

# Development of a Biomembrane Platform for the Study of Virus Infection

**CNF Project Number: 1686-08**

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*Primary CNF Tools Used: Heidelberg DWL2000 Mask Writer, ABM Contact Aligner, Odd Hour E-beam Evaporator, Oxford PECVD, PT-740 etcher, Disco Dicing Saw, Bruker AFM, Malvern NS300 NanoSight*

## Abstract:

As a “label-free” alternative to optical sensing, electrical sensing represents a more feasible, reproducible, and scalable detection method for many biological processes.<sup>1-2</sup> Among various electrical sensing techniques, the non-invasive electrochemical impedance spectroscopy (EIS) technique is especially suitable for accurately quantifying the bio-recognition events occurring at a variety of biointerfaces,<sup>3-4</sup> such as bacterial, viral, cellular and synthetic lipid membranes. Our group has pioneered a microelectrode system that will support the self-assemble of lipid vesicles (SLBs) on the electrode surfaces, and their electrical properties (resistance, capacitance) can be extracted by applying an alternating voltage and recording the current response.<sup>5-7</sup> In the past year, we reported a robust protocol to form SLB on such platform<sup>8</sup> and we leveraged such platform to recreate viral infection of host cell and screen antibodies targeting different viral entry steps.<sup>9</sup>

## Summary of Research:

To fabricate the microelectrode devices, photomasks were created using the Heidelberg DWL2000 Mask writer and used with the ABM Contact Aligner to pattern photoresist that was spun onto a fused silica wafer. A first layer of gold contact pad was patterned following the developing of S1813 photoresist and the deposition of Au thin film. A thin layer of SiO<sub>2</sub> insulating layer is then deposited directly on top of the Au contact pad using Oxford PECVD. Electrode area was then patterned on SiO<sub>2</sub> following the spin-coating and developing of the second layer of photoresist. PT-740 etched was then used to etch the exposed SiO<sub>2</sub> until Au contact pad has been exposed. A conductive polymer, PEDOT:PSS was then spun over the fused silica wafer followed by the deposition of a Germanium hard mask (odd hour e-beam evaporator). A third layer of photolithography was performed on a layer of negative photoresist (nLOF

2020) spun on top of Ge, where all resists above Ge at areas except active electrode surface were developed. Unprotected Ge and PEDOT:PSS underneath were then etched using PT-740. Ge on top of active electrode area was then etched in water bath overnight. Once the microelectrode device was fabricated, a PDMS well was stamped directly on top to create a reservoir for SLB self-assembling and allow following EIS measurement.

## Conclusions and Future Steps:

The facilities and technical infrastructure provided by the Cornell NanoScale Science and Technology Facility (CNF) have been essential to the development of our SLB-integrated microelectrode platform. These capabilities directly supported our progress in establishing robust SLB formation, recreating virus–host membrane interactions and evaluating therapeutics targeting viral infection. Continued access to CNF will be critical as we further miniaturize the devices, improve fabrication reproducibility, and expand the platform toward higher-density and multiplexed bioelectronic sensing.

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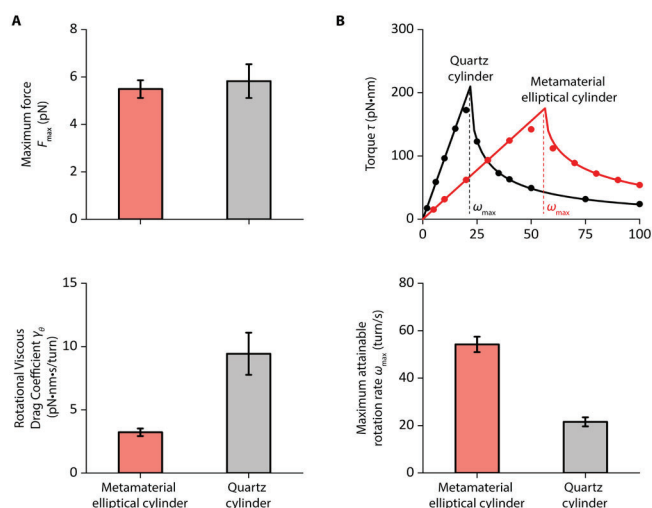


*Figure 3: Trapping properties and maximum rotation rate of metamaterial elliptical cylinders. (A) Measurements of the maximum trapping force  $F_{max}$  (top panel) at 30 mW laser power before the objective and rotational viscous drag coefficient  $\gamma\theta$  (bottom panel). (B) Method to determine the maximum trapping torque  $\tau_{max}$  (top panel) and the maximum rotation rate  $\omega_{max}$  (bottom panel) without slippage at 30 mW laser power before the objective.*

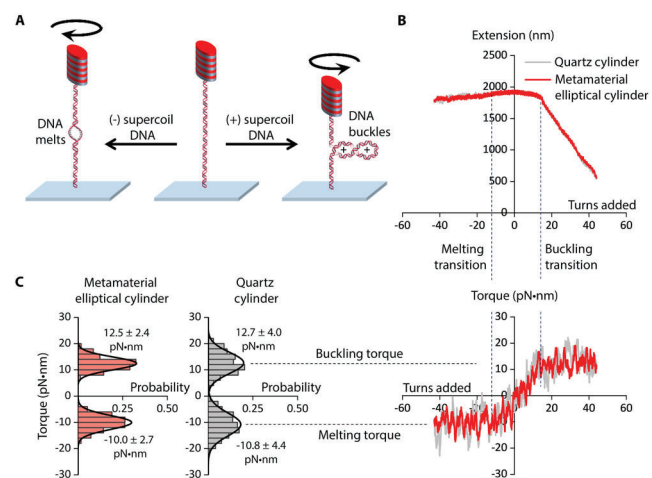


*Figure 4: DNA torque measurements using a metamaterial elliptical cylinder; in comparison with those from a quartz cylinder. (A) experimental configuration for the measurements. (B) Measured DNA extension and torque as a function of turns added to DNA under 1 pN force. (C) Histograms of measured torque upon (+) DNA buckling and (-) DNA melting. Each histogram is fit by a Gaussian, with the mean and the SD of the fit shown.*

# Biocompatible Cylinders for Studying Protein Torsional Dynamics

**CNF Project Number: 1738-08**

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*Primary CNF Tools Used: ASML Deep Ultraviolet Stepper, Oxford 81 Etcher, Oxford 82 Etcher, Oxford PECVD, SC4500 Odd-Hour Evaporator, SC4500 Even-Hour Evaporator, Zeiss Supra SEM, Zeiss Ultra SEM*

## Abstract:

The angular optical trap (AOT) is a powerful instrument for measuring rotational mechanics of fundamental biological processes. The AOT can simultaneously measure the force and torque exerted to a DNA substrate, while applying rotational motion to it. One of the key components of the AOT is its trapping particle, which is designed with optical torque generation. So far, we have developed two types of particles that can generate torque, namely the metamaterial elliptical cylinder (torque generated via shape anisotropy) and the quartz cylinder (torque generated via birefringence).

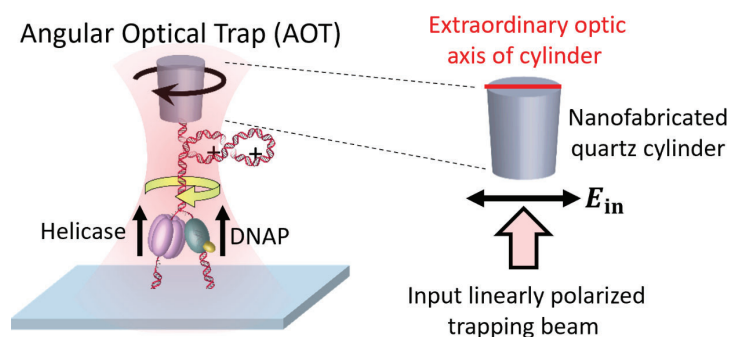


Figure 1: An experimental configuration to track replisome rotation relative to DNA using the AOT.

## Summary of Research:

We have applied the metamaterial elliptical cylinders and the birefringent quartz cylinders to many fundamental biological studies, including studying the DNA torsional mechanics (1, 2), DNA replication (3), and transcription (4). The optical force and torque generation capacity of those cylinders were optimized via COMSOL simulations (1, 2, 5). Those cylinders were nanofabricated via a deep-ultraviolet-lithography-based process (6) and functionalized through a chemical reaction with (3-Aminopropyl)triethoxysilane (7). In the past year, we have used those cylinders to reveal the mechanisms of torsion-mediated replication process (Fig. 1) (3) and transcription-through-nucleosome process (Fig. 2) (4).

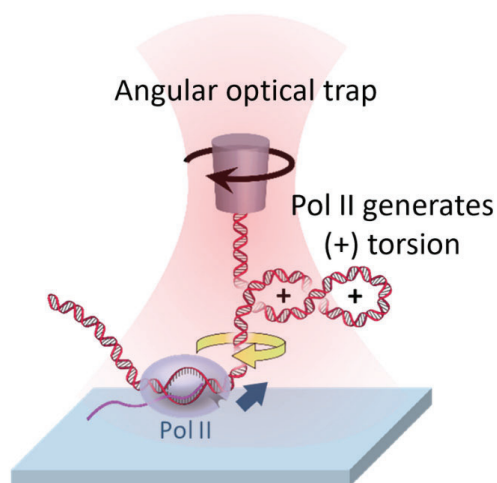


Figure 2: An experimental configuration to track RNA polymerase rotation relative to nucleosomal DNA using the AOT.

## Conclusions and Future Steps:

During DNA replication, the replisome can rotate rapidly relative to the DNA substrate. For example, 50 – 100 turns/s in bacteria (8). In such a system, the existing cylinders may be challenging to keep up with such a fast rotational speed (which is determined by the maximum achievable optical torque and the rotational viscous drag coefficient). To enable the detection of faster rotational motion of a replisome, we are working on developing metamaterial elliptical cylinder with a higher cross-section eccentricity to further increase the maximum optical torque while remaining the rotational viscous drag coefficient.

