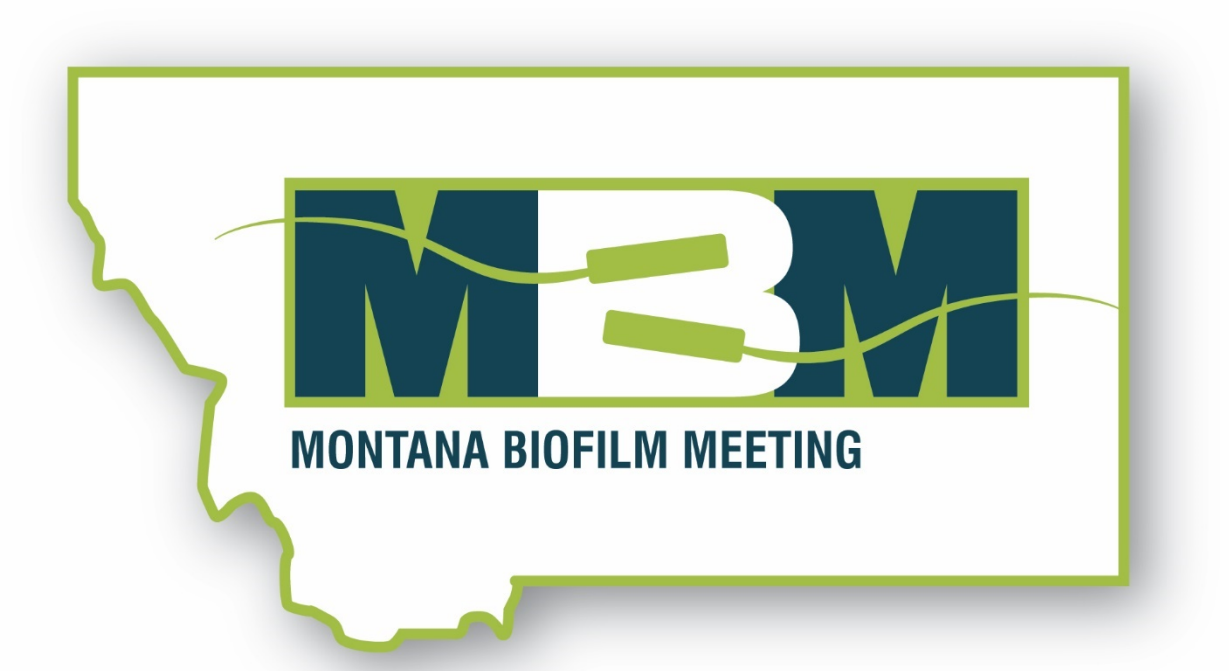


PROCEEDINGS

JULY 16-18, 2019

Bozeman, Montana

Table of Contents

SESSION 1: Biofilm Methods

SESSION 2: Wound Biofilms

SESSION 3: Alternative Biocides

Table of Contents

Session 6: Biofilms in Space

Special: Joint Biofilm Workshop

Poster Abstracts

Guest Posters

Center for Biofilm Engineering Posters

Table of Contents

SESSION 1: Biofilm Methods

Proposed standard method for antimicrobial urinary catheters: ruggedness test results

Presenter: **Darla M. Goeres**[1,2], Associate Research Professor
Co-authors: Jennifer Summers[1], Philip S. Stewart[1,2], Garth James[1], Albert Parker[1], Paul Sturman[1], K. Scott Phillips[3]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
[3]US Food and Drug Administration, Silver Spring, Maryland, USA.

Urinary catheters are a critical medical device in modern medicine, used in almost every healthcare setting worldwide. Catheter associated urinary tract infections (CAUTIs) account for 37% of all healthcare associated infections. Many surface modifications, such as antimicrobial coatings, have been proposed in the literature, although we are not aware of any at present that have been shown to effectively reduce CAUTI. A variety of test methods exist to evaluate the efficacy of surface modified urinary catheters, but there is no validated *in vitro* standard method. The Intraluminal Catheter Model (ICM) was developed to evaluate the efficacy of surface modifications to inhibit biofilm growth on the catheter lumen. The ICM was subjected to a rigorous statistical evaluation of its ruggedness, specifically assessing how the bacterial log density and log reduction changed with small adjustments to key operational factors. Five operational factors were varied: inoculum concentration, flow of medium through the catheter, pH of the artificial urine medium (AUM), temperature of the incubator, and biofilm removal technique. The results of the analysis highlighted that biofilm growth is most sensitive to changes in pH, which indicates that the growth medium must be optimized to increase the method's ruggedness. The analysis also demonstrated that sonication was more efficient than scraping as a means to harvest biofilm from the catheter surface. With further optimization of the procedure, the ICM has the potential to become a useful tool to evaluate the efficacy of surface modified urinary catheters. The development of a standardized *in vitro* method which better simulates some of the key physiological parameters necessary for bacterial pathogenesis of CAUTI may aid in the development of new anti-biofilm technologies, as well as providing a standard method that regulators could require for more accurate preclinical testing of devices prior to costly animal studies.

Monitoring hand hygiene and its effects on healthcare-associated infections

Presenter: **Albert Parker**[1,2], Biostatistician
Co-Authors: James Arbogast[3]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Mathematical Sciences, Montana State University, Bozeman, MT, USA.
[3]GOJO Industries, Akron, Ohio, USA.

The spread of microbes, including multi-drug resistant microbes, occurs mostly via contaminated health care workers' hands and surfaces (WHO 2019). Thus, healthcare workers (HCW) are required to perform hand hygiene at several different moments when interacting with patients. HCWs are hand hygiene compliant when they perform hand hygiene at all of the required moments. Clearly, it is important for healthcare facilities to maintain high hand hygiene performance rates (defined as the total number of hand hygiene events divided by the total number of moments requiring hand hygiene). Unfortunately, in the absence of rigorous hand hygiene programs, HCW hand hygiene performance rates can be low. A 4-year retrospective observational study was performed in a single 93-bed hospital to determine whether an automated hand hygiene monitoring system (AHHMS) plus 3 supplementary strategies increased hand hygiene performance rates. Time series analysis, that accounted for seasonal trends in the data, showed that just implementation of the AHHMS did not yield a sustained improvement in hand hygiene performance rates. But implementation of an AHHMS, when combined with supplementary strategies as part of a multimodal program, did result in an 85% increase ($P < .0001$) in hand hygiene performance rates (Figure 1) (Boyce et al. 2019). Over the same period, the incidence density of non–*Clostridioies difficile* healthcare-associated infections decreased by 56% ($P = .0841$), while *C. difficile* infections increased by 60% (P =

.0533). Vector autogressive models were applied to assess possible time-lagged relationships and Granger causality between hand hygiene performance rates and healthcare associated infection rates. Failure to find a clear relationship between increased HH performance rates and reductions in HAIs underscores that there are many factors that contribute to HAIs, for example antimicrobial stewardship activities, compliant glove use when caring for patients with *C. difficile* infections, handwashing practices, and surface disinfection protocols (Boyce et al. 2019).

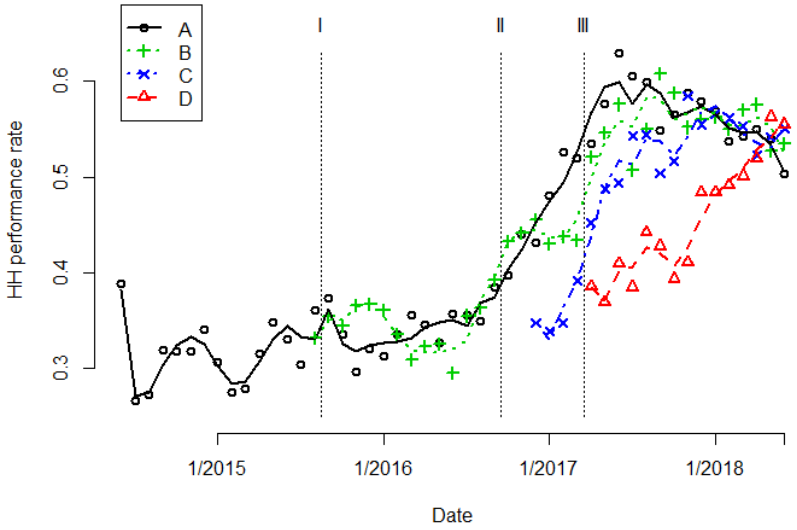


Figure 1: Hand hygiene performance rates over 3 different interventions. HH performance rates for 4 different units in the same hospital are indicated by different colored symbols. Trend of the HH performance rates are indicated by different colored curves. The vertical dashed lines indicate when 3 interventions occurred.

Product-specific method development to assess microbial contamination

Presenter: **Christopher J. Jones**, Director
Affiliation: Research and Development, Sharklet Technologies, Aurora, CO, USA.

