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2B

CHEMISTRY, BIOCHEMISTRY, AND MATERIALS SCIENCE

Session Moderator: Sharona Gordon, Physiology and Biophysics

MGH 228

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Heterogeneous Production of C-Di-GMP during Early Stages of *P. aeruginosa* Biofilm Formation

Jessica Lynn Parker, Senior, Microbiology
Mentor: Matthew Parsek, Microbiology
Mentor: Catherine Armbruster, Microbiology

Biofilms are surface-associated aggregates of bacteria encased in a protective, extracellular matrix. Biofilm bacteria resist antibiotic treatment and killing by the host immune response, leading to persistence in a variety of chronic infections. *Pseudomonas aeruginosa* is an opportunistically pathogenic bacterium and a model organism for studying biofilm formation. One major factor that drives biofilm formation in *P. aeruginosa* and other bacteria is the intracellular second messenger signaling molecule cyclic diguanylate monophosphate (c-di-GMP). Elevated c-di-GMP levels promote cell surface adhesiveness by up-regulating production of biofilm extracellular matrix components and down-regulating motility genes. In contrast, planktonic *P. aeruginosa* cells are known to have comparatively lower c-di-GMP levels than their biofilm counterparts. While factors contributing to the formation of mature biofilms have been well-characterized, early biofilm formation, when a bacterium first senses a surface and transitions from a planktonic state to a surface-attached state, remains largely understudied. Using a fluorescent, transcriptional reporter of intracellular c-di-GMP and confocal microscopy, we have monitored the dynamics of c-di-GMP production at the single cell level. Our major finding is that c-di-GMP levels are elevated in only a subpopulation of *P. aeruginosa* cells (30-69%) during this time. We have used flow-assisted cell sorting (FACS) to separate surface-attached cells with high and low c-di-GMP, to examine ways in which these populations are physiologically distinct. We have found by lectin staining and by qRT-PCR that surface-associated cells with high intracellular c-di-GMP produce more polysaccharide than their surface-associated, but low c-di-GMP counterparts. We hypothesize that the heterogeneity in c-di-GMP observed during *P. aeruginosa* surface sensing represents a specialization among the genetically homogenous population into subpopulations of non-motile, early polysaccharide producers and motile, surface-exploratory cells, both of which contribute to downstream biofilm maturation.

Studies of the Gating Mechanism of the Pain-Sensing Ion Channel TRPA1

Amanda Qu, Senior, Biochemistry
Levinson Emerging Scholar, Mary Gates Scholar, NASA Space Grant Scholar, UW Honors Program
Mentor: Sharona Gordon, Physiology and Biophysics
Mentor: Gilbert Martinez, Physiology and Biophysics

The protein Transient Receptor Potential Ankyrin type 1 (TRPA1) is an ion channel found in nociceptive (pain-sensing) sensory neurons. TRPA1 is activated by several noxious compounds, including those found in mustard plants, garlic, smoke, and tear gas, among others. It is responsible for the sensation of irritation and pain that these compounds cause, as well as some related chronic pain disorders. A better understanding of TRPA1 could lead to novel therapeutics against chronic pain. For this reason, the mechanism by which TRPA1 activates is an active area of research. TRPA1 contains a coiled-coil domain at its C-terminal end and several ankyrin repeat domains (ARDs) at its N-terminal end. These are both very common repeating protein motifs; ARDs in particular often modulate protein-protein interactions. Many channels in the Transient Receptor Potential (TRP) family, which includes TRPA1, contain these domains, but their role in channel activation is not fully understood. However, a recently solved atomic structure of TRPA1 provided some key insights. The structure showed that TRPA1's coiled-coil domain is tightly enveloped by its ARDs, and they appear to interact with each other. No other TRP channel with a known structure exhibits this unique structural arrangement. My project aims to better characterize the interaction between the ARDs and coiled-coil. I am studying a mutation in the ARDs of the human TRPA1 channel, which is at its interface with the coiled-coil. The mutation, K591E, changes a

key lysine amino acid residue, which is positively charged, to a negatively charged glutamate. It is found in rattlesnake TRPA1, which unlike the human protein is activated by temperatures above 27 C. Current results show that the K591E mutant is active at room temperature, even without any other compounds. A stronger understanding of TRPA1's activation mechanism will be vital to the development of effective next-generation pain therapies.

