SCHOOL OF MEDICINE & DENTISTRY
UNIVERSITY of ROCHESTER MEDICAL CENTER

2022 PREP Symposium

June 14, 2022

Program Directors

Jacques Robert, PhD & Elaine M. Smolock, PhD

Presentations

Time	Name	Title; *Advisor*
1:00pm-1:15pm		**Introduction**
1:15pm-1:30pm	**LSLC Internship overview**	***Advisor: Danielle Alcena-Stiner, PhD***
1:30pm-1:45pm	**Helen Chan**	**Evaluating the Efficacy of MB-PDT against Gram-Positive and -Negative Bacteria in Hydrogel and Silicone Models** ***Advisor: Martin Pavelka, PhD***
1:45pm-2:00pm	**Bianca Flores**	**Azoles May Target Underlying Mechanism(s) of Localized Provoked Vulvodynia** ***Advisors: Megan Falsetta, PhD***
2:00pm-2:15pm	**Jaclyn McCoy**	**The Effects of *V. cholerae* Type Three Secretion System Activities on Host Cell-Cell Junctions** ***Advisor: Michelle Dziejman, PhD***
2:15pm-2:30pm	**Tanique McDonald**	**Developing a Voluntary Head Restraint Method to Visualize Orientation Domains in V1 of Macaques** ***Advisor: Farran Briggs, PhD***
2:30pm-2:45pm	**Jia Mei**	**Antimicrobial Activity of a Triple Antibiotic Combination Toward Ocular *Pseudomonas aeruginosa* Clinical Isolates** ***Advisor: Paul Dunman, PhD***
2:45pm-3:00pm		**Questions and Reception**

Evaluating the Efficacy of MB-PDT against Gram-Positive and -Negative Bacteria in Hydrogel and Silicone Models

Helen Chan and Martin Pavelka

An abscess is the formation of a “wall” to encapsulate polymicrobial bacterial and fungal microorganisms to further prevent infection. A deep tissue abscess could arise from post-operative infection, abdominal trauma, or the rupture of organs. Depending on the severity of the abscess, the effects of this infection could range from mild morbidity to mortality. If left untreated, the mortality rate could range from 45-100%. The current treatment for deep tissue abscess is the combination of ultrasound/CT guided drainage and broad-spectrum antibiotics. If the state of the abscess does not improve with standard therapy, there could be a risk of antibiotic resistance and serious sequela. Thus, the need to find an alternative therapeutic is crucial. Photodynamic therapy (PDT) is the use of a non-toxic photosensitizer, light, and molecular oxygen to inactivate microorganisms. One mechanism by which photodynamic therapy creates oxidative burst is the type I mechanism. In the type I mechanism, electrons are transferred to surrounding substrates to produce oxygen radicals such as hydrogen peroxide and superoxide radicals to oxidize biomolecules. A large production of reactive oxygen species in the cell could lead to oxidative burst. An advantage of this treatment is that the photosensitizer molecules specifically damage the targeted species without harming the host cells. Many studies have looked at the effects of this treatment against planktonic and biofilm bacterial states. However, not many have looked at the potential of this treatment in an anatomical model such as an abscess. In the present study, we explored the hypothesis that methylene blue photodynamic therapy is effective against Gram-positive and -negative bacteria in an artificial 3-D abscess model. To test this, we grew mature biofilms on hydrogels and sterile food-grade silicone then subjected them to photodynamic therapy treatment. In contrast to control experiments with planktonic cells, both gram-positive and -negative bacteria biofilms are showing resistance to PDT treatment. In this study, we hope to better understand the advantage of using an alternative treatment model to treatment deep tissue abscesses.