Microbial contamination of surfaces serves as a vector of transmission, contributing to the spread of disease. Sharklet has developed a microtexture that can be applied to surfaces in order to limit the microbial transfer and contamination on abiotic surfaces. The ultimate goal of Sharklet microtexture is to reduce transfer of pathogens and limit the spread of disease. Standard test methods are useful for ensuring equivalence of protocols and results across multiple replicates, operators, and laboratories. Several internal standard test methods have been developed to assess the efficacy of the Sharklet microtexture to reduce microbial transfer and contamination. These methods aim to replicate the normal use-case and mimic the microbial contamination of common surfaces. The test methods can be loosely grouped by the methods of inoculation and sampling. Samples are contaminated with microbes through touch-transfer, immersion, or aerosol. Sampling of surfaces occurs via touch-transfer, immersion with agitation, or sampling with swabs. Each of these methods has advantages and disadvantages, and selection of method is driven by final product use, size, shape, microbial contaminants, and repeatability. Sharklet microtexture efficacy was determined by comparing the performance of Sharklet and smooth flat silicone films utilizing four internal test methods. Each surface was tested with a variety of microbes representing a wide range of pathogenic contaminants. These methods are useful for evaluating surface technologies and mimic many of the common uses for Sharklet products, however not all products consist of two-dimensional surfaces. As such, it is essential to adapt these protocols to test three-dimensional products as well. These product-specific adaptations can result in the development of novel standard test methods. In order to test touch-transfer of microbes onto three-dimensional objects, we developed a bead-based microbial transfer method. This method has high repeatability, measured by the standard deviation of contamination on a smooth prototype product with several species of microbes. These data indicate that the bead-based method is a suitable and reliable approach to touch-

transfer contamination of three-dimensional objects. Testing of production samples demonstrates the efficacy of Sharklet micropattern on these products. In addition, these methods can be utilized to reliably contaminate surfaces with a variety of microbes, which is useful in testing many types of treatments designed to reduce microbial transfer and the spread of disease.

Interlaboratory study results for the drip flow reactor

Presenter: **Diane K. Walker**, Research Engineer
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Precision and bias statements are an important component in standardized methods as they provide information about the variability that can be expected when a method is used across laboratories. The objective of an interlaboratory study (ILS), therefore, is to collect data that describe the repeatability and reproducibility of a test method and the bounds within which the data is considered acceptable. The results of a recently conducted ILS for ASTM E2647 Standard Test Method for Quantification of a *Pseudomonas aeruginosa* Biofilm Grown Using a Drip Flow Biofilm Reactor with Low Shear and Continuous Flow will be presented, along with earlier ruggedness testing results collected during method development.

Understanding your production facility's microbiome using 16S metagenomics

Presenter: **Michele Sayles**, Executive Director
Affiliation: Food Safety and Quality, Diamond Pet Foods, Meta, Missouri, USA.

While advances in next generation sequencing (NGS) continue to push our understanding of isolates, pathogens, and foodborne illness, there are additional NGS applications that can improve aspects of how we manage food safety and food quality. This presentation will provide unique and real-world examples of using next generation sequencing applications to improve the food supply chain. NGS applications include techniques such as metagenomics, targeted amplicon sequencing, antimicrobial resistance, and food authenticity. One of our biggest challenges with NGS is finding practical applications of use. This presentation will include plant applications in which some aspect of NGS, specifically 16S metagenomics is used to understand a production facility's microbiome and the impact of a novel application method of probiotics in the production environment. A central theme of these applications will be metagenomics. Metagenomics is used to identify key changes in a microbiome. This presentation will explore the use of 16S metagenomics to help understand plant mapping and the impact of environmental application of probiotics. The presentation will briefly review why metagenomics was chosen, the environmental application of probiotics and dive into a case study and share objectives, results and future planned actions.

SESSION 2: Wound Biofilms

Architecture and phylogenetic structure of chronic wound biofilms

Presenter: **Garth James**[1], Associate Research Professor
Co-Authors: Elinor DeLancey Pulcini[1], Steve Fisher[1], Philip S. Stewart[1,2], Daniel Gibson[3], Gregory Schultz[3], Jungmin Park[3], Michael Weaver[3], Jung Kim[3], Susan Millan[3], Debra Lyon[3], Joyce Stechmiller[3].
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
[3]University of Florida, Gainesville, FL, USA.

Chronic wounds typically harbor polymicrobial biofilms, which have been implicated in prevention of healing. As part of the project, "Biobehavioral mechanisms underlying symptoms and healing outcomes in older individuals with chronic venous leg ulcers (VLU)" funded by the National Institute of Nursing Research, the CBE is examining wound specimens to evaluate the presence of biofilms and their architecture as well as the community structure. Here, architecture refers to the spatial arrangement of biofilm and particular species within the wound tissue, while phylogenic structure refers to the types and abundances of bacteria within the wound specimens. Biofilm

presence and general biofilm architecture are being evaluated using epifluorescent and confocal scanning laser microscopy on wound specimens stained using SYTOX® green (DNA) and wheat germ agglutinin conjugated with Texas Red® (WGR-TR, carbohydrates). The former stains bacteria and host nuclei, while the latter stains host extracellular matrix and the extracellular polymer matrices of some species of bacteria. The 3-D spatial relationships between species will be evaluated using specimens stained using 16S-targeted probes and the Double Labeling of Oligonucleotide Probes for Fluorescence In Situ Hybridization (DOPE-FISH) approach. Targeting of species will be guided by the results of community structure analysis. This analysis is being conducted by 16S sequencing. The study design is longitudinal with specimens collected from each wound at presentation (Week 1) and 2, 4, 6, and 8 weeks thereafter. Each subject receives a standardized therapy for chronic VLU. To date, biofilm was detected using microscopy in 21% (n=11) of Week 1 specimens, 17% (n=12) at Week 2, 22% (n=9) at Week 4, 20% (n=6) at Week 6, and 25% (n=4) at Week 8. Preliminary analysis of phylogenetic structure has been conducted for 42 VLU specimens from 13 subjects. The number of bacterial species in each specimen with greater than 1% relative abundance ranged from 1-9, with a mean of 4.8, indicating that most of the wounds harbored poly-bacterial communities. The most abundant genus was *Corynebacterium*, present in 81% of specimens, which was followed by *Staphylococcus* (71%), *Pseudomonas* (40%), and *Streptococcus*. (29%). Anaerobic bacterial genera included *Anaerococcus* (33%), *Finegoldia* (29%), and *Ralstonia* (29%). For individual subjects, there were shifts in the phylogenic structure of bacterial communities in the wound specimens over time. Future research will evaluate a larger number of subjects and evaluate the 3-D relationships between bacterial species of biofilms within the wound tissue.

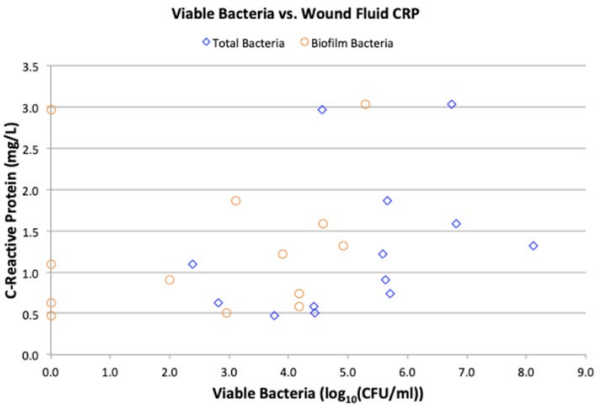
10

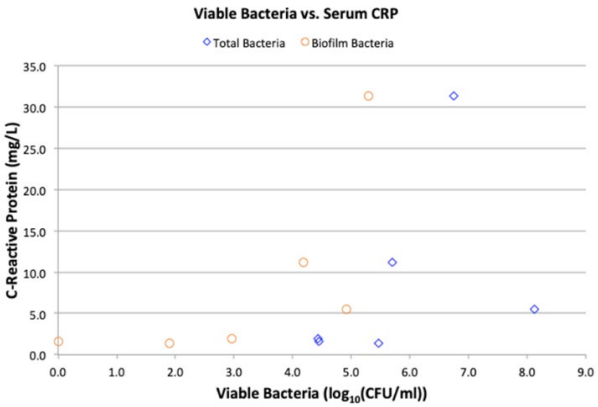
Integrating symptom science with innovative molecular measures: Focus on understanding the trajectory of healing vs. non-healing in chronic venous leg ulcers

Presenter: **Joyce K. Stechmiller**[1], Associate Professor
Co-Authors: Debra Lyon[1]; Susan Millan, MD[2]; Daniel Gibson[3]; Michael T. Weaver[1]; Diana Wilkie[1]; Christiaan Leeuwenburgh[4]; Garth James[5]; Phil Stewart[5]; Debra Lynch Kelly[1]; Jung Kim[1], JoAnne Whitney[6]; Gregory Schultz[3]
Affiliation: [1]College of Nursing, University of Florida, Gainesville, FL, USA.
[2]Health Wound Care and Hyperbaric Center, University of Florida, Gainesville, FL, USA.
[3]Obstetrics and Gynecology, College of Medicine, University of Florida, Gainesville, FL, USA.
[4]Institute on Aging, College of Medicine, University of Florida, Gainesville, FL, USA.
[5]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[6]School of Nursing, University of Washington, Seattle, WA, USA.