Correcting for Mass Transport Effects in Bacterial Adhesion Studies

Kayla Marie (Kayla) Hogan, Senior, Bioengineering
Mary Gates Scholar, NASA Space Grant Scholar
Mentor: Wendy Thomas, Bioengineering

With the rising prevalence of drug resistant bacteria, there is a pressing need to develop novel approaches which do not rely upon antibiotics. Anti-adhesive therapies target the ability of bacteria to adhere to tissue via adhesin receptor interactions. Yet, commonly used methods for characterizing these interactions involve parallel plate flow chambers (PPFCs), and are consistently done without consideration of mass transport effects. Here, we demonstrate that measurements of adhered bacteria taken within a large adhesive spot can vary significantly based upon the location measured. A computational model of a PPFC experiment is developed and validated in order to use partial differential equations to determine how various parameters influence spatial variation in adhesion. Use of a computational model allows investigation into a wide range of parameter values which would be impractical to experimentally test. Based upon these results, analytic models are established to accurately predict how transport conditions affect adhesion measurements. Interestingly, the measurement which best demonstrates a spot's affinity for a bacterial ligand is the difference between the concentration of bound bacteria at the start of the spot and the consistent concentration reached well into the spot, according to these models. This is validated through PPFC experiments. Additionally, we demonstrate that within a multispot array, the adhesion in the spot of interest is highly affected by the preceding spot. It is vital that experimenters recognize this issue while interpreting their data. Guidelines to help experimenters set up and interpret their data in a manner that accounts for mass transport effects are described. Ultimately these guidelines could help reduce or negate the systematic error which mass transport introduces to adhesion experiments, and lead to better anti-adhesive therapies.

Hexanoyl-Chitosan-PEG Copolymer Coated Iron Oxide Nanoparticles for Hydrophobic Drug Delivery

Guanyou Lin, Senior, Bioen: Nanoscience & Molecular Engr
Levinson Emerging Scholar, Mary Gates Scholar, UW Honors Program
Mentor: Miqin Zhang, Materials Science & Engineering

Paclitaxel (PTX) is one of the most commonly applied chemotherapeutic drugs to treat cancers. PTX inhibits mitotic spindle assembly and chromosome segregation so that cancer cells cannot proliferate normally. While highly effective in arresting cancer development, PTX is extremely hydrophobic and insoluble in physiological environment. Hence loading PTX onto a bio-compatible and water soluble drug carrier is important. Here we report a nanoparticle formulation that can effectively load and stabilize PTX, and transport it into cells. To synthesize the nanoparticle drug delivery systems, iron oxide nanoparticles were first nucleated, grew and capped with oleic acid to form oleic acid coated iron oxide nanoparticles (IONP-OA). Amphiphilic triblock copolymer hexanoyl-chitosan-PEG (CP6C) was made by attaching methoxy polyethylene glycol and hexanoic anhydride to chitosan backbone. CP6C was coated onto IONP-OA through hydrophobic interaction between oleic acid and hexanoyl groups. PTX was loaded in the hydrophobic region between the two groups. Then, chlorotoxin (CTX) as the targeting ligand for brain tumor was conjugated onto nanoparticle through a heterobifunctional linker. The desired product structure (CTX-PTX-NP) was confirmed by TEM, FT-IR and 1H NMR. Characteristic FT-IR and 1H NMR peaks of hexanoyl and mPEG were observed. The product nanoparticles were stable in size (˜50 nm) after a 4-week incubation. Importantly, the PTX loading efficiency was 31.3% PTX/Fe weight to weight ratio determined by HPLC and ferrozine assay. The product showed only minimum drug leak in aqueous solution, indicating that the hydrophobic drug was successfully encapsulated and stabilized in nanoparticle. Furthermore, when applied to glioma cells U118MG, the nanoparticles showed robust cellular uptake and were able to inhibit 80% cell growth in vitro. In summary, our nanoparticle can successfully load hydrophobic drug PTX and delivery it into glioma cells effectively.

Development of a Nanofiber Micronization Process for Water-Soluble Drug Delivery

Christina Nhan, Senior, Bioengineering
Mary Gates Scholar, NASA Space Grant Scholar
Mentor: Kim A. Woodrow, Bioengineering
Mentor: Rick Edmark, Bioengineering

Current approaches to deliver prophylactic drugs against HIV are ineffective for many reasons, including drug leakage, low drug loading, and lack of drug release tunability. To protect against HIV infection, a versatile prophylactic drug deliv-

