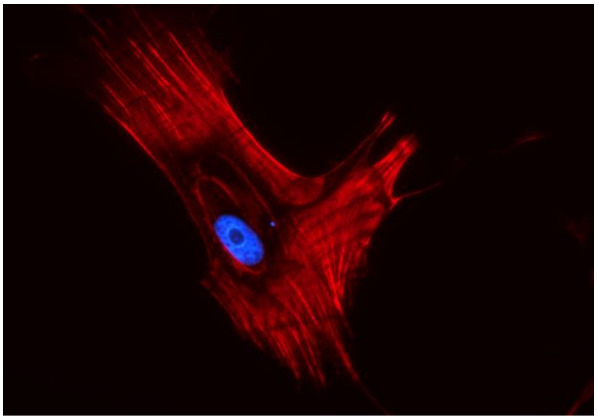
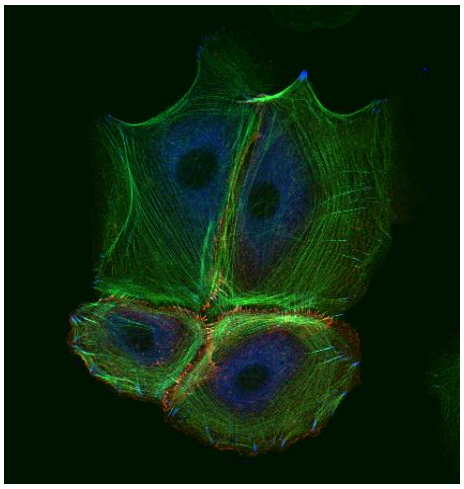
Soft Interfaces IGERT @ Syracuse University





Research Projects

Academic Year 2012-2013

IGERT
INTEGRATIVE GRADUATE EDUCATION AND RESEARCH TRAINEESHIP

NSF

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A Biomimetic Membrane System to Dissect the Role of TNF-α in Inflammatory Disease

Rebecca Bader, Chemical and Biomedical Engineering
Martin B. Forstner, Physics

The tumor necrosis factor alpha (TNF-α) and its receptor play a critical role in the pathogenesis of autoimmune and/or systemic inflammatory diseases, such as rheumatoid arthritis (RA), ankylosing spondylitis, psoriatic arthritis, and Chron's disease. As a result, TNF-α blockade is currently being explored as a treatment strategy to alter the course of these disease states. Despite some promising results, there is considerable variation in regards to efficacy and side effects. For example, only 60% of RA patients respond to TNF-α blockers. Likely, this variability is related to the many unanswered fundamental questions regarding the mechanism of TNF-α signaling. One of which is the relationship between the presentation of TNF-α and the corresponding cellular response. The particular questions that this experimental project tries to answer in the context of the response of RA synovial fibroblasts (Figure 1), the so-called conductors of joint destruction, are: How is cellular response such as cytokine secretion and mRNA expression dependent on the concentration of membrane bound TNF-α? How is this response dependent on the ratio of soluble to membrane bound TNF-α? What kind of dynamic reorganization does the TNF-α receptor undergo upon binding? What effects do specific kind and quantities of TNF-α blocker have on the cellular responses?

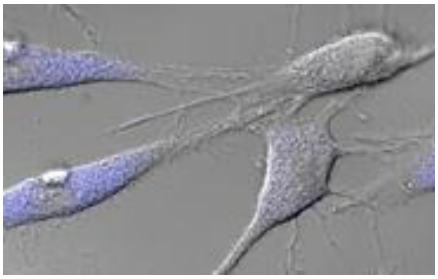


Figure 1: Confocal image of synovial fibroblasts - often called "the conductors of joint destruction" in rheumatoid arthritis.

The design of these experiments draws from the toolbox of several disciplines. For one there is the site specific lipidation of proteins recently developed by the Forstner and Bader Lab. This technique that is based on the insertion of a non-standard amino acid, allows for the creation of large membrane surfaces that present to cells proteins that are membrane bound but yet mobile within the plane of the interface (Figure 2). Since these artificial membranes are supported on planar substrates such as glass, they are ideally suited for investigations involving light based techniques such as quantitative light and fluorescence microscopy. Since this cell-artificial membrane hybrid allows to adjust the content and concentration of the artificial membrane this model for cell-cell contact is perfect for quantitative studies of cellular interactions in a controlled and quantitative way. While strong responses such as apoptosis can be directly assessed under the microscope, the more complex patterns of cytokine secretion and mRNA expression can be readily assessed using multiplex immunoassay on the Luminex Platform.

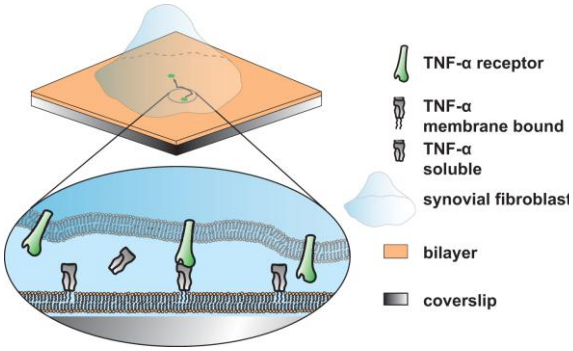


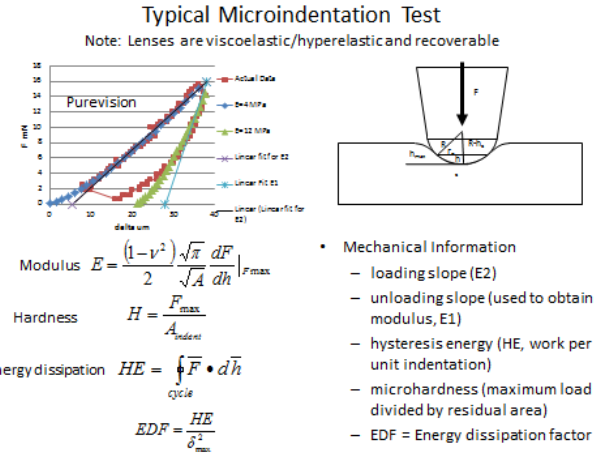
Figure 2: Schematic of the live-cell/supported membrane hybrid. Cells can be exposed to controlled amounts of surface bound and soluble TNF-α