Azoles May Target Underlying Mechanism(s) of Localized Provoked Vulvodynia

Bianca Flores and Megan Falsetta

Vulvodynia is a crippling disease that drastically reduces the quality of life in women, defined as chronic pain localized to ring of tissue surrounding the vaginal opening known as the vulvar vestibule. Vulvodynia is a diagnosis of exclusion; diagnosis is based on otherwise unexplained pain lasting greater than 3 months. Thus, the mechanisms of disease are poorly understood. We have new evidence suggesting the cytochrome P450 (CYP450) family of enzymes is involved in the vulvodynia mechanism and may represent a new therapeutic target. We aimed to narrow down the list of possible enzymes within this nearly 50-member family that might relieve vulvar pain if targeted. We used a validated primary human fibroblast culture system where each woman serves as her own control. In this model, fibroblasts are cultured from affected and unaffected sites in patients. In addition, fibroblasts are cultured from the same sites in women without the disease. Proinflammatory mediators prostaglandin E_2 (PGE_2) and interleukin-6 (IL-6) serve as surrogate measures of pain in this model. We evaluated the role of CYP450 in vulvar pain by antagonizing specific family members to (1) determine if CYP450 is a reasonable target for therapeutic intervention, (2) pinpoint family members of interest, and (3) evaluate if azoles used to treat vulvovaginal yeast infection, which are known to target CYP450, would have therapeutic effect for vulvodynia. We found that miconazole, which is commonly used to treat vulvovaginal yeast infection, reduces pro-inflammatory mediator production in fibroblasts. Miconazole targets CYP2C9, CYP2C19, CYP3A4, and CYP3A7, suggesting their involvement. As we uncover additional pieces of the vulvodynia mechanism, we identify new avenues for improved patient care. Based on published clinical data and our in vitro findings, azoles may have a role in treating vulvodynia.

The Effects of *V. cholerae* Type Three Secretion System Activities on Host Cell-Cell Junctions

Jaclyn McCoy, Katharine Tomberlin, and Michelle Dziejman

Cholera is an infectious disease that induces watery diarrhea and vomiting, causing severe dehydration that can lead to hypovolemic shock and death. The disease is caused by the Gram-negative bacterium, *Vibrio cholerae*, and usually infects the human host via contaminated drinking water. *V. cholerae* strains are very diverse and can be classified based on the O-antigen, a highly variable component of the lipopolysaccharide. Strains from the O1 and O139 serogroups can cause epidemic cholera, but strains from non-O1 and non-O139 serogroups can also cause cholera. While pilus mediated colonization (by the toxin co-regulated pilus, TCP) and secretory diarrhea caused by cholera toxin (CT) are characteristic virulence strategies found in O1 and O139 serogroup strains, some pathogenic non-O1 and non-O139 serogroup strains encode a Type 3 Secretion System (T3SS) as an essential virulence factor instead of TCP and CT. Our studies focus on the T3SS-positive *V. cholerae* strain named AM-19226, which was clinically identified in 2001 in Bangladesh. We are interested in studying T3SS-mediated pathogenic mechanisms of *V. cholerae* and defining host responses in order to understand how the T3SS facilitates colonization and disease in the absence of TCP and CT.

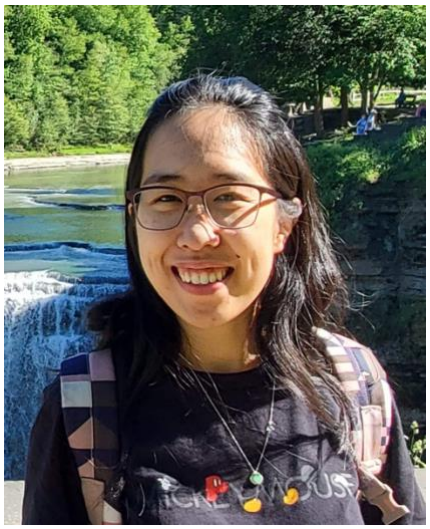
We performed an RNA transcriptome analysis of Caco2-BBE cells co-cultured with strain AM-19226, to identify T3SS dependent changes in Caco2-BBE gene expression. Co-culture with a T3SS-null strain was used for comparison. Bioinformatic analyses revealed several interesting categories of genes that were differentially regulated. We focused further studies on eight genes of interest, which encode proteins involved in the formation, maintenance and reorganization of intestinal epithelial cell tight junctions, adherence junctions, and focal adhesions. We are interested in investigating cell-cell junction disruption as a component of T3SS-mediated disease in CT-negative strains of *V. cholerae* as some bacteria, such as *Yersinia pseudotuberculosis*, cause diarrheal disease in part by disrupting intestinal cell homeostasis and altering junctional integrity.

We used two approaches to further investigate the eight genes of interest. RT-qPCR was completed to verify that genes identified as differentially regulated by transcriptome studies showed similar, T3SS dependent changes in expression levels. Western blot analysis was then performed to determine whether associated protein levels were changed. Based on our findings, at least one gene, THBS1, important for maintaining focal adhesions, shows T3SS dependent differential expression. The results suggest that one strategy used by T3SS-positive *V. cholerae* to cause disease involves targeting focal adhesion proteins to alter epithelial cell dynamics.