ery platform that offers flexible dosing schedules would be an attractive and empowering option for many women. In the Woodrow Research Group, we have developed a fiber-in-fiber (FIF) drug delivery platform that addresses these requirements. The primary benefit of FIF is that it provides both immediate and prolonged drug release to protect against HIV acquisition. To fabricate the FIF platform, two nanofibers are used: a rapidly dissolving burst release fiber with encapsulated, slowly-degrading sustained release nanofibers. To preserve the release properties of the sustained release nanofibers and to ensure that they are of an imperceptible size to the user, we micronize the sustained release nanofiber in a blender. While this process is effective for water-insoluble drugs, water-soluble drugs like tenofovir leak out during micronization. Thus, improving the encapsulation of water-soluble drugs in the sustained release nanofiber component is needed to expand the functionality of FIF. I have developed a new strategy using dry micronization to improve the encapsulation of water-soluble drugs, allowing the FIF system to deliver diverse classes of drugs to protect against HIV acquisition. Retention of water-soluble drug drastically increased from 0% using previous strategies to 90% with dry micronization. Current experiments are assessing the drug release profiles of the dry micronized fibers compared to those intact nanofibers. SEM images of dry micronized fibers show that nanofiber architecture still exists, meaning that the benefits of nanofiber technology apply to dry micronized fibers. Therefore, dry micronized fibers have the potential to be their own drug delivery platform that can be delivered orally, intravenously, as well as intravaginally as a part of the FIF system.

Immune Modulation of Electrospun Nanofibers on Dendritic Cell Activation

Namratha Potharaj, Senior, Bioengineering
Levinson Emerging Scholar, Mary Gates Scholar, UW Honors Program
Mentor: Kim A. Woodrow, Bioengineering
Mentor: Jaehyung Park, Bioengineering

Vaccines save approximately 2.5 million lives every year, and vaccine development is an ongoing area of research in immunology. A vaccine is formulated with antigen and adjuvant, the latter of which enhances the host immune response to an antigen via stimulation of antigen presenting cells (APCs) like dendritic cells (DCs). Only a few adjuvants are currently approved for clinical use, posing a significant obstacle for new vaccine development. Multiple studies have investigated a diverse array of biomaterials and highlighted chemical analogues of pathogen associated molecular patterns recognized by immune cells. However, the immunogenic effects of physical properties like stiffness and porosity, which play an important role in cell function, are not fully understood. The goal of this project is to investigate how the stiffness and porosity of electrospun nanofibers modulate DC activation states. Recent studies showed that macrophages exhibit higher activation on stiffer substrates. Both macrophages and DCs are APCs derived from myeloid progenitors, thus motivating the hypothesis that stiffer nanofiber meshes could also induce higher DC activation. We investigated poly (vinyl alcohol) (PVA) and chitosan (CS) nanofibers that were cross-linked to modulate stiffness and porosity while also improving stability in water for cell studies. PVA and CS nanofibers were both thermally cross-linked at 150C. PVA nanofibers were additionally treated with methanol for 8 hours. Thermal treatment did not cause a significant change ($p>0.05$) in the stiffness of the PVA nanofibers whereas combined methanol and thermal treatment for 20 minutes produced PVA fibers that were twice as stiff. Cross-linked nanofibers will be incubated with murine bone marrow-derived DCs for cell viability and DC activation studies. DC activation state will be measured by cytokine secretion along with CD86 and CD80 surface marker expression. The results from this study have the potential to guide engineering of immune-modulating biomaterials for novel adjuvant development.

Characterization of High Internal Phase Emulsions

Andreas M. Averkiou, Senior, Chemical Engineering
UW Honors Program
Mentor: John Berg, Chemical Engineering

This work introduces a new format for topical skin care products since current products have an oily feel and often leave an unpleasant residue. This new format is known as a High Internal Phase Emulsions (HIPE) with an aqueous dispersed phase and silicone continuous phase. Due to its unique structure and responses to shear forces (i.e. the type of force used when applying lotion to skin) this novel liquid structure spreads on the skin instantaneously which produces a pleasant cooling feeling and leaves behind no residue. The dispersed phase (i.e. water) in HIPEs occupies between 74% - 96% of the total volume as droplets, while the continuous phase (i.e. silicone) is pushed into thin films or even small droplets between the dispersed phase, producing a system of structured water droplets. Since the favorable spreading properties of HIPEs arise from this unique packing of water and silicone, determining the size distribution of the water droplets and how this affects its response to shear forces, allows me to quantify what makes the application of these HIPEs pleasant. I prepared HIPEs that were 80% and 90% by volume water at different speeds of mixing (800-1200 RPM) using a planetary mixer. After the HIPEs were made I imaged them using differential interference contract (DIC) microscopy and cross polarized microscopy. Using these images I measured the bubble diameters and statistically analyzed the data set with the computer softwares ImageJ and JMP respectively, to obtain size distributions. I found that as the speed of mixing increased the average droplet size decreased. I then

probed the HIPE's responses to shear forces of different magnitudes and frequencies using oscillatory rheology. I have related the HIPE's internal structure to how it responds when it is spread on the skin for different size distributions and different volume fractions.