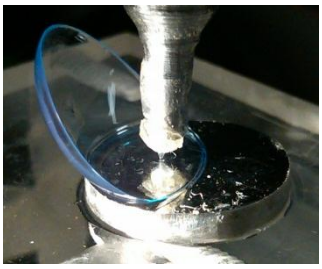
After the successful completion of this research project several milestones and insights will have been reached. On the engineering side a new live cell-membrane hybrid for the quantitative study of RA synovial fibroblast interactions will have been developed and put to use, giving added knowledge of RA pathogenesis. We will have learned about the details of the competitive interactions of soluble and membrane bound TNF-α and the resulting cellular response. In a broad context, these insights can be expected to bring about a new understanding of the role of TNF-α in diseases that are characterized by chronic inflammation.

Title of project*: Surface micro- and nano-mechanics of hydrogel polymers used in contact lenses: Adhesion, friction, modulus and viscoelasticity during dehydration.*

Names and departmental affiliation of faculty involved: Jeremy L. Gilbert, Ph.D., Department of Biomedical and Chemical Engineering, Julie M. Hasenwinkel, Ph.D., Department of Biomedical and Chemical Engineering

Project description: The hydrogel-based polymers used in contact lens applications are a complex mix of multiple different monomers. These silicone and vinyl based hydrogels can have between 40 and 80% water in the fully saturated state. Contact lenses are required to meet a significant set of complex conditions for appropriate long-term performance including good oxygen permeability, stable hydration, some adhesion to the substrate cornea and low friction interaction between the eyelid tissue and the anterior lens surface. Additionally, lens solutions, which are themselves a complex mix of water, boric acid, hyaluronic acid and other constituents including surfactants, may be taken up by the lens and affect performance. To date, the contact lens community does not have suitable surface micromechanical testing methods available to measure local surface mechanics.





The primary goal of this project is to develop new testing methods to directly assess surface micromechanics and nanomechanics of contact lenses. The surface mechanics involved include indentation testing to obtain modulus, hardness and viscoelastic characteristics. These tests, using micron-scale indenters, are highly capable of local property measurement, profiling of properties across lens surfaces, and tracking changes in surface mechanics with hydration state. This project will also develop indentation-based methods to measure frictional interactions and adhesive interactions of the lens surface with an indenter.

This work will be primarily experimental and developmental. The methods to be developed are new for hydrogel lenses. The project will involve test method development, interfacing data acquisition and control software with micromechanical devices (sensors, actuators, etc.) and development of modeling techniques that bring concepts of viscoelasticity to the understanding of surface micromechanical behavior. Additional structural and mechanical characterization methods will be required including scanning electron microscopy, atomic force microscope, dynamic mechanical analysis, and other methods as appropriate.

The structure and properties of the surface of these complex polymer systems in the partially hydrated and continuously changing conditions of the eye are poorly understood. Tear composition varies widely amongst the population with varying amounts of proteins and lipids that may alter surface behaviors. Additionally, the amount of adhesion and the micromechanical properties exhibited by hydrogels, are highly dependent on hydration state and time of contact with the opposing surface. Another element of behavior includes the variation in the polymer structure and properties with location about the surface of the lens and through its cross section. This work will seek to address many of these issues by studying lenses under controlled hydration states and exposed to known environmental conditions.

How Cells Deal with (Mechanical) Stress

James Henderson, Chemical and Biomedical Engineering
Martin B. Forstner, Physics

Mechanical forces acting on a living cell can have a profound impact on its biological function. From embryonic development to controlled cell death, cellular responses to the mechanical properties of the environment have been recognized to play an important role in a plethora of biological functions and cellular regulation. Not surprisingly, perturbations of the mechanisms that couple mechanical signals into the biochemical signaling pathways of cells are indicative of many diseases. In addition, the detailed understanding of cellular mechano-biology in the context of stem cell differentiation and tissue formation is pivotal to make significant progress in tissue and prosthetics bioengineering. Yet, currently there are still many open fundamental questions regarding the mechano-biology of living cells. One of which is the coupling between external mechanic stimuli, changes in cellular mechanics, and cell behavior. The particular questions that this experimental project tries to answer are: How are the stress fields within a cell distributed in space and how do they change over time? How fast and where do cells dissipate stresses arising from external stimuli and changes in their environment? How do these responses depend on the mechanical history?

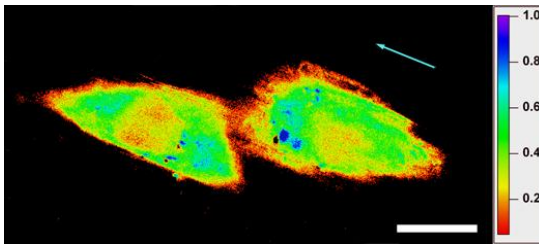


Figure 1: False color image representing the relative stresses in a cell growing on a surface with parallel grooves (groove direction indicated by arrow). Scale bar: 15µm.

The successful execution of this project necessitates the synergetic application of methods from Biology, Physics, and Material Sciences. Firstly, the cellular stress fields need to be determined with high spatial and temporal resolution. To that end, a fluorescence based sensor is introduced in cells of interest. Using multicolor fluorescence microscopy and image analysis one can thus visualize local stresses within those cells (Figure 1). Secondly, in order to induce mechanical perturbation, bio-functionalized active cell culture surfaces of shape memory polymers will be used (Figure 2). These surfaces will change their surface from flat to grooved depending on temperature. This allows for well controlled changes in surface topography and, consequently, mechanical action on surface adhered cells.