## References:

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# Development of a 3D Microfluidic Platform for Dynamic Compression of Tumor Spheroids

**CNF Project Number: 2068-11**

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*Primary CNF Tools Used: AMST MVD100, Oxford 81 Etcher, Oxford 82 Etcher, Unaxis 770 Deep Silicon Etch, YES EcoCean Asher, KLA P7 Profilometer, ABM Mask Aligner, Hamatech Hot Piranha*

## Abstract:

Solid tumor progression is accompanied by changes in both extrinsic and intrinsic mechanical forces within the tumor microenvironment[1]. One of the defining extrinsic mechanical cues is the compressive stress imposed by the surrounding tissue on a growing tumor[1, 2]. To date, most studies investigating mechanical compression have focused primarily on its effects on tumor cell behavior, with relatively little attention given to how compression influences the ability of immune cells to infiltrate and kill tumors[1, 3]. In addition to extrinsic forces, the intrinsic mechanical properties of tumor cells have also been shown to regulate cellular behavior and influence therapeutic responses[4], highlighting the need for improved methods to characterize and quantify the physical properties of tumors. These knowledge gaps persist, in part, because there is a lack of experimental platforms that can both characterize tumor mechanics and support immune–tumor interactions within a physiologically relevant 3D environment. In this study, we developed a microfluidic microrheometer that is able to apply dynamic compression to quantify the intrinsic physical properties of tumor spheroids and investigate how mechanical compression regulates tumor–immune interactions.

## Summary of Research:

Summary of Research: A previously developed microfluidic compression platform [5] is used as the foundation for quantifying tumor mechanics and investigating tumor–immune interactions (Fig. 1). The device consists of three functional layers: (1) a cell culture layer containing tumor spheroids and immune cells embedded within a three-dimensional (3D) extracellular matrix (ECM), referred to as Layer 1 (L1);

(2) a top-hat-shaped PDMS membrane that functions as a compression piston, referred to as Layer 2 (L2); and (3) a pressure control layer that uses fluid pressure to deflect the PDMS piston downward and compress the tumor spheroids, referred to as Layer 3 (L3) (Figure 1A). L2 and L3 are plasma-bonded together to form a pressure control unit, which is assembled above L1 and sealed between a Plexiglas manifold and a stainless-steel frame. Application of pressure to the control chamber deflects the PDMS piston downward by a distance,  $\Delta h$ , producing a precisely controlled compressive strain ( $\Delta h/h$ ) on the tumor spheroids (Figure 1B). Figure 1C shows representative brightfield and GFP fluorescence images of a tumor spheroid before and after the application of 120 mbar pressure, illustrating the deformation induced by compression. The relationship between applied pressure and membrane displacement was established by tracking fluorescent beads attached to the underside of the membrane. Detailed calibration procedures and compressive strain calculations have been reported previously[6]. The platform is capable of applying controlled compressive strains of up to 50% to tumor spheroids 100–200  $\mu\text{m}$  in diameter with Young's moduli of approximately 1250 Pa. More recently, we expanded the capabilities of this platform by incorporating a thin polyacrylamide (PAA) gel layer onto the surface of L1, enabling the device to function as a microfluidic microrheometer capable of quantifying the stiffness of intact tumor spheroids[5]. During compression, deformation of the calibrated PAA gel provides a quantitative measure of the compressive force applied to the spheroid. By combining the measured force with the applied strain, the stiffness of the tumor spheroid can be determined. A detailed description of the microrheometer design, calibration, and stiffness calculations has been reported previously[5].

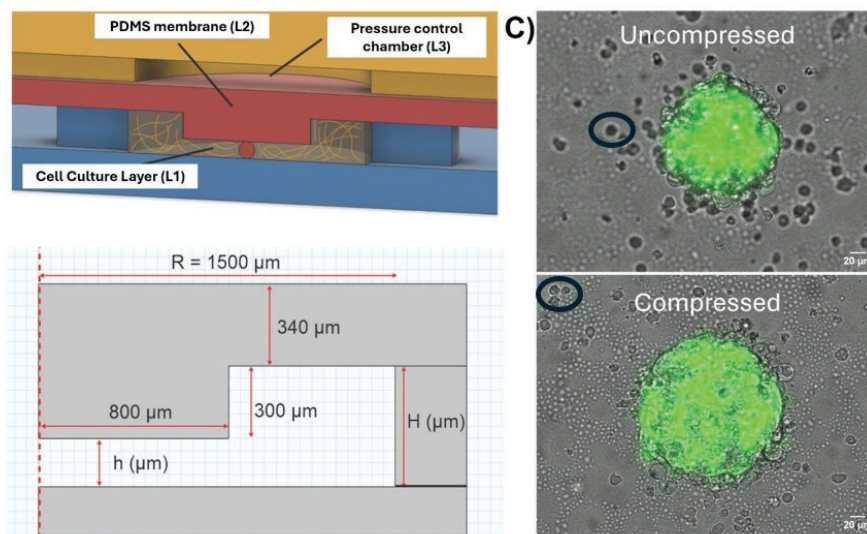


Figure 1: Three-layer PDMS microfluidic device for spheroid compression. A-B) Cross-sectional view of the three layers (initial  $h = 200 \mu\text{m}$ ). C) Representative merged brightfield and GFP fluorescence images of a GFP-labeled MCF-7 breast tumor spheroid with NK cells embedded in 1.5 mg/mL collagen before and after the application of 120 mbar compression. Representative NK cells are indicated by the blue circles (scale =  $20 \mu\text{m}$ ).

## Conclusions and Future Steps:

The microfluidic microrheometer provides a unique platform for simultaneously characterizing the mechanical properties of intact tumor spheroids and investigating tumor-immune interactions under controlled mechanical compression. Initial studies demonstrate its ability to quantify the stiffness[5] and viscosity[6] of breast tumor spheroids. Future work will use this platform to determine how compressive stress alters NK cell migration, tumor infiltration, and cytotoxic activity, thereby providing new insights into the role of mechanics in regulating anti-tumor immune responses.

## References:

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# Integrating Nanofabricated Microfluidic Mixers and Flow Cells for Time-resolved Studies of RNA

**CNF Project Number: 2158-12**

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*Primary CNF Tools Used: Nanoscribe, Heidelberg DWL2000, Oxford PECVD, ABM mask aligner, Oxford RIE, Unaxis 770 Deep Silicon Etch, Suss SB8e Substrate Bonder, DISCO dicing Saw*

## Abstract:

RNA molecules serve many important biological functions, and they interact in interesting ways with other molecules, from ions to proteins to other nucleic acids. In order to get insight into RNA's biological roles, it is important to understand how RNA conformations change when they bind with other molecules. To investigate these structural changes we integrate 3D-printed microfluidic mixers with fabricated microfluidic channels, building flow cell devices that allow us to use the same type of mixer for both time-resolved fluorescence and time-resolved x-ray scattering measurements of RNA.

## Summary of Research:

RNA interacts with RNA-binding proteins to form complexes that are involved in regulating various cellular processes [1-2]. Measuring how the structure of an RNA molecule changes when it interacts with a binding partner can help us understand the functional role of the RNA. Two useful techniques for characterizing these dynamic molecules are small angle x-ray scattering (SAXS) and fluorescence correlation spectroscopy (FCS). SAXS measures overall size and shape of the molecule, as well as the amount of association/aggregation within the sample, by detecting the x-rays scattered from molecules in solution. FCS provides information about the size and interactions of molecules in solution by measuring the fluctuations in emitted photons from fluorescently labeled molecules as they diffuse in and out of a laser confocal volume [3].

In order to capture the intermediate stages of an interaction between an RNA and its binding partner, we turn to time-resolved methods. Microfluidic mixers allow us to initiate reactions between biological

molecules and then probe the system at different positions along an observation region in order to measure a range of timepoints in the reaction. In past work we developed Nanoscribe 3D-printed microfluidic mixers which enable the rapid mixing of two input solutions. This type of chaotic advection mixer, called a Kenics, consists of a channel containing alternating helical elements[4]. A schematic of the Kenics mixer is shown in Figure 1. As the two solutions flow through the helical elements, they are repeatedly stretched, split, and stacked in a process known as a baker's transformation. This results in increasingly thin layers across which even relatively large biological molecules, like RNAs and proteins, can rapidly diffuse. The Kenics efficiently initiates reactions between RNAs their binding partners and has been used to conduct time-resolved SAXS (TR-SAXS) experiments [5]. In addition we have previously fabricated microfluidic channels that interface with our lab-built confocal microscope [6].



Figure 1: Schematic of a flow cell with a Kenics mixer.

Now, we have put these pieces together, integrating the 3D printed Kenics mixers with fabricated channels, enabling us to perform both TR-SAXS and TR-FCS using the same microfluidic mixer. Figure 2 shows flow cell devices for both techniques. With these microfluidic devices, TR-FCS and TR-SAXS can be used in complementary ways. For example, since FCS provides similar size information to SAXS through a different method, it can serve as a cross-validation. Also, TR-FCS can be performed in our lab to characterize a particular RNA system, allowing us to identify measurement conditions and time-scales of interest before taking the system to a synchrotron x-ray source. We have used these devices to collect data on RNA systems, using TR-FCS in our lab and TR-SAXS at the Advanced Photon Source (APS) at Argonne National Lab.

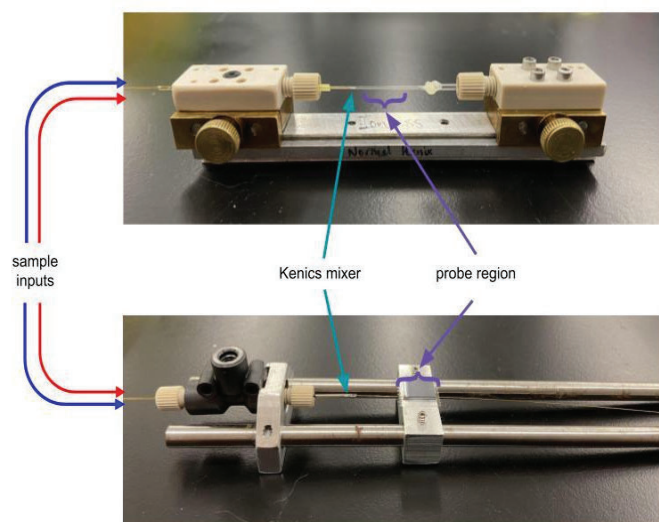


Figure 2: Top: Kenics flow cell device for TR-SAXS. Bottom: Kenics flow cell device for TR-FCS.

