Figure 2b and c shows a TEM image of NP2-uncoated and NP1-silica/probe. The DLS results demonstrating the change in size as a function of time during the coating procedure can be seen in Figure 3a. The absorption (Figure 3b) and emission spectra were recorded on a spectrophotometer and spectrofluorometer, respectively for NP1 uncoated, PVP-coated and silica coated NPs. NP1-silica/probe sample exhibited an emission peak around 800 nm that indicates the targeted imaging probe LS301 was successfully incorporated into the nano-construct.

The internalization of the silica-probe coated NP1 was investigated in A431 cancer cells. The relative fluorescence of the NMOF particles at different time point is shown in Figure 4. The high intensity of fluorescence after one hour shows that NP1 rapidly internalized in the cells. Following this peak intensity, the fluorescence intensity gradually decreased with time until it plateaued after four hours. This decrease in fluorescence was probably caused intracellular degradation of the particles, which was accompanied by the drug release. The observed fluorescence enhancement at one hour incubation could be attributed to sequestration of the dye in the silica shell [4]. Subsequent degradation of the silica shell resulted in the release of the LS301 molecular probe, with the attendant loss of fluorescence enhancement by the silica shell.

Conclusions and Future Work:
To summarize, we successfully synthesized a high payload cisplatin-based NMOF anti-cancer drug construct with targeted tumor imaging capability. The controlled drug delivery and targeted tumor imaging were demonstrated in both in vitro and in vivo studies. In the future, more drugs or co-drug will be examined to form this kind of NMOF construct to explore the better drug delivery and therapeutic effect.

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References:
System Automation for High Throughput Biosensor

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Abstract and Introduction:
Photonic crystal (PC) microcavities have demonstrated the highest sensitivities among label free, chip based, optical biosensors. These high sensitivities coupled with the study of binding kinetics have important implications in the fields of biomarker and drug discovery for pharmaceutical industries. Chip based biosensors permit miniaturization, leading to the possibility of economical personal diagnostic assays for detecting biomarkers of cancer [1].

A holistic approach for studying binding kinetics requires studying the effects on the biosensor of several samples with various biomolecule concentrations. Real-time data acquisition, concentration modification, and sample/sensor interaction control was automated using LabVIEW. See Figure 1 for the front panel of our program showing synthesized data, which delivered a step by step procedure of analysis.

Multiplexed biosensor devices have the ability to detect multiple biomolecules by functionalizing their conjugate pair (receptor) onto the microcavities of the different arms of the multiplexed device. See Figure 2 for basic device structure. Bovine serum albumin (BSA) is commonly used to block non-specific binding, thus securing the specificity of the device. As a function of time, biomolecules will bind to their respective receptor, change the index of refraction, and appropriately induce a red shift for the resonance wavelength of the transmission spectrum. Upon red shift saturation, a buffer wash is introduced. Upon buffer saturation, a net red shift is produced, denoting the difference between the final resonance wavelength and the initial resonance wavelength. A Scatchard regression of the plot of net red shift versus biomolecule concentration produces a line whose absolute value of the slope is $K_a$; $\Delta G$ can be found by using the Gibb’s free energy equation for the inverse of the $K_a$.

Experimental Procedure:
Troubles with biomolecule contamination lead to the characterization of our automation program to be preformed allowing the biosensor to detect changes in molar concentration of sugar water. Fiber optic cables were coupled with the gratings of our biosensor. The input and output cables were connected to a tunable laser and photodetector, respectively. Initially, 40 $\mu$L of deionized water was introduced to the sensor. After

Figure 1, left: Screen shot of LabVIEW program made to automate data collection and analysis. Synthesized data was placed into the graphs in order to demonstrate how the program would operate while making biosensing measurements. (1) Transmission spectrum of each laser sweep. (2) Resonance Wavelength vs. Time. (3) Resonance Wavelength vs. Time for each sample concentration. (4) Net Red Shift vs. Concentration. (5) Scatchard linear regression of Net Red Shift vs. Concentration. Note the net red shifts in (2) and (3).

Figure 2, right: SEM showing an L13 microcavity coupled to a W1 PC waveguide [1].
160 seconds, 5 µL of 200 M sugar water was added on top of the deionized water every 80 seconds. The idea was to slowly increase the molar concentration of the sample without making noticeable changes to the sample size.

Before microfluidics could be implemented into our biosensing, characterization had to be made. An enclosed microfluidic channel mold was bonded to a dummy wafer with UV-curing optical adhesive. See Figure 3. The dummy wafer was used because it perfectly matched the device the mold would be used with in the future, but would save ruining actual devices. Two syringes fitted to two syringe pumps were connected with a “Y”-joint, whose output was connected to the input of the microfluidic channel. The output of the microfluidic channel was lead to a waste receptacle. Using an additional, independent program to automate syringe pump control, adhesive application techniques, adhesive strength, and maximum flow rate were then tested. Thorlabs NOA61 and NOA81 optical adhesives were used.

**Results and Conclusions:**

Our biosensing automation program worked successfully using chemical sensing. A real-time plot showing the change in resonance wavelength versus time and easy control over the setup was demonstrated. See Figure 4.

A maximum flow rate of 1 mL/min was achieved with the NOA81 optical adhesive. It was determined that the NOA61 optical adhesive had too weak of a bond strength to be used in conjunction with our microfluidic pressure conditions. The syringe pump control program worked successfully. A new mold with input and output ports in the horizontal direction rather than the vertical direction was submitted to our local machine shop. This design should further reduce pressure inside the mold, lessening the chances of leaking and contaminating the stage. Due to time constraints, a mold of this design was not able to be tested during this internship.

**Future Work:**

The next step would be to integrate the syringe pump control program with the data collection and analysis program. This would create a singular program that would control almost every aspect of the experiment all from one panel. Furthermore, once the biomolecule problem is fixed, biosensing will resume. The singular program can then be used to begin taking data and studying binding kinetics in order to determine $K_a$ and $\Delta G$.

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**References:**

Engineering Three-Dimensional Biological Scaffolds Using a Modified Rotary Jet Spinning System

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Abstract:
Protein fibers with nanoscale diameters comprise the extracellular space in tissues and organs in the body. Current tissue engineering approaches often fail to recapitulate the three-dimensional nanoscale geometry of extracellular matrix fibers. Here, we have designed and developed modifications to a rotary jet spinning (RJS) system for fabricating nanofibers. Traditionally, the RJS produces two-dimensional sheets of polymer nanofibers at high speeds by solution extrusion through a perforated reservoir. Due to the nanometer thickness of the elongated polymer droplets, the nanofibers quickly dry and solidify and then are collected on a mandrel. The focus of this project is to engineer an automated nanofiber production and mandrel collection system in order to (a) easily create anisotropic scaffold tissue, and (b) generate small three-dimensional basic organ structures. The RJS system was constructed with multiple points of actuation for precise control, a computer program that allows for various parameter inputs to alter the fabrication process, a specially made support structure, and a custom designed interfacing circuit to allow for digital controls over high-voltage inputs and outputs. Currently, the RJS system is nearing the completion of its engineering process. With these modifications, we will be able to replicate the three-dimensional nanostructures of tissues and organs.

Introduction:
Advancements in biological research have created a demand for new substrates to be utilized for cellular development and testing. New biological scaffolds are needed to test cell dynamics in a three-dimensional environment and provide a structure for tissue development. This new structure would allow for more effective techniques in tissue experimentation in vitro [1]. It was hypothesized that the structure of these biological scaffolds could be engineered using the process of rotary jet spinning (RJS) [2]. This process can be visualized in Figure 1.

Experimental Procedure:
The configured RJS system produced thin sheets of the scaffold nanofibers. The process needed to be changed to include a more accurately controlled environment and a nanofiber collection system to build three-dimensional biological scaffolding. The leading idea involved using a rotating mandrel collection system that could be motioned linearly through the developing thin sheets of nanofibers. This design allowed for varying speeds of collection and different scaffold structures. Figure 2 has been removed from this report.

This RJS system was designed to be run by computer program that interfaced with the motor controls of actuating part of
In order to accurately design the interface, the respective motors needed to be chosen. For the center RJS motor that produces the nanofibers, an 80,000 rpm Nakanishi motor was selected to allow for a large range of centrifugal forces to aid in the development of smaller nanofibers. A Maxon motor was chosen for the collection mandrel. The Maxon motor can achieve speed rates up to 6,000 rpm, but it was reduced to 2,285 rpm to keep the collected nanofibers from being spun off of the mandrel.

The linearly actuating motor is used to move the collection mandrel through the nanofiber extrusion zone for collection. Different speeds of the linear motor cause the collection process to produce various scaffold structures. To allow for a wide range of scaffolding options, a high-speed/high-torque system was needed. For this task, a Misumi linear stepper motor actuator was chosen.

Results and Conclusions:

The program for the system was written in LabView with a simple Graphical User Interface (GUI) that displays the proper operation of the system to the end user as seen in Figure 3. To interface between the computer program and motor controls, a circuit was constructed to translate the digital signals of the computer into high-voltage signals and controls over the motors.

With the parts that were received, the RJS system center motor was constructed, as seen in Figure 4. When the center motor and the collection mandrel motor were connected to the main circuit board and tested, both worked in accordance to their respective operations in the program. These two were the only ones tested since the linear motor had still not been received.

Future Work:

The RJS system requires the integration of the linear motor system. After this is complete, the system can be tested operationally. The RJS system was designed with many variables in the system set to constant rates. This was done for simplicity in the engineering process. After the RJS system has been completely assembled and tested, an upgraded version has been devised that allows for the set variables to be varied both automatically through the operating program and manually.

This will allow the system to fully test differing extrusion and collection scenarios to pinpoint optimum nanofiber extrusion techniques for building ideal anisotropic biological scaffolding for different solutions.

Acknowledgments:

I would like to thank my principal investigator, Prof. Kevin Kit Parker, for opening his lab and giving me the opportunity to learn. I’d also like to thank my mentor, Dr. Sung Jin Park, for his guidance and support. A special thanks is needed for Matthew Hemphill, Grant Gonzales, and Josue Goss for their help around the lab and great advice. I would like to thank Harvard University, the School of Engineering and Applied Science, the Disease Biophysics Group, Dr. Kathryn Hollar, Ms. Melanie-Claire Mallison, the National Nanotechnology Infrastructure Network Research Experience for Undergraduates (NNIN REU) Program, and the National Science Foundation (NSF) for the opportunity to receive this experience.

References:

Fabrication of Nanofluidic Devices for DNA Confinement

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Abstract:
Confining deoxyribose nucleic acid (DNA) in a nanochannel affords the elongation necessary for DNA barcoding, a promising genomic mapping technique. Therefore, our objective was to fabricate nanofluidic devices that would allow for efficient confinement of genomic DNA. To limit DNA sticking to the channel walls, fused silica, an inherently hydrophilic material, was used as the device substrate. After overcoming many fabrication hurdles, these nanofluidic devices were successfully fabricated; however, DNA sticking to the nanochannels proved to be a significant issue during device loading. Scanning electron microscopy (SEM) images of the nanochannels indicated that channel roughness was likely the cause. The fabrication process was further refined, in regards to electron-beam lithography, to minimize this roughness.

Introduction:
Accurate, low-cost genome sequencing will have broad implications in a wide variety of fields such as personalized medicine [1]. Next-generation sequencing methods have made tremendous strides over the past decade in accuracy and read length. However, these ‘short read’ shotgun sequencing methods possess inherent limitations for entire genome sequencing. Repetitive genomic regions, which account for half of the human genome, cannot be directly mapped [2], and technologies that rely on ensemble measurements are unable to detect rare genomic variations [3].

Confining genomic DNA to a nanochannel can afford numerous sequence analysis techniques without the need for amplification or DNA fragmentation. If DNA can be loaded into a channel ~ 50 nm wide, the DNA molecules cannot fold back on themselves and are forced by physical confinement to be in an elongated, linearized state [3]. If the DNA is fluorescently labeled at specific sequences, the confined DNA creates a distinct optical pattern, resembling a barcode that provides a large-scale view of the genomic DNA. This technique known as DNA barcoding provides a scaffold for de novo construction of sequence data obtained from next-generation sequencing methods, allowing for entire genome sequencing [1].

Thus the objective of the project was to establish a robust method for fabricating nanofluidic devices that effectively confine genomic DNA. Specifically, we wanted to use fused silica, which is inherently hydrophilic, as the substrate material to limit DNA sticking to the nanochannel walls [3].