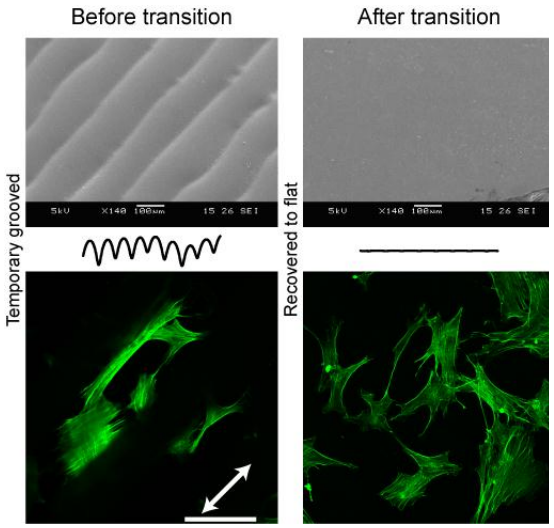
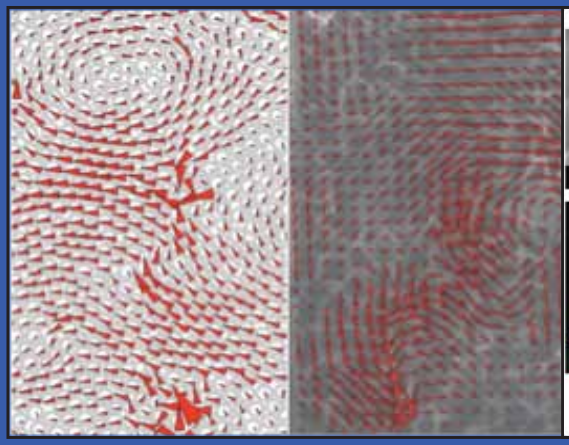


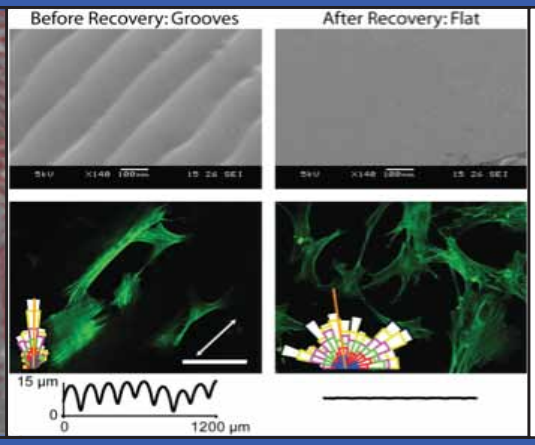
Figure 2: Active cell culture substrate mechanically perturbs cells. Top: Electron micrographs of an active cell culture substrate that can be triggered to transition from a grooved topography to a flat surface. Bottom: Confocal images of stained cells on a temporary grooved topography show microfilaments aligned with groove direction (white arrow) before transition. After transition, microfilaments have rearranged, are randomly oriented, and exhibit apparent stress fiber formation. Scale bar is 100 µm. Traces are profilometry scans of representative samples.

With the successful completion of this research project, several aspects of cellular biomechanics will have been illuminated. Firstly, we will have arrived at general insights in cellular stresses and how they relate to functions such as motility. Furthermore, we will have gained insight in the spatial and temporal mechanisms of externally induced stress dissipation in living cells. These insights are expected to provide unprecedented new understanding of tissue development, maintenance, and disease states. As a result, important new advances in diagnosis and treatment of diseases as well as regenerative medicine can be anticipated.

How do cells move in response to mechanical changes in their environment and interactions with other cells?

Many critical biomechanical events—from early embryonic development to tumor formation—involve biomechanical stimuli changing in complex, non-repeating patterns over periods of minutes, hours, or days. In this project, students will use recently developed shape changing and stiffness changing cell culture substrates to mimic and study biomechanical stimuli experienced by cells during tissue development, disease, and repair. They will be part of a team that cultures cells on shape memory polymer (SMP) substrates, develops image analysis tools to quantify the motility and shapes of cells, applies methods from statistical physics to describe motion and emergent behavior, and generates theoretical and computational models to understand observed behavior and make new predictions. Our long term goal is to improve our ability to control cell behavior during tissue repair/regeneration and increase our fundamental understanding of developmental biology, disease pathogenesis, and wound healing.





We have **positions available** for graduate students who are interested in performing any combination of the following on our interdisciplinary, collaborative team:

- theoretical calculations
- simulations
- experiments

Prof. Jay Henderson (henderson.syr.edu)
Department of Biomedical and Chemical Engineering and Syracuse Biomaterials Institute (SBI)

Prof. Lisa Manning (www.phy.syr.edu/~mmanning)
Department of Physics and SBI

Prof. Cristina Marchetti (www.phy.syr.edu/Marchetti_files/)
Department of Physics and SBI

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Tools and techniques

- biological cell culture
- polymer synthesis
- image analysis and cell tracking
- simulations and "active matter" modeling
- analytical calculations for diffusion rates and phase transitions

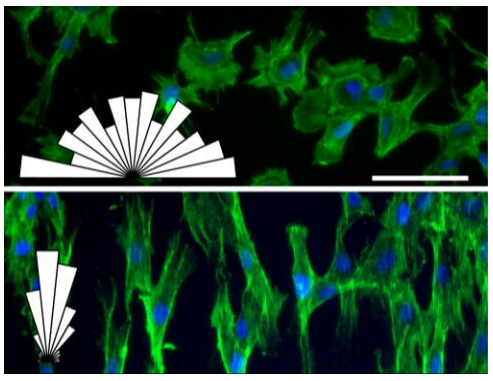
Gradients Smart Materials (**GradSmarts**)

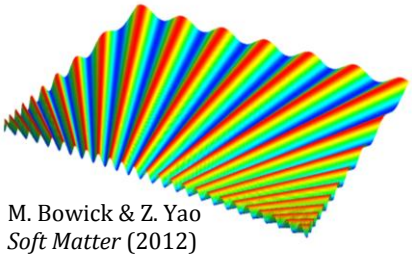
FACULTY MENTORS

James H. Henderson ■ Biomedical and Chemical Engineering
Patrick T. Mather ■ Syracuse Biomaterials Institute
Mark Bowick ■ Physics

Study shape-memory cell culture materials that combine topographic gradients with biochemical gradients.