## Conclusions and Future Steps:

We have built Kenics microfluidic devices for fluorescence applications and begun to use TR-FCS in combination with TR-SAXS to investigate RNA structure and dynamics. Further experiments will apply these methods to RNA-protein interactions to learn about how RNA structure changes in phase-separating systems. Future work may also include improving the time-point range of the TR-FCS device. Currently, the fastest accessible timepoint is limited to a few hundred milliseconds by the low flow speeds needed for TR-fluorescence measurements. However, timepoints in the tens of milliseconds or less could be reached by redesigning the channel and mixer geometry.

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# Platform for Live Cells Infrared Chemical Imaging

**CNF Project Number: 2472-16**

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*Primary CNF Tools Used: JEOL 9500, SC4500 evaporator, Zeiss Ultra Scanning Electron Microscope, Oxford Plasma Enhanced Chemical Vapor Deposition, PlasmaTherm 740, Glen Resist Strip, DISCO dicing saw, Heidelberg MLA 150, Schott IR inspector, OEM Endeavor M1*

## Abstract:

Our group has been developing infrared spectroscopy and microscopy for live cells analysis by engineering nanostructured antennas on infrared transparent materials and coupling antenna resonances to molecular vibrations. The standard techniques and materials (e.g. e-beam lithography, calcium fluoride substrates, antenna made of gold) used in our device are not scalable due to usage of expensive tools/materials and CMOS incompatible metals. In this report, we focus on replacing our standard substrate, calcium fluoride, used in previous studies with a silicon wafer.

## Summary of Research:

Infrared (IR) spectroscopy is a common non-destructive, label-free technique to identify chemical substances. Previously, we have demonstrated devices (MEIRS [1], 3D-ITS [2], 3D-MEIRS [3]) which are based on coupling plasmonic resonances of nanoantennas to molecular vibrations of chemical components. This allows us to monitor live cell activities such as intracellular activities, cholesterol depletion and cell adhesion. Additionally, by using a mid-IR quantum cascade laser (QCL) light source, our group designed a laser-scanning inverted confocal microscope. The QCL emission is focused on a diffraction-limited spot and scanned across the metasurface through movement of a motorized microscope stage. A liquid-nitrogen-cooled mercury-cadmium-telluride (LN-MCT) mid-IR detector collects modulated reflection from the interaction of the analyte with the metasurface near-fields [4]. Recently we utilized this technique to study metabolic cell differentiation [5]. The vibrational contrast from amide II and lipids (Figure 1) clearly show the high surface sensitivity of our device.

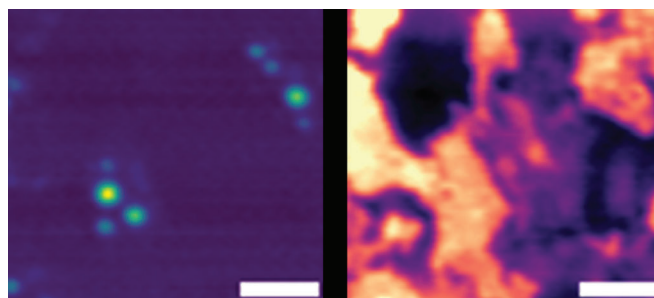


*Figure 1: Vibrational contrast of 3T3-L1 at 12C=O ester (left) and amide II (right) bands (scale bar: 40μm).*

However, the current substrate (CaF<sub>2</sub>) is very fragile and poses difficulty if one wants to use larger microplates, typically used for drug discovery studies. This work focuses mostly on replacing CaF<sub>2</sub> with a simple silicon wafer with an oxide layer.

The device is made of gold nanoantennas on a double-side polished 4-inch Si wafer with an aluminum nitride spacer. First, the RCA cleaned silicon wafers were deposited by a 1.6 μm thick AlN layer. To pattern nanoantennas, a thin layer of PMMA was spin-coated followed by the e-beam exposure with JEOL9500. After developing, SC4500 evaporator was used to deposit a 5nm Ti adhesion layer followed by 70nm layer of Au to form antennas. Since we use the backside of the substrate to focus IR light, back etched marks located exactly under the metasurface arrays were made by backside alignment using Heidelberg MLA 150 for patterning and PT740 for etching. The last step is to lift-off gold by soaking the wafer/pieces into acetone overnight. Schott IR inspector was used to observe both sides of the wafer, top side having metasurfaces and bottom one having marks (Figure 2).

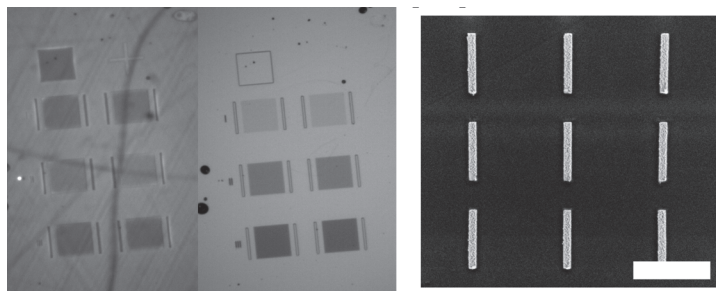


Figure 2: Left and middle figures show the bottom and top side of the wafer, respectively. SEM image of the antennas (right figure, scale bar: 3  $\mu\text{m}$ ).

The metasurfaces are then brought to the lab where we culture cancer cells on top of them and acquire images and spectrum. Figure 3 shows the IR spectra of cells in newly fabricated metasurface. The absorbances magnitudes (vibrational contrast) of amide peaks are comparable to our previous designs, thus making it possible to replace fragile and expensive  $\text{CaF}_2$  with an unexpensive and easy to make  $\text{AlN}$ -on-Si substrates.

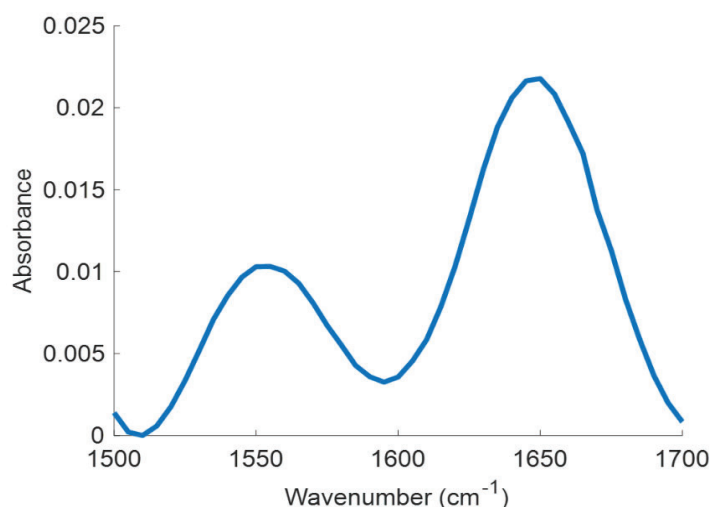


Figure 3: Absorbance spectrum of 3T3-L1 cell measured on the metasurface.

## Conclusions and Future Steps:

We have demonstrated that our plasmonic metasurface-based devices can also be made with simple Si unlike a standard IR-transparent  $\text{CaF}_2$  which paves the way for scaling up. We plan to replace expensive e-beam lithography with cheaper and more scalable photolithography (e.g. ASML DUV stepper) for metasurface patterning to large-area microplates. Plus, gold is not a compatible CMOS metal, so we also envision to consider different metals like TiN or Al.

## References:

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# Influence of Geometric and Flow Complexity on Sperm Migration Dynamics

**CNF Project Number: 2827-19**

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*Primary Source(s) of Research Funding: Internal funding*

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*Research Group Website: <https://abbaspourradlab.com/>*

*Primary CNF Tools Used: ABM Mask Aligner, KLA P7 Profilometer*

## Abstract:

Successful fertilization requires sperm to navigate complex reproductive environments shaped by fluid flow and geometric confinement. This report summarizes two studies exploring bovine sperm migration across distinct hydrodynamic and structural conditions. First, we investigated sperm rheotaxis in radial flow fields, revealing that moderate flows drive upstream migration, while higher flows induce rotational rheotaxis, where sperm follow stable curved trajectories around the flow origin. These findings enabled the development of a unidirectional navigation chip that enriches highly motile sperm with up to 98% output motility. Second, we examined sperm transport through confined obstacle lattices, demonstrating that geometric confinement regulates scattering behavior and surface interactions, with a transport model predicting enhanced effective diffusivity. Together, these studies reveal how flow and architecture independently regulate microswimmer navigation and provide principles for designing bioinspired sperm selection platforms.

## Summary of Research:

This report summarizes two related studies investigating how bovine spermatozoa navigate physically structured microenvironments that reproduce key features of the female reproductive tract [1], and how the resulting behavioral insights can be translated into functional sperm-selection technology for assisted reproductive technology. For each study, a soft lithography technique was used to fabricate the microfluidic devices. The master molds were fabricated by coating SU-8 photoresist on a silicon substrate, exposing the masked wafer to UV light, and developing.

The first study, published in *Lab on a Chip*[2], combined simulations and experiments to investigate sperm rheotaxis in an outward radial flow. While moderate flow rates induced upstream migration toward the flow origin, higher flow rates produced a previously unreported behavior, rotational rheotaxis, in which sperm swirled around the flow origin while continuing to migrate inward. This behavior arises from the coupling between intrinsic helical swimming and the non-uniform shear field of radial flow.

These findings informed the design of a selection device (Fig. 1), the sperm unidirectional navigation chip, which integrates the radial flow geometry with strategically positioned constricted flow channels to reinforce unidirectional progression and exclude non-progressive cells. Evaluated on raw bovine semen, the sperm unidirectional navigation chip achieved output motility of up to 98%, recovered approximately 30% of motile sperm from the input sample, and produced a selected population swimming roughly 50% faster on average than raw semen. These results demonstrate that radial flow geometry, when appropriately tuned, can be exploited as an active selection mechanism rather than functioning merely as a passive environmental feature, offering a low-complexity, centrifugation-free alternative for assisted reproductive technology sperm preparation.