Device Fabrication:
The nanofluidic devices were fabricated on 10 cm diameter, 500-µm-thick fused silica wafers. Each device contained an array of nanochannels between two parallel microchannels with reservoirs for loading (Figure 1). To fabricate the nanochannels, electron-beam lithography was used. Since fused silica is an insulating material, 30 nm of aluminum was deposited on the wafers to prevent charging. An electron beam resist layer was spin-coated on top of this aluminum layer, and standard techniques were used to define, develop, and etch the device patterns. This process produced channels 60 nm deep and 50-150 nm wide (depending on the predetermined electron beam exposure level). The microchannels and reservoirs were patterned by contact photolithography and were etched by a wet and dry etch sequence. Sandblasting was then used to establish the through holes on the back of the wafer. After thorough cleaning, the etched wafer was physically pressed onto a 250-µm-thick wafer and fusion-bonded overnight.

Initial attempts to fabricate the devices using two 250-µm-thick wafers (Figure 1) were unsuccessful because it was difficult to establish a good bond between the wafers. Analysis indicated that significant warping in the wafers, most likely due to undesired etching, was the possible cause.
**Device Evaluation:**

The device was filled with 2.5X tris, borate, ethylenediaminetetraacetic acid (TBE) aqueous buffer containing B-mercaptopethanol (5% w/w), ascorbic acid (0.07% w/w), and polyvinylpyrrolidone (0.01% w/w). Genomic DNA from virus phage λ, dyed with fluorescent label YOYO-1, was pipetted into the reservoir, pumped through the microchannel, and subsequently forced into the nanochannels by an applied voltage. However, as DNA moved through the nanochannels, a large portion of the DNA was sticking to the channel walls (Figure 2).

SEM micrographs of the device indicated that there was significant roughness on the bottom and sides of the nanochannels which is most likely the reason for the DNA sticking (Figure 3). To reduce channel roughness, the electron-beam lithography process was modified by depositing the 30 nm aluminum layer above the electron beam resist instead of below it. This modification allowed for a more complete removal of the aluminum layer, thereby creating a cleaner fused silica etch. Nanochannels produced following this protocol were significantly smoother (Figure 4); however, more analysis and testing is needed to confirm that channel roughness has been reduced.

**Future Work:**

After establishing a robust fabrication procedure that produces smooth channel walls, the next step would be to load DNA into the nanochannels to determine if the modified procedure has reduced DNA sticking. These nanofluidic devices will be used to further our understanding of how confined DNA behaves, which will ultimately help enable better genomic mapping technologies.

**Acknowledgments:**

I thank Dr. Julian Sheats, Prof. Kevin Dorfman, and the staff of the University of Minnesota Nanofabrication Center for helping with the project, and the NNIN REU Program and the NSF for financial support.

**References:**


Conformable Conducting Polymer Electrodes used with an Ionic Liquid Gel for Electroencephalography

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Introduction:

Electroencephalography (EEG) is a non-invasive and relatively inexpensive diagnostic tool that typically uses metallic electrodes on the surface of the scalp to measure electrical activity in the brain. Commercial silver/silver chloride (Ag/AgCl) electrodes used in EEG require a liquid electrolyte to decrease the electrode-skin impedance. This electrolyte not only often dries out quickly, preventing recording over longer time periods, but it also can sometimes cause short circuits if it leaks between two electrodes in a high density electrode array [1].

One path to improving EEG electrodes is having a flexible electrode, which improves the contact with the skin and thus decreases the electrode-skin impedance [2]. In a similar effort, conducting polymers are also being researched as a material to be used due to their flexibility, high conductivity, rough surface area (and thus lower impedance), and biocompatibility [3]. Additionally, polymerized ionic liquids are appealing for use as a quasi-solid-state electrolyte in combination with an electrode because they do not run or dry out, solving two of the main problems with electrolytes currently used in EEG [4].

In this project, we improved upon current electrodes by decreasing the electrode-skin impedance and improving time stability using a combination of a conformal substrate, conducting polymer, and an ionic liquid gel. We show that the ionic liquid gel improves the performance and time stability compared to commercial electrodes.

Experimental Procedure:

Figure 1 illustrates the fabrication process for the devices. Gold electrodes were patterned using a standard shadow mask evaporation process onto a two-micron layer of Parylene C. We then insulated the electrodes with two additional layers of Parylene C. Subsequently, standard photolithography was used to expose only the electrode sites to etching. Following etching, the conducting polymer poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) — PEDOT:PSS — was spun over the entire wafer. The top layer of Parylene C was removed from the electrodes and contact pads, leaving the remaining Parylene C to insulate only the interconnects. This process yields conformal electrodes with a thickness of only 4 µm.

The holder was laser-cut out of Kapton®, and gold was then deposited. This holder was attached to the thin electrode, preventing it from curling up into itself and providing a more stable contact pad than the delicate Parylene C. Figure 2 demonstrates the flexibility of the device.

The ionic liquid gel was made using an ionic liquid (1-ethyl-3-methylimidazolium ethyl sulfate), a monomer (poly(ethylene glycol) diacrylate), and a photo initiator. It was then coupled with the electrode as a quasi-solid-state electrolyte.
Results:
We tested the impedance of the ionic liquid gel on electrodes, along with several different controls. We fabricated electrodes with PEDOT:PSS only, gold only, PEDOT:PSS plus the ionic liquid gel, and gold plus the ionic liquid gel. We also tested a commercially available Ag/AgCl electrode used for clinical purposes. Figure 3 shows the electrical impedance spectroscopy for each of these electrodes. The electrode with only PEDOT:PSS demonstrated a better impedance than the gold only, the electrode that performed the worst. The increased surface area of the PEDOT:PSS provides an increased capacitance, thus lowering the impedance. The ionic liquid gel significantly improved the performances of both the gold and the PEDOT:PSS electrodes to a range similar to commercially available electrodes.

Conclusions:
In conclusion, we developed a process for the fabrication of conducting polymer EEG electrodes with a thickness of only 4 µm. These electrodes are incredibly conformal, improving their recording quality and decreasing their electrode-skin impedance. We also used an ionic liquid gel coupled with these electrodes, which further reducing their impedance. We showed that the ionic liquid gel did not dry out, even over three days, compared to the commercially available gel. This provides a means of recording data with EEG over extended periods of time, which is often a necessity. The gel also functions as a solid-state electrolyte, resulting in less chance of leakage and a short circuit. This solves two of the biggest problems clinicians face when using EEG. These improvements to current electrodes could provide a path to better and more accurate EEG recordings, meaning clinicians could rely less heavily on more invasive diagnostic techniques.

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References:
Development of Microfluidic Devices for Use in Immunophenotyping

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Abstract:
Immunophenotyping presents significant promise in the diagnosis and prognosis of immune system disorders. While a means of detection of diseases including HIV/AIDS, tuberculosis and sepsis, current methods (flow cytometry and enzyme-linked immunosorbent assay or ELISA), lack specificity, as well as time and sample-volume efficiency. Microfluidic devices may present the solution to such limitations. Through the integration of a high-porosity, polydimethylsiloxane (PDMS) microfiltration membrane (PMM), with a microfluidic microfiltration platform, microbead filtration may be used to achieve antigen-specific cellular isolation. The PMM was fabricated via semiconductor microfabrication techniques including traditional lithography and reactive ion etching (RIE). The silicon molds for components of the microfiltration platform were fabricated using photolithographic techniques, followed by deep reactive ion etching (DRIE). The PDMS components were then fabricated via soft lithography and layered to construct a complete device for microfiltration and chemiluminescence assay application for highly sensitive subpopulation characterization. This microfluidic device may drastically reduce time, costs and inaccuracies of immunophenotyping, yielding more efficient detection, evaluation, and treatment of disorders.

Introduction:
The immune system has various leukocyte constituents within the blood, maintaining particular functions in disease combating. Infections and other obstructions cause abnormalities amongst constituents, characterized in the numbers, proportions or functional responses of leukocyte subpopulations. For example, human immunodeficiency virus (HIV) causes CD4+ T cell depletion. Thus, the identification of disproportionately-low CD4+ T cells and overall T-cell proportions provide an indication of acquired immunodeficiency syndrome (AIDS) [1]. Immunophenotyping technology is used to identify qualitative abnormalities in immune cell subpopulations for use in diagnosis and prognosis. The current challenge in the design of immunophenotyping devices is overcoming the time, cost, and sample-size inefficiencies, while increasing characterization specificity. A microfluidic device is proposed with the integration of a high-porosity PMM within a microfluidic chamber, consisting of cell culture and immunoassay components (see Figure 1). The PMM is designed for efficient bioparticle separation. Microbeads are coated with cell-specific antibodies, resulting in the selective capture of the cell subpopulations via bonding of cell surface proteins to beads. Pore diameters are designed to trap only bead-subset groups above the PMM. The PMM was fabricated with traditional lithography and RIE [2], then layered between microchamber components [1]. This device will isolate cell subsets and characterize populations using a no-wash chemifluorescence assay (AlphaLISA) [3].

Experimental Methods:
PMM Micromachining. PMM fabrication was completed using a surface micromachining technique for soft materials [2]. A silicon wafer was O₂-plasma activated and silanized with (tridecafluoro-1,1,2,2,-tetrahydrooctyl)-1-trichlorosilane vapor for later PMM release. The PDMS prepolymer was prepared with a 1:10, curing agent: base monomer ratio, spun onto silanized wafers at 7,000 rpm for 30 s, then cured at 60°C overnight. The PDMS surface was plasma activated for 5 min, with coating of AZ 9260 photoresist immediately following. A soft-bake process at 90°C for 10 min and traditional exposure
and development followed. The wafers were processed with RIE using SF$_6$ and O$_2$ mixtures to anisotropically pattern PDMS layers.

**Device Construction.** The device consisted of cell culture and immunoassay layers fabricated from silicon molds made via photolithography and deep reactive ion etching (DRIE) [1]. The cured PDMS structures sandwiched the PMM atop a glass slide via a O$_2$ plasma-assisted bonding process.

**Device Testing / Functional Immunophenotyping.** Device testing included capture efficiency of the PMM, quantified with ImageJ. The cultured cells were labeled and captured according to previous protocol [1]. Microbeads and cells were quantified via hemocytometer, then imaged via fluorescence microscopy (see Figure 4). After microfiltration of cells, LPS addition induced cytokine secretion. AlphaLISA signal detection was facilitated by AlphaLISA bead interaction with cytokines [1, 3].

**Results and Discussion:**

The PMM was successfully fabricated and integrated into the device (see Figures 2 and 3). Some membranes were corrupted during the micromachining process due to separation between photoresist and PDMS layers following exposure. Moderate success rates during PMM fabrication suggest a need for improvement in the micromachining process, including an adjustment in soft-baking or plasma-activation steps to ensure adequate photoresist-PDMS annealing. High capture purities (~95%, whole and lysed) confirm that the PMM successfully filters cells. Percentage of monocytes were at 4% and 6% for whole and lysed blood, respectively, within the range of CD14+ monocyte content for a healthy individual (see Figure 4), confirming that the correct subpopulation was isolated. The multi-component device facilitates multi-step processes with high specificity and reduced time (10-fold reduction), sample volume (5 µL / assay) and effort of processes, confirming it as a superior immunophenotyping device.

**Conclusions:**

We successfully fabricated a PDMS-based, bioparticle separation membrane that is easily integrated into a microfluidic, immunophenotyping device. Immune cells were isolated and characterized for use in functional immunophenotyping. The testing of microfiltration capabilities revealed that the membrane had a high capture yield and capture purity. The same device yielded successful characterization of immune cells via AlphaLISA biosensing with a vast reduction in process time. With the shortened assay time, heightened sample efficiency, and the ability to determine the functional status of subpopulations of immune cells, the PMM-integrated device is a novel approach to diagnosis and prognosis of immune diseases and disorders.

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**References:**

Cell Polarization on Circular Topography with Various Radii of Curvature

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Abstract:
Recent studies have shown that cells orient along micro/nano-scale grooves that model the extracellular matrix (ECM) and provide a bidirectional cue for migrating cells [1]. Using ultraviolet (UV)-assisted capillary lithography techniques, sub-cellular sized arrays with varying radii of curvature were fabricated as a model system to probe cellular migration on curved substrata. Chinese hamster ovary (CHO) cells were cultured and plated on substrates with and without the micro-scale circular patterns. From time lapsed live cell imaging, the results demonstrated more explicitly that the cells polarized along the direction of topographic feature, and preliminary data showed that cell elongation was greater on the patterned surface versus cells on the flat surface.

Introduction:
The topographical and mechanical properties of the structure a cell adheres to are key factors in determining cellular response. These features are important for controlling the direction and speed of migration during cellular growth [2]. Better understanding of how cells sense and react to the physical features of their substrata can lead to optimized methods for tissue and muscular regeneration. With advances in fabrication techniques of biomaterials that has enabled stronger control over micromechanical and environmental properties such as local rigidity and curvature of the ECM components, micro-scale circular concentric ring patterns were constricted to model the natural curvatures in the ECM. We hypothesized that Chinese hamster ovary (CHO) cells will react to the micrometer range topographical features of the patterns by sensing the curvature and migrating along the grooves.