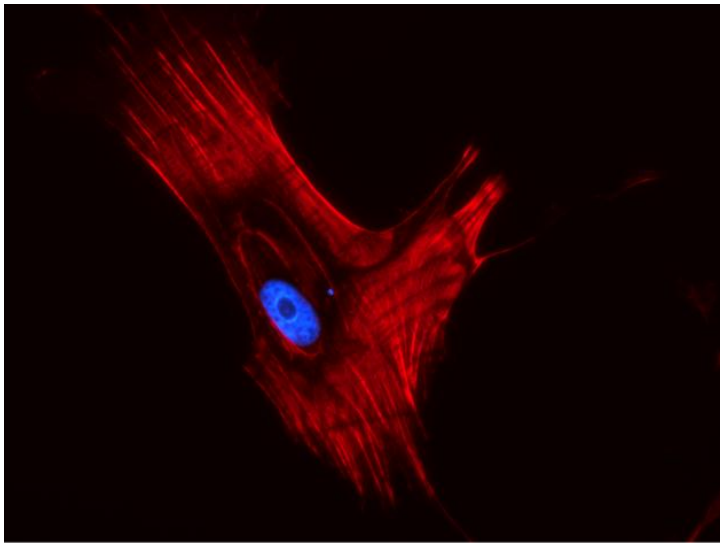
- It is known that properties of the material substrate, such as surface topography, can direct cell behaviors.
- One form of topography that drives droplet or cell motion is a gradient-wrinkle surface (see lower right).
- The combined effects of topography and biochemical signals are poorly understood.
- The labs of the faculty mentors have recently developed the first shape memory polymer substrates that can be programmed to change shape with attached and viable cells.
- The trainee will prepare shape memory polymers that exhibit controlled and switchable topography and gradients of cell-signaling molecules, while modeling the phenomena to guide the work.
- By culturing differentiated (e.g., cartilage cells) or progenitor (e.g., stem cells) cells on such unique materials, the trainee will reveal the combined effects of topography and biochemical gradients on cell behaviors important to tissue development and tissue engineering.
- Outcomes are anticipated to have positive and broad impact in the areas of biomaterials science, cell mechanobiology, tissue engineering, and medical device design.





M. Bowick & Z. Yao
Soft Matter (2012)

We seek ambitious graduate students with interests in biomaterials and biomechanics and interfacial physics



Methodology and expertise to be gained:

- Cell and tissue culture
- Interfacial physics theory
- Cell and molecular biology
- Polymer Science
- Live cell imaging
- Shape memory polymer science
- Experimental techniques
- Computational modeling

The trainee will be well prepared to pursue:

- Research in an academic, industrial, or governmental setting
- Research across multiple fields of engineering, physics, and biology
- Intellectual property development and translation

~ More information is available at henderson.syr.edu, mather.syr.edu, and www.phy.syr.edu/~bowick/ ~

Interplay between tissue mechanics and biochemical signaling during spinal cord development

In normal wild-type zebrafish embryos (and in all other vertebrates), the spinal cord contains distinct classes of neuronal cells that form at precise locations and express specific combinations of transcription factors. In zebrafish, the temporal pattern of differentiation is also important; normal neuronal cells form slowly over time as the surrounding tissue grows, divides, and changes its morphology, and develop a robust "salt and pepper" pattern (Top two panels on the right).

In contrast, "*mindbomb*" (*mib*) mutants have neuronal cells that differentiate early and all at the same time. These embryos lack an important signaling pathway which is conserved across many vertebrates, the Notch signaling pathway. In these mutants, the neuronal cells do not end up in the right pattern (Bottom two panels). An interesting question is whether the difference in differentiation timescales means that the cells experience different mechanical environments, which in turn influences neuronal cell patterning.

In this project, graduate students will work as part of an interdisiplinary team to quantify the spatio-temporal expression patterns of transcription factors, analyze images to quantify cell and tissue shapes, and develop a mechanical and biochemical model that makes predictions about cell patterning. Students can choose to work mostly on experiments, mostly on mathematical modeling, or some comination of the two.

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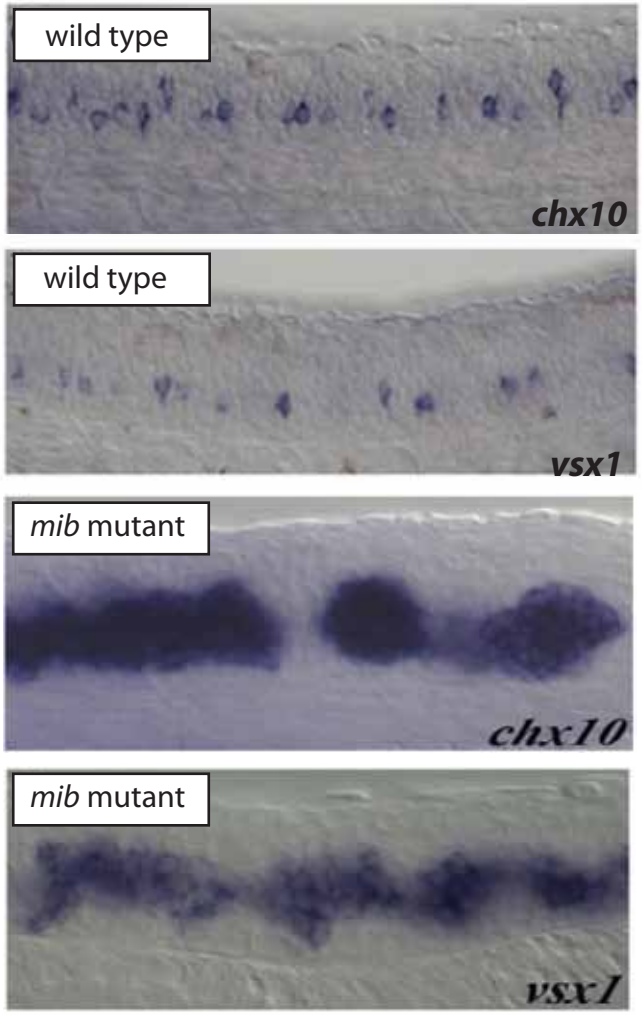


Pattern and morphologies of neuronal cells in developing spinal cords in zebrafish. Staining indicates expression of two different types of transcription factors found in neuronal cells. Top two panels: wildtype embryos show a "salt and pepper" pattern of neuronal cells. Bottom two panels: *mib* mutants have a thick band of neuronal cells.