The second study, currently available as a bioRxiv preprint[3], investigated sperm navigation in confined, lattice-like environments that mimic the folded fallopian epithelium. Using microfabricated obstacle arrays (Fig. 2a-c), sperm trajectories and scattering dynamics were quantified experimentally, and the resulting data were used to develop a mathematical transport model describing sperm migration through structured environments.

The central finding was a passive adaptive sensing mechanism regulating sperm-surface interactions

as a function of local confinement. Scattering angle varied systematically with lattice spacing, and surface-interaction duration decreased under tighter confinement, indicating that cells disengage from surfaces more rapidly in more confined settings. The transport model predicted that this confinement-dependent scattering behavior could enhance effective diffusivity by up to ninefold relative to obstacle-free, free swimming (Fig. 2d).

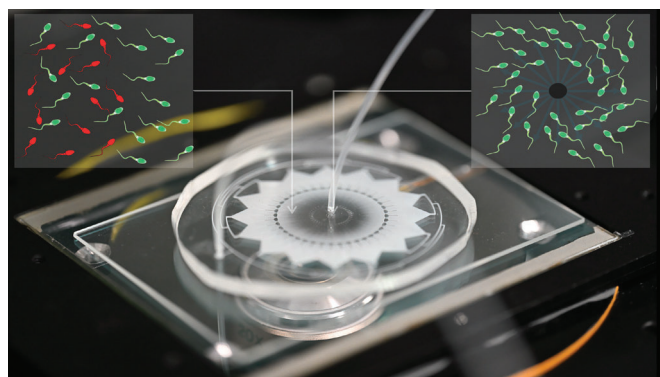


**Figure 1:** Microfluidic sperm unidirectional navigation chip for selective enrichment of highly motile sperm. Fluid enters through a central inlet and flows radially toward distributed outlet channels. Highly motile sperm swim upstream and become trapped near the center by rotational rheotaxis, whereas non-motile sperm, debris, and damaged cells are carried downstream and removed by the flow.

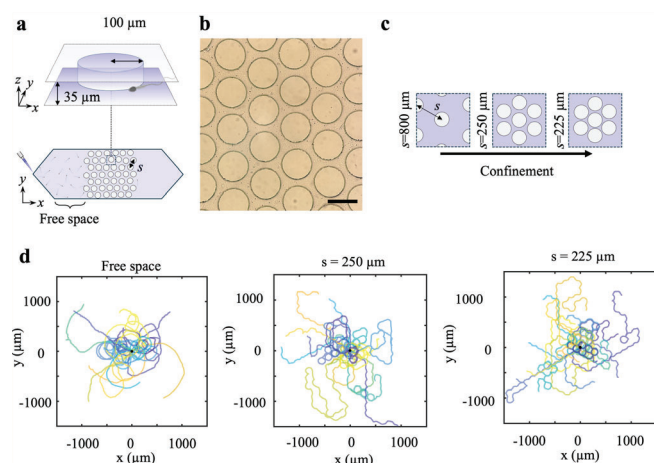


**Figure 2:** Microfluidic obstacle lattice for studying sperm navigation under confinement. (a) Schematic of the circular obstacle array. (b) Microscopic image of the fabricated obstacle lattice (scale bar: 200  $\mu\text{m}$ ). (c) Lattice confinement is systematically increased by reducing the center-to-center obstacle spacing,  $s$ . (d) Representative sperm trajectories in an obstacle-free environment and in obstacle lattices with progressively stronger confinement, illustrating the effect of lattice geometry on swimming behavior.

## Conclusions and Future Steps:

Taken together, these two studies establish that both hydrodynamic and geometric features of the reproductive tract actively shape sperm transport, rather than serving as passive obstacles. The radial flow study demonstrates that flow-field structure can be engineered into a functional, quantifiably effective selection platform, while the confinement lattice study demonstrates that geometry alone modulates transport efficiency through an adaptive, passive surface-sensing mechanism robust to changes in motility state. Collectively, these findings support treating geometry and hydrodynamics as tunable design parameters for microfluidic device development.

Several directions warrant further investigation. First, the sperm unidirectional navigation chip's motile sperm recovery yield ( $\sim 30\%$ ) leaves room for optimization; future iterations should explore alternative constriction placements and channel dimensions to improve throughput without sacrificing selection purity. Second, the mechanistic basis of rotational rheotaxis merits deeper theoretical characterization, particularly the transition between directed and rotational regimes. Third, the adaptive sensing mechanism identified in the lattice study should be examined at a molecular or biomechanical level to clarify whether it arises from flagellar mechanics alone or involves additional membrane- or surface-mediated processes. Fourth, integrating the two device paradigms, radial flow selection and confinement-based transport enhancement, into a single platform may yield synergistic gains in selection efficiency and throughput. Finally, validating these findings in human sperm samples will be essential to assess translational applicability, given that the current work was conducted exclusively in a bovine model. Addressing these questions will help move this work from proof-of-concept demonstrations toward clinically viable assisted reproductive technologies.

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- [3] A. Karimi, X. Jiang, and A. Abbaspourrad, Adaptive surface sensing enables mammalian sperm navigation in complex environments, *bioRxiv* 2025.11.16.688464 (2025).

# Vibration-Assisted On-Chip Oocyte Denudation

**CNF Project Number: 2827-19**

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*Research Group Website: <https://abbaspourradlab.com/>*

*Primary CNF Tools Used: ABM Contact Aligner, Heidelberg Mask Writer - DWL2000, Dicing Saw - DISCO, P7 Profilometer - Versalaser Engraver/Cutter Tool*

## Abstract:

We developed an open-surface microfluidic platform for on-chip removal of cumulus cells and oocyte denudation through vibration-induced flow. Unlike traditional methods that depend on repeated manual pipetting—labor-intensive, operator-dependent, and potentially stressful for oocytes—our technique employs precisely controlled fluid movement around microscale structures. This method effectively detaches cumulus cells while keeping the oocytes securely within the chip.

## Summary of Research:

Recent advances in micro/nanofabrication and microfluidics have improved the precise manipulation of biological cells. We engineered an open-surface microfluidic platform for on-chip removal of cumulus cells and denudation of mouse oocytes, utilizing vibration-induced flow (VIF). (Favakeh et al.) This system features a microstructured chip positioned on a computer-controlled XY piezoelectric stage. The stage was custom-designed on a PMMA substrate using a VersaLASER tool, with two piezoelectric actuators embedded along the X and Y axes. Applying two sinusoidal signals with a 90° phase difference to these actuators produces circular vibrations and localized streaming around the micropillars, as shown in Figure 1.

Figure 2 illustrates that the open-surface chip can be manufactured through various microfabrication methods, such as direct SU-8 photolithography on glass, PDMS soft lithography, hot embossing with polystyrene, and 3D printing. These techniques facilitate the creation of cost-effective, open-surface devices that simplify the loading, observation, and recovery of oocytes with a pipette.

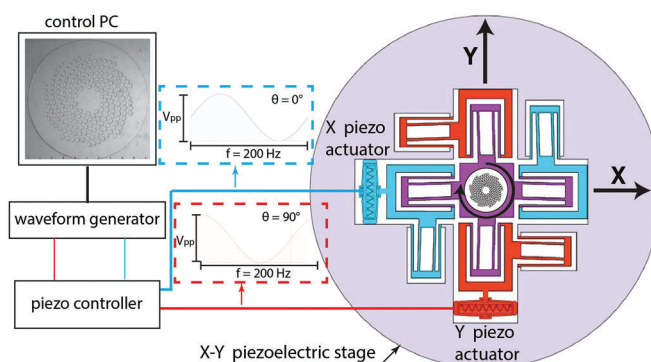


*Figure 1: Vibration-induced flow platform. Schematic of the experimental setup, including the control computer, waveform generator, piezoelectric controller, custom XY piezoelectric stage, and open-surface microstructured chip. Two sinusoidal signals with a 90° phase difference are applied to the X- and Y-axis piezoelectric actuators to generate circular vibration.*

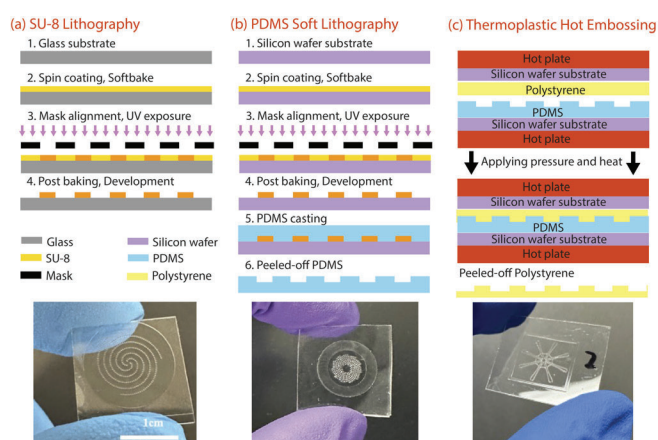


*Figure 2: Fabrication methods for the open-surface microstructured chip. Schematic illustration of chip fabrication using (A) direct SU-8 photolithography on glass, (B) PDMS soft lithography from an SU-8 master, and (C) hot embossing of polystyrene using a PDMS mold.*

Figure 3 illustrates the flow field and size-based separation mechanism. Each micropillar vibrates circularly, creating a localized whirling flow. The interactions between neighboring pillars' flow fields facilitate fluid movement across the micropillar array. The pillar diameter, spacing, and arrangement were optimized so that larger cells stay confined in the central chamber, while smaller detached cells and debris pass through the gaps and are transported outward.