Experimental Procedure:
Liquid polyurethane acrylate (PUA, 301 MPa) was drop-dispersed onto treated cover glass (~ 10 µL/cm²). A mold with the circular concentric patterns was pressed down on the PUA and rolled flat to form a uniform PUA layer on the cover glass. This combination was then exposed to UV light for 20 seconds, after which the mold was carefully peeled off from the cover glass, and an identical circular concentric array remained on the cover glass. The fabricated cover glasses were then placed under a UV lamp overnight to completely cure any remaining liquid PUA. Each cover glass was subsequently attached to the bottom of a cell culture device for cell plating.

Prior to plating the cells, each well of the cell culture device was coated with a 1.0 mL solution of Collagen Type I (50 µg/mL) for six hours. To detach the CHO cells from their culture dishes, 2.0 mL of trypsin base (0.05%) was pipetted into each dish for three minutes. The detached cells were washed down and added to 6.0 mL of Dulbecco’s modified eagle medium (10.0% fetal bovine serum, 1.0% penicillin strip). One milliliter of this solution was added to each well of the cell culture device. The CHO cells were given 24 hours to adhere to the surface of the cover glass. Time-lapse image series were acquired from an electronic, inverted microscope (Nikon).

Results and Conclusions:
As seen in Figures 1 and 2, the cells on the flat substrate had random orientation while the cells on the patterned substrate aligned along the direction of the micro-scale grooves. After quantifying our data (Figure 3), the results confirmed that the cells on the flat substrata had a substantial amount of directional deviation from the curvature of a circle, while the cells that migrated on the patterned substrata had a very small average directional deviation from the curvature of a circle. Our protocol allowed us to successfully polarize cells along the grooves of our micro-patterned arrays.

As shown in Figure 4, cell elongation was greater for the cells on the patterned surface compared to the cells on the flat surface. Our preliminary data also suggested that cell elongation slightly increased as distance from the center of the concentric rings increased. This may be due to factors involving focal adhesions to increasing degrees of curvature. Our results confirmed that the topographical features of the micropatterns increased the elongation of the cells that migrated on them, and the elongation of a cell may be dependent upon the degree of curvature of the substrata underneath.
Future Steps:
Studying initial adhesion processes, focal adhesions, and the attachment/detachment of cells will allow us to better understand how cells are able to sense the topographical features of the substrata they grow on. Observing cell skeletal fiber alignment and alpha-actin stress fiber distribution will help us better understand the mechanisms behind cellular migration.

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References:

Figure 1, top: Cells on flat substrata after 24 hours of plating.
Figure 2, bottom: An entire micro-patterned array after 24 hours of plating. Cells appear to polarize along the concentric arrays.

Figure 3: Cells on the concentric arrays showed much less directional deviation from curvature compared to the cells on flat substrata.

Figure 4: Cells on the concentric arrays had greater elongation than cells on flat substrata. Also, there seems to be a correlation between increasing distance from the center of the concentric arrays and increasing elongation.
Fabrication of “Barcode” Nanowires for Multiplexed Detection in Biological Assays

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Abstract and Introduction:
“Barcode” nanowires, composed of multilayers of gold (Au) and nickel (Ni), suggest a possibility of replacing the fluorophore cell labeling technique used today, which will introduce a “macro” magnetic detection and classification method for multiplexed biological assays. Low cost of fabrication [1] and a huge variety of magnetic signatures give barcode nanowires great advantage over fluorophores, which are costly and limited by the spectrally resolvable wavelengths. The nanowires can be functionalized with antibodies, so that different cell types can be tagged with unique barcode nanowires. The ferromagnetic properties of nickel are employed for nanowire characterization and manipulation, e.g. separation from the supernatant in the experimental procedure. Here, fabrication and characterization of barcode nanowires and their application in biological multiplexing studies were investigated. Two cell lines, A549 (human lung carcinoma) and HFF (human foreskin fibroblast), were incubated with two types of barcode nanowires that were conjugated with antibodies targeting the respective cells. The result demonstrated successful specific targeting of cells by the corresponding barcode nanowires. Together with quantitative magnetic characterization, successful functionalization of our barcode nanowires showed much promise in various cell detection applications.

Experimental Procedure:
Nanowire Fabrication. Nanowires were grown through sequential electrodeposition in gold (HS-434 RTU, Technic, Inc.) and nickel (NiSO₄) electroplating solutions. A porous anodized aluminum oxide (AAO) template was sputtered with a copper conductive layer on one side and used as a working electrode. The working electrode, reference electrode, and counter-electrode were all immersed in a plating solution, and electrical charges were applied for metal deposition in the pores. The length of each metal segment was controlled by electrodeposition time. Once the nanowires were grown, the back copper contact was etched by ion-milling process and then freed by dissolving the AAO template in sodium hydroxide. The nanowires were suspended in deionized water until use.

Nanowire Characterization. While still in the template, the nanowires were characterized by vibrating sample magnetometry (VSM) at different angles ranging from 0° to 180°, with 0° being parallel and 90° perpendicular with respect to the magnetic field. Once the nanowires were freed and suspended in an aqueous solution, the nanowires were observed through scanning electron microscopy (SEM) for dimensions (Fig. 1).

Multiplexed Detection. Two types of barcode nanowires, Au-Ni (0.306 µm, 3.104 µm) and Au-Ni-Au (0.126 µm, 1.8 µm, 0.27 µm), were functionalized with primary antibodies, each specifically targeting A549 and HFF cells, respectively. Then, Au-Ni nanowires were conjugated with a green fluorophore, fluorescein isothiocyanate (FITC), to be distinguished from non-fluorescent Au-Ni-Au nanowires. Both A549 and HFF cells were conjugated with blue nuclear stains, Hoechst 33342. Plasma membranes of only HFF cells were tagged with wheat germ agglutinin with green Alexa Fluor 488 conjugate to optically distinguish the two cell types. The nanowires suspended in corresponding cell media solutions were titrated into cell cultures, in which A549 and HFF cells were plated 24 hours in advance, and incubated for six hours at 37°C. The cell media was aspirated, and formalin was added for cell fixation. Finally, formalin was replaced with a phosphate buffered saline (PBS) solution.

Figure 1: SEM of barcode nanowires (Au-Ni-Au). Minnesota Nano Center.
Results and Conclusions:
The VSM measurements successfully characterized each nanowire type, providing a specific magnetic signature of coercivity versus angle curves. Coercivity is the magnitude of magnetic field required for any magnetic materials to be demagnetized after reaching the saturation moment, and it can be measured at different angles discussed in the procedure. This way, our characterization methods rely on the nanowires’ magnetic properties rather than optical imaging.

Three nanowires with different Ni deposition times, 10, 15, and 20 minutes, gave coercivities of 212.24, 261.17 and 285.37 Oe, respectively, all measured at 0°, and 131.54, 180.09, and 366 Oe, respectively, at 90° (Figure 2).

In multiplexing studies, the fluorescent nanowires, which were coated with antibodies targeting A549 cells, were observed to be tagged onto A549 cells only (Figure 3). Equivalently, the non-fluorescent nanowires, which were conjugated with antibodies targeting HFF cells, were found tagged to only HFF cells that were distinguished from A549 cells with their fluorescent plasma membranes (Figure 4). Through this experiment, specific cell-targeting by barcode nanowires was successfully tested and observed. The applications of barcode nanowires in biological assays seem highly viable with our magnetic characterization and antibody-functionalization techniques.

Future Work:
More experiments with various cell lines need to be conducted to ensure standardization of our nanowire-tagging methods. The possibility of nanowires being internalized after the tagging can also be looked into, in comparison to prior research findings of non-specific internalization of barcode nanowires in osteosarcoma cells [2]. The magnetic characteristics of our barcode nanowires suggest more possibilities of research topics, such as manipulation of cell matrix with influence of magnetic field gradient on the internalized nanowires.

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References:
Effects of Cellular Architecture on MDA-MB-231 Breast Cancer Cell Motility

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Abstract:
Over 200,000 women living in the United States will be diagnosed with breast cancer this year. Of those whose cancer metastasizes, wherein the cells migrate away from the initial tumor, only 23% will survive [1]. Cellular mechanics and architecture drive a wide range of cellular behaviors, such as contractility and apoptosis [2, 3]. Here, we assessed the role of cellular architecture in breast cancer motility. MDA-MB-231 breast cancer cells were micropatterned into two sizes of four shapes. We explored cytocoellular architecture, measured lamellipodia extension as a function of that architecture, and investigated cellular invasion into a collagen gel. Results suggest that cellular architecture plays a role in determining the likelihood of tumor cell metastasis.

Introduction:
Metastasis of a tumor to vital organs greatly decreases chances of survival [1]. The factors that drive tumor metastasis are poorly understood. Cellular architecture has been shown to dictate certain cellular processes indicative of migration intent [4]. Thus, we hypothesized that cellular architecture plays a role in tumor metastasis.

We studied the correlation between cellular architecture and breast cancer cell motility. MDA-MB-231 breast cancer cells were micropatterned into two sizes of four shapes each. Cells patterned at 100% area were patterned on fibronectin microfeatures, which displayed 100% of the cross-sectional area attributed to a single MDA-MB-231 cancer cell on an unpatterned substrate. Immunofluorescence confocal microscopy was used to examine cytocoellular architecture. We also measured lamellipodia extension as a function of cytocoellular architecture to determine cell motility with respect to cell shape. Finally, we assessed the effect of cytocoellular architecture on cell migration into a collagen gel overlay.

Experimental Procedure:
As per Figure 1, casts for production of polydimethylsiloxane (PDMS ) stamps were fabricated from SU-8 photoresist spun on a silicon wafer via standard photolithography technique. Casts were coated with Sylgard 184 polydimethylsiloxane (PDMS ) (10:1 base: curing agent), degassed, and baked at 90°C for four hours.

Stamps were coated with fibronectin (50 mg/mL in H2O), an extracellular matrix protein, and incubated for one hour. The fibronectin was then stamped onto PDMS-coated glass coverslips. A suspension of MDA-MB-231 human breast cancer cells (75,000 cells/mL) was seeded onto the coverslips. Overnight incubation at 37°C and 5% CO₂ allowed cells to settle and conform to the fibronectin microfeatures.

Micropatterned cells were fixed with 4% paraformaldehyde and permeabilized with 0.05% Triton-100 (in PBS at 37°C). Cells were stained with AlexaFlour 488 Phalloidin and 4’, 6-diamidino 2-phenylindole (DAPI) in PBS, then imaged with an Olympus IX81ZDC inverted confocal microscope.

Figure 1: Photolithography and Microcontact Printing Methods.
1. Photomask aligned on SU-8 coated wafer.
2. Microfeatures developed on the wafer via selective exposure and a chemical etching process.
3. PDMS poured onto the wafer and heat-cured.
4. PDMS stamp with microfeatures is peeled off the wafer.
5. PDMS stamp coated with extracellular matrix (ECM) protein, fibronectin (FN).
6. FN coated PDMS stamp is stamped onto a coverslip transferring FN to the coverslip, providing guidance cues for cell shape.
Results and Discussion:
Microcontact printing reliably produced the confocal images shown in Figure 2.

Time-lapse photography acquisition every five minutes for one hour of micropatterned cells allowed for quantification of cell area change. The lamellipodia protrusion parameter (LPP), which is the standard deviation of the normalized area measurements spanning this hour, was used to quantify lamellipodia extension.

Figure 3 indicates a relatively large LPP for the 100% triangle cell. This was attributed to the high concentration of actin filaments found at the cellular periphery (Figure 1). It was noted that, for all but the triangle, the smaller cell size produced a higher LPP. However, further data acquisition is required to validate the results.

Future Work:
Attempts at a collagen invasion assay adapted from previous work by Goswami et al. [5] were made. Methods for the assay can be found in Figure 4. Imaging techniques are yet to be perfected. We aim to solidify a protocol and imaging technique that allows us to quantify degrees of invasion with respect to cellular shape.

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References:
Microfluidics for the Study of Breast Cancer

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Abstract:
We present a microfluidic system for determining the efficacy of chemotherapy drugs on breast cancer. The volume of the device is approximately 12 µL total, including a control chamber and an experiment chamber. A protocol for fabrication, preparation for cell introduction, culture and introduction of the experimental and control reagents is described. We were able to use the protocol to prepare MCF-7 human breast cancer cells in the device. The cells were able to adhere to the culture surface, proliferate and survive the culture protocols. However, the assessment of the efficacy of the chemotherapy drug tamoxifen was not successful due to the crystallization of the drug as the temperature in the device dropped during the observation periods.