Goal:
Develop quantitative model based on experimental data to explain how neuronal cell patterning depends on mechanical interactions and signaling

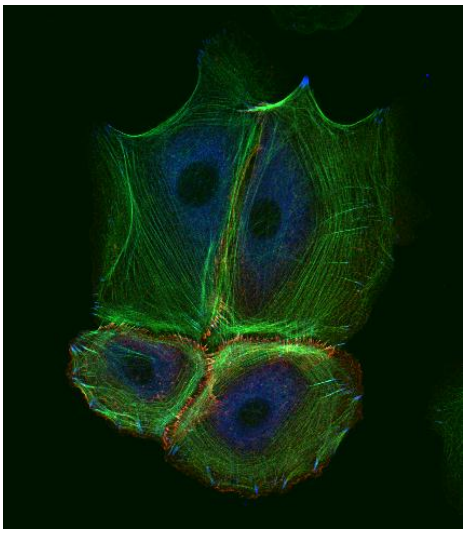
Techniques used by our team:
- zebrafish husbandry
- in situ hybridisation and immunohistochemistry
- image analysis
- computer simulations
- mathematical modeling

Project Leaders:
Prof. Kate Lewis
(biology.syr.edu/faculty/lewis/lewis.htm)
Department of Biology, Syracuse University

Prof. Lisa Manning (www.phy.syr.edu/~mmanning)
Department of Physics, Syracuse University

What controls surface tension and cell sorting in tissue formation and morphogenesis?

Living tissues are soft materials with well-defined mechanical properties, largely unexplored. A key question in morphogenesis is understanding how embryonic structures and organs are formed by segregation of different cell types and the formation of large-scale boundaries among cell compartments. In passive materials, intermolecular forces yield the condensation of molecules into a dense phase and manifest themselves as an emergent material property called surface tension.



Similarly, cohesive interactions between cells, mediated by cadherins and other adhesive molecules, cause them to form dense colonies. The origin of the effective surface tension of cohesive cells is, however, more complex than conventional surface tension. Like in liquids, traction forces in cells and cell colonies appear to be concentrated at the cell or colony boundary, and there is evidence that other mechanisms contribute to the surface tension of the colony, including the contraction of the cytoskeletal actomyosin network, cell migration and biochemical signaling. In addition, some tissues transition from liquid-like behavior to solid-like behavior, allowing buckling phenomena and generating new boundary conditions. In this theory project the student will use a combination of numerical and analytical tools to explore the interplay of all these mechanisms in controlling cell sorting and the emergence of tissue segregation. The goal is to gain a quantitative understanding of the mechanisms that control morphogenesis and to increase our ability to control tissue formation in vitro.

Project Leaders:

- Prof. Lisa Manning (www.phy.syr.edu/~mmanning) *Department of Physics*
- Prof. Cristina Marchetti (www.phy.syr.edu/Marchetti_files/) *Department of Physics & SBI*

Tools:

- Numerical simulations
- Continuum mechanics of "active matter"
- Theory of phase transitions
- Close collaboration with experimentalists

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Soft Nanostructured Interfaces

Mathew M. Maye, Department of Chemistry
Mark Bowick, Department of Physics
Radhakrishna Sureshkumar, Department of Biomedical and Chemical Engineering

In this project, students will first synthesize inorganic nanomaterials, like semiconductive quantum dots or metallic nanoparticles in the chemistry department using standard air-free or aqueous colloidal synthesis procedures. The surface of each nanoparticle will then be modified with an assortment of biomaterials, such as; oligonucleotides (ssDNA), polypeptides, or engineered proteins, using a number of surface chemistry approaches. Then, students will utilize the molecular recognition capabilities of the biological interface to induce 'self-assembly' of the nanoparticles into larger multi-particle assemblies. Some of the main goals in this project include; controlling the interparticle spatial properties (distances), tailoring assembly morphology, phase, or symmetry, and to monitor assembly in-situ. The role of the underlying nanoparticle shape will also be explored. This research thus encompasses the design, fabrication, and implementation of these 'biomimetic' nanoparticles. Due to its broad scope, this project calls for graduate student who wishes to explore both empirical and theoretical experiments in materials chemistry, soft condensed matter physics, and chemical/biological engineering.

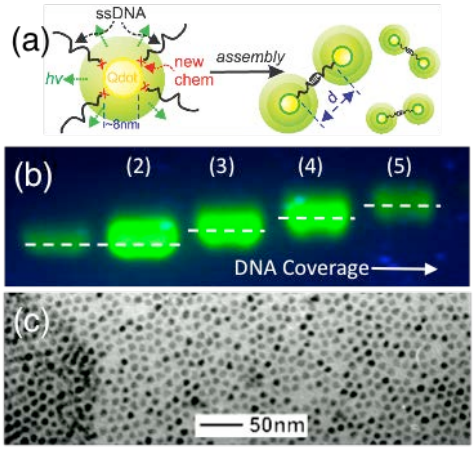


Fig.1: (a) A schematic of the DNA-modified qdots fabricated. (b) Gel result showing tailored DNA-coverage. (c) TEM micrograph of the large qdots. Maye Group. Modified from Reference 1-4.

While the inorganic synthesis of the nanoparticles uses established "hard" chemistry and physics, the functionalization of the nano-interface with biomaterials is less understood by comparison, and involves understanding and modeling "soft" interactions that occur when many biomaterials are bound to a nano-interface in close proximity. Interaction forces, molecular crowding, and cooperative phenomena are often observed. These soft interactions must be both overcome, and harnessed, if successful control of the self-assembly behavior is to occur.