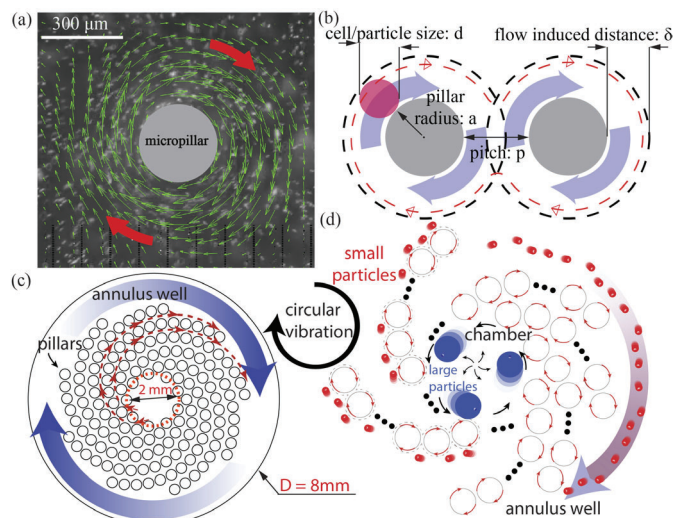


Figure 3: VIF-based flow generation and size-selective separation. (A) Circular vibration generates localized whirling flow around each micropillar. (B) Transportation flow caused by circular vibration through the array of pillars. (C) Topographical feature of induced flow by circular vibration through the spiral pillar array for the proposed chip. (D) Particle size separation using VIF.

Figure 4 depicts the on-chip denudation process. Cumulus–oocyte complexes are initially loaded into the central chamber. Applying circular vibration creates vortical flows around the micropillars, which transport and rotate the complexes within the array. This controlled navigation ensures the oocytes move through the micropillars in the loading chamber area, while detached cumulus cells are effectively separated. The denuded oocytes are confined and easily collected from the open-surface device. These results indicate that VIF provides a controllable platform for automated oocyte processing.

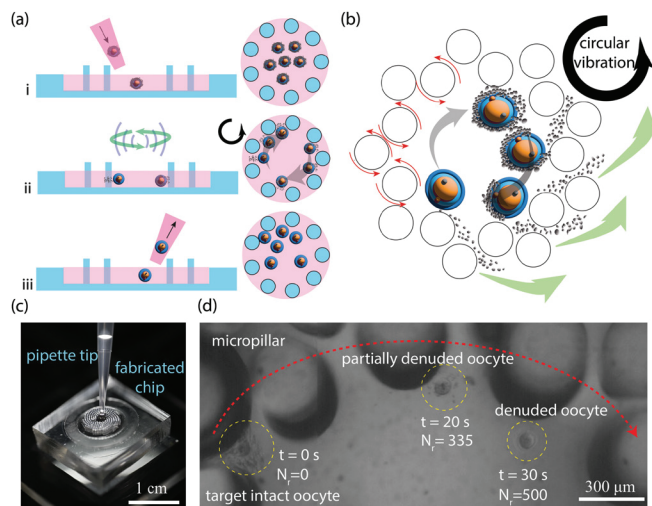


Figure 4: On-chip cumulus removal and oocyte denudation procedure. (A) Cumulus–oocyte complexes are loaded into the central chamber, (B) transported and rotated by vibration-induced flow, and exposed to hydrodynamic forces that detach the surrounding cumulus cells. The denuded oocytes remain confined within the device and (C) can be recovered using a pipette. (D) Stacked images of freeze/thaw oocyte denudation within the loading chamber at different times ( $t$ ) and different numbers of rotations ( $N_r$ ).

## Conclusions and Future Steps:

We developed an open-surface VIF platform for on-chip cell separation and mouse oocyte denudation. Circular vibration produced localized flow around micropillars, enabling oocyte transport, rotation, and removal of surrounding cumulus cells. The device kept denuded oocytes in the central chamber while moving smaller cells and debris outward. This shows that VIF provides a simple, low-cost, contact-minimized method for oocyte denudation, potentially reducing manual pipetting.

## References:

- [1] Favakeh, Amirhossein, et al. "On-Chip Oocyte Cumulus Removal Using Vibration-Induced Flow." *Lab on a Chip*, vol. 25, no. 21, Oct. 2025, pp. 5551–62.

# Investigating the Effect of the Tumor Microenvironment on Metastatic Progression using Micro and Nano-scale Tools

**CNF Project Number: 2912-20**

**Principal Investigator(s): Claudia Fischbach<sup>1</sup>**

**User(s): Kylie Persson<sup>1</sup>, Siyoung Choi<sup>1</sup>, Jack Crowley<sup>2</sup>**

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*Primary Source(s) of Research Funding: The work described was supported by the National Science Foundation Graduate Research Fellowship DGE 2139899 (Kylie Persson), 1R01CA297166-01A1 and the Center on the Physics of Cancer Metabolism through Award Number 1U54CA210184-01 from the National Cancer Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health.*

*Primary CNF Tools Used: ABM Contact Aligner, Heidelberg DWL2000, Hamatech 9000, Malvern NS300 NanoSight*

## Abstract:

Breast cancer is the second leading cause of cancer-related death for women in the United States<sup>1</sup>. Breast cancer mortality is driven by metastasis, where cancer cells disseminate from the primary tumor to seed distant tissues such as the bone. During bone metastatic disease, cancer cells interact with their microenvironment consisting of a mineralized, collagen-rich extracellular matrix and other cell types including mesenchymal stem cells (MSCs), macrophages, and osteoclasts. These cells are known to participate in reciprocal signaling with cancer cells to influence tumorigenesis through the exchange of soluble factors<sup>2,3</sup> and extracellular vesicles (EV). However, the mechanisms by which soluble factor and EV signaling influence tumor cell invasion and the development of a pro-tumorigenic microenvironment remain unclear due to the lack of models that enable systematic study. To this end, CNF tools were used to investigate various stages in metastasis: EV-mediated formation of a pre-metastatic niche in bone and tumor cell invasion into bone. For pre-metastatic niche development studies, we leveraged EVs derived from breast cancer cells and bone-mimetic engineered substrates to investigate how EV binding to bone is regulated by components of their glycocalyx (sugar-coating on the outside of EVs) and matrix mineral content. For invasion studies into the bone, we developed a microfluidic model of the bone microenvironment with mineralized collagen microchannels and have

shown that tumor cell invasion is inhibited when co-cultured with macrophages, osteoclasts, and MSCs seeded in a mineralized microchannel.

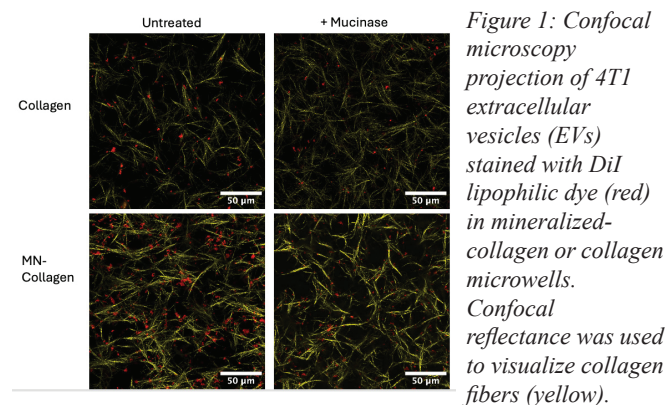
## Summary of Research:

*Isolation of breast cancer-derived extracellular vesicles and interactions with bone matrix*

Prior to arriving at distant metastatic sites, tumor cells can release soluble factors and extracellular vesicles (EVs) into circulation to prime the microenvironment of distant target organs for subsequent development of organotropic metastasis. EVs are gaining appreciation as stable vehicles of cell-derived cargo contributing to tumorigenesis and pre-metastatic niche (PMN) formation. In collaboration with Matt Paszek's Lab, we have successfully isolated and characterized EVs from murine 4T1s breast cancer cells using the Malvern NS300 NanoSight. Currently, we are investigating differences in 4T1 EV binding to bone-mimetic engineered substrates. These bone-mimetic models were developed at the CNF using SU-8 photolithography to create a micropatterned silicon wafer used to fabricate PDMS "microwells". These PDMS microwells are filled with a collagen type I matrix and subsequently mineralized using the polymer-induced liquid precursor (PILP) method to mimic bone extracellular matrix, as previously described<sup>5</sup>. Through this engineered system, we can systematically explore differences in EVs binding in the presence or absence of mineral in bone. Furthermore, we are examining how EV characteristics, such as glycocalyx composition, will affect binding to bone and whether this is dependent on matrix mineral content. Our preliminary data suggest that EV binding is increased in mineralized collagen substrates, and that

removal of mucins from the glycocalyx reduces their ability to bind (Figure 1). Future work will incorporate MSCs in addition to EVs to probe the interplay between bone-resident cells, bone matrix and EVs. Overall, these studies will help unravel the mechanisms by which EVs and key components of the bone microenvironment regulate the onset of bone metastasis.

#### *Effects of mineralized collagen on breast cancer cell invasion using a microfluidic model of the bone*



During bone metastasis, tumor cell invasion and subsequent seeding of osteogenic niches<sup>4</sup> can be influenced by macrophages. Macrophages not only exert immunomodulatory effects, but also have the potential to differentiate into osteoclasts, the primary cell type driving osteolysis in bone metastasis patients. Using SU-8 photolithography in conjunction with the ABM Contact Aligner and a photomask generated by the Heidelberg DWL2000, we have created a dual channel microfluidic devices that enables co-culture breast cancer cells and macrophages encapsulated in a 3D collagen matrix. To model these interactions within a bone-like microenvironment, the microfluidic device was modified using the PILP method to include selective and controlled collagen mineralization of the device microchannels<sup>5</sup>. We have shown that macrophages promote the invasion of breast cancer cells regardless of collagen mineralization and that this effect occurs without cell-cell contact, suggesting that it was caused by soluble factors secreted from macrophages. However, it was determined that collagen mineralization inhibits the ability of macrophages to promote tumor cell invasion compared to non-mineralized collagen co-culture conditions (Figure 2). Similarly, when macrophages were differentiated into osteoclasts within a mineralized collagen channel, tumor cell invasion was also inhibited compared to non-mineralized collagen devices (Figure 3). Lastly, MSCs cultured within mineralized collagen also decreased tumor cell invasion compared to non-mineralized conditions (Figure 4). Future work includes device materials characterization and validating our

findings by incorporating breast cancer cells with multiple resident bone cells to create tri-culture models.

Figure 2: Confocal microscopy projection of non-mineralized collagen device (top) and mineralized (MN) collagen device (bottom) seeded with MDA-MB-231 breast cancer cells (green) and RAW264.7 macrophages (red). DAPI was used to stain nuclei (blue).