Introduction:
Early diagnosis and treatment are a cancer patient’s best chance of survival. To that end, microfluidic systems show great promise for developing point of care diagnostic technologies and quick evaluation of treatment options. Microfluidic systems allow precise manipulation of cells in a controlled environment, and can be cheaply and easily fabricated using standard soft lithography techniques. Microscale systems use smaller sample and media volumes, and provide fast results. Testing chemotherapy drugs on a biopsy within a microfluidic system can give patient-specific results so high efficacy treatment can begin as soon as possible.

The objective of this project was to determine the time for a known chemotherapy drug to have an observable effect on cancer cells in a microfluidic device. The cells used were from the MCF-7 cell line, immortalized human breast cancer epithelial cells. The cells were dosed with 20 µM tamoxifen, a chemotherapy drug proven to be effective against MCF-7 cells after a minimum of seven days with traditional methods [1].

Methods:
A microfluidic device was fabricated using standard soft lithography replica molding. A polydimethylsiloxane (PDMS) microfluidic structure was bonded to a glass microscope slide to form a device with two identical 5.95 µL chambers, experiment and control, separated by a central channel. This design allowed mixing between the two chambers initially to establish uniform cell growth in the experimental and control chambers. Diffusion between the chambers could be prevented simply by the flow of fluid through the central channel at a rate ten times higher than the flow into the chambers. The functionality of the design was confirmed by filling the chambers with food coloring and running water through the central channel. This effectively separated the chambers, as shown in Figure 1.

To set up the experiment, each input and output of the microfluidic device was connected to Tygon® Microbore tubing with 20 gauge blunt needles. A three-way stopcock was attached to the end of each tube to facilitate input changes. Three-milliliter syringes were used at the side inputs In1 and In2 to fill the chambers shown in Figure 2. The cell culture chambers were initially filled with gelatin to facilitate cell attachment. Phosphate-buffered saline (PBS) was used to wash out the excess gelatin.
and wet all the surfaces. The PBS was then replaced with cell culture media, Dulbecco’s modified eagle medium, plus 5% fetal bovine serum and 1% penicillin-streptomycin. MCF-7 cells were introduced into the chambers, and the microfluidic device was placed in an incubator until the cells adhered to the surface, forming a monolayer.

When healthy cell cultures had been established in each chamber, a syringe pump was used to run media through the middle channel and separate the experimental and control groups. Another syringe pump was then used to introduce 20 µM tamoxifen into the experimental chamber and the same amount of tamoxifen solvent, ethanol, into the control chamber. Cell viability of the two groups was then compared over the next 24-hour period. The flow rates into the chambers were 1 mL/h for 50 min to introduce the tamoxifen, then 2 µL/h complete media to maintain the cells. The corresponding flow rates for the central channel were 10 mL/h and 20 µL/h. These flow rates were shown to maintain the separation of fluids and provide sufficient media for cell growth without introducing harmful levels of shear stress to the cells.

Results and Conclusions:

After 14 hours with tamoxifen outside of the incubator, the MCF-7 cells still appeared to be healthy. The microfluidic device was placed in the incubator. Six hours later both the control and experimental groups showed new cell growth (Figure 3). Tamoxifen crystals were visible in the experimental chamber, indicating conditions were too cool outside the incubator (Figure 4).

The MCF-7 cells failed to exhibit inhibited growth or cell death within 24 hours of tamoxifen introduction. Removing the cells from the incubator for observation cooled the tamoxifen causing crystals to form that the cells couldn’t absorb.

Future Work:

Future work includes improving the experimental procedure to prevent crystallization of tamoxifen and broadening the scope to simultaneously test different chemotherapy drugs at varying concentrations.

Acknowledgments:

I would like to thank my PI, Prof. Jennifer Blain Christen, my mentor Tao Luo, and Dixie Kullman for all of their help and guidance. Thanks to the National Nanotechnology Infrastructure Network Research Experience for Undergraduates Program, the National Science Foundation, and the Center for Solid State Electronics Research at Arizona State University for research support and funding. I would also like to thank Prof. Karen Anderson and Eva Amouzougan from the Biodesign Institute Center for Personalized Diagnostics for providing the cells and reagents.

References:

Design of Synthetic Protein Membranes Using Droplet Microfluidics

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Abstract and Introduction:
Protein conjugated beads and particles are often used in biomedical processes [1]. However, these particles suffer a number of limitations. Proteins are chemically attached to fixed locations on the particle surface, and cannot move independently of the particle, even in the presence of external stimulation. This can also lead to partial protein denaturation and loss of activity [2]. The goal of this work was to overcome the limitations of solid particles by studying the behavior of proteins on a liquid-liquid interface. By engineering artificial membrane proteins using the PURExpress in vitro protein synthesis platform from New England BioLabs (NEB) and attaching them to droplets using droplet microfluidics, the detrimental effects of denaturation can be greatly mitigated or avoided completely. Preliminary measurements and observations show that protein droplets are uniformly coated with mobile, active proteins. These characteristics distinguish protein droplets from solid particle systems and make them a promising solution to problems in drug delivery, biosensing, as well as the study of protein interactions. Of particular interest is the application of protein droplets to the study of apoptosis.

Experimental Methods:
Using template DNA provided by NEB, protein-transmembrane helix complexes were synthesized as single proteins using their PURExpress synthesis kit [3]. For the purpose of this work, membrane versions of green fluorescent protein and streptavidin were studied.

Droplets were made using PDMS microfluidic devices, as in Guo, et al. [4], and by simple emulsification. Protein membranes were observed on both water-in-oil and oil-in-water droplets. Droplets were incubated at 37°C after formation to allow proteins to be synthesized and coat the droplet interface.

The structure and properties of these protein droplets were studied using confocal microscopy. Engineered transmembrane green fluorescent protein was used as a model system to study protein droplets and membranes. Fluorescent biotin dyes allowed the use of streptavidin protein drops to demonstrate protein activity.

Results and Conclusions:
Protein Density. While protein-conjugated solid particles vary widely in their degrees of coverage, protein droplets show uniform coatings on the entire surface of the drop. Preliminary measurements also show that droplets are covered with a higher density of proteins than are commercially available particles. Commercially available streptavidin particles are coated with approximately 1.5 ng/cm², but preliminary data shows that streptavidin droplets could have coverage levels above 700 ng/cm².

Protein Mobility. Unlike proteins bound to solid particles, proteins attached to droplets were observed to move in small groups along the plane of the membrane. This could lead to engineered proteins that can respond to stimuli and assemble into complex structures, similar to proteins on the cell membrane.

Protein Activity. To determine if attachment to the droplet interface severely damaged the proteins, streptavidin droplets were placed in a solution of biotinylated dye, which only active streptavidin would bind. Fluorescence microscopy demonstrated that the entire surface of the streptavidin drop had the dye molecule bound to it. Further testing is needed to determine the degree of activity and assess the potential of protein droplets as biosensors.

Future Works:
One of the most promising applications of protein droplets is to the study of apoptosis. Malfunctions of apoptosis, the natural process of programmed cell death, play a major role in the development and progression of cancer [5]. However, the study of apoptosis requires costly and specialized cell culture conditions and soluble synthetic substitutes have proven to be inefficient.

Natural apoptosis occurs when a cell presents a complex of transmembrane proteins called the FAS ligand on its surface, which binds to corresponding receptor on the target cell and induces cell death. Droplets coated with the FAS ligand proteins have the potential to act as synthetic cellular analogs, since they are densely coated with active proteins that can move to assemble into the complete ligand complex.
Droplet protein technology has the potential to greatly reduce the cost barrier of entry for researchers hoping to study apoptosis for the development of cancer therapies.

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References:
Curved Functionalized Microfluidic Channels for the Study of Cell Dynamics

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Abstract:
There have been studies in which physical environments are simulated by growing cells on different stiffness substrates to observe changes in migration speed, cell stiffness, etc [1]. However, these flat, two-dimensional systems do not accurately simulate in vivo environments in which cells exist. The vast majority of tissue in the human body exhibits at least some additional parameter, whether it is depth, curvature, etc. The objective of this study was to make observations on how curvature and stiffness affect cell proliferation, growth, and orientation preferences in channel-like structures. Polyacrylamide/ bisacrylamide gels with different substrate stiffness were fabricated with cylindrical voids of varying channel diameters. Two-dimensional gels with similar substrate stiffness were also observed as a control for the system. Initial observations indicate curvature prompts fibroblasts to migrate along the lumen (length) of the channel as well as aggregate.

Introduction:
Many groups have simulated wound healing by performing scratch assays on a group of fully confluent cells on a flat surface and observing migration towards the wound. However, these substrates do not accurately simulate in vivo environments, as most tissues exhibit some additional parameter, whether it is depth, curvature, etc. Substrate stiffness has also been seen as a factor that influences cell migration velocities [2]. The aim of this study was to see how curvature affects cell migration on different substrate stiffness with the purpose of creating a curved wound-healing environment.

Experimental Procedure:
Substrates were made by polymerizing a mixture of 8 wt% and 4 wt% polyacrylamide with 0.3 wt% bisacrylimide solutions. 8 wt% / 0.3 wt% polyacrylamide/bisacrylamide gels yielded a Young’s modulus of 25 kPa (stiff), while 4 wt% / 0.3 wt% polyacrylamide/bisacrylamide gels yielded a Young’s modulus of 5 kPa (soft). Channels were made with diameters of 736 µm, 384 µm, and 114 µm. Flat substrates with the same composition served as the control. Gels were embedded with a synthesized GRGDSP acrylamide to promote cell adhesion to the substrate surface. Human foreskin fibroblasts from the BJ line were transfected with green fluorescent protein to observe migration patterns.

Migration was tracked using confocal microscopy with stacks starting and ending where green fluorescent protein detected. Projections were made along the Z axis in order to average the fluorescent signals for two-dimensional tracking. Migration was tracked using an ImageJ plugin, MtrackJ. Total average velocity calculated along with velocities in the X and Y vectors.

Results:
In looking at the migration patterns on the stiffer substrates, there was no statistically significant difference in total velocity between the control verses the curved environments. Additionally, there was no difference in total velocity between the 114 µm diameter channel and the 384 µm channels in the soft gel. However, cells that were seeded within the 768 µm channel in the softer gel exhibited a higher total velocity verses the cells on the two-dimensional substrate.

To better understand how the curvature of the substrate affects the migration patterns of the cells, the velocity along the major axis of the channel and around the curvature of the channel were measured. The total average velocities were broken down into their subsequent X and Y components. From our initial observations, fibroblasts within the 736 µm channel preferred...
to move along the channel, having a higher average X velocity than average Y velocity. Fibroblasts within the 384 µm diameter channel did not have a preference for movement along one axis or the other. Within the 114 µm channel, fibroblasts had a higher X velocity, indicating faster movement along the channel.

Cells within curved channels had a tendency to aggregate and stay together, whereas fibroblasts that were seeded on the two-dimensional substrates exhibited little to no aggregation. During the course of the two-dimensional substrate experiment, fibroblasts that did come into contact with each other would instantaneously repel each other, whereas fibroblasts in the curved channel would aggregate together and stay together for the entire duration of the experiment.

Conclusions and Future Works:
There are indications that curvature has a minimal effect on the total velocity of fibroblasts. However, once these velocities have been broken down, there is a clear indication that fibroblasts grown on the curved channels have a preference to move along the major horizontal axis rather than moving around the curvature of the channel. In looking at the X and Y velocities in the stiff 384 µm channel, there was no difference between them. In qualitatively analyzing the migration patterns of the cells, the vast majority tend to also move along the major horizontal axis of the channel. This disparity may simply be attributed to having a small sample size, as there were only 49 samples measured.

Aggregation in the curved environments was quite unexpected, as fibroblasts are contact inhibitive cells. In future studies, we would like to see how the cells pull on their environment through traction force microscopy, as there are obvious differences in movement between both axes. We would also like to observe how cells pull on the substrate as well as each other on curved environments using traction force microscopy [3].

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References:
Identifying the Biomechanical Effects of UV Resistant Molecules and Nanoparticles on Human Skin

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Abstract:
The stratum corneum (SC) is the outermost layer of the epidermis and experiences significant amounts of mechanical stress. A lipid matrix surrounding layers of protein-filled cells forms the stratified SC, which is essential for proper functioning at the nano- and micron-scale. Ultraviolet (UV) radiation due to sun exposure can alter the organization and structural integrity of the SC components. The administration of UV-absorbing chemical compounds and nanoparticles, namely titanium dioxide (TiO2) and zinc oxide (ZnO), can reduce damage. However, it is not fully understood how these UV-resistant treatments interact with the SC, and their efficacy at protecting biomechanical properties from UV exposure is unclear. In this work, we investigated the ability of various topical sunscreens containing UV-resistant molecules and nanoparticles to protect the biomechanical properties of UV-irradiated human SC. We examined the tissue’s resistance to crack propagation, given by the intercellular delamination energy. We also used attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) to study UV inhibitor diffusion into the SC as well as structural changes that occurred from UV exposure. We found that the UV-resistant treatments successfully protected the biomechanical properties up to relatively large UV dosages.

