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# Thermo mechanical studies of cells with nano probes on Si Substrate

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## ABSTRACT:

Interpreting the role of mechanical forces and geometric constraints is crucial to understand cellular function and development. It has been seen that the type and direction of mechanical stimuli and alignment by topography can lead to significant differences in cell behavior but the mechanisms are not completely understood. We apply long term cyclic tensile stretch (CTS) using substrates with micro-patterned adhesive lines to study its effect on the structure and function of C2C12 skeletal myoblasts. Photolithography and micro-contact printing were used to pattern fibronectin on a passivating NCO-sP(EO-stat-PO) surface in a Polydimethylsiloxane (PDMS) chamber. C2C12 cells were seeded in the chambers on patterns of 30  $\mu\text{m}$  line width at 0°, 45°, and 90° orientations relative to the strain direction. Adhesive micro-patterns and passivation were maintained for more than 4 days of CTS with no signs of degradation. Our results indicate that CTS and micro-patterned lines strongly affected actin stress fiber orientation resulting in the highest organization on lines transverse to the applied strain with decreasing alignment as line orientation approached the strain direction. Nuclei aspect ratio was also significantly affected, resulting in elongation. Morphologically, myotube formation was incomplete after 4 days. Interestingly, actin striations were observed exclusively on the 45° line patterns subjected to CTS.

## INTRODUCTION

Cells adhere to one another to form tissues and organs. Cell adhesion plays a fundamental role on a variety of cell functionality, such as growth and cell division, as well as on disease progression such as angiogenesis. Until today there is very little knowledge on the cooperative arrangements and synergistic interactions between adhesion sites, significance of their cluster size, shape, their characteristic length scales, and their dynamics. This project will address some of the yet unanswered questions on cell adhesion and provide fundamental insight on the relation between cell mechanics and disease progression. Furthermore, a video series will be developed with three episodes for the promotion of science and bio-nano-technology among school students.

The objective of the current NSF project is to develop a novel Si substrate and a 3D force sensor to investigate thermo-mechanical behavior of single cells at the scale of a single adhesion site. The cellular investigations include: (a) size and strength of single adhesion sites; (b) thermal activities during formation of the sites; (c) inter and intra-cellular response of cells due to thermal stimuli applied at the sites. Prof. Joachim Spatz's lab at the Max Planck Institute, Stuttgart, Germany has pioneered some of the most advanced techniques in controlling cell adhesion at the single focal adhesion site scale. Several of the ideas proposed in the current NSF project originated from publications from the Spatz group.

Before going to conduct research under the IREE grant, Wylie Ahmed was an undergraduate student in the Department of Mechanical Sciences and Engineering (MechSE) at UIUC. He worked with two graduate students, one from MechSE and the other in Cell and Developmental biology. Together with the graduate

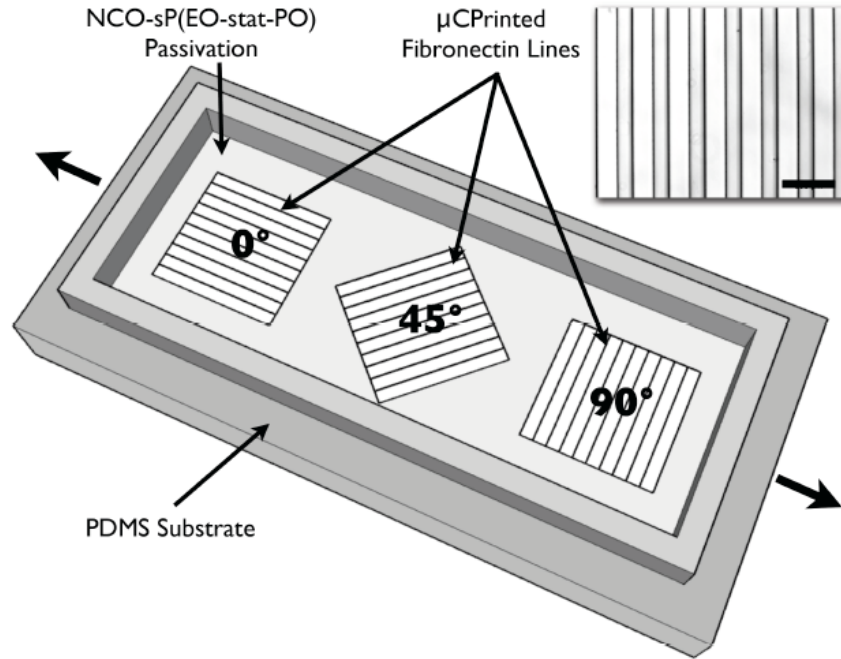
students, Wylie studied the adhesion between an axon and muscle cells. The graduate students recorded the fluorescent images of the axons highlighting neurotransmitters during the adhesion process. Confocal microscopy was used for imaging. These images contain large volume of data. Wylie's task was to analyze the data and correlate the neurotransmitter dynamics with mechanical tension in axon produced by the adhesion. In just about 3 months, Wylie performed a statistical analysis, and showed a strong correlation between tension and neurotransmission accumulation at the adhesion site. His performance as an undergraduate student had far exceeded any expectation for which he was chosen to conduct research in Germany. He postponed his graduation by 6 months for the trip.