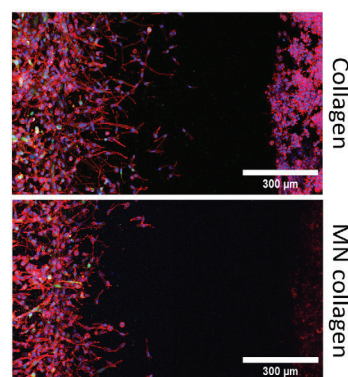
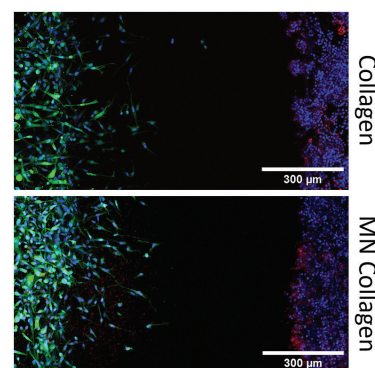
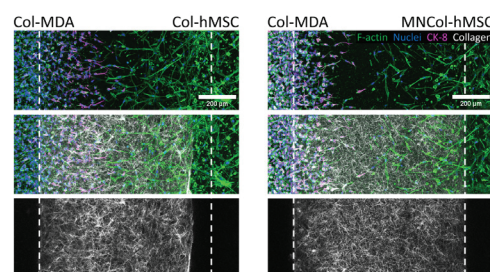


Figure 3: Confocal microscopy projection of non-mineralized collagen device (top) and mineralized (MN) collagen device (bottom) seeded with MDA-MB-231 breast cancer cells (green) and RAW264.7 osteoclasts. F-actin and nuclei are shown in red and blue, respectively.

Figure 4: Confocal microscopy projection of MDA-MB-231 cells and human MSCs invading into the central matrix of mineralized and non-mineralized collagen devices, representative z-slice of collagen reflectance, and overlay images shows invading cells and matrix.



## References:

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# Fabrication of Microelectrode Arrays for Oxygen Generation to Support Cellular Growth in Implantable Devices

**CNF Project Number: 3066-23**

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*Primary Source(s) of Research Funding: Advanced Research Projects Agency for Health (ARPA-H), Resilient Extended Automatic Cell Therapies (REACT)*

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*Primary CNF Tools Used: AJA Sputterers, Oxford 81/82, ABM Contact Aligner, Odd-Even hour evaporators*

## Abstract:

This project aims to develop a microelectrode array for wireless generation of oxygen to support cell culture in implantable, therapeutic devices. To achieve this goal, we have fabricated Platinum (Pt) and Gold (Au) microelectrodes using tools available at Cornell Nanofabrication Facility (CNF). The electrodes are fabricated directly into flexible substrates (e.g., polyimide (PI) and PET thin films) and Iridium oxide (IrOx) films are electrochemically deposited. IrOx catalyzes watersplitting, leading to oxygen evolution at a lower potential ( $\sim 1.2$  V) compared to bare Pt ( $\sim 1.7$  V). Based on the oxygen generation performance of the Pt/IrOx electrodes, the microelectrode design have been revised to optimize oxygen generation for cell growth. In future, the Pt/IrOx electrodes will be integrated with NFC chips to allow for wireless operation of the devices.

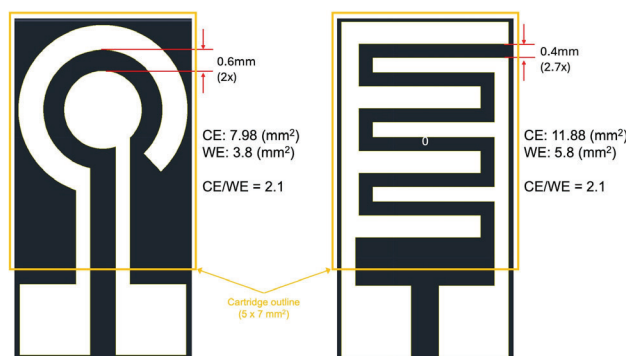


Figure 1: Updated design of microelectrode to enhance counter electrode area.

of the microelectrode array has been optimized to account for the potential drop in 2-electrode setup for seamless integration with the final implantable device. In 2-electrode system, the onset potential for oxygen generation is shifted to higher values due to the unavoidable potential drop arising from the current-driven polarization on the surface of the counter electrode continuously altering the reference [1]. To balance this, we have updated the design of our microelectrodes for a higher counter/working electrode surface area and decreasing the distance between them to enhance conductivity (Figure 1). We have successfully redesigned the mask and fabricated Pt and Au microelectrodes with higher counter electrode area on commercially available PI as previously (Figure 2).

## Summary of Research:

The Ti/Pt and Cr/Au microelectrode array is fabricated using much simpler approach compared to previous iterations. Commercially available PI and PET thin films are treated with oxygen plasma and primed with HDMS followed by photolithography for patterning microelectrode arrays. Pt microelectrodes are fabricated using lift-off process while Au microelectrodes are fabricated using wet etching. This year, the design

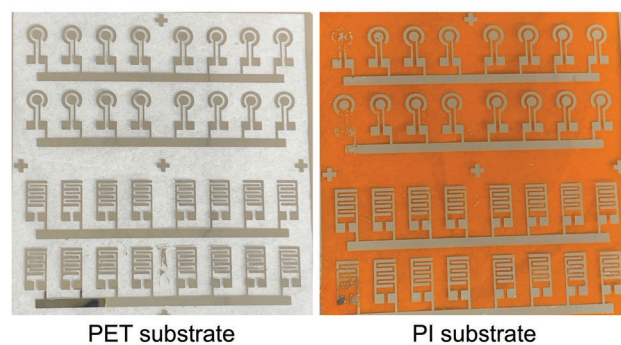


Figure 2: Pt microelectrodes fabricated on polyimide (PI) and PET films.

## Conclusions and Future Steps:

Based on our preliminary experiments using the microfabricated devices, the designs have been updated to enhance performance. We are currently exploring and investigating micro-coil antenna designs to integrate the microelectrodes into a wireless oxygen generation system.

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# Using Microfluidic Devices to Understand the Growth and Migration of *S. Aureus* within Submicron Channels

**CNF Project Number: 3229-24**

**Principal Investigator(s): Christopher J. Hernandez**  
**User(s): Jay VanDelden**

*Affiliation(s): Department of Orthopaedic Surgery, University of California, San Francisco*

*Primary Source(s) of Research Funding: Center for Disruptive Musculoskeletal Innovations Research Grant (UCSF)*

*Contact: Christopher.Hernandez@ucsf.edu, jvd@eigenphase.com*

*Research Group Website: <https://www.hernandezresearch.com/>*

*Primary CNF Tools Used: AJA Sputter Deposition Tool, Gamma Automatic Coat-Develop Tool, ASML Deep UV stepper, Gamma Automatic Coat-Develop Tool, Oxford 82 Tool, Plasma-Therm 770 ICP tool, Oxford 100 Tool, Versalaser, AFM, Profilometer, Zeiss Ultra 55 SEM microscope*

## Abstract:

Microfluidic devices were created to understand the growth and migration of the human pathogenic bacterium, *Staphylococcus aureus* through nanoscale channels. This work was inspired by human clinical studies, which have found that *S. aureus* is able to colonize channels in the bone tissue matrix that are 2-3 times smaller than cell diameter during infection. It is unknown how *S. aureus* enters channels given that the bacterium is normally non-motile. It has been proposed that cells may enter via growth – such that daughter cells form within channels and then proliferate down the channel length. Using microfluidic devices, we tested the idea that *S. aureus* could enter channels smaller than cell width via growth and division. We found that proliferation could facilitate migration, but only to cell deformations of 600 nm (65% cell width). Below 600 nm, mechanical confinement significantly slowed or completely inhibited division. Thus, *S. aureus* is unlikely to traverse nanoscale channels in bone via division. In contrast, we found that physiologically relevant fluid pressures generated around bone in vivo could force cells into channels smaller than cell width.

## Summary of Research:

The Hernandez Research Group has developed a microfluidic device, which can be used to deform single bacterial cells to submicron widths<sup>1</sup>. The device is manufactured from etched silica wafers, and consists of a series of tapered channels, that narrow in width from ~1.5 to 0.3mm (Figure 1). When a suspension of bacteria is flowed into the device, individual bacteria are trapped within tapered channels by a pressure differential ( $\Delta P$ ) generated between the channel inlet and outlet. There are 4 distinct loading pressures generated within one

device due to the parallel, ladder-like arrangement of channel sets. Greater pressure differences cause cells to travel further into the channel and experience greater deformation. Our prior works have shown that cell deformation within channels activates mechanosensitive stress response systems and can be used to infer information on cell stiffness<sup>2,3</sup>.

Recently, we used devices to understand how *S. aureus* colonizes the bone tissue matrix during infection<sup>4</sup>. Specifically, microscopic studies have found *S. aureus* within channels in bone, known as canaliculi<sup>5</sup>. Canaliculi range in width from 300 to 900nm, requiring *S. aureus* to deform to only 1/3 its original size to navigate. The ability of *S. aureus* to colonize canaliculi is likely a major contributor to treatment failure and chronic infection, as bacteria become protected from immune cell clearance and antibiotic therapy within nanochannels. It is generally unknown how *S. aureus* colonizes canaliculi, but it has been proposed that cells may enter through division<sup>5,6</sup>. Therefore, to test this idea, we determined if proliferation could lead to *S. aureus* migration within our microfluidic device.

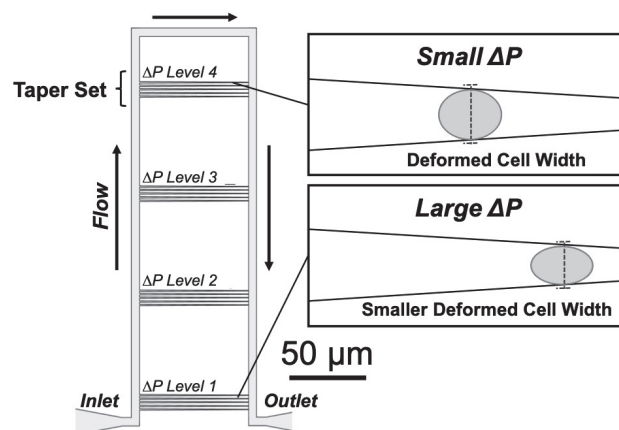


Figure 1: Schematic illustration of the microfluidic device.