Venous leg ulcers account for 70–90% of ulcers found in the lower leg, affect 2 million persons annually, including nearly 4% of people over age 65 years, and create a high symptom burden of wound-related symptoms and symptoms of pain, depression, anxiety, fatigue and cognitive dysfunction, collectively labeled as “psychoneurologic symptoms” (PNS). Our central hypothesis is that the interrelated cellular and molecular mechanisms whose immune activation contributes to the development and persistence of chronic venous leg ulcers (CVLU) may also lead to the development and severity of sickness behaviors (SB) (pain, fatigue, depression). To achieve the aims, we will longitudinally examine 200 older adults (age $\geq$55) who receive standardized wound treatment biweekly across eight weeks’ time. We will characterize patient-host characteristics (age, comorbidities, sex, race/ethnicity, BMI, nutritional status, lifestyle habits, and wound treatment [pressure therapy, debridement, antibiotics]); systemic inflammatory activation (C-reactive protein and cytokines); wound microenvironment factors (local inflammation [Matrix metalloproteinase (MMP) enzymes C-reactive protein, cytokines], biofilm, and micro RNAs); symptoms (PNS [cognitive dysfunction, pain, fatigue, and depressive/anxiety symptoms] and wound-related); and wound characteristics and healing trajectory at the five time points. Blood was collected from patients at 0, 4, and 8 weeks and the serum was assayed for CRP via ELISA. Wound swabs and curettage were collected weekly. The curettage was immediately processed and assayed for viable total and biofilm bioburdens. The swabs were extracted with PBS-Tween and assayed for CRP via ELISA. SB were measured with instruments and interviews. Due to similar collection frequency, there were more points to correlate between the wound CRP and viable bacteria. The wound fluid CRP values ranged from 0.0 – 11.8 mg/L with a mean of 2.02 mg/L and median of 1.27 mg/L. The data are highly scattered with a noisy trend towards higher CRPs being associated with higher

bioburden. The results are a "research in progress." Preliminary findings on nine patients with elevated serum or wound fluid CRP also had elevated bioburdens. The wound fluid CRP data were more varied and lower than have been previously reported. We found that the total protein values for the collected wound fluid were abnormally low, and therefore CRP may be understated due to inadequate fluid collection and over dilution during collection. SB findings revealed mild to moderate symptoms. This study is on-going and will yield more samples to determine if the emerging patterns remain and correlations with SB. Furthermore, additional multi-molecular assays will be performed once enough samples have been collected; which may also help explain the biological variance we have seen.





Chronic wounds are chronic infections caused by biofilm

Presenter: **Randall Wolcott**, MD, Medical Director
Affiliation: Southwest Regional Wound Center, Lubbock, TX, USA.

Chronic wounds are often categorized by the comorbidities associated with them and yet the primary cause of chronic wounds is biofilm phenotype microbiota. Early microscopic studies demonstrating biofilm in chronic wounds have now been confirmed. Biofilm is associated with all chronic infections and interestingly only takes hours to a day to develop. At a molecular level biofilm produces cellular senescence and hyper-inflammation to produce a parasitic-like persistent infection. In animal models it is necessary to seed the wound with bacteria to produce the exudate, slough and nonhealing associated with a chronic wound. Clinically, we see that chronic wounds are stalled and accumulate slough (acute wounds do not) and by pursuing Koch's model we can prove that it is the microbiota that causes this behavior. The European Infectious Disease Society has clearly stated that biofilm causes chronic infections like chronic wounds and offers clinical as well as microbiologic guidelines for establishing the presence of biofilm. Their guidelines go on to state that treatments and products with multiple antibiofilm strategies should be employed when these criteria for chronic infections are established, especially in a chronic wound. Most wound care today is done by trial and error with use of monotherapies used in a serial fashion. In biofilm-based wound care, multiple concurrent strategies are used aggressively early in the management of the wound and then stepped down until advanced therapies can be effectively utilized. Changing the wound care paradigm to primarily focusing on the wound microbiota and secondarily managing host comorbidities will lead to better wound healing outcomes.

SESSION 3: Alternative Biocides

Consumer product preservation: sustaining product microbial quality in a dynamic environment

Presenter: **Charles A. Pettigrew**, Principal Scientist
Affiliation: Beauty and Grooming Microbiology, Procter and Gamble, Cincinnati, OH, USA.

PandG's current product preservation approaches have a long history of safety, efficacy, and cost effectiveness. However, there is an increased scrutiny of several core preservative systems from a range of external stakeholders, including NGOs, regulators, and retailers. These external trends could result in de-selection pressures that limit preservative choice and increase potential microbial health risks. Alternative preservation solutions are needed to provide potential mitigation options and to better meet the needs of our consumers. While these challenges are not unique to PandG, and multi-stakeholder discussions on this topic are already underway, we are undertaking a focused Sustainable Preservation Program to communicate and collaborate with our external partners to help spur innovation and ensure access to key technologies needed for the future. In this talk, we will look at preservation as a holistic process, and provide case studies which demonstrate how, by adopting new preservation technologies, we are able to continue producing robust consumer products which successfully resist microbial contamination.

Modern solutions for product protection

Presenter: **Edward Rolls**, Global Account Director
Affiliation: Symrise GmbH, Holzminden, Germany.

Within the cosmetics industry, preservatives play a key role in protection against microorganisms in both manufacturing and consumption of the product. Available to formulators are annexed preservatives (available on a positive list in the EU (under annex V), or in the Japanese standards for cosmetics, Chapter 3) and now an ever growing list of multifunctional ingredients. Over the last 15 years changes within legislation as well as pressure from NGOs, consumer trends and retailers have all played a part in severely limiting the choice of preservatives available to formulators. In the past a formulator had a free choice of annexed preservatives such as formaldehyde donors, parabens, isothiazolinone, organohalogens, aromatic alcohols and organic acids. Now nearly all of these classes are in some way restricted due to the factors mentioned above to avoid or reduce use of them. This makes the job of the formulator much more complicated. In order to choose the right solution for product protection there are many areas to consider. Of highest importance is the broad spectrum activity of the preservatives and/or multifunctional ingredients selected. With the increasingly restricted list of options available, finding a single ingredient that can offer protection against all types of bacteria, yeast and molds is very difficult; therefore, use of blends is becoming an ever increasing area of innovation. Another key area is water solubility; protection of the aqueous phase is priority as this is where the microorganisms will grow. However, many preservative and multifunctional ingredients are oil soluble therefore a strategy to pull them back into the water phase is key. This demonstrates how a system is needed rather than a single ingredient just being dropped into the formula to solve a problem. Added to this are other factors such as oil/water partition coefficients that are key when formulating emulsions. Finally, other concerns such as temperature stability, pH range, packaging and compatibility with other ingredients all need to be considered to find the perfect fit solution. Taking all of the considerations from above I will review the latest innovations from Symrise GmbH and explain how our multifunctional ingredient classes can be used. Finally, I will explain how to select the right combinations depending on the product type in order to have broad spectrum protection of products whilst, at the same time adhering to new legislation and latest consumer trends and needs.

Alternatives for preservation

Presenter: **Julie Vaughn Biege**[1] and **Rosanna Stokes**[2]
Affiliation: [1]Global Business Development Director for Industrial Products and Business Development, Emerald Performance Materials, Vancouver, WA, USA.
[2]Manager for Consumer Products, Emerald Performance Materials, Vancouver, WA, USA.

This presentation will provide an overview of current challenges in preservation and different approaches being taken to address these from a supplier's perspective. Today's palette of available preservatives continues to shrink, with regulatory pressure, consumer preferences, label changes, and supply disruptions creating many challenges. Emerald will present new solutions to work within that palette, meet consumer demand, and achieve the needed preservation efficacy in a variety of formulations. Recent developments in preservation include preservation options, boosters and multifunctional additives—in both consumer and industrial applications. Approaches that take a full formula approach can help to optimize performance.

Biocidal-free future in EU. Wood protection and coating. Why and how?

Presenter: **Berit Lindegaard**, Product Manager
Co-Authors: Jonas Stenbæk, Microbiologist and Thomas Sørensen
Affiliation: Building and Construction, Danish Technological Institute, Taastrup, Denmark.