Introduction:
The stratum corneum is essential for wound healing, infection prevention and equally important, protecting underlying layers of skin from solar radiation. UVB radiation in particular has proven harmful to the skin, not only on a cellular level but also on a biomechanical level. Irradiation damage can produce changes in the lipid matrix and cellular structure, leading to disrupted barrier function. This obstructs the skin’s ability to desquamate, or shed, as well as provide mechanical strength. The effects of this include cracking and chapping, as well as other cosmetic defects [1].

Nanoparticles such as TiO2 and ZnO possess UV-inhibiting qualities, making them ideal components of commercial sunscreens. However, it is unknown how they interact with and protect the stratum corneum from harmful radiation.

Experimental Procedure:
We obtained samples of SC through blunt dissection of human cadaveric tissue from Caucasian female donors. To analyze the mechanical properties of the SC, double cantilever beam (DCB) testing was employed to yield the delamination energy, Gc (J/m2), which reflects the extent of cellular cohesion within the SC. Figure 1 displays the DCB sample configuration. Nanoparticle sunscreen (SS) was applied to SC samples to obtain an even coating of 2 mg/cm2 before exposure to 500 J/cm2 of broadband UVB radiation.

Fractured samples were repeatedly delaminated by attaching a new polycarbonate beam to remove lower layers of cells from the original beam with adhered SC. This yielded second and third delamination energies, which increased with depth due to greater intercellular cohesion in the SC.

We used attenuated total reflectance Fourier transform infrared spectroscopy to characterize fractured samples. Changes in height and location of characteristic C-H lipid peaks further revealed how nanoparticle sunscreen interacted with the SC.

Results and Conclusions:
We employed dynamic light scattering particle sizing to obtain an estimate of the nanoparticle diameter. An average diameter of
135 nm was seen, which was larger than expected. This discrepancy was likely due to agglomerations caused by the sunscreen ingredients.

We obtained the delamination energies of samples with no treatment, 500 J/cm² UVB only, sunscreen only, and sunscreen plus 500 J/cm² UVB. As seen in Figure 2, samples irradiated without nanoparticle sunscreen have significantly lower delamination energies due to reduced intercellular strength. However, the sunscreen-treated and UVB-irradiated samples possess delamination energies that are not significantly different than that of the unexposed samples. This indicates that the sunscreen is indeed protecting the SC from UV damage.

Figure 3 shows that underlying layers of the SC are protected in the same fashion, as the second and third energies of sunscreen-treated samples were greater than the untreated plus irradiated samples.

ATR-FTIR analysis showed peak shifts, which suggest changes in SC lipid fluidity due to emollient diffusion into lower layers [2]. The lack of ZnO and TiO₂ peaks near 2275/2350 cm⁻¹ and 1645/3400 cm⁻¹, respectively, (not shown) suggest there was no nanoparticle diffusion.

Overall, it was determined that nanoparticle sunscreen effectively protects the biomechanical properties of the stratum corneum at relatively high broadband UVB dosages. Diffusion of the emollient was suggested by peak shifts of the second and third delamination lipid C-H stretches. However, nanoparticle diffusion was not observed, in agreement with the unexpectedly large particle size. This is fortunate, as unwanted nanoparticle diffusion is a potential harm of using such sunscreens.

**Future Work:**

We hope to further this study by exploring how nanoparticle sunscreen can affect previously UV-damaged tissue, wherein the barrier function has already been significantly compromised. ATR-FTIR analysis of sunscreen-treated damaged tissue will also help us understand the way nanoparticles diffuse into lower layers of the SC.

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**References:**


Fabrication of Nano Ion Pumps for Retinal and Neural Prosthesis

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Abstract:
In some cases, permanent vision loss may be treated using retinal prostheses; however, current methods of neural stimulation pose potential health risks. To overcome these risks, an approach based on ion stimulation rather than electrical stimulation may be more appropriate. To this end, fabrication techniques were investigated for anodized aluminum oxide (AAO) membranes for use in electrically-gated, ion-selective nanoscale pumps. Additionally, track-etched polycarbonate (PC) membranes were functionalized using crown-ether compounds and their ion selectivity was characterized. Fabrication of AAO membranes was successfully completed, and a K⁺-selective compound was determined.

Introduction:
Age-related macular degeneration is the leading cause of permanent vision loss in the United States [1]. It and similar conditions may be treated using retinal prostheses [2]; however, current prostheses rely on electrical stimulation, which can be neurotoxic and can lead to tissue-damaging electrolyzing [3]. To overcome these risks, an approach based on ion stimulation may be used. In this route, neural firing is triggered via injection of sequestered potassium cations (K⁺). This method has been shown to be viable, and the activation threshold and response latency in rabbit retinal cells has been investigated [4, 5].

To facilitate implementation of this approach, fabrication techniques for an electrically-gated, ion-selective nanoscale pump were investigated. In order to fire neurons when desired, the pump must be capable of both sequestering and releasing ions as directed; this is accomplished using a gating system in which ions are driven through a membrane by an applied voltage across the device. The membrane must also be ion-selective so only usable K⁺ ions are stored and released; this will be accomplished through functionalization of pores in the membrane. An overview of the device is shown in Figure 1.

In this study specifically, fabrication techniques were investigated for anodized aluminum oxide (AAO) membranes, which are used in the pumps due to their biocompatibility, previously investigated preparation, and ability to be functionalized for ion selectivity. Additionally, track-etched polycarbonate (PC) membranes were functionalized with crown ethers and their ion selectivity was characterized using cyclic voltammetry.

Experimental Procedure:
The general fabrication procedure for a suspended AAO membrane is shown in Figure 2. A dielectric barrier layer was first deposited on one side of a double-side polished silicon wafer using atomic layer deposition (ALD). Next, Al...
was deposited using electron beam evaporation followed by another ALD dielectric layer. A mask of photoresist (PR) was created and the dielectric was etched in the open windows using inductively coupled plasma reactive-ion etching (ICP-RIE). The exposed aluminum was then anodized in a solution of sulfuric acid. On the backside, dielectric barrier layers were added via ALD, masked, and etched using ICP-RIE to provide windows for the Bosch etch. Finally, the wafer underwent a Bosch etch until AAO membranes were suspended over each window. A phosphoric acid etch was used to open nanopores blocked by barrier layers.

Functionalization studies were completed on track-etched polycarbonate (PC) membranes treated with crown ethers. Membranes were mounted between two polymer gaskets between two reservoirs. Reservoirs were loaded with 0.01 M solutions of NaCl, KCl, and CaCl₂, and electrodes were inserted. Applied potentials ranged from -4 V to 4 V.

Results and Conclusions:
Using the techniques described above, AAO membranes containing nanopores were successfully fabricated. Figure 3 shows the cross-section of such a membrane imaged on a scanning electron microscope (SEM). From this image, it can be seen that the nanopores extend completely through the membrane and are open on both ends.

Figure 4 shows the result of one functionalization study. The figure compares cyclic voltammetry results for PC membranes that are untreated and treated with a specific crown ether compound. Functionalization with this compound results in selectivity for K⁺. Results from the untreated membrane roughly align with expectations based on diffusion coefficients; in the untreated membrane, however, more K⁺ is transported than Na⁺, and comparatively little Ca²⁺ is transported.

Future Work:
In the near future, functionalization studies will be performed using AAO membranes prepared using the techniques investigated in this project. Beyond this, work will begin on fabricating complete pump devices.

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References:
Differentiation of Human Mesenchymal Stem Cells to Schwann Cells on Electrospun Nanofibers

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Abstract:
Injuries to the peripheral nervous system (PNS) are irreparable even with surgical intervention. Tissue engineering strategies use a combination of cellular therapies and biocompatible scaffolds in an effort to restore full function to damaged nerves. In this study, electrospin nanofibers were used as the substrate for investigating the differentiation of the human mesenchymal stem cells (hMSCs) into Schwann cell lineage for nerve repair. We investigated the effects of fiber diameter and cell seeding density on the differentiation process. Poly-((e)-caprolactone) nanofibers of various diameters were fabricated using the electrospinning technique and their average diameters were assessed via scanning electron microscopy. The hMSC-derived Schwann cells were visualized through immunofluorescence microscopy for S100, a widely recognized Schwann cell marker.

Introduction:
The human body possesses very limited regenerative capabilities. For example, severe damage to the peripheral nervous system is irreparable even with surgical intervention due to the limited availability of Schwann cells (SCs), a type of glial cell that supports the extension of axons and communication between synapses. Therefore, this project aims at supplementing hMSC-derived SCs to facilitate the repair of damaged nerves to their full function in combination with biocompatible scaffolds, constructs that provide an encouraging environment for the regeneration.

The electrospinning technique is a simple technique requiring little equipment, but has the ability to produce fibers from biocompatible materials with diameters in the nanometer range. These nanofibers resemble the structure of the extracellular matrix (ECM) making them an effective scaffold. Electrospin nanofibers are particularly attractive for use with nerves and other anisotropic tissues because the electrospinning technique readily allows for uniaxial alignment of nanofibers, mimicking their anisotropic anatomy. In this study, poly-((e)-caprolactone) (PCL) electrospin nanofibers were used as the substrate for investigating the differentiation of hMSCs into Schwann cell lineage for nerve repair. Bone marrow derived hMSCs were chosen particularly due to their clinical relevancy. We investigate the effects of fiber diameter and cell seeding density on the differentiation process, two parameters chosen due to their correlation to conditions governing the initial development of the PNS.

Methods:
Uniaxially aligned electrospin nanofibers were constructed using the electrospinning technique outlined in Figure 1 from PCL, a biocompatible polymer, dissolved in various solvent systems and spun at various parameters to achieve three distinct diameter sizes. Samples of each fiber type were assessed for size...
and morphology using scanning electron microscopy, which is shown in Figure 2. PCL nanofibers were sterilized with ethanol and coated with collagen for cell seeding.

The hMSCs were cultured up to P5 in Dulbecco's modified eagle medium (DMEM). Once confluent, cells were detached by trypsin and seeded on the PCL nanofibers at $1 \times 10^5$ cells/mL for the fiber diameter study, and both $1 \times 10^5$ and $1 \times 10^4$ cells/mL on the intermediate fiber for the cell density study.

The differentiation of hMSC was carried out over a 2.5 week period using an established technique prescribing neuronal inducing factors including β-mercaptoethanol, retinoic acid, platelet derived growth factor AA, nerve growth factor, and forskolin as supplements in DMEM media. The hMSC-derived Schwann cells were visualized through immunofluorescence microscopy for S100, a cytoplasmic protein widely used as a Schwann cell marker.

Results and Discussion:
Differentiation to Schwann cell lineage was observed on all fiber types (Figure 3), but clear advantage could be seen in the thick fibers, which provided a larger surface area. We predict this larger surface area would promote myelination in co-culture with neurons. In the case of cell density (Figure 4), advantage was obvious in $1 \times 10^5$ cells/mL, but the upper limit of cell density should be tested. Further optimization of the culture system should be investigated, but, even in its current state, this system has great promise. The ability to culture healthy Schwann cells for cellular therapies without introducing donor site morbidity could be highly impactful in achieving full PNS nerve repair.

Future Work:
In the future, we seek to apply this approach to nerve repair in model animals for in vivo studies in rats with the eventual goal of successful nerve regeneration in human subjects.

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References:
Electrohydrodynamic Jet Printing on Hydrogel Substrates for Cell Culturing Applications

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Abstract:
Patterning cell cultures on a biologically compatible surface has the potential to significantly extend cell biology knowledge. Electrohydrodynamic jet (e-jet) printing has the capability to pattern surfaces with various biological compounds. This project focused on the use of an e-jet printer to pattern a protein complex, fibronectin, used for cell attachment, on the surface of polyacrylamide hydrogels. E-jet has the potential to print fibronectin ink droplets as small as 2 µm. Polyacrylamide gel stiffness can be modified to mimic various biological tissues. This research has the potential to make advancements in cellular biology knowledge, three-dimensional cellular patterns or bio-arrays, and tissue engineering.