Developing a Voluntary Head Restraint Method to Visualize Orientation Domains in V1 of Macaques

Tanique McDonald, Marvin Doyley, and Farran Briggs

Orientation domains describe the columnar organization of orientation-selective neurons that by definition exhibit peak activation when presented a light stimulus (i.e., bars and lines) at a specific orientation. Although orientation domains are a unique facet of early visual processing, they continue to remain enigmatic with respect to the *in vivo* functional architecture. In the traditional approach to studying vision, invasive immobilization of the head is utilized for primate behavioral training on simple visual fixation tasks. Here, we describe a novel, non-invasive, voluntary head restraint method for primate behavioral training. With this method, we demonstrate the successful performance of visual fixation tasks with a non-head restrained primate. We propose that the voluntary head restraint method is a viable behavioral training technique that can likely be used to construct three-dimensional maps of orientation domains in awake macaques using functional ultrasound.



Antimicrobial Activity of a Triple Antibiotic Combination Toward Ocular *Pseudomonas aeruginosa* Clinical Isolates

Jia Mei, William Johnson, Bailey Kinn, Emily Laskey, Lydia Nolin, Pratham Bhamare, Charlotte Stalker, Pauls Dunman, and Rachel Wozniak

Purpose: *Pseudomonas aeruginosa* is a leading cause of corneal infections. Recently, we discovered an antimicrobial drug combination, polymyxin B/trimethoprim (PT) + rifampin, that displayed impressive efficacy toward *P. aeruginosa* in both in vitro and in vivo studies. As such, this combination was further evaluated as a potential keratitis therapeutic through testing the combination's efficacy against a diverse set of *P. aeruginosa* clinical isolates. **Methods:** Minimum inhibitory concentrations (MICs) of moxifloxacin, levofloxacin, erythromycin, tobramycin, PT, polymyxin B (alone), trimethoprim (alone), and rifampin were determined for 154 ocular clinical P. aeruginosa isolates, 90% of which were derived from corneal scrapings. Additionally, the efficacy of PT + rifampin was evaluated utilizing fractional inhibitory concentration (FIC) testing. **Results:** While 100% of isolates were resistant to erythromycin (average MIC 224 ± 110 μg·mL-1) and trimethoprim (alone) (206 ± 67.3 μg·mL-1), antibiotic resistance was generally found to be low: moxifloxacin (2% of isolates resistant; average MIC 1.08 ± 1.61 μg·mL-1), levofloxacin (3.9%; 1.02 ± 2.96 μg·mL-1), tobramycin (1%; 0.319 ± 1.31 μg·mL-1), polymyxin B (0%; 0.539 ± 0.206 μg·mL-1), PT (0%; 0.416 ± 0.135 μg·mL-1), and rifampin (0%; 23.4 ± 6.86 μg·mL-1). Additionally, FIC testing revealed that PT + rifampin eradicated 100% of isolates demonstrating additive or synergistic activity in 95% of isolates (average FIC index 0.701 ± 0.132). **Conclusions:** The drug combination of PT + rifampin was effective against a large panel of clinically relevant *P. aeruginosa* strains and, as such, may represent a promising therapeutic for *P. aeruginosa* keratitis.

Publication: Mei JA, Johnson W, Kinn B, Laskey E, Nolin L, Bhamare P, Stalker C, Dunman PM, Wozniak RAF. Antimicrobial Activity of a Triple Antibiotic Combination Toward Ocular Pseudomonas aeruginosa Clinical Isolates. Transl Vis Sci Technol. 2022 May 2;11(5):26. doi: 10.1167/tvst.11.5.26. PMID: 35612831; PMCID: PMC9145016.

Thank you to everyone involved in URMC-PREP!

Mentors, Bench Mentors, & Committee Members

Administration
Chloe Wise, Daisy Bird Geer, & Benjamin Lovell

Life Sciences Learning Center
Danielle Alcena-Stiner, PhD & Dina Markowitz, PhD

Office of Equity and Inclusion
Adrienne Morgan, PhD and John Cullen, PhD

SCHOOL OF MEDICINE & DENTISTRY
UNIVERSITY of ROCHESTER MEDICAL CENTER