During the past decade, the European wood protection and coatings industry has experienced a serious challenge in relation to BPR (Directive 98/8/EC (BPD) or Regulation (EU) No 528/2012(BPR)). This challenge has been extra noticeable within some product types, especially, Product Type (PT) 8 (wood protection). Although the active ingredients in PT8 are listed as approved biocides, there is no guarantee that these ingredients remain on the list forever. There are numerous alternative products, however, these are often less effective or more costly to use. Approval of new biocides and biocidal products is a complex procedure and it is often rendered difficult when environmental authorities in individual countries have different attitudes towards the properties and effects of products on humans and the environment. Experience shows that a considerable amount of man hours is used on communication and documentation in relation to the specific attitudes of the individual countries. Having a wood preservative on the market in the PT8-group can be connected with such a financial strain that it does not correspond to the profit performance. A parallel problem is expected within PT6 (in-can preservation) and PT7 (film conservation), where the industry faces a similar challenge. There are no easy solutions to this challenge. The Danish Technological Institute (DTI) has followed the development of the biocidal regulation closely in the latest decades. In this time frame, we have observed many different initiatives in relation to finding alternative strategies and products. Typically, a supplier tries to develop a strategy and technology that can solve the problem with one ingredient or one process; e.g. new biocides, modification of wood or other moisture reducing technologies. The conclusion has until now been that there is no simple, full solution which is as affordable as existing products. One alternative strategy cannot stand alone. Several techno-economic challenges as well as regulatory barriers remain to be met in order to implement new technologies and actual industrial production. A large group of technology suppliers and industrial manufactures of coating and wood protection are joined to develop new and lasting solutions, aiming for a Biocidal-Free Future.

HCPA microbiology preservative subcommittee (MPS) supporting and enhancing the microbiological quality of consumer, household, and industrial products

Presenter: **Tony Rook**, Associate Director of Research and Development
Affiliation: Global Microbiology Resource Center, The Sherwin-Williams Company, Cleveland, OH, USA.

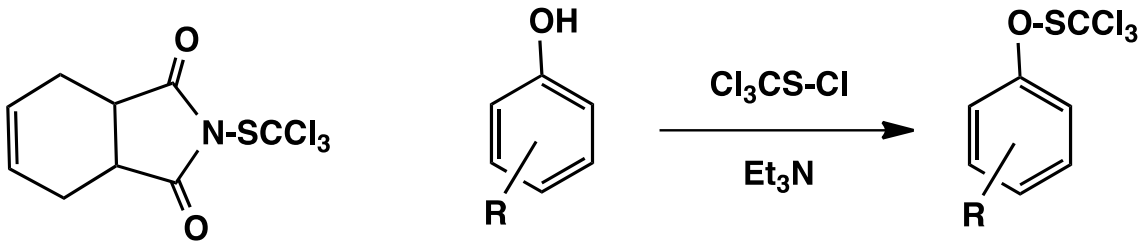
The global supply of preservatives intended for the protection of consumer products has become increasingly threatened due to pressures from governmental regulatory actions, retailer imposed limitations and supply disruptions of key preservative feedstocks. The consumer, household and industrial products industries are grabbling with a number of regional regulatory actions regarding preservatives including recent actions by the Health Canada's Pest Management Regulatory Agency (PMRA) and upcoming packaging and labeling requirements within the European Chemicals Agency limiting the use level of key preservatives approved by the EU Biocidal Products Regulation (BPR). Subsequently, retailers have begun to develop internal sustainability programs aimed

to limit lists of chemicals types based on their own internal criteria. In addition, the industry has struggled with supply chain disruptions of key preservatives due to lack of availability of precursor chemistries because of new environmental regulations and chemical plant explosions within China. The Household and Commercial Product Association's (HCPA) Microbiology Preservative Subcommittee (MPS) is committed to establishing best practices and acceptable standards to address the increasing concern for microbiological quality within consumer, household and industrial products. A Preservation Stewardship Task Force was commissioned within the MPS to communicate the necessity of effective preservative strategies to prevent product spoilage and the positive impact which responsible preservation strategies have on the overall sustainability of consumer, household and industrial products. This presentation is intended to provide an overview of the accomplishments of the HCPA's MPS and its current agenda towards driving sustainable preservation within consumer, household and industrial products.

The design, synthesis, and evaluation of new classes of antimicrobials for the control of biofilms

Presenter: **Thomas S. Livinghouse**[1], Professor
Co-Authors: Danica J. Walsh[1,2], Philip S. Stewart[2,3]
Affiliation: [1]Chemistry and Biochemistry, Montana State University, Bozeman, MT, USA.
[2]The Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[3]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.

The elucidation of new, highly potent antimicrobial agents for the control of colonized bacteria remains a central goal of the chemical and biological sciences. A prominent and long-standing obstacle to the realization of this objective involves the identification of antibiotics that exhibit high selectivity with respect to toxicity toward the target organism. Phenols (i.e., 2) constitute a sizeable class of compounds, which have been shown to exhibit antimicrobial properties against numerous bacteria, spanning the range from Gram-positive to Gram-negative organisms. The N-sulfenylimide Captan (1) has been used for many years as a commercial fungicide for the protection of fruits for human consumption. Its mode of action has been ascribed to the inhibition of normal cell division in a broad spectrum of microorganisms. Although several of the corresponding phenyl sulfenate esters (i.e., 3) have been reported, none have been investigated as antimicrobials toward biofilm colonies. This presentation will focus on the antibacterial activity of several low-molecular weight phenols against *S. epidermidis* and *P. aeruginosa* in the planktonic and biofilm states as well as the significant activity enhancements that are realized upon conversion of the parent phenols 2 to the corresponding trichloromethanesulfenate esters 3. In addition, a novel method for achieving selective cell uptake and antimicrobial sequestration via a pro-drug approach will be described.



1 2 3

SESSION 4: Young Investigators

Oral biofilm-stimulated human gingival epithelium differentially regulates inflammatory responses in co-cultured immune cells

Presenter: **Jason L. Brown**[1], Research Assistant
Co-authors: W. Johnston[1], C. Delaney[1], J. Butcher[2], S. Khan[3], D. Bradshaw[3], S. Culshaw[1], G. Ramage[1]
Affiliation: [1]Oral Sciences Research Group, Glasgow Dental School, School of Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK.
[2]Life Sciences, School of Health and Life Sciences, Glasgow Caledonian University, Glasgow, UK.
[3]Oral Healthcare RandD, GlaxoSmithKline Consumer Healthcare, Weybridge, UK.

Oral diseases including gingivitis and periodontitis (PD) arise through a dysregulated immune response to microbial challenge. The gingival epithelium provides the first line of defence against the oral microbiota, acting as a physical and immunological barrier in the oral cavity. Communication between the gingival epithelium and the immune cells patrolling the epithelial barrier is vital in maintaining symbiosis between the host and microbiota. Here, we combined a 3D model of immune cells, gingival epithelium and complex biofilms in order to study the bacterial-host cell interactions (Figure 1). *In vitro* grown 'health-associated', gingivitis-associated' and 'periodontitis-associated' bacterial biofilms were co-cultured with the human gingival epithelium (HGE) to investigate the host inflammatory response to different biofilms. Immune cells (peripheral blood mononuclear cells (PBMCs) and CD14+ monocytes) were also cultured with or without un-stimulated and stimulated HGE. Epithelial/immune cell gene expression and protein release were assessed by quantitative PCR and ELISA. The morphology and integrity of the epithelium was evaluated by histological staining; the associated biofilms were visualised by scanning/transmission electron microscopy (SEM/TEM) and confocal scanning laser microscope (CSLM) imaging. HGE used in the study formed 6-8 layers, which histologically demonstrated characteristics of gingival epithelium. The different multi-species oral biofilms containing "health-associated", "gingivitis-associated" and "periodontitis-associated" microbiota possessed unique composition, architecture and morphologies as assessed by quantitative PCR and SEM, TEM and CSLM imaging. We observed unique inflammatory responses in HGE cultured with multi-species oral biofilms. The "health-associated" biofilm had minimal impact on the epithelium whilst "gingivitis-associated" and "periodontitis-associated" biofilms induced a pro-inflammatory response, including elevated interleukin-8 gene expression and increased protein release. Biofilm-stimulated HGE differentially regulated inflammatory responses in co-cultured PBMCs and CD14+ monocytes. Our results show 3D epithelial models may offer a substantial advantage for *in vitro* study of host-pathogen interactions. These tissue models recapitulate similar inflammatory responses and cell signalling interactions observed at the oral mucosa in certain diseases like gingivitis and PD. This platform allows *in vitro* dissection of the impact of oral biofilms cultured with epithelial tissue and immune cells. Findings from this 3D model may contribute to our understanding of the pathogenesis of oral inflammatory diseases *in vivo*.

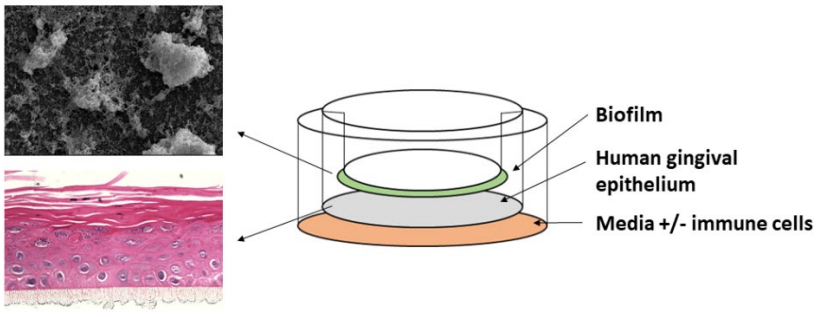


Figure 1: Schematic diagram depicting the 3D human gingival epithelium (HGE) and biofilm co-culture set-up. Images represent a scanning electron microscopic image of the "periodontitis-associated" biofilm and haematoxylin and eosin stained HGE tissue section.