Introduction:
Cell cultures are affected by many different factors. Being able to control and manipulate the environment (biocompatible substrates) and location of a cell culture can provide important information to cell biology knowledge [1]. The ability to place a single cell on a substrate in a flexible pattern has the potential to affect many areas of research. Electrohydrodynamic jet (e-jet) printing can be used for high-resolution (< 10 µm) free form patterning of biological materials on a wide range of substrates [2]. A schematic of the e-jet printer can be seen in Figure 1. The main components of the system are a charged nozzle and a grounded substrate that create an electric field. As the electric field is increased, the ink in the nozzle tip is drawn into a conical formation and onto the substrate [3]. The system is computer controlled through LabVIEW [2]. The system can be run on a direct current mode for continuous jetting or a pulsed printing mode for drop-on-demand printing [4].

The e-jet printing system was used to pattern fibronectin on the surface of polyacrylamide hydrogels for cell culturing applications.

Experimental Procedure:

Fibronectin Production. Fibronectin was made from a 40 µg/mL fibronectin concentrate [1]. The fibronectin was made with filtered 1X phosphate buffered sulfate (PBS) [5].

Polyacrylamide Hydrogel Production and Activation. The hydrogels were made with 8% acrylamide and 0.3% bis-acrylamide solution in double distilled water (ddH2O) along with 1M HEPES stock solution buffer and 0.2% acrylic acid [1]. To initiate polymerization of the gel, 10% ammonium persulfate and 0.05% tetramethylethylenediamine (TEMED) were added. Then 35 µL of the liquidous (and quickly polymerizing) hydrogel was placed between an indium tin oxide (ITO) microscope slide and a standard glass coverslip. After the gels were fully polymerized, the gels were soaked in an n-hydroxysulfosuccinimide (NHS) and n-(3-dimethylaminopropyl)-n′-ethylenediamine hydrochloride (EDC) bath to activate the surface for printing by reacting with acrylic acid within the gel [1]. The gels were then stored in ddH2O to keep hydrated before printing.

Printing Protocol. Hydrogels on ITO slides were placed on the vacuum chuck of the e-jet printer. The syringe was filled with 1-3 drops of fibronectin and initialized by pressurized air pushing the fibronectin to the end of the nozzle. The nozzle was positively charged until jetting occurred and parameters were set...
Results and Conclusions:
The fibronectin was successfully patterned on the hydrogel surface and cells were subsequently seeded on those patterns, Figure 3. Various patterns were developed using both electronic and manual techniques. Ink droplet size was characterized using an Olympus BX51 microscope. The droplet size was varied by changing either the stand-off height (the distance between the charged nozzle and the substrate) or the pulse width (the duration of time that the nozzle is charged in one location). The pulse width provided more consistent size variations. Figure 4 shows the relationship between pulse width and droplet size. As the pulse width increased the droplet size increased exponentially. The vertical bars on this graph show the standard deviation from the average droplet size. As can be seen, the standard deviation increased as pulse width increased; mostly likely, a function of increased variation due to the larger number of droplets released in a given period.

Future Work:
The next phase of this project will focus on cell culturing. During this research phase, the cell cultures did not uniformly adhere to the patterned fibronectin. This requires further investigation, including looking into different ink materials like collagen. Printing quality and properties using variations in hydrogel stiffness and water content is another potential research avenue.

Acknowledgements:
I would like to thank my principal investigator, Prof. Kira Barton, as well as my mentor Leo Tse, along with members of the partnering lab, Prof. Allen Liu, Xinyu Tan, and Elizabeth Steel for their extensive help during the duration of this project. I want to also commend the University of Michigan NNIN site coordinators, Sandrine Martin, Brandon Lucas and Trasa Burkhardt for their assistance within the program. I also want to extend a huge thanks to the National Nanotechnology Infrastructure Network Research Experience for Undergraduates Program and the National Science Foundation for providing the opportunity to participate in this program.

References:
Surface Ligand Density Dependence of Collective Cell Migration on Photoactivatable Self Assembled Monolayers

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Abstract:
Cell migration is an important biological process that can be influenced by many extracellular factors. In this study, the effect of surface density of an extracellular matrix (ECM) ligand on collective migratory characteristics was explored using a tunable, photoactivatable substrate. The substrate exposes cyclic arginine-glycine-aspartic acid (cRGD) ligand at a given surface density upon photoirradiation. HeLa cervical cancer cells and Madin Darby canine kidney (MDCK) cells were photopatterned on circular regions (r = 75 µm) for 5 h and migration was induced by irradiating surrounding regions. Results show an increasing speed for MDCK cells as the cRGD ratio decreases (p < 0.001) and a constant migration speed for HeLa cells on all substrates.

Introduction:
Cell migration is an important component of cancer metastasis and the wound healing process, where the collectivity of migration can be influenced by many factors such as the composition of ECM, soluble factor gradients, etc. [1, 2]. Previous studies have also shown the dependence of patterned substrates on various cellular functions during in vitro experiments [3]. Here, the effect of cRGD surface density on collective migratory characteristics was explored in HeLa and MDCK cells using a photoactivatable substrate. As MDCK cells expressed a stronger cell-to-cell binding mechanism than HeLa cells (E-cadherin versus N-cadherin), it was expected that with varied concentrations of surface cRGD, a faster migration speed would be observed for HeLa cells in comparison to MDCK cells.

Experimental Procedure:

Polymer Synthesis. Photocleavable poly(ethylene glycol) (PCP) was synthesized through a 2:1 mixing of alpha-methoxy-omega-amino poly(ethylene glycol) (PEG, MW = 12K) and Bis(12-(4-(1-(succinimidloxy)carbonyloxy)ethyl)-2-methoxy-5-nitrophenoxy)disulfide (DSNBD) and reacted overnight at room temperature. Commercially available bis-1-(11-[2-{2-(2-hydroxy-ethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-undecyl) disulfide (EG) was used as the diluting agent for cRGD. All disulfide compounds were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 50 µM.

Substrate Fabrication. Substrates were fabricated on quartz glass with a 5 nm titanium adhesion layer and 20 nm gold layer. EG was used to dilute cRGD to the studied concentrations and then each mixture of cRGD:EG was diluted in a 4:1 ratio of PCP:[cRGD:EG]. The final mixture of 4:1 PCP:[cRGD:EG] was mixed and 15 µL of the solution was pipetted onto the substrate. After an overnight incubation, the substrates were immersed in a DMSO bath and rinsed of unfunctionalized disulfides. Next, substrates were sterilized in 70% ethanol and prepared for photopatterning.

Cell Culture. HeLa and MDCK cells were seeded on circular photopatterned substrates (r = 75 µm) for 2 h in serum-free Dulbecco’s modified eale medium (DME) after which the media was exchanged to DMEM containing 10% fetal bovine serum (FBS). The cells were then incubated for 3 h at 37°C before being exposed to a secondary UV irradiation (Figure 1).

Results and Conclusions:
HeLa cells were cultured on a range of cRGD:EG concentrations (1:10 to 1:100,000,000) to test cell attachment and shape. Attachment was observed on concentrations ranging between 1:10 and 1:10,000 with the number of attached cells decreasing with lower concentrations of cRGD. Additionally, the shape...
of attached cells shifted from a spread to a more rounded morphology as cRGD density decreased (Figure 2).

Next, cell migration from the circular cluster was examined. After confinement cells were released via secondary UV irradiation and representative images of migration were collected every fifteen minutes for four hours (representative snap shots shown in Figure 3). MDCK and HeLa migration speeds were evaluated using time profiles of the average cluster radius change after confinement release and compared at each cRGD concentration. While MDCK cells show an inversely proportional relationship between cRGD density and speed (p < 0.001), HeLa cells show no significant difference at any cRGD concentration (Figure 4). When compared between cell lines, HeLa cells show a significantly higher migration speed at cRGD densities of 1:100 (p < 0.001) and 1:1,000 (p < 0.05), with no difference at 1:10,000.

In conclusion, it was found that HeLa cells selectively attach to a certain range of cRGD densities. After testing this range in MDCK and HeLa cells, an inversely proportional relationship between speed and cRGD density was observed in MDCK cells while no relationship was observed in HeLa cells. These results indicate a correlation between receptor type (E- versus N-cadherin) and migration speed at high cRGD densities which could help predict migration characteristics in new cell lines based on cell-to-cell adhesion type.

Future Work:
In this study, a high throughput testing bed for cell migration was fabricated. By using this substrate for testing cell migration, the migratory behavior of cell lines can be determined at specific surface ligand concentrations. For future studies, cells will be fixed three hours after release and stained for E- and N-cadherin junctions. By quantifying cell-to-cell adhesion, the importance of receptor type on migration speed can be determined.

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References:
Micro-Scale Microbial Fuel Cell: Petroleum Biosensing

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Introduction:
A microbial fuel cell (MFC) employs a certain type of bacteria categorized as exoelectrogenic, meaning they allow electrons to be transported from the inside to the outside of their cell membranes, via their catabolic reactions [1]. This process produces electricity as the electrons flow over an external load as shown in Figure 1. The anode and cathode chambers must be completely separate and no oxygen can enter the anode chamber, as the bacteria we explore are an anaerobic species. The final electron acceptor in the cathode is generally an inorganic catholyte [1], i.e. potassium ferrocyanide. The most common bacteria used are proteobacteria, especially those from the genus Shewanella and Geobacter [1]. An MFC has multiple applications such as water sustainability, clean energy, and biosensing. In this research, the MFC will be considered micro-scale, with a 1 cm² anode.

The main goal of this study was to test the effectiveness of an MFC as a biosensor in order to sense organic pollutants. The idea behind the sensor relies on a change in current generated by the MFC or series of MFCs. The bacteria in the MFC were able to oxidize organic materials such as acetate in order to produce current depending on the size of the MFC and the thickness of the biofilm [1]. When a pollutant was introduced into the anode, the bacteria were unable to break it down in the same way as the normal organic substance, causing a change in current that was detectable after knowing the previous baseline data [2].

The basis of this research project was to see if the micro-scale MFC had the ability to sense for petroleum without destroying the biofilm of bacteria. Oils spills are a major concern in any aquatic environment and it can impact the water quality for years after the initial spill. Other problems can be economic or socially problematic, such as a reduction in the tourist industry [3].

Experimental Procedure:
The micro-scale microbial fuel cells in this research were set up in a manner consistent with the micro-scale devices described by Ren [4]. The anode and cathode were glass slides with gold thin-film electrodes. Silicone gaskets prevented leakage and a proton exchange membrane (PEM) allowed protons to flow from anode to cathode. Nanoports allowed the anolyte and catholyte to reach the anode and cathode respectively at a controlled flow rate of 2 µL/min. Geobacter sulfurreducens enriched inoculum was used to form biofilm on the anode. The MFCs were started and allowed to reach a steady current before any testing began. The concentration of sodium acetate in the anolyte was...
25 mM. In order to test the sensitivity of the MFCs to the petroleum, petroleum was diluted into the anolyte solution through a solvent bridge of toluene and ethanol. The final concentrations used were 0.5 mM and 1 mM. The anolyte used was either the 25 mM acetate with the petroleum or a buffer solution with the same ionic characteristics as the anolyte but containing no acetate. This was flowed into the MFCs at the main flow rate for several hours each and data points were taken every minute.

Results and Conclusions:
A transient curve of current density over time was taken from a working MFC in order to see the effects of the buffer solution with 0.5 mM petroleum being added, as shown in Figure 2. The results indicated in each case that the MFCs were sensitive to petroleum. If petroleum was added without other organic substrates, the MFCs could not oxidize the petroleum and the current was drastically reduced as shown in Figure 3. However, if the petroleum was added in addition to the 25 mM acetate, the MFCs were able to oxidize both the acetate and petroleum as shown in Figure 4. Previous research indicates that Geobacter sulfurreducens might be able to break down benzene, a large component of petroleum, via the reaction \( \text{C}_6\text{H}_6 + 30\text{Fe(III)} + 12\text{H}_2\text{O} \rightarrow 30\text{Fe(II)} + 6\text{CO}_2 \). More research would be required to determine if this was a consistent response.

The MFCs were able to recover from a lack of organic substrates after being starved for multiple hours. The experiments show the MFCs can be re-used after a series of spiking petroleum samples.

Acknowledgments:
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References:
Introduction:

Quorum sensing is an essential communication mechanism in bacteria. By transmitting small molecules to each other, bacteria can sense their own population and respond accordingly. Coupling such mechanisms with synthetic biology can lead to a wide range of potential applications, including microbial detection of heavy metals [1]. Although many quorum sensing mechanisms have been characterized at the molecular level, variations in population-level responses are not well understood.

We engineered the luxI/luxR quorum sensing mechanism from V. fischeri into E. coli cells such that the cells functioned as fluorescent receivers of a signal molecule, an acyl homoserine lactone (AHL) [2]. The cells were studied in a microfluidic device to allow for constant population density and dynamic media variation, to introduce signal molecules. Finally, we created and extended a deterministic mathematical model to characterize the responses of the fluorescent experiments. Utilizing the model’s predictions will allow for the use of quorum sensing in practical applications.