We used fluidic pressure to deform 322 individual cells to varying widths within microfluidic channels. We observed that deformation slowed *S. aureus* division, as cells that were more deformed took longer to divide (Figure 2A). When cells were deformed more than 606nm, no divisions were detected. We speculated that mechanical confinement may inhibit proliferation if the division plane is positioned parallel to the channel walls, restricting cell growth across the width of the channel. However, fluorescent labeling of proteins localized to the division plane showed that most cells had planes aligned perpendicular to the channel walls in the orientation least likely to impair division (Figure 2B). Thus, mechanical deformation inhibits *S. aureus* growth through an unidentified stress response, making it unlikely that bacteria enter canaliculi in bone through division.

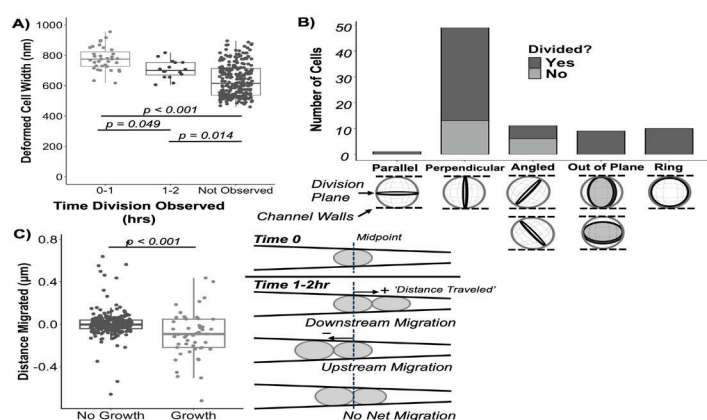


Figure 2: (A) The deformed width of cells that did or did not divide within the device is shown ( $n = 322$ ). (B) Orientation of the division plane is shown relative to channel walls for cells within the device ( $n = 80$ ). (C) The distance migrated for cells that divided within channels ( $n = 51$ ) and for cells that did not divide ( $n = 271$ ) is shown with an illustration of the analysis method.

To further verify that *S. aureus* colonization of canaliculi via growth was unlikely, we determined whether cell division within channels resulted in cell migration. We hypothesized that division would result in the net migration of cells towards the narrower end of the channel if cells could preferentially sense and divide into canaliculi as proposed<sup>5,6</sup>. We found, however, that growth and division had a marginal effect on cell positioning. On average, dividing cells migrated only  $97 \pm 215$ nm and showed a slight bias (56% of cells studied) to migrate upstream towards the wider end of the channel (Figure 2C).

## Conclusions and Future Steps:

Given our observation of impaired growth in deformed cells, coupled with our findings that proliferation does not lead to significant migration, we conclude that *S. aureus* is unlikely to enter canaliculi in bone through growth and division. As *S. aureus* is non-motile, external forces would be required to transport cells into canaliculi. Therefore, we speculate that *S. aureus* transport in bone may be facilitated by fluid pressures. Based on our studies investigating *S. aureus* deformation as a function of applied pressure, we estimate that only 9kPa would be needed to force cells into channels measuring 300nm. Fluid pressures generated in bone during normal physical activity have been reported to be as high as 8 to 20kPa<sup>7</sup>. Thus, the ability of bacteria to colonize canaliculi may be more closely related to physical loads on the bone and the mechanical properties of the bacteria rather than growth. Future work will determine if differences in cell stiffness between clinical isolates of *S. aureus* effect canalicular colonization and subsequent infection severity.

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# Silicon Nitride Cantilevers for Muscle Myofibril Force Measurements

**CNF Project Number: 1255-04**

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**Primary Source(s) of Research Funding:** Canadian Institutes of Health Research

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**Research Group Website:** <https://kinesiology.ucalgary.ca/research/labs-and-centres/human-performance-lab>

**Primary CNF Tools Used:** Primary tools: GCA 5X Stepper, SUSS MA6-BA6 Contact Aligner, Photolith spinners, Oxford 81 ion etcher, Reynolds Tech KOH Hood

## Abstract:

The production of custom silicon-nitride force nano-levers allows for the measurement of nanometre-level forces in individual myofibrils [1]. Using the optical displacement of cantilevers attached to individual myofibrils or isolated sarcomeres permits the quantification of mechanical parameters associated with basic muscle properties (Figure 1). Recently, our lab has used cantilever-based force measurements in isolated myofibrils to investigate the behaviour of the sarcomeric Z-line under stress, and the behaviour of the gigantic spring-like protein titin. Briefly, for the first time, we have established that the sarcomeric Z-line may undergo extreme and reversible expansion at extreme sarcomere lengths (Figure 2). Notably, this expansion appears to be restricted to sarcomeres from type II muscle fibres and may constitute an injury prevention mechanism in skeletal muscle at extreme sarcomere lengths (Figure 2). Second, following our recent advances in immunolabeling techniques of titin (see [2]), we have mechanically quantified segmental extension of titin in passive myofibrils. This has led to a couple of distinctive insights: first, the distal immunoglobulin domains of titin appear to be extensible when titin is in its in situ sarcomeric configuration (Figure 3); second, that at long sarcomere lengths ( $>3.8\mu\text{m}/\text{sarcomere}$ ), we confirmed that titin does indeed pull out of the A-band (Figure 3). Last, in collaboration with the High-Resolution Macromolecular Imaging Facility at the University of British Columbia, we have developed a unique system that allows for imaging of individual sarcomeres and myofibrils using cryo-electron tomography (Cryo-ET) after mechanical testing is performed on those exact same myofibrils (Figure 4). This system will allow for the correlation of different mechanical states of sarcomeres with angstrom-level resolution of protein structures within those same sarcomeres and therefore has the potential to provide significant insight into the

molecular mechanisms of muscle function. Importantly, all recent advancements have been made possible by the use of the custom silicon-nitride force nano-levers that allow for measurements of force production in individual sarcomeres and myofibrils.



Figure 1: Example myofibril held between a glass needle and force nanolevers imaged under phase contrast.

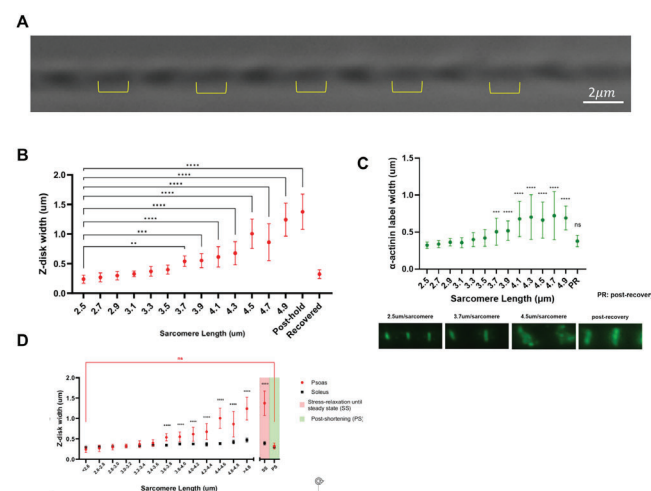


Figure 2: (A) Example myofibril held at  $\sim 4.5\mu\text{m}/\text{sarcomere}$  showing significant apparent Z-line expansion. (B) Significant Z-line expansion occurs in rabbit psoas myofibrils at a length  $\geq 3.7\mu\text{m}/\text{sarcomere}$  ( $n \geq 8$  sarcomeres per point, one-way ANOVA, Dunnett's multiple comparisons test). (C)  $\alpha$ -actinin label width as a function of sarcomere length shows significant expansion also at lengths  $\geq 3.7\mu\text{m}/\text{sarcomere}$  that appears recoverable after a slack period of 10 min ( $n \geq 30$  sarcomeres per point, one-way ANOVA, Dunnett's multiple comparisons test). (D) Z-line expansion is type II fibre-specific and is absent in soleus (type I).

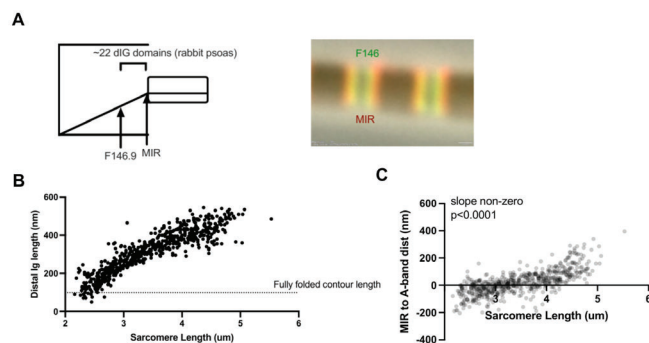


Figure 3: (A) Location of distal PEVK (F146.9) immunolabel and A-band insertion label (TTN-MIR), visualized with AF488 and AF647 secondary antibodies, respectively. (B) Distal Ig domains clearly demonstrate extension past fully folded contour length indicating unfolding of Ig domains occurs. (D) Location of TTN-MIR immunolabel relative to the A-band shows a significant non-zero slope indicating titin is pulled out of the A-band at long ( $>3.8$   $\mu\text{m/sarcomere}$ ) sarcomere lengths ( $P < 0.001$ ,  $F$ -test).

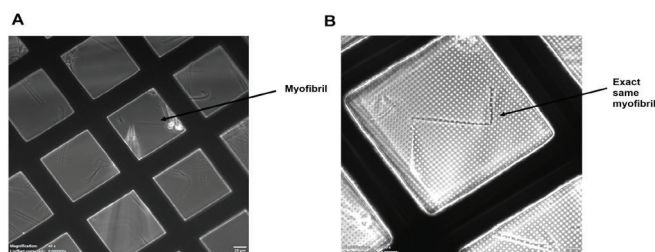


Figure 4: (A) Myofibril attached to mechanical testing apparatus suspended above Cryo-ET grid immediately prior to placement. (B) The same myofibril visualized in A post-placement on the grid and following grid removal for plunge freezing. The myofibril retains its sarcomere length and can be found intact exactly as it was placed on the grid.