Investigation of synovial fluid induced *Staphylococcus aureus* aggregate development and its impact on surface attachment and biofilm formation

Presenter: **Matthew J. Pestrak**, Postdoctoral Researcher
Co-Authors: Devendra H. Dusane, Doug V. Guzior, Paul Stoodley
Affiliation: Microbial Infection and Immunity, The Ohio State University, Columbus, OH, USA.

Periprosthetic joint infections (PJIs) are a devastating complication that occurs 2 to 5% of patients following joint replacement. These infections are costly and difficult to treat, often requiring multiple corrective surgeries and prolonged antimicrobial treatments. In 2012 in the United States, approximately 15,000 hip and 50,000 knee PJIs were reported. These infections were predicted to cost nearly $566 million, and annual costs continue to rise as the incidence of infection increases. In addition to their financial burden, these infections result in lengthy periods of disability and frequently result in death within 5 years of the initial surgery. The gram-positive bacterium *Staphylococcus aureus* is one of the most common causes of PJIs, and it is often resistant to a number of commonly used antimicrobials. This drug resistance can be partially attributed to the ability of *S. aureus* to form biofilms. Biofilms associated with the surface of indwelling medical devices have been observed on components removed during chronic infection, however, the development and localization of biofilms during PJIs remains unclear. Prior studies have demonstrated that synovial fluid (SF), in the joint cavity, promotes the development of bacterial aggregates with many biofilm-like properties, including antibiotic resistance. We anticipate these aggregates have an important role in initiating biofilm development during PJIs. Therefore, we sought to determine specifically how SF promotes aggregate formation and the impact of this process on surface attachment. Using flow cytometry and microscopy, we quantified the aggregation of various clinical *S. aureus* strains following exposure to purified synovial fluid components. We determined that fibrinogen, fibronectin, and serum albumin promoted bacterial aggregation, while eDNA and hyaluronic acid did not promote aggregation. To determine how SF mediated aggregation affects surface attachment, we utilized microscopy to measure bacterial attachment over time under various shear stress conditions. Surprisingly, we found that SF exposure significantly impeded bacterial surface attachment to a plastic flow cell. Finally, we determined that fibrinogen, fibronectin and serum albumin were sufficient to inhibit *S. aureus* surface attachment. We conclude from these data that the protein components of SF have a crucial role in promoting bacterial aggregation and preventing surface attachment during PJI. Therefore, we propose that SF has an important defensive role for the host by reducing attachment and subsequent biofilm formation during PJI, however this may be offset in part by the formation of protected bacterial aggregates that could lodge in surface features of implants and host tissue.

Evolution of *Pseudomonas aeruginosa* in a chronic burn wound model

Presenter: **Erin S. Gloag**[1], Postdoctoral Researcher
Co-Authors: Christopher W. Marshall[2], Marvin Whiteley[3], Vaughn S. Cooper[2], Daniel J. Wozniak[1]
Affiliation: [1]Microbial Infection and Immunity, The Ohio State University, Columbus, OH, USA.
[2]Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.
[3]School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA.

Chronic infections persist despite extensive treatment strategies. These infections are commonly caused by bacterial biofilms. *Pseudomonas aeruginosa* is often implicated in chronic infections, especially in immune-compromised individuals. Further complicating chronic infections are the ability of bacteria to adapt, usually by evolving variants that are more fit and persistent. Despite our current understanding of the divergent phenotypes of adapted variants, studying their emergence in an infection is challenging, as there are few animal models that mimic what is observed clinically. Our lab has developed a porcine full-thickness chronic burn wound model where bacterial burden and wound healing can be monitored kinetically for up to 35 days. In this model, wild type *P. aeruginosa* establishes localized infections with a high bacterial burden. Matrix encased biofilm aggregates are detected throughout the wound. The porcine full-thickness chronic burn wound model is a clinically relevant model that permits the study of chronic infections over time. Using this model, we analyzed how *P. aeruginosa*

evolves and adapts in response to a chronic infection. Porcine wounds were inoculated with six different *P. aeruginosa* strains (two lab strains and four clinical/environmental isolates). The bacterial burden was then examined on 3, 14 and 28 days post infection. As early as 3-d post infection, both lab strains, PA14 and PAO1, dominated, outcompeting the 4 other strains in the infection. Hyper-biofilm forming rugose small-colony variants (RSCVs) were isolated from the wounds, and were the earliest and predominant phenotypic variant. RSCVs that evolved from PA14 and PAO1 were identified, with PA14 RSCVs prevailing over PAO1 RSCVs across all time points. Whole genome sequencing revealed that PA14 RSCVs acquired driver mutations exclusively in the wsp chemosensory pathway. PA14 RSCVs also acquired CRISPR-Cas adaptive immunity against prophages from the *P. aeruginosa* wound isolate B23 present in the infection. In contrast, PAO1 RSCVs acquired recombination of prophage elements from *P. aeruginosa* strains B23 (wound isolate), S54485 (UTI isolate) and MSH10 (water isolate), where the insertion event disrupted genes involved in c-di-GMP regulation. Therefore, the porcine full-thickness chronic burn wound model is an important model for studying chronic infections, as it allows for the analysis of bacterial evolution and adaption in an infection. We predict that hyper-biofilm RSCVs are common, early adaptations during chronic infections.

CBE-NBIC scientific and collaborative opportunities

Presenter: **Jeremy Webb**[1,2], Principal Investigator
Co-Authors: Mark Richardson, CEO[1]; Cait MacPhee, Co-director[1], Professor[3]; Rasmita Raval, Co-Director[1], Professor[4]; Miguel Cámara[1], Professor[5]; Jo Slater-Jefferies, Operations Director[1]
Affiliation: [1]National Biofilms Innovation Centre (NBIC), UK.
[2]University of Southampton, Southampton, UK.
[3]University of Edinburgh, Edinburgh, Scotland, UK.
[4]University of Liverpool, Liverpool, England, UK.
[5]University of Nottingham, Nottingham, England, UK.

Partnerships between international biofilm centers provide opportunities to develop and strengthen the global research and industry community through international collaboration in biofilms. The recently established National Biofilms Innovation Centre (NBIC) is a partnership of four core-funded UK Universities (Southampton, Edinburgh, Liverpool, Nottingham) together with a network of an additional 37 partner research institutions, an industry hub, and partnering with government agencies and national infrastructure for science. NBIC provides a focus for industry partners to access biofilm research, expertise and capabilities across the UK, connecting the right expertise, simplifying knowledge transfer and catalyzing collaboration. It also facilitates these collaborations by providing targeted funding calls aimed at joint industry-academic projects. This presentation will overview the major scientific themes and goals within NBIC with exemplars of current research projects. It will also discuss the opportunities for collaboration and synergy between CBE and NBIC including opportunities for leveraging strategic international funding opportunities to promote collaboration.

SESSION 5: Biofilms and Host Response

Mechanics of biofilm infection

Presenter: **Philip S. Stewart**, Distinguished Professor
Affiliation: Chemical & Biological Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

This presentation examines a likely basis of the tenacity of biofilm infections that has received relatively little attention: the resistance of biofilms to mechanical clearance. One way that a biofilm infection persists is by withstanding the flow of fluid or other mechanical forces that work to wash or sweep microorganisms out of the body. Examples related to the cystic fibrosis lung, the oral cavity, indwelling catheters, and phagocytosis by leukocytes are considered. Video microscopy of biofilms undergoing conventional antimicrobial treatments underscores a common outcome: partial killing of biofilm microorganisms but negligible biofilm removal. As

materials, biofilms exhibit viscoelasticity and poorly characterized failure modes. The fundamental criterion for mechanical persistence is that the biofilm failure strength exceeds the external applied stress. Mechanical failure of the biofilm and release of planktonic microbial cells is also important *in vivo* because it can result in dissemination of infection. The apparent contradiction for a biofilm to both persist and disseminate is resolved by recognizing that biofilm material properties are inherently heterogeneous. The possibility of alternative therapies for treating biofilm infections that work by reducing biofilm cohesion could: 1) allow prevailing hydrodynamic shear to remove biofilm, 2) increase the efficacy of designed interventions for removing biofilms, 3) enable phagocytic engulfment of softened biofilm aggregates, and 4) improve phagocyte mobility and access to biofilm.

Early recruitment of neutrophils prevents *Staphylococcus aureus* biofilm formation

Presenter: **Brian Pettygrove**[1,2], PhD Candidate
Co-authors: Rachel M. Kratofil[3], Kyler B. Pallister[2], Paul Kubes[4], Jovanka M. Voyich[2], Philip S. Stewart[1,5]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Microbiology and Immunology, Montana State University, Bozeman, MT, USA.
[3]Immunology, Cumming School of Medicine, University of Calgary, Calgary, Alberta, CA.
[4]Snyder Institute of Infection, Immunity, and Inflammation, University of Calgary, Calgary, Alberta, CA.
[5]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.