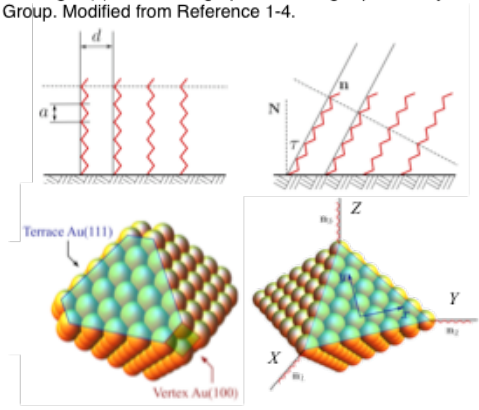


Fig.2: (Top Panel) A schematic of thiol-terminated Alkyl chains at a surface (Top Panel), and illustration of gold nanoparticles with octahedral symmetry. Modified from Reference-5.

In addition to synthesizing the nanomaterials studied, students will also perform modeling of the orientation of the organic or biological monolayer at the nano-interface using models such as the Ginzburg-Landau model (5). The dependence on monolayer phase behavior will be related to the underlying nano-interface faceting, symmetry, and binding chemistry.

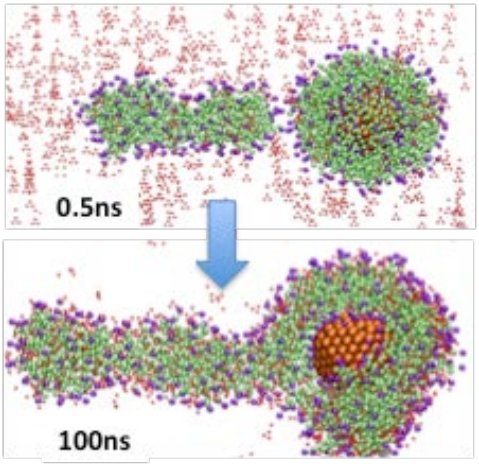


Fig.3: Recent molecular dynamic simulations Modeling surfactant/nanoparticle interactions. See Reference-6 (Sureshkumar Group).

The functionalization and assembly process will also be studied using molecular dynamics simulations. These studies give a time resolved picture that allow for better understanding of how materials are organized at the interface, as well as interactions between the molecules, as well as solvent interactions (6).

Working on this project will allow the student to gain valuable experience in the following fields:

- **Nanomaterial Synthesis & Functionalization**: Synthesis of nanomaterials via traditional colloidal routes as well as modern inorganic and organometallic routes. These particles will be functionalized with; Self-Assembled Monolayers (SAMS), macromolecules/polymers, oligonucleotides, polypeptides, and engineered proteins (Maye Group, Fig.1, Ref 1-4).
- **Morphological & Interfacial Characterization**: Nanoparticle morphology will be studied using: Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Powder X-ray Diffraction (XRD), Atomic Force Microscopy (AFM), UV-visible Spectroscopy (UV-vis), and Fluorescence Spectroscopy. The organic or biological interface will be studied using Infrared Spectroscopy (FTIR), 1D & 2D Nuclear Magnetic Resonance (NMR), dynamic light scattering (DLS), and gel electrophoresis.
- **Self-Assembly*:*** The self-assembly will be monitored and characterized in-situ using dynamic light scattering (DLS), small angle X-ray Scattering (SAXS), TEM, confocal microscopy, and Forster Resonance Energy Transfer (FRET).
- **Modeling the Effect of Nanoparticle Shape on Assembly**: It is expected that the faceted nature of the nanoparticle surface influence the ordering of nanoparticle coatings. In this project we will model and examine the role of edges and vertices as preferential locations for binding and the effect on self-assembly routes (Figure 2, Bowick Group, Ref 5.).
- **Molecular Dynamic Simulations**: The organization of surfactants, monolayers,and biomaterials at the nanoparticle interface will also be modeled using molecular dynamic (MD) simulations. Using coarse-grained (CG) potentials the potential mean force (PMF) values will be calculated, which allow or insights into the time-resolved organization of the coatings at the nanoparticle interface. These new simulations will be used to evaluate long-ranged electrostatic interactions, and solvent polarizability. (Fig. 3, Sureshkumar Group, Ref 6.)

References: (1) *Chem. Mater.* **2011**, 23, 4975–4981; (2) *Langmuir,* **2011**, *27,* 4371–4379; (3) *Nature Nanotech.***, 2010,** *5,* 116-120; (4) *Nature Mater.*, **2009**, *8*, 388-391; (5) arXiv:1111.2244 [cond-mat.soft] (to appear in EPL Letters); (6) *Langmuir* **2012**, (In-Press, dx.doi.org/10.1021/la203745d)

Single-molecule detection of biomolecules using engineered nanopores

Advisor: Prof. Liviu Movileanu, Department of Physics, Syracuse University,

E-mail: lmovilea@physics.syr.edu, Phone: 315-443-8078

Co-advisor: Prof. Phillip Borer, Department of Chemistry, Syracuse University,

E-mail: pnborer@syr.edu, Phone: 315-443-5925

A nanopore may act as an amazingly versatile single-molecule probe that can be employed to reveal several important physical and chemical features of biomolecules, such as nucleic acids and proteins. The underlying principle of nanopore probe techniques is simple: the application of a voltage bias across an electrically insulated membrane enables the measurement of a tiny picoamp-scale transmembrane current through a single hole of nanometer size, called a nanopore. Each molecule, translocating through the nanopore, produces a distinctive current blockade, the nature of which depends on its physical properties of the translocating molecules as well as the molecule-nanopore interaction.

Such an approach proves to be quite powerful, because single small molecules and biopolymers are examined at very high spatial and temporal resolutions. This project will envolve recent developments in molecular genetic engineering, single-molecule biophysics and nanotechnology. The long-term goal of the project is designing hybrid nanofluidic devices that include protein and synthetic nanopores.

From a practical point of view, this methodology shows promise for the integration of engineered nanopores into portable instruments, which would provide a new generation of research tools in nanomedicine and high-throughput devices for molecular biomedical diagnosis.