## Summary of Research:

Imaging of myofibrils was performed on an inverted Olympus IX83 microscope in both phase contrast and fluorescent channels (where applicable). Images were analysed using CellSens® Dimensions software. Myofibrils were attached to a glass needle and custom silicon-nitride force-nanolevers; the glass needle was displaced using a piezoelectric motor, and force was measured using optical displacement of the force nanolevers based on known spring constants.

First, following observations of Z-line expansion at extreme sarcomere lengths in unrelated myofibril testing (Figure 2A), we investigated the behavior of the sarcomeric Z-line in rabbit psoas (type II) myofibrils.

We found significant expansion of the Z-line through both phase contrast imaging (Figure 2B) and using  $\alpha$ -actinin immunolabels specific to the Z-line (Figure 2C) at extreme sarcomere lengths. Notably, after a 10 min recovery period at slack length, Z-lines returned to normal width, suggesting that this undescribed phenomenon is reversible. We attempted similar experiments in rabbit soleus (type I) myofibrils and found significantly reduced Z-line expansion, suggesting that this phenomenon is fibre-type specific (Figure 2D).

Second, we placed titin immunolabels at the distal end of titin's PEVK and at titin's main immunogenic region (MIR), located at the A-band insertion of titin (Figure 3A). This allowed for the quantification of extension of the distal immunoglobulin domain extension in isolated myofibrils. Results suggested that these domains were highly extensible (Figure 3B), in opposition to assumptions in the field that they were fixed [3]. We also noted that at long sarcomere lengths  $>3.8$   $\mu\text{m/sarcomere}$ , titin's insertion into the A-band, measured by the MIR immunolabel, pulled out of the A-band (Figure 3C).

Third, we have developed a unique protocol where a myofibril may be tested mechanically using the custom force-nanolevers, then placed directly onto a Cryo-ET grid (Figure 4A) while preserving the mechanical state of the myofibril. Then, the Cryo-ET grid can be removed for immediate plunge freezing, and the same myofibril subsequently identified (Figure 4B) for imaging using Cryo-ET. This method will allow, for the first time, correlation of mechanical properties at the sarcomeric level with protein structures in individual sarcomeres and thus can significantly advance mechanistic understanding of muscle function.

## Conclusions and Future Steps:

In conclusion, the use of custom force nanolevers for quantification of myofibrillar forces has been critical to developing our understanding of muscle mechanics. Using immunofluorescent and phase contrast imaging coupled with simultaneous force measurements of myofibrils has permitted the identification of novel phenomena, such as Z-line expansion, and the development of novel techniques such as mechanically coupled Cryo-ET of individual sarcomeres.

## References:

- [1] M. E. Fauver, et al. IEEE Trans Biomed Eng 45(7):891-898, 1998. DOI: 10.1109/10.686797
- [2] D. Desai, et al. J Exp Biol 229(2): jeb250931. <https://doi.org/10.1242/jeb.250931>
- [3] P. Bennett, et al. J Struct Biol 120(1): 93-104. <https://doi.org/10.1006/jsbi.1997.3898>

# Fabrication Patterned Substrates for Cell Alignment

**CNF Project Number: 3302-25**

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*Primary Source(s) of Research Funding: Start up funding*

*Primary CNF Tools Used: Primary tools: ABM Contact aligner and DWL2000*

## Abstract:

Microcontact printing ( $\mu$ CP) is a soft lithography technique that allows for the controlled patterning of cells in an organized fashion. Here, we were interested in better understanding how surface geometry and tissue alignment affect myoblast differentiation and cellular contraction. To study this, we used Cornell's NanoScale Science & Technology Facility (CNF) to design and develop  $\mu$ CP substrates containing microgrooved patterns with single-micron features that could promote tissue morphogenesis and cell alignment. These were fabricated using SU-8 photolithography to form patterned silicon wafer masters with the DWL2000 Mask Maker and ABM Contact Aligner. The wafer masters were then used to create PDMS stamps, which were applied to gelatin-coated acrylic sheets to generate grooved cell culture surfaces. Throughout the project, we explored several fabrication parameters, including SU-8 thickness, baking conditions, and UV exposure time, while troubleshooting issues such as incomplete photoresist coverage and mask adhesion during exposure. After establishing a reliable fabrication process, the patterned substrates were used to culture  $C_2C_{12}$  skeletal muscle cells, allowing the formation of aligned cells that grew parallel to the tissue patterning direction, suggesting that local topological cues play an important role in guiding cellular organization.

## Summary of Research:

The goal of this project was to investigate how microscale surface geometry promotes muscle cell alignment. To create patterned cell culture substrates, we used photolithography techniques as available at the Cornell NanoScale Science & Technology Facility (CNF), fabricating silicon wafer masters containing parallel line patterns. These designs consisted of two patterned regions with 4  $\mu$ m line widths, 20  $\mu$ m spacing, and a 14 mm gap between the regions. The wafers were then fabricated using SU-8 photoresist, the DWL2000 Mask Maker, and the ABM Contact Aligner.

Briefly, to enable device fabrication, we investigated optimizing the photolithography process to allow for precise definition of micron scale features. Initial challenges included incomplete SU-8 coverage, manual edge bead removal, mask adhesion during exposure, and determining appropriate baking and UV exposure conditions. After iterating upon these parameters, we established a reliable process. This allowed for the consistent production of high-quality wafer masters, which could be used in the development of micropatterned tissue chips, enabling the controlled formation of aligned muscle tissues.

Gelatin tissue chips were then fabricated using PDMS micromolding. This allowed us to evaluate how microscale topologies could be used to guide cellular organization. Here, master wafers were used as molds to form PDMS stamps, which in turn could serve as repeatable, soft-lithographic molds for use in gelatin chip manufacture. Next, the PDMS stamps were pressed into gelatin-coated acrylic sheets to generate a micro patterned surface (Figure 1). These were then seeded with  $C_2C_{12}$  skeletal muscle cells, an immortalized mouse myoblast cell line. After culturing for fourteen days,  $C_2C_{12}$  formed aligned, multinucleated myotubes. As evidenced by brightfield microscopy, these showed that the cells elongated and aligned in the direction of the patterned substrates (Figure 2), demonstrating that surface patterning can provide effective cues for promoting skeletal muscle differentiation and alignment. These results suggest that engineered geometries can influence cell behavior and may serve as useful tools for studying tissue regeneration and cellular organization.

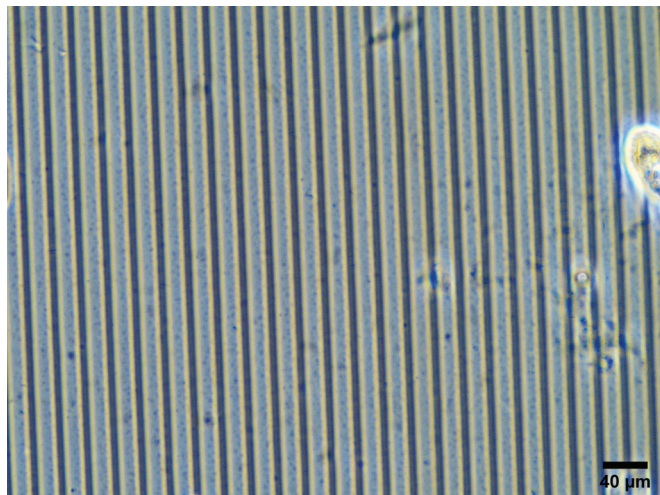


Figure 1: Gelatin micromold produced using a PDMS stamp derived from the SU-8 patterned silicon wafer. Parallel grooves with a line width of 4 μm and spacing of 20 μm were successfully transferred onto the gelatin surface.

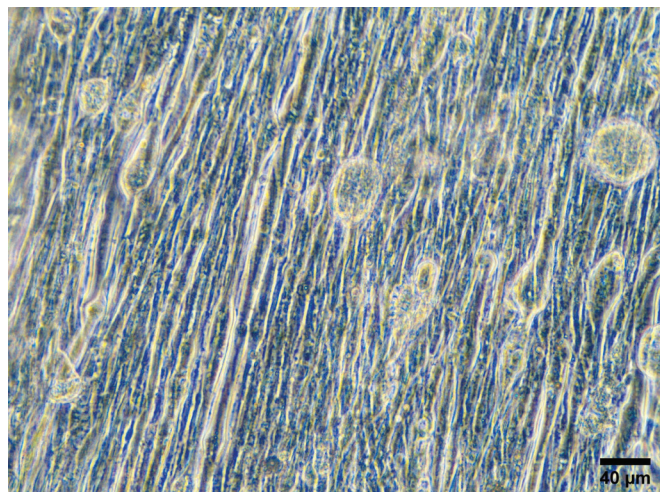


Figure 2: C<sub>2</sub>C<sub>12</sub> skeletal muscle cells cultured on the patterned gelatin substrate. Cells exhibit elongation and alignment parallel to the direction of the grooves, demonstrating that the engineered surface geometry provides effective guidance that influences cell organization.

## Conclusions and Future Steps:

Collectively, this project successfully established a workflow for fabricating micropatterned substrates using CNF's photolithography equipment. Through multiple rounds of iteration, high-quality SU-8 wafer masters and PDMS stamps were produced. C<sub>2</sub>C<sub>12</sub> cells cultured on the patterned gelatin surfaces showed clear alignment along the pattern, demonstrating that surface geometry can effectively guide cellular organization.

Going forward, we hope to use these processes as a basis for developing geometrically controlled tissue chips to study how infarct geometry and tissue alignment impact action potential propagation in myoblasts following ischemic injury. By establishing a baseline photolithography process, we plan to use this as a platform for varying further construct parameters, including pattern spacing, relative alignment, and overall geometric patterning. By doing this, we hope to better understand how these features impact emergent cell behaviors, such as tissue conductance, syncytium formation and myoblast differentiation. In using this approach, our goal is to better understand how these basic structure-function relationships can give rise to aberrant physiological behaviors following ischemic injury.