Staphylococcus aureus commonly causes nosocomial infections on implanted biomaterial surfaces. Mechanisms of immune evasion in the early stages of *S. aureus* biofilm formation are not yet clear. The objective of this work was to determine parameters important in the ability of host defenses to eradicate contaminating microorganisms from a biomaterial surface and thereby prevent establishment of a biofilm-based infection. Clearance of newly attached *S. aureus* bacteria from a serum-coated glass surface by human neutrophils was investigated *in vitro* using time-lapse confocal microscopy and quantitative image analysis. Additionally, recruitment of neutrophils to a contaminated surface was investigated using intravital imaging. In control experiments in which the surface was inoculated with bacteria but no neutrophils were added, attached bacteria grew rapidly (mean specific growth rate 0.95 ± 0.04 h^{-1}) and formed discrete aggregates. When neutrophils were added to the system, they migrated on the surface and discovered, phagocytosed, and killed attached bacteria. The surface density of neutrophils needed to be high enough such that attached aggregates were discovered and eliminated quickly, as undiscovered *S. aureus* formed aggregates that became resilient to neutrophil clearance. Significant reductions in bacterial burden were observed when the neutrophil to bacteria ratio was greater than one, however total clearance of bacteria from the surface occurred only at much higher ratios. To investigate the effect delayed neutrophil recruitment would have on clearance of contaminating bacteria, attached *S. aureus* was given one, two, or three hours to grow on the surface prior to neutrophil addition. We observed formation of aggregates 7.87 ± 3.72 μm^2, 61.74 ± 23.60 μm^2, and 80.64 ± 42.30 μm^2 in size, respectively. Neutrophils failed to effectively clear these larger aggregates while still readily killing similar numbers of single cell *S. aureus* in control experiments. In experiments where bacteria were pregrown for two or three hours, less than a log reduction in bacterial biomass compared to control wells was observed following neutrophil challenge. Single cell controls and one-hour head-start conditions resulted in significantly greater reductions in biomass, demonstrating that neutrophils more easily clear smaller aggregates or single cells. We used propidium iodide staining to investigate cytotoxic damage to neutrophils during these interactions. Concurrent with reduced clearance of bacteria, significantly higher rates of cytotoxic damage to neutrophils were measured in experiments where *S. aureus* aggregates were larger- greater than 30% of all interactions observed in the three-hour head-start experiments resulted in neutrophil lysis. This suggests a threshold *S. aureus* aggregate size wherein the bacteria can not only resist clearance by, but also actively antagonize neutrophils, resulting in persistence. Preliminary experiments were conducted using intravital imaging to investigate the *in vivo* timescale of neutrophil recruitment to a contaminated surface. In most experiments, neutrophil recruitment to the surface was limited within the three-hour experiment, indicating that there exists a window for contaminating bacteria to form more resilient aggregates before neutrophils reach the site of infection.

Together, these results suggest that neutrophils have the potential to eliminate a nascent biofilm, but they must be recruited in sufficient numbers and quickly enough to achieve this outcome.

Pseudomonas aeruginosa biofilms and adaptations during chronic infections

Presenter: **Daniel J. Wozniak**[1,2], Professor
Co-Authors: Erin Gloag[1], Matthew Pestrak[1], Mohini Bhattacharya[1,4], Vaughn Cooper[3], Marvin Whiteley[5], Victor Torres[6]
Affiliation: [1]Departments of Microbial Infection and Immunity, The Ohio State University, Columbus, OH, USA.
[2]Microbiology, The Ohio State University, Columbus, OH, USA.
[3]University of Pittsburgh, Pittsburgh, PA, USA.
[4]University of Chicago, Chicago, IL, USA.
[5]Georgia Institute of Technology, Atlanta, GA, USA.
[6]New York University, New York, NY, USA.

Pseudomonas aeruginosa and *Staphylococcus aureus* cause devastating infections in immunocompromised individuals. Once established, these infections become incredibly difficult to treat due to the development of antibiotic tolerant, aggregated communities known as biofilms. Understanding the dynamic interactions between biofilm grown bacteria and the immune system is a goal of these studies. In one study, a hyper-biofilm forming clinical variant of *P. aeruginosa*, known as a rugose small-colony variant (RSCV), is frequently isolated from chronic infections and is correlated with poor clinical outcome. While prior studies suggest RSCVs could survive by evading the host immune response, our study reveals infection with the RSCV, PAO1Δ*wspF,* stimulated an extensive inflammatory response that caused significant damage to the surrounding host tissue. In both a chronic wound model and acute pulmonary model of infection, we observed increased bacterial burden, host tissue damage, and a robust neutrophil response during RSCV infection. Given the essential role of neutrophils in *P. aeruginosa*-mediated disease, we investigated the impact of the RSCV phenotype on neutrophil function. The RSCV phenotype promoted phagocytic evasion and stimulated neutrophil reactive oxygen species (ROS) production. We also demonstrate that bacterial aggregation and TLR-mediated pro-inflammatory cytokine production contribute to the immune response to RSCVs. Additionally, RSCVs exhibited enhanced tolerance to neutrophil-produced antimicrobials including H_2O_2 and the antimicrobial peptide LL-37. Collectively, these data indicate RSCVs elicit a robust but ineffective neutrophil response that causes significant host tissue damage. This study provides new insight on RSCV persistence, and indicates this variant may have a critical role in the recurring tissue damage often associated with chronic infections. The virulence mechanisms associated with *Staphylococcus aureus* biofilms are only being investigated in our group. Neutrophils play a crucial role in the innate immune response to bacterial infections, including *S. aureus*. In a second study, we describe two major virulence mechanisms that contribute to the ability of *S. aureus* biofilms to evade killing by neutrophils. Specifically, we show that while neutrophils exposed to *S. aureus* biofilms produce extracellular traps (NETs) and phagocytose bacteria, both mechanisms are inefficient at clearance of biofilm biomass. This is attributed to the leukocidin LukAB, that allows for *S. aureus* survival during phagocytosis (Fig. 1). We also show that the persistence of biofilm bacteria trapped in NETs is facilitated by *S. aureus* thermonuclease (Nuc) mediated NET DNA degradation. This second study therefore describes key aspects of the interaction of neutrophils with *S. aureus* biofilms and provides an explanation for how *S. aureus* evades the neutrophil response to persist during chronic infections.

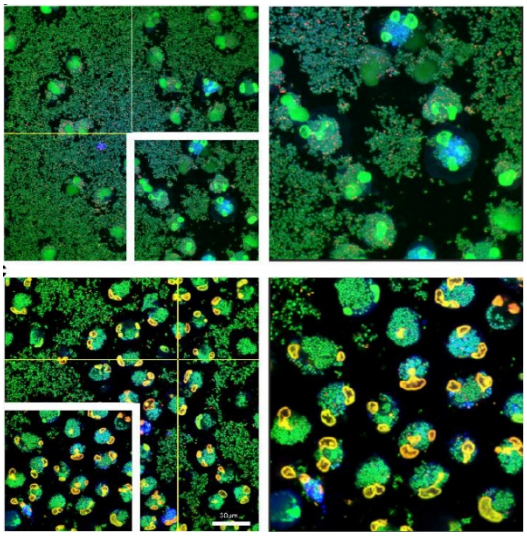


Figure 1: *LukAB contributes to bacterial survival when biofilms are in contact with neutrophils. Upper panels, wild type* S. aureus*, lower panels,* S. aureus ΔlukAB*.*

Breaking down the immunobiology of implant fibrosis/foreign body response

Presenter: **Joshua Doloff**, Assistant Professor
Affiliation: Biomedical Engineering and Materials Science Engineering, Translational Tissue Engineering Center and Institute for NanoBioTechnology, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Implanted biomedical devices comprise a major component of modern medicine and are essential for many clinical applications ranging from tissue repair/reconstruction, electrical pacing/stimulation, controlled release, glucose sensing, and cell transplantation. However, a huge impediment to their therapeutic performance and lifespan, host immune-mediated foreign body response, results in their being walled off behind dense layers of fibrotic scar tissue. Systems biology technologies to elucidate core players in this rejection response will be presented in the context of identifying next generation drug targets. Different implant sites, biomaterials, multi-component devices, and animal models, including non-human primates and a new humanized variant, will be considered. Lastly, examples of leveraging this information toward the design of next generation technologies for successful prevention of implant rejection will be discussed.

SESSION 6: Biofilms in Space

Development of nanoengineered materials for organisms (NEMO) to resist biofilm formation in space

Presenter: **Kasthuri Venkateswaran**[1], Senior Research Scientist
Co-Authors: Ganesh Babu Malli Mohan[1], Ceth Parker[1], Camilla Urbaniak[1], and Thomas Higgins[2]
Affiliation: [1]Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA, USA.
[2]GoldShield, Locust Valley, NY, USA.