Experimental Procedure:

SU-8 master molds were fabricated via a two-step photolithography process in order to produce 10 µm tall channels and 5 µm deep chambers. Wafers were silanized with tridecafluoro-1,1,2,2-tetrahydrooctyl-1-dimethylchlorosilane in a vacuum dessicator.

The polydimethylsiloxane (PDMS) devices were fabricated by pouring a mix of 10:1 polymer to cross-linking agent (65 g total weight) onto the SU-8 mold then cured for four hours at 50°C, then removed.

COMSOL software was used to create a model of the fluid dynamics and transport processes in the channel and chambers, and a time frame for AHL transport into the chamber from the channel was determined.

Fluorescent microscopy using 2 µM fluorescent bovine serum albumin (Alexa Fluor 488) pumped through the chip using 5 mL syringes and a charge-coupled-device (CCD) camera were utilized to validate the devices. Results were compared against COMSOL simulation, and confirmed the accuracy of the simulation’s predictions of mass transfer in an empty chamber, as seen in Figure 2.

The mathematical model was set up to capture the following biochemical interactions: External AHL diffuses into the cells, reversibly binds with luxR to form a dimer, which acts as a transcription factor for the luxI promoter. Upon binding of this transcription factor, transcription and translation of the gfp gene is initiated. The green fluorescent protein (GFP) undergoes chemical maturation before fluorescing. All species degrade over time.

The model was programmed into MATLAB, using generalized mass action to capture the species mentioned above vs. time. Euler’s method for 1st order linear differential equations was used, and parameter estimation was coded in order to estimate...
the rate constants governing each term. The mean-squared-error was minimized to best-fit the model to experimental data.

**Results and Discussion:**

Figure 3 depicts the model’s fit to an AHL pulse length of fifty minutes at varying concentrations, using identical rate constants for all experiments, varying only protein production rates. Our model clearly captured the fluorescent responses for most of our experiments, with some deviations, as the 25 µM curve shows. We believe this is due to the wider peak generated in the 25 µM experiment, for unknown reasons. Finally, Figure 4 applies our model to data taken from literature and directly compares our fit with theirs [3]. Since their experiment setup is different, allowing for unbounded growth, some of the rate constants were adjusted accordingly.

One major improvement that needs to be made includes accounting for the delay in the response in our data. In quorum sensing, transcription levels are basal until a critical concentration of the signal molecule is reached, upon which a burst of transcription occurs [2]. This is likely due to some form of internal feedback control. Still, these fits prove that our model’s ability to characterize a variety of experimental setups, and has promising potential to predict future experiments.

**Future Work:**

Our model will be extended to fit other growth conditions. Once the model has been validated with a sufficiently wide range of experiments, we will begin testing the model by predicting experiments with specific signal pulse profiles and concentrations.

We will use transmitter cells that produce AHL and emit it via diffusion, and run experiments with both transmitters and receivers. These experiments more closely represent quorum sensing in nature. We can then begin moving towards genetically engineering bacteria for practical applications such as the biosensor mentioned earlier for detecting toxic heavy metals in water. Our work will open the door for many applications of quorum sensing in the bioengineering field.

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**References:**


Fabrication of Flexible, Implantable Probes for in vivo Recording of Neural Activity

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Introduction:

In order to study the brain activity of living organisms, electrode arrays can be implanted into a rat brain and can determine the source of an electrical signal down to the firing of a single neuron. Commercial probes containing these arrays are available; however, most of these probes contain an inflexible silicon backbone. Because of the large discrepancy in flexibility between the silicon probe and the brain tissue, the probe is seen as a foreign object and is encapsulated during the body’s immune response.

In order to perform long term neural recordings, flexible materials that better match the Young’s modulus of brain tissue (approximately 100 kPa) [1] must be used as the probe backbone. In addition, a conducting polymer coating made of poly(3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate) (PEDOT:PSS) can be coated on top of the electrodes to improve the performance of the probe.

Probe Configuration:

In this study, flexible neural probes (with the configuration shown in Figure 1) were fabricated. The probe backbone (Figure 1-A) was made of a Parylene-C film. On top of the backbone, metal contacts to interface with a data acquisition device (Figure 1-B), electrodes to collect the neural signals (Figure 1-E), a temperature control pad to adjust for thermal drift (Figure 1-D), and the wires connecting all of the components were patterned. A layer of PEDOT:PSS was coated on top of the electrodes, then a layer of DE1 permanent photoresist (Orthogonal, Inc.) was added on top of the probe to insulate everything except for the electrodes and metal contacts.

Materials and Fabrication:

Probe Backbone: Parylene-C. Parylene-C (PaC) is an FDA approved biocompatible polymer that is electrically insulating, durable, and conformable to most surfaces, while having a relatively simple fabrication process [2]. Using a PDS 2010 Labcoater (Specialty Coating Systems), PaC dimer can be vaporized and deposited as a uniform film onto a surface at room temperature. The film thickness is controlled by the amount of dimer loaded into the instrument. For this project, four grams of dimer were deposited onto silicon wafers, resulting in a film thickness of approximately 2 µm.

Electrodes, Contacts, and Wires: Metal. Metal was patterned by spin-coating photoresist onto the PaC-coated wafers, exposing the pattern using an MJB4 mask aligner (SUSS Microtech), coating the surface of the substrate with metal using an Auto500 metal evaporator (BOC Edwards), then lifting off in an acetone bath. The acetone dissolved the remaining photoresist and removed the metal above it, leaving only the patterned metal (as shown in Figure 2-1).

Electrodes (Conducting Polymer): PEDOT:PSS. Poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS) is an electrochemically stable conductive polymer that is able to translate ionic signals into electronic signals by moving holes in the polymer structure. When coated on an electrode, the “fuzzy”
polymer structure increases the surface area, thus decreasing the impedance (due to better contact with the target tissue) and improving the performance of the electrode [1].

Rather than using a traditional PaC lift-off technique to pattern the PEDOT:PSS, a subtractive technique was used in order to prevent damage to portions of the probe fabricated in prior steps while also significantly reducing fabrication time. The fabrication process began by spin-coating a layer of PEDOT:PSS on top of the substrate (a silicon wafer coated in PaC with the metal patterned on top). The electrode pads were then masked using a layer of OScO4000 photoresist (Orthogonal, Inc.) (Figure 2-2). The substrate was then etched in an Oxford Plasmalab 80+ reactive ion etcher (RIE) to remove the unprotected PEDOT:PSS (this process is shown in Figure 2-3). Finally, the remaining OScO4000 photoresist was stripped off the surface using a fluorinated stripper (Orthogonal, Inc.), leaving only the PEDOT:PSS on the electrode pads (Figure 2-4).

**Insulating Coating: DE1 Photoresist (Orthogonal, Inc.).** DE1 photoresist from Orthogonal, Inc. is a permanent, biocompatible photoresist. It was used as an insulating layer for the probe (to prevent damage and expose only the electrodes to the brain tissue) and was patterned via standard photolithography (result shown in Figure 2-5).

**Results and Future Work:**

Each component of fabrication was tested and optimized; however several steps must be taken prior to the completion of the electrode and probe production process. First, a stiff “shuttle” layer must be added to aid in the insertion of the probe into the rat brain. The shuttle must attach to the probe, minimize insertion damage to the brain, maintain flexibility of the probe, and must allow for accurate insertion (a flexible, removable, or dissolvable shuttle). After the shuttle is completed, the probes must be tested *in vivo* by implanting into a rat brain and recording neural signals over time. A successful probe will not be walled off or otherwise damaged in the immune response and will not wear over time.

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**References:**


The Study of Disposable Substrates in Surface Acoustic Wave Devices

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Here we show that SAW devices, whose components were coupled through 25 µm double-sided tape or petroleum jelly, can perform the same functions as a typical SAW device. Polydimethylsiloxane (PDMS) channels, which are normally directly bonded to a lithium niobate (LiNbO₃) piezoelectric substrate with deposited gold interdigital transducers (IDTs), were instead bonded to glass slips and coupled to gold IDT-deposited LiNbO₃. The modified SAW device retained the ability to manipulate particles, given an increase of power. This study shows that coupled SAW devices can be as effective as typical devices, allowing more widespread use.

Introduction:
SAWs are efficient cell and microparticle manipulators [1]. The concept behind particle manipulation is simple: A function generator sends an electrical signal to an IDT pair. The IDTs then convert that signal into SAWs on the surface of a LiNbO₃ substrate. The two identical SAWs propagate toward each other and interfere to form a standing SAW within a PDMS channel between these two IDTs. The pressure nodes in the standing SAW apply an acoustic forces on particles, guiding them toward pressure nodes, allowing particles suspended in the PDMS channel to be manipulated [2]. Our approach is similar, except that SAWs will travel from LiNbO₃ through a coupling agent and a glass slip before reaching the channel. The advantage is that the bonded channel and glass can easily be removed from LiNbO₃, allowing the device to be disposable and re-used.

Experimental Procedure:
Standard lithography procedures were used to deposit IDTs on a LiNbO₃ wafer. The wafer was spin-coated with SPR 3012 photosresist at 4000 rpm at 45 seconds (s) and then baked at 65°C, 95°C, and again at 65°C for 60 s each. Wafers were then exposed to ultraviolet light for 18 s and baked using the same parameters. The product was developed using a CD26 developer for approximately 60 s and cleaned using de-ionized water and nitrogen. The wafer was further cleaned using a plasma cleaner, with 1000 sccm O₂, and 50 sccm He after vacuum-down at 600 torr and 200 W for four minutes. After, a 5 nm adhesion layer of chrome and a 50 nm layer of gold were coated on the wafer to produce the IDTs.

Abstract:
Surface acoustic wave (SAW) devices have been widely used in microfluidics to manipulate fluids and particles. Applications include single cell manipulation, particle focusing, particle patterning, cell separation and cell sorting. However, in typical SAW devices, if channels are defective or if channels become contaminated after use, the device cannot be reused because the channel component is directly bonded to the SAW device substrate.
The wafer-IDT composite was submerged in PG remover overnight; a sonicator was used with the IDT-wafer composite with isopropyl alcohol to remove excess gold.

The fabrication procedure for the PDMS channel was similar. Silicon wafers were coated with a SU-8-50 photoresist, spun at 2000 rpm for 45 s, and then baked at 65°C and 95°C for 5 min and 20 min, respectively. The wafer was exposed to ultraviolet light for 65 s and baked at the same temperatures for 2 min and 8 min. The product was developed by submerging in SU-8 developer for 3 min, and cleaned using isopropyl alcohol. The wafer was then baked at 150°C for 10 min. The PDMS channel was created by mixing one part elastomer curing agent to ten parts elastomer base, poured over the pattern, and incubated at 65°C for half an hour. Then channels were drilled to create inlets and outlets and bonded (via plasma induced bonding) to 250 µm glass.

Coupling the wafer-IDT with the channel-glass composite was accomplished using either petroleum jelly or 25 µm thick double-sided tape as a coupling agent. Figure 2 shows a schematic of each major step.

Results:
Coupling results showed that our coupled SAW device retained the ability to pattern stationary particles. Four and ten micron polystyrene beads aligned along pressure nodes after the SAW was toggled, using a frequency of 24 MHz and a power of 17.0 dBm. Figures 3 and 4 show patterning results for each coupling agent. In addition, our device was also able to separate those 4 and 10 µm diameter beads if the power was large enough; for tape coupling, the power (19.5 dBm = 89.1 mW) was nearly twice as large compared to the power needed to separate particles with petroleum jelly coupling (17.0 dBm = 50.1 mW).

Conclusions and Future Work:
We have demonstrated that coupled SAW devices can pattern and separate particles and can be as effective as traditional SAW devices, despite needing a higher input power, and that petroleum jelly is a more efficient coupling agent than 25 µm tape. In the future we hope to quantify the coupling efficiency (i.e. how much power is required to separate beads using coupling agents of varying density), examine different coupling agents, and expand the experiment by manipulating cells instead of beads.

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References:
The Impact of MEMS-Produced Micro-Electrode Material Coating on Dental Plaque Biofilm Growth

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Abstract:
The development of dental biofilms can create damaging acidic environments due to the production of metabolic byproducts. A micro pH electrode has been created (Figure 1) using microelectromechanical (MEM) fabrication techniques to continuously measure the pH at different distances from saliva-coated surfaces on which biofilms can develop. A coating was needed for this micro pH electrode to prevent the environment of the biofilm and oral cavity from altering the functionality of the pH sensor. Three different material coatings were selected for their known biocompatibility with living cells: parylene, silicon dioxide, and silicon nitride. To determine which coating was best suited for this purpose, the micro electrode was tested in micro flow cells and batch cultures inoculated with fluorescent Streptococcus gordonii bacteria. The biofilm was then imaged in three dimensions using a fluorescent microscope.