Project title: Inter-kingdom signaling in cell-surface interactions

Faculty members: Dacheng Ren and Rebecca Bader, Department of Biomedical and Chemical Engineering, Syracuse Biomaterials Institute, Syracuse University

Project description:

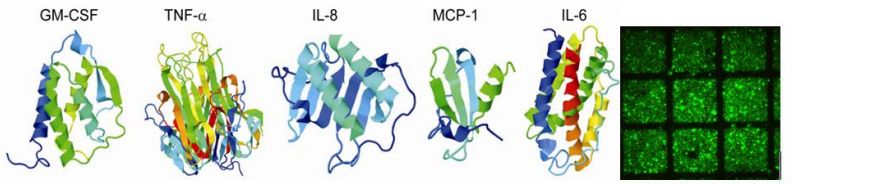
Motivation: The capability of adhesion to surfaces and subsequent proliferation are essential for all cell types, especially during infections of bacterial cells in a eukaryotic host. It is well documented that bacteria can form multicellular structures, known as biofilms, on both abiotic surfaces and host tissues. Interaction between invading bacteria and host cells decide the outcome of the infection and the fate of both organisms. In addition to the formation of multicellular biofilms, it is also well documented that bacteria can enter a dormant stage (due to phenotypic variations, not mutations) and form persister cells, that are extremely tolerant to a variety of unrelated stresses such as antibiotics, heavy metal and heat. Persister formation increases significantly in biofilms, allowing the population to survive the challenge of almost all classes of antibiotics. The surviving persister cells can relapse to normal cells when the antibiotic therapy is discontinued, presenting a great challenge to treatment of infectious diseases.

Inter-kingdom signaling processes play critical roles in bacteria-host interactions. Thus, better understanding and controlling of such processes in biofilm formation and persistence are important for both fundamental study and microbial control. However, these complex systems and processes are associated with structural heterogeneity and temporal and spatial variation in gene expression, both of which are unmet challenges in research.

Research work: Recently, we discovered that some host immune signaling factors have potent effects on persister control. For example, the growth factor granulocyte macrophage-colony stimulating factor (GM-CSF) can render more than 97% of the persister cells of the human pathogen *Pseudomonas aeruginosa* sensitive to antibiotics. In addition, the Ren lab has obtained promising results in biofilm research using patterned biofilm formation by tailoring surface chemistry and surface topography.

In this *experimental* project based on collaboration between the Ren lab and Bader lab, the IGERT fellow will learn state-of-the-art technologies such as surface engineering, bioconjugate chemistry, immunological assays, microscopy, and co-cultures of bacteria and eukaryotic cells. The goals of this project are: (1) characterize the effects of multiple immune factors and reveal the associated mechanisms at both cellular and molecular levels; (2) characterize the effects of synergy between immune factors and other biofilm control agents such as alginate lyase; and (3) modulate the effects of the immune factors through covalent conjugation to poly(ethylene glycol).

This project fits in the area of *Biomaterial Interfaces* of the IGERT program. The results from this project will significantly improve our understanding and control of bacteria biofilm formation and antibiotic resistance.



Project title: Diagnosis and Controlling Chronic Bacterial Infections by Targeting Biofilms

Faculty members: Dacheng Ren (Department of Biomedical and Chemical Engineering, Syracuse Biomaterials Institute, Syracuse University), Juntao Luo (Department of Pharmacology, SUNY Upstate Medical University), Parul Goyal (Department of Otolaryngology, SUNY Upstate Medical University)

Project description:

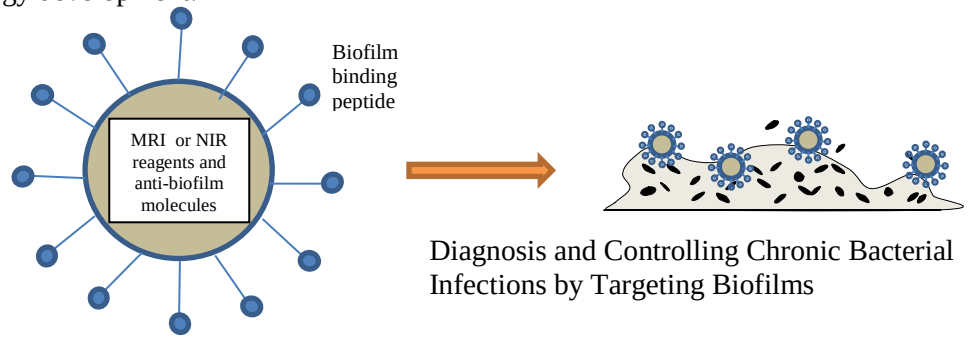
Motivation: The rapid development and spread of multidrug resistant infections present an increasing challenge to public health and disease therapy. As an intrinsic mechanism of drug resistance, formation of surface-attached multicellular structures, known as biofilms, renders bacteria up to 1000 times less susceptible to antibiotics than their planktonic (free-swimming) counterparts of the same genotype. Excessive antibiotic treatment of biofilm infections at sublethal concentrations has been shown to generate antibiotic-tolerant strains. Thus, such intrinsic mechanisms also facilitate the development of resistance through acquired mechanisms that are based on genetic mutations or drug resistance genes. Consistently, biofilms are responsible for 80% of human bacterial infections, especially chronic infections, with high mortality and morbidity.

In addition to the challenges in biofilm therapy, biofilm infections are also difficult to diagnose. Because biofilm cells are attached to host tissues rather than floating in body fluids, currently there is no effective method to detect biofilms non-invasively.

Research work: To address these grand challenges, we will develop new approaches to achieve better diagnosis and treatment by directly targeting biofilms. This project is based on existing collaboration among the faculty members with expertise in biofilm physiology and bacteriology (Dr. Ren), nanotechnology and nanomedicine (Dr. Luo), and first-hand clinical experience (Dr. Goyal). The three faculty members have developed technologies to create micelle systems with functional ligands for specific targeting, and to control biofilm formation through synergistic effects between antibiotics and other chemical and physical factors.