The principles that govern how different cell types interact and self-organize into complex tissues or structures are poorly understood, but are being actively investigated by NASA. Microgravity would be expected to induce significant effects in biofilm formation and molecular mechanisms behind how microorganisms react, interact, and self-organize in space conditions are not well established. The NanoEngineered Materials that resist Organisms (NEMO) project, a multi-disciplinary team, is developing and testing materials that prevent biofilm formation in microgravity conditions. The formation of biofilms on surfaces, with consequent biofouling of space hardware and life-support systems, is of significant concern to NASA. It is also a growing health concern on Earth. The International Space Station (ISS)–Microbial Tracking experiments led by the Jet Propulsion Laboratory (JPL), along with other investigations conducted between 2000 and 2015, have revealed the presence of potentially harmful microorganisms in the crew cabin, the Environmental Control and Life Support System (ECLSS), and drinking water containers on the ISS. Materials that resist biofilm formation are a critical necessity for long-duration spaceflights, when such microorganisms might have time to develop their harmful effects. The NEMO team's approach is to develop novel coating technologies that can significantly change the adhesion properties of surface materials to inhibit biofilm formation. The GoldShield (GS) has developed technologies that are unique in their performance because of compounds that create a residual performing action when applied, with protection that continues for years. The residual activity is borne from the chemistry forming a covalent bond to virtually any substrate. The GS chemistry positively charges the substrates to which it is applied through a nitrogen compound, which attracts the negatively charged microbes. As microbes are attracted to the substrate, they are killed through the reaction of a long carbon chain that destroys their cell structure. This chain remains active for extended periods, with evidence showing activity that ranges from months to more than two years in some cases. The antimicrobial properties of GS products were tested in a hospital system and also went through a clinical trial at the Oakwood Hospital System, in Dearborn, MI. Our preliminary results show the suppression of biofilm formation in *Staphylococcus aureus* cells on GS-products coated aluminum. *Staphylococcus aureus* is an opportunistic pathogen and also one of the biosafety level 2 bacteria isolated from the ISS environmental surfaces. *S. aureus* cells were reported to establish biofilms on medical implants and host tissues infections, play a critical role in the persistence of chronic infections. A pilot study was carried out using *S. aureus* IF4SW-P1 for their ability to form biofilm on spacecraft qualified aluminum metal coupons. It has been demonstrated that the GS matrix used as a

coating for the aluminum coupon significantly reduced biofilm formation (>95%) of *S. aureus* IF4SW-P1 isolated from ISS environmental surfaces. Epi-fluorescence microscopy (qualitative) and ImageJ (quantitative) assays were used to measure the biofilm formation. Furthermore, field emission scanning electron microscopy with energy-dispersive x-ray analysis mapping confirms that when GS was not coated on aluminum coupons *S. aureus* cells formed more biofilms than on GS coated coupons. Systematically measuring changes in "omics" and analyze molecular mechanism(s) associated with biofilm formation is needed to understand why ISS microbes are producing more biofilms and develop countermeasures to prevent biofilm formation. The delivery of NEMs that could resist and prevent biofilm formation in spaceflight conditions will potentially benefit future NASA life support systems.

Management of biofilms in the operation of the ISS water recovery and management system

Presenter: **Layne Carter**, Water Subsystem Manager for the International Space Station (ISS)
Affiliation: NASA Marshall Space Flight Center, Huntsville, AL, USA.

Biofilm growth is a significant concern for NASA's current and future water systems. The International Space Station (ISS) Water Processor Assembly (WPA) produces potable water from a combination of humidity condensate and urine distillate. After two years of operation, the WPA experienced a significant failure (clogged solenoid valve) due to biofilm growth in the waste tank that collects these two waste streams. The WPA waste tank now requires significant management to prevent biofilm from impacting downstream components. This issue is magnified for future NASA manned missions due to the need to place the vehicle's life support system in a dormant state during uncrewed operations (e.g., when vehicle is in Mars orbit during surface mission). The urine distillate and humidity condensate are also expected to be an issue during dormancy, especially where these waste streams originate (the condenser in the Distillation Assembly of the Urine Processor) and the Water Separators that collects and delivers the humidity condensate removed from the atmosphere. To address these concerns, NASA is performing an ongoing research task to a) identify viable methods for inhibiting growth of biofilms, b) develop design solutions for implementing these various methods, c) perform a trade study to select methods (taking into account the design solutions), and d) evaluate effectiveness in ground test prior future missions.

Design considerations for mitigating biofilm growth on the ISS and future missions

Presenter: **Mononita Nur**, Aerospace Engineer
Affiliation: NASA Marshall Space Flight Center, Huntsville, AL, USA.

As NASA's manned space program aims to venture beyond low earth orbit, researchers at the agency are conducting an ongoing effort to describe and ameliorate the challenges associated with long duration or long dormancy operation for life support systems. Throughout operation, the Water Processor Assembly on the International Space Station has experienced significant biofouling and biofilm growth in water tanks and associated hardware that has resulted in system interruption and loss of operation. NASA is pursuing an ongoing activity to develop concepts to manage biofilm growth in the wastewater to preclude biomass impacts to the flow paths while also insuring the system is sufficiently clean to support a pre-dormancy flush. Selected research to tackle this issue found both within NASA and in literature will be detailed in terms of potential efficiency of biofouling management. The greatest share of the focus has been on chemical additive means of prevention or mitigation - current biocides under consideration include silver, silver dihydrogen citrate, iodine, bromine, orthophthaldehyde, ozone, and peroxide. Physical and material considerations and potential hardware configuration changes will also be outlined. These primary points provide context and a framework for a subsequent panel discussion and question and answer session.

Joint Biofilm Workshop Conducted by NASA

NASA workshop on wastewater systems for extended spaceflight

The ISS wastewater distribution bus collects condensate from two primary waste streams, including crew urine and humidity condensate (from respiration/perspiration). Urine is treated with a strong oxidant and acid to prevent microbial growth and facilitate the urine distillation process. The distillate from this process is combined with the humidity condensate and processed through the Water Processor Assembly. Neither the urine distillate nor the humidity condensate have a biocide to inhibit microbial growth. As a result, biofilm has formed in the wastewater distribution plumbing and the feed tank to the Water Processor. This biofilm has created operational issues on ISS that require specific controls to manage the release of biomass that can subsequently obstruct flow paths. Further, the biofilm introduces complications now that NASA is defining concepts for implementing dormant operations in future missions (e.g., during Mars orbit while crew is on surface mission). The baseline approach proposed for dormancy is to flush the system with clean water, though this approach requires minimal biofilm growth in the system prior to implementing the flush. NASA is pursuing an ongoing activity to develop concepts to manage biofilm growth in the wastewater to preclude biomass impacts to the flow paths while also ensuring the system is sufficiently clean to support a pre-dormancy flush. A detailed overview of the current issues and relevant design considerations will be given during the Thursday morning session, followed by an afternoon workshop to discuss viable concepts and design solutions that may be implemented by NASA. The following questions will be discussed in the afternoon workshop. Current biocides under consideration include silver, silver dihydrogen citrate, iodine, bromine, orthophthaldehyde, ozone, and peroxide. Are there limitations with these biocides that would reduce their effectiveness? Are there other biocides that you have experience with that may be more effective? What are the known methods in which these biocides can be introduced? Various coatings are currently being considered that will limit microbial growth and/or biofilm adhesion. What operational experience do you have that would help NASA with implementation, including the long-term functionality in the given environment? Can these coatings be readily applied to tubing and/or bellows tanks? Be prepared to discuss alternate design concepts that would eliminate the bellows tank, keeping in mind the high-level requirements to receive wastewater at various flow rates from the condensing heat exchangers and the Urine Processor. Be prepared to discuss alternate concepts that focus on integrating biofilm growth without impacting downstream processes (due to release of biomass) or the transition to dormancy. Within the existing design, what design or operational modifications would improve the existing situation?

Guest Posters

Texas State University

Date: 07/2019
Title: **Polymicrobial biofilm experiment preparation for the International Space Station**
Authors: **Starla G. Thornhill**[1], Jiseon Yang[2], Jennifer Barilla[2], Cheryl A Nickerson[2], C Mark Ott[3], and Robert JC McLean[1]
Affiliation: [1]Biology, Texas State University, San Marcos, TX, USA.
[2]Biodesign Institute, Arizona State University, Tempe, AZ, USA.
[3]Johnson Space Center, NASA, Houston, TX, USA.

The International Space Station (ISS) is a built environment that has been continuously inhabited since November 2000. Living with the resident crew are the microorganisms carried to the ISS as flora with the astronauts and accidentally introduced in supplies. These microorganisms have established a biofilm in the water filtration unit of the Environmental Control and Life Support System (ECLSS) that recycles urine and humidity to provide drinking water for the ISS crew. In addition to potential health problems for the crew, biofilms may also induce corrosion on the stainless steel piping. To investigate biofilm biology in space, we are sending an experiment to the ISS using a Biocell plate designed by Bioserve (University of Colorado, Boulder). Here, a mixed culture of *Escherichia coli* and *Pseudomonas aeruginosa* will be grown on a stainless steel surface in an artificial urine medium in microgravity. Our intention is to investigate biofilm formation, the relative distribution of *E. coli* and *P. aeruginosa* within the biofilms, their susceptibility to disinfection, and their potential for stainless steel corrosion. Samples will be compared to simultaneously tested ground controls (full gravity). To date, we have identified and chosen bacterial strains (*P. aeruginosa* PAO1 and *Escherichia coli* F11) and inserted *gfp* and mCherry genes so that the organisms can be distinguished using confocal microscopy. We are also testing media stability, the preservation of cultures prior to launch; culture time points for biofilm growth; disinfection protocols; stability following biofilm formation, fixation and post-flight microscopy, and reproducibility.