Medical Relevance:
The oral cavity provides an opportunity for microbes to develop very complex and potentially harmful biofilms. These biofilms can contribute to the formation of dental caries lesions through the production of acidic metabolic byproducts. The longer this biofilm is left undisturbed through substandard dental hygiene, the more likely it is that the caries lesion will worsen [1].

A deeper understanding of the metabolic and acidic conditions in dental biofilms would allow the scientific community to produce better dental hygiene products. Micro-electrode sensors placed within these biofilms could allow for real time data collection of the pH conditions in these micro environments.

Experimental Process:
The micro pH chips were fabricated on silicon wafers. Each chip contained four sets of sensors placed at staggered heights in order to measure pH at different positions within the biofilm. Photolithography techniques such as spinning, evaporation, liftoff, masking, and etching were used to pattern the chips with iridium sensors. The electrochemical sensing sites were then activated by the conversion of iridium to iridium oxide through exposure to an electrically pulsed signal in a sulfuric acid solution (Figure 1).
A 3D printer was used to fabricate micro flow cells. A syringe pump modification was also designed to accommodate additional flow channels necessary for efficient testing. The micro flow cells were designed to contain 100 µl of a mixture of human saliva and bacterial growth medium in which the biofilm was cultured (Figure 2).

Discussion:
The micro pH chips produced can successfully signal a change in pH, with an intensity of approximately 20 millivolts per pH unit change (Figure 3). This response can theoretically be improved to between 60 and 80 millivolts per pH unit change following the Nernst Equation and documented iridium oxide electrode responses in the literature [2].

These coatings were then tested in growth media inoculated with S. gordonii expressing mcherry, a red protein. Batch experiments conducted on the micro pH chips showed bacterial growth on all materials. Further tests and quantification are needed to determine if there is a significant difference in growth for any material.

Conclusions:
Micro pH sensing chips were produced that can detect a difference in pH conditions in a chemical solution. Three different coatings were tested on these pH micro-electrodes; silicon dioxide, silicon nitride, and parylene. Tests conducted under flow cell conditions showed that S. gordonii will grow in the developed flow cell in saliva mixed with growth medium (Figure 4).

Additionally, a new biofilm testing system was developed, consisting of a ten chamber flow cell which could be connected to a syringe pump fitted with a ten syringe expansion attachment. The ten chamber flow cell was custom designed to fit the pH sensing chips into the top of the flow cell. The entire apparatus could then be imaged from below by a confocal microscope to obtain images of biofilm formation on the pH sensing chips in real time (Figure 2).

Future Work:
Future studies will involve improving the accuracy and sensitivity of the sensors, as well as obtaining sufficient biofilm growth for 3D imaging in the flow cell. Eventually the micro pH sensors could be connected to a bluetooth device with an antenna which could be placed into an intraoral retainer to study dental plaque in real time under clinical conditions. The work done during the course of this project serves as preliminary research to produce this type of pH sensor for use in humans.

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References:
Fabrication of Microchemical Field-Effect Transistor

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Abstract:
The commonly manufactured metal-oxide field-effect transistor (MOSFET) amplifies electrical signals applied to the gate electrode. If one replaces the metal gate of a MOSFET with a selectively permeable membrane, it enables transistors to amplify electrical signals based on the ion concentration of a solution. Such devices are known as chemical field-effect transistors (ChemFETs). A research group at Minnesota envisions these micro-scale ChemFETs could analyze sweat droplets as small as one millimeter in diameter. This will allow noninvasive tests to detect diseases such as cystic fibrosis, osteoporosis, diabetes, and other conditions, as part of a larger program to use the mapping of sweat production to detect loss of neural function due to diabetes, chemotherapy, industrial or defense-related exposure to toxins, alcoholism, HIV, and other conditions. Casting of poly(2-hydroxyethyl methacrylate) and including ionophores, valinocycin and ETH 2120 in the membrane enhances the selectivity and responses of ChemFETs.

Introduction:
The ultimate goal of this project is to find a way to quantify sweat gland function to detect peripheral neuropathy. Peripheral neuropathy is damage of the peripheral nervous system; its symptoms include decrease of sensory sensitivity, random pain, and itching in the damaged region. Although these symptoms are definitive, the severity can be different based on the other parameters like ages and pain tolerance. In order to prevent peripheral neuropathy, precise monitoring of nervous function is required. The Minnesota group is developing a way to electrically measure sweat production. This determines sudomotor neural function, a good replacement for the function of the central nervous system. However, if sweat were to be measured, it would be useful to measure the content of sweat. It is possible to detect many conditions through a simple noninvasive test.

A transistor amplifies electric signals according to the voltage applied to the device. For most transistors, they contain three major regions: the source, the drain, and the gate. Source and drain are where the electric current is being amplified, while the gate is where the external voltage is provided. For a chemical field-effect transistor (ChemFET), a selective membrane replaces the gate oxide. The membrane, with positive charge ionospheres, would work similarly to an external voltage and amplifies the electrical signal according to positive ion concentration in a solution.

Experimental Procedure:
Prior to the fabrication process, a design of wafer map was completed with L-Edit programs. Every wafer included twelve chips, shown in Figure 1. Each chip included 120 ChemFETS,
120 n-type metal oxide semiconductors (nMOS), and four actives regions. The ChemFET and nMOS channel lengths varied from 5 to 10 µm. One 100 µm capacitor was used as well.

Thermal oxidation of silicon wafers was performed with steam at 1000°C for 100 minutes. Active regions were opened with the first lithography step and followed by wet etching. Etch completion was determined by the hydrophobic nature of Si compared to SiO₂. Impurities were induced by diffusion; a phosphorous-containing liquid was cast onto the wafer followed by heating at 1000°C for 10 minutes. The presence of the impurity was confirmed using four-point probe. The middle oxide was removed with the second mask. Growth of the gate oxide was achieved by dry thermal oxidation for 15 minutes. The contact region was opened with the third mask. Aluminum was deposited by thermal evaporation or sputtering and patterned with the fourth mask. Finally, annealing was performed at 450°C for five minutes.

An nMOS functionality was used to verify the fabrication procedures. Each transistor was tested by sweeping the drain voltage while keeping the gate at a constant voltage between zero and eight volts (Figure 3). Once fabrication validity was confirmed, silylation was carried out by submerging the wafer in a mixture of 2.5 ml of 3-(trimethoxysilyl) propyl methacrylate and 22.5 ml of toluene at 90°C for four hours. The 40 mg of polyHEMA crystal was dissolved within 2 ml of reagent alcohol overnight. Finally, 71.2 mg of 2,2 dimethoxyphenylaceto phenone photoinitiator was added to the polyHEMA mixture and cast onto the wafer, followed by UV light exposure for two minutes.

Results and Conclusions:

The validity of fabrication techniques was confirmed. The first successful fabricated chip has 44% of functioning devices, while the latest chip has 100% yields. The increased in yield is believed to be due to changing the dopant diffusion cycle from 950°C for 15 minutes, to 1000°C for 10 minutes. Based on the preliminary result, the higher diffusion temperature increased the doping concentration. The anneal process proven to be essential to the functionality of the device. Without annealing, the yield of successful devices in a chip was zero.

The final dimension of the device is roughly 450 µm in width and 800 µm in length. This is important considering the smallest test droplet produced from a syringe is 1000 µm in diameter. Although the hydrogel was visible under microscope, it is unclear whether the bonding between the gate oxide and hydrogel is covalent or mechanical (Figure 4). More tests are required.

Future Work:

Investigating the bonding mechanism of the hydrogel is essential to the functionality of our device. Hence, testing the mechanism by ethanol washing should be performed. The selective membrane casting should be the following step of fabrication. Once the membrane is attached characterization of ChemFETs can begin.

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Exploring the Effects of Theophylline on Neutrophil Function in Inflammatory Diseases

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Abstract:
Neutrophils play a key role in the human immune system as the first cells to migrate to sites of inflammation. Several common respiratory diseases, such as chronic obstructive pulmonary disease (COPD) and asthma, are characterized by excessive chemotaxis and damaging apoptosis of neutrophils around infection. Theophylline, one potential therapeutic candidate, was employed to examine drug effects on neutrophil function. The chemotactic behaviors of neutrophils were monitored using a microfluidic platform after incubation with various concentrations of theophylline for different time periods. Theophylline worked to limit the motility of neutrophils in small concentrations and long incubation times; however, there was no influence on the polarization of neutrophils. Meanwhile, theophylline induced a tremendous decrease in neutrophil viability based on colorimetric assay. This work provides new insights on how theophylline affects neutrophil function in vitro, potentially guiding drug application for the treatment of neutrophilic inflammation in vivo.

Introduction:
Chemotaxis is a dynamic cellular process by which cells migrate to inflammatory areas in the presence of chemical gradients composed of signaling molecules called chemokines [1]. Abnormal excessive chemotaxis and failure in apoptotic pathways are the main contributors for neutrophil accumulation around infection sites. Neutrophilic inflammation is resistant or poorly responsive to some traditional drugs targeted for chronic respiratory diseases, such as corticosteroids. Theophylline has been successfully utilized as a pharmaceutical treatment for neutrophilic inflammation, but the detailed mechanisms are not clear. In this work, the drug effects of theophylline on neutrophil function were studied to explain the mechanism of drug-cell interaction and provide direction for the design of novel anti-inflammatory approaches.

Microfluidic technology is unique in its ability to mimic in vivo process in vitro by maintaining a flow vital to observing biological processes. More importantly, microfluidic devices are able to provide stable chemokine gradients with high spatiotemporal resolution and quantitative data for describing chemotactic behaviors in different conditions. A gradient microfluidic device containing parallel serpentine channels and a cell culture chamber was employed in this study (Figure 1) [2].

Method:
Microfluidic device molds were fabricated through photolithography methods with SU-8 photosresist spun onto a silicon wafer. Polydimethylsiloxane (PDMS) was poured on the mold and cured overnight, followed by permanently bonding PDMS and glass slides using oxygen plasma.

Human blood samples were layered on separation medium and centrifuged to isolate neutrophils. Cell mixtures were washed with Hank’s balanced salt solution (HBSS) buffer and cell lysis buffer, then pure neutrophils were counted and diluted to the appropriate concentration.

Prior to injecting neutrophils into the device, the cell culture chamber was incubated with fibronectin. Next 5-10 µL of neutrophil suspension (3-5 × 10⁶ cells/mL) in HBSS buffer was introduced into the device (Figure 2). Three different
theophylline concentrations were tested: 1 mM, 100 µM, and 10 µM, for durations of 30 minutes, 90 minutes, or 150 minutes. A chemokine gradient was created by mixing buffer and 10 µg/mL interleukin-8 (IL-8) solution in the serpentine channels under 100 µL/h flow rate.

Neutrophil activity was assessed for 20 minutes by calculating the motility index (MI), chemotactic index (CI), and effective chemotactic index (ECI) with Metamorph software. MI quantifies the overall movement of the neutrophils, and CI analyzes how much cells moved in the gradient direction. ECI is the product of MI and CI, examining the effectiveness of neutrophil migration.

The cytotoxicity of theophylline was monitored using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. In the 96-well plate, 100 µL of neutrophil suspension (6 × 10⁵ cells/mL) was incubated with each drug concentration for the various time periods. After incubation, neutrophils were mixed with 100 µL MTT solution (0.5 mg/mL) for two hours to produce precipitation, and 100 µL DMSO was added to dissolve otherwise insoluble crystals. Finally, optical density was measured at 570 nm, with 655 nm as reference, using a microplate reader.

Results and Conclusions:

As shown in Figure 3, 10 µM theophylline and long incubation times (90 and 150 minutes) induced a significant decrease in motility of neutrophils; however, larger concentrations (1 mM and 100 µM) had no impact on MI. There was no influence on the polarization and effectiveness of neutrophil chemotaxis under any of the conditions. Meanwhile, theophylline caused an obvious drop in neutrophil viability based on the MTT assay in a time-dependent manner. Cell viability was reduced to 50% compared to the initial viability after 150 minutes of incubation for all concentrations of theophylline.

These preliminary results displayed that theophylline is not an effective regulator of neutrophil chemotaxis except at the lowest concentration considered, contradicting previous findings that theophylline attenuated neutrophil chemotaxis in the traditional chamber-based assays [3]. A reasonable explanation is that theophylline has slight effects on neutrophil chemotaxis in short term. Instead, high cytotoxicity was observed, implying that theophylline acts as a potent treatment by inducing cell death, which was not taken into account in current literature (Figure 4). This work supplied a better understanding of the relationship between drug effects and neutrophil function relevant in multiple diseases.

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