The Spatz lab is outfitted with state-of-the-art equipment and is an excellent intellectual resource that Max Planck Institute brings together from all over the world. The lab is an extremely dynamic research center with more than 50 research students, post docs, and scientists. There is an abundance of ideas in the group. Wylie not only learned the techniques from Spatz group, but was also inundated with research ideas that will form the foundation of his future research. This international collaboration (January – July 08) allowed Wylie to work closely with research scientists and graduate students from various disciplines including physics, chemistry, and biology to gain exposure to a multitude of research projects and new techniques. The highly multidisciplinary environment served as an introduction to the world of bio-nanotechnology and was an ideal gateway to graduate studies. Wylie completed his B.S. in Mechanical Engineering and is now continuing his education as a Ph.D. student in Prof. Taher Saif's lab.

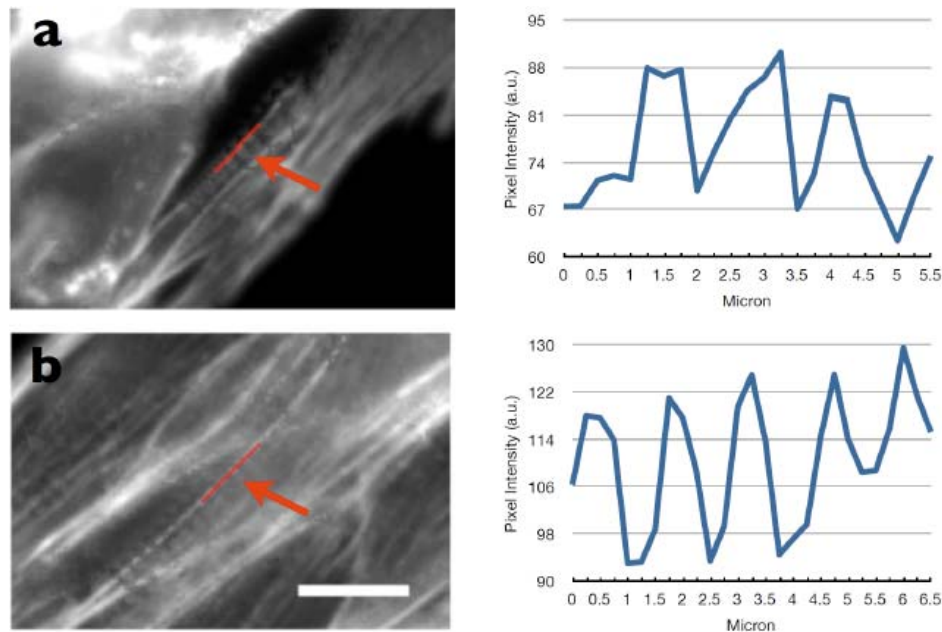
## **RESEARCH ACTIVITIES AND ACCOMPLISHMENTS OF THE INTERNATIONAL COOPERATION**

Wylie worked in Professor Joachim Spatz's group in the Department of New Materials and Biosystems at the Max Planck Institute in Stuttgart, Germany under the supervision of Dr. Ralf Kemkemer. The current proposal looks at the thermo-mechanical behavior of cells on silicon substrates. The scientific question it addresses is how cells sense their mechano environment, and what functionality change do they undergo. This IREE project looks at the mechanosensitivity of myoblasts and their differentiation to myotubes under mechanical stretching.

Wylie worked closely with Dr. Ralf Kemkemer's group, which specializes in cellular mechanostranduction. During the first phase of the collaboration Wylie learned biology and chemistry laboratory techniques, advanced imaging, and microfabrication from the experts in the Spatz group. In the second phase of the research experience Wylie worked closely with Dr. Kemkemer to develop a research plan to investigate the anisotropic mechanosensing of myoblasts. The research environment was very fruitful and encouraged creativity and sharing of ideas. As an introduction Wylie learned many biology and chemistry laboratory techniques such as cell culture and chemical synthesis. The high quality laboratories of the Spatz group also aided in training of advanced imaging techniques. Wylie was able to be trained and gain extensive experience with fluorescent and confocal microscopes, AFM, SEM, Cryo-SEM, and microfabrication. In the second phase of Wylie's research experience he was able to focus on two projects. First, he worked with a PhD student to develop a UV optical curing system for generating stiffness gradients in PEG-DA hydrogels. Then he was able to develop his own project under the supervision of Dr. Ralf Kemkemer. He worked with a stretching system developed by the Spatz group in conjunction with a stretchable patterned substrate developed at RWTH Aachen to investigate the anisotropic mechanosensing of muscle satellite cells as shown in fig. 1. It was found that there is an interdependence of cytoskeletal structure and nuclei morphology on mechanical stretch and that specific stress states promote myogenesis (fig.2) (**paper submitted to Biomaterials**)[1].