The IGERT fellow will have opportunities to learn molecular biology, nanotechnology, cultures of bacteria and eukaryotic cells, microscopy and work on an animal model. Specifically, the research goals include: (1) Identifying peptides with high binding affinity to bacterial biofilms using phage display; (2) Conjugate these peptides to free dyes and micellar nanoparticles loaded with MRI contrast reagents or fluorescence dye to label biofilms for diagnosis with non-invasive approaches such as MRI and endoscopy; (3) Test the labeled systems for diagnosis of biofilms and targeted drug delivery (anti-biofilm molecules). This will be tested in a co-culture system and a rabbit model to mimic sinusitis as a disease model. The effects of viscosity on biofilm formation, drug resistance and the efficacy of the systems developed in Aims 1 and 2 will be evaluated.

This *experimental* project fits in the research areas of *Biomaterial Interfaces* and *Nanostructured Interfaces* of the IGERT program. It will lead to significant improvement in diagnosis and treatment of chronic bacterial infections. The IGERT fellow will obtain intense training in multidisciplinary research and technology development.



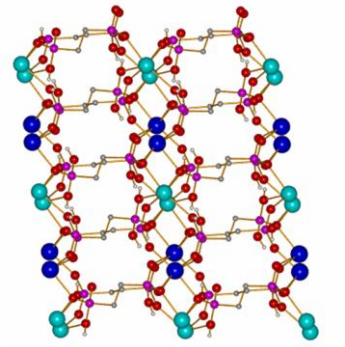
Diagnosis and Controlling Chronic Bacterial Infections by Targeting Biofilms

Biomimetic and bioactive bone templating materials and additives to bone cement based on magnesium and calcium phosphonates

Karin Ruhlandt, Department of Chemistry,
Julie Hasenwinkel, Department of Biomedical and Chemical Engineering

A collaborative project, based on the combined expertise in the preparation of microporous alkaline earth metal phosphonates (KR) and PMMA based bone cement (JH) will yield novel biomimetic materials with wide ranging applications in the biomedical field. Synthetic work as well as characterization of the solids (X-ray crystallography, thermal properties) will be done in the Chemistry Department, while the testing of the novel material, including performance as bone cement additives, biocompatibility and activity will be done in the Biomaterials Institute (JH).

Based on the biocompatible nature of the alkaline earth metals, and the likelihood of biocompatibility in the resulting materials, we are developing new materials for bone templating and as additives to bone cement. We will make use of the flexibility of the phosphonate ligand (i.e., linker length, nature and rigidity) to introduce biomimetic functionality, and design materials exhibiting differently sized pores, to obtain materials for a range of medical applications. We are especially intrigued by the potential of bioactivity, which enables a strong connection between an implant and bone.



The combination of expertise on the preparation of phosphonates (KR, for an example of a recently prepared compound see above) with that on PMMA based bone cements (JH) will significantly increase the application base of the materials, as the phosphonates may be molded into specific shapes for bone templating applications.

The bioactive materials will be included in two-solution bone cements to enhance the connection between the cement, the bone and implants. This material will also be tested for the treatment of vertebral fractures, or enhanced strength of bone cement for total joint replacement fixation though introduction of the phosphonates. It is expected that incorporation of phosphonates in two-solution bone cements will improve the biocompatibility of the cements.

Questions? Please contact:

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Project title: Probing mechanisms of bacterial infection through computer simulations

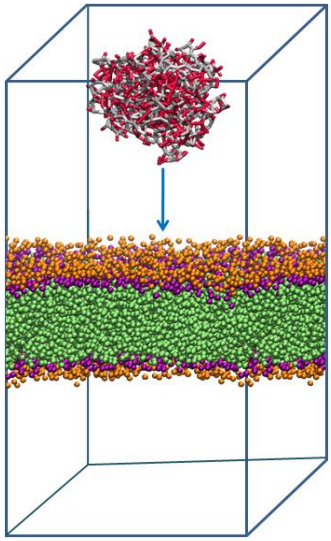
Faculty members: Radhakrishna Sureshkumar, Dacheng Ren and Shikha Nangia, Department of Biomedical and Chemical Engineering, Syracuse University

Project description:

Motivation: A major cause of infection in living cells is the development of a bacterial biofilm in the host tissue. Biofilms are aggregates of bacteria surrounded by a polymeric matrix that can adhere to almost any surface including blood vessels, medical implants, and surgical devices. Once inside the living tissue, the bacterial biofilms interact with the host cells through chemicals known as signaling factors. The signaling factors are typically relatively small polypeptides that have profound effects on the cellular activities of the host. The signaling process is quite complex. Our present understanding of the interactions between the signaling factors and the host cell is far from complete. A better understanding of the physical and chemical mechanisms underlying the signaling process could provide alternative ways to prevent infections.

We use computer simulations to identify the key interactions between the signaling factors and the cell membrane of the host cell. We aim to develop a capability to predict how the structure and composition of the polymeric matrix of the biofilm and chemical structure of the signaling factor affect signal translocation through the biofilm and across host cell interface. Insights from the simulations will be critical in developing prototypes for antibacterial agents.

Research work: In this project, molecular dynamics simulations are currently being used to study the interactions between model cell-membranes and α-tumor necrosis factor (α-TNF), a common signaling factor. To keep the computational cost low, the molecular dynamics simulations are carried out in a coarse-grained representation, which is benchmarked against more detailed atomistic ones. In order to identify the energy barrier associated with the translocation of the α-TNF through the cell-membrane, we perform potential of mean force calculations. Similar calculations are being performed from the α-TNF in a model biofilm matrix.



Initial MD simulation setup for α-TNF and cell membrane interactions. Water molecules are not shown for clarity.

In continuation of the this *computational project*, the IGERT fellow will extend our methodology to (1) other signaling factors, such as- G-CSF, GM-CSF, MCP-1, IL-6, and IL-8, (2) calculate potential of mean force of the binary interactions, and (3) identify similarities in structures and trends in the amino-acid sequence of the signaling factors that enhance the interactions with the host cell.

This computational approach could provide unprecedented molecular level insight into the signaling process between the bacterial biofilm-cell membrane interfaces. This project is a good fit for the *Biomaterial Interfaces* division of the IGERT program.