**Fig.1:** Schematic diagram of the PDMS stretchable substrate. The entire surface of the culture medium reservoir (70x 50 x 5 mm) was passivated with a NCO-sP(EO-stat-PO) coating. PDMS stamp shown in upper right hand corner (scale bar = 100 μm) was used to micro-contact print functional lines of bovine Fibronectin (5 μg/mL) onto the NCO-sP(EOstat-PO) surface at 0°, 45°, and 90° orientations relative to the strain direction. Outward pointing arrows represent strain direction.



**Fig.2:** Image of actin striations (phalloidin staining) observed in myoblasts subjected to CTS. Actin striations were observed in many different cells but strikingly only on the 45° micro-patterns. The red arrows in (a) and (b) indicate the region from which the intensity profile was measured. Both show a periodicity of approximately 1.5 μm. Actin striations suggest the induction of differentiation pathways to functional skeletal muscle. (scale bar = 10 μm).

Wylie was involved full time in Spatz's lab for six months, learning the necessary art of cell culture, substrate preparation, prevention of contamination, florescent techniques, biosensors, and single molecule imaging. These techniques will assist the current project significantly in its exploration of the thermo-mechanical characteristics of focal adhesions. The proposed international collaboration has a direct impact on the continuing NSF project. It extends the scope of the current project to visualization of individual FACs at a molecular scale, and hence monitor their dynamics in response to local thermal excitations.

### **BROADER IMPACTS OF THE INTERNATIONAL COOPERATION**

While at the Max Planck Institute, Wylie took a German language course (offered at the Max Planck Institute). Max Planck also arranges, for each research group, industrial tours. Wylie participated in such tours. The Max Planck Institute also organizes cultural evenings for its scientists and visitors. It invites both local and national artists to perform at the shows. Wylie was invited, as a Max Planck guest, to these cultural shows. Prof Spatz was extremely excited to host the student from UIUC and initiate an active collaboration with my group. One of the staff scientists at Spatz group, Dr. Ralf Kemkemer, was also very interested to work with me through the joint student. With the support from Spatz group, and their many years of work on cell adhesion at a molecular scale, we envision that the joint project can be extended to address various fundamental questions. The scope of the original award was limited to fibroblast and endothelial cells. The IREE expands the scope to muscle cells. Furthermore, it expanded the experience of the student both technically and socially. Thus the student has learned a broad range of advanced techniques for exploring cell mechanics.

### **DISCUSSION AND SUMMARY**

The NSF IREE Grant allowed an undergraduate student to travel to the Max Planck Institute for Metals Research in Stuttgart, Germany to gain exposure to the multidisciplinary field of nano-biotechnology. The student experienced an accelerated introduction to top caliber research in an environment that fostered creative thinking and ingenuity. This 6 month research project introduced the student to basic biology and chemistry laboratory techniques, advanced methods in imaging, and allowed the student to conduct two highly independent projects: 1) Developed a UV optical system for creating steep stiffness gradients in PEG-DA hydrogels. 2) Investigated the role of anisotropic mechanosensing in muscle satellite cell structure and development [1]. This experience helped the undergraduate student establish a firm research foundation and provided a smooth transition to graduate studies. Wylie is currently pursuing his Ph.D. with Prof. Taher Saif in the Department of Mechanical Sciences and Engineering at the University of IL at Urbana-Champaign. Additionally this international collaboration has established a long-term relationship between Prof. Saif and Prof. Spatz's research labs which will allow the free exchange of research ideas and future work. Based off this experience, it is recommended for "best practices" of future operation of the IREE Program that the highest preference should be given to foreign labs with history of outstanding research experience.

### **ACKNOWLEDGEMENTS**

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## BRIEF BIOGRAPHIES OF RESEARCHERS

**Wylie Ahmed** received his B.S. degree in Mechanical Engineering from the University of IL at Urbana-Champaign in 2008. He is currently pursuing his Ph.D. in Mechanical Engineering under the supervision of Prof. Taher Saif. Research interests include cellular mechanics, and more specifically, the mechanics of neurotransmission.

**Taher Saif** received both his B.S. and M.S. in Civil Engineering from Bangladesh University of Engineering and Technology and Washington State University in 1984 and 1987, respectively. He received his PhD in Theoretical and Applied Mechanics from Cornell University in 1993. He then held a postdoctoral position in the Department of Electrical Engineering at the National Nano-Fabrication Facility at Cornell University from 1993-1996. He is currently a Gutsgell Professor of Mechanical Sciences and Engineering at the University of IL at Urbana-Champaign. Professor Saif's research focuses on the mechanics of microelectromechanical systems (MEMS), fracture mechanics, submicron materials behavior and bio-MEMS. He uses MEMS devices, often of his own design, to explore the mechanics of extremely small things-from nanocrystalline metal films to living cells.