Primordial Genetics

Date: 06/2019
Title: **Developing bacteriophages as sanitizing agents for controlling *E. coli* O157:H7 biofilms**
Authors: **Karen D. Xu**, Alan Greener, Justin Stege and Helge Zieler
Affiliation: Primordial Genetics Inc., San Diego, CA, USA.

E. coli O157:H7 is an important food pathogen that can cause serious diseases in adults and children, such as hemolytic uremic syndrome (HUS). It is transmitted by contaminated food products such as meats and leafy vegetables. In recent years, there have been multiple well-publicized outbreaks, usually accompanied by severe illness and occasional deaths, that have required large-scale food recalls in some cases involving millions of units of contaminated product. This pathogen is difficult to treat, and prognoses for patients with severe sequelae are poor. Eliminating *E. coli* O157:H7 bacterium from the food supply has proven very challenging. Bacteriophages are attractive options as prophylactic antibacterials because they are highly host-specific, safe and nontoxic for all multicellular organisms, and much less disruptive of the microbiome than antibiotics and biocides. However, few bacteriophage products have been developed because of low efficacy and inconsistent killing of target bacteria. Specifically, bacteria challenged with bacteriophages rapidly develop resistance to bacteriophage infection, and bacterial populations rapidly recover from a bacteriophage challenge. In addition, bacteria present in biofilms are naturally more resistant to bacteriophage infection. Primordial Genetics has developed novel bacteriophage technology that enables the development of bacteriophages with much longer-term inhibition of bacterial growth than natural bacteriophages. This technology has been validated using a wild type *E. coli* strain under laboratory conditions. The company is in the process of applying this technology to pathogenic *E. coli* strains such as O157:H7 and testing whether the technology is capable of killing bacteria within biofilms. Our goal is to develop effective and long-lasting prophylactic bacteriophage-based products as effective antibacterial agents for controlling *E. coli*

O157:H7 biofilms and as sanitation agents for the food industry. Primordial Genetics' bacteriophage capabilities will enable numerous other phage-based solutions for combatting pathogenic or contaminating bacteria in crops, animals, humans and various industrial settings.

Mississippi State University

Date: 07/2019
Title: **Treatment of multi-drug resistant infections using poloxamer-407/antibiotic compounds vs. conventional management in veterinary patients**
Authors: **Elizabeth A. Swanson**[1], Gallaher HM[1], Rochat MC[2], Hixon LP[1]
Affiliation: [1]College of Veterinary Medicine, Mississippi State University, Mississippi State, MS, USA.
[2]College of Veterinary Medicine, Purdue University, West Lafayette, IN, USA.
Sponsored by: Funded in part by an MSU CVM Office of Research and Graduate Studies House Officer Grant

As with human medicine, multiple-drug resistant (MDR) infections are a growing problem in veterinary patients and hospitals and are associated with limited treatment options and significant patient morbidity. The purpose of this retrospective study was to compare the effectiveness of a novel topical compound in treating MDR infections compared to traditional treatment methods. We searched the medical records at 2 institutions to identify cases with MDR infections that were treated with and without poloxamer gel compounds. Patient signalment (species, breed, age, sex), type and location of infection, bacterial culture and susceptibility (CandS) results, type and duration of treatment, and final outcome were recorded. Our search identified 14 patients with MDR infections that were treated with the poloxamer-407/antibiotic compounds (11 dogs, 2 horses, 1 donkey, and a tortoise). 10 patients (9 dogs and 1 horse) were identified whose infections were treated conventionally. Tissues infected included wounds (n=12), bones (osteomyelitis and physitis) (n=9), and dermatologic (skin and ears) (n=3). The most common bacterial species isolated included *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, and *Staphylococcus intermedius*. All infected tissues were treated by debridement and systemic antibiotics selected from culture and susceptibility results. Orthopedic implants were removed where appropriate. For the patients also treated with poloxamer gel, mean duration of treatment prior to gel application was 25 days (range: 6-68 days). Gel compounds consisted of 25% poloxamer gel with antibiotic choice based on CandS results (vancomycin, amikacin, clindamycin). Frequency of application varied from once to every 2-7 days, with most cases at 2-3 days (at times of bandage changes). Mean duration of gel treatment was 10 days (range 1-17 days), until infection was resolved for a total mean treatment time of 36.17 days. All soft tissue infections resolved with gel application. 2 orthopedic cases (1 dog and 1 horse) resolved with gel treatment; 2 cases were lost to follow up. For infections treated conventionally, mean duration of treatment was 46 days. All soft tissue infections in dogs resolved, but 1 horse with distal limb wounds was euthanized when treatment failed. We concluded that poloxamer gel-antibiotic compounds were a safe and effective therapy for chronic, MDR, soft tissue infections that failed to respond to conventional treatment, and that they resulted in faster resolution of MDR infection and tissue healing compared to conventional methods. It is unclear if these compounds are consistently effective in orthopedic infections.

CBE Posters

CBE Poster #749

Date: 07/2019
Title: **Chickensplash! Exploring the health concerns of washing raw chicken**
Authors: **Caitlin Carmody**[1,2], Benjamin Grodner[1,3], Stephanie McCalla[4], James Wilking[1,3]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Mechanical & Industrial Engineering, Montana State University, Bozeman, MT, USA.
[3]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
[4]Mathematical Sciences, Montana State University, Bozeman, MT, USA
Sponsored by: NSF/USP/INBRE

The Food and Drug Administration (FDA) recommends against washing raw chicken due to the risk of transferring dangerous pathogens through splashed drops of water. Many cooks continue to wash raw chicken despite this warning, however, and there is a lack of scientific evidence outlining the danger of microbe transfer through splashing. Here we use large agar plates to confirm that bacteria can be transferred from the surface of raw chicken through splashed water drops. We also show that faucet height, surface angle, and flow type affect splash height and distance. Using high speed imaging and MATLAB particle tracking to analyze splash trajectories, we found that increasing faucet height increases splash height and that angling the splash surface decreases the splash height in the direction the platform is angled. We are currently working to identify the bacteria transferred using 16s sequencing. We anticipate that the information found in these experiments can be used to recommend safe household practices for washing raw chicken.

CBE Poster #750

Date: 03/2019
Title: **A model to quantify the enhanced mass transfer of CO_2 into high alkalinity algae culture medium**
Authors: **Nickolas Avila**[1,3], Agasteswar Vadlamani[2], Mohammadmatin Hanifzadeh[2], Brahmaiah Pendyala[2], Sasidhar Varanasi[2], Robin Gerlach[1,3], Sridhar Viamajala[2]
Affiliation: [1]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
[2]Chemical Engineering, University of Toledo, Toledo, OH, USA.
[3]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: US Department of Energy, National Science Foundation, MSU Undergraduate Scholars Program, W.M. Keck Foundation

Microalgae can autotrophically utilize bicarbonate ions (HCO_3^-) in solution as a carbon source. At a high pH (>10) and high alkalinity, algae cultures can take advantage of an enhanced mass transfer of CO_2 from the atmosphere as well as provide a reservoir of bicarbonate for fixation; culturing under these conditions has been shown to increase biomass productivity and reach levels achieved otherwise only with high concentration CO_2 supplies. We have developed a mathematical model that describes this enhancement of CO_2 mass transfer by using the hydroxyl ion concentration, diffusion coefficients, total alkalinity, equilibrium constants of the carbonate/bicarbonate equilibrium equations, and the volumetric mass transfer coefficient (k_La), while also accounting for the effects of ionic strength and variable temperature of the media. Most of these parameters are well known from the literature and their changes at varying ionic strengths and temperatures have been previously described. Changes in hydroxyl ion concentrations are easily measured as pH. Our approach allows us to estimate the volumetric mass transfer coefficient by fitting data relating pH and time to an ordinary differential equation that describes this unsteady-state mass transfer. We determined the volumetric mass transfer coefficient for the transport of atmospheric CO_2 into paddlewheel-mixed open alkaline ponds by fitting pH vs. time data to the model using an algorithm developed in the programming language Python. Temperature vs. time data and ionic strength data determined the time-dependent change in the model parameters. The algorithm results in good fits to seven experimental data sets over a range of total alkalinities (17.0 and 135 mEq), temperatures and pH values. These generally good fits provide a strong basis for optimizing the operation of high-alkalinity cultivation processes of microalgae in the presence of only atmospheric CO_2 to increase algal biomass productivity.

CBE Poster #751

Date: 07/2019
Title: **Precision control of oxygen diffusion using hydrogels**
Authors: **Jason Zeng**, PhD Student
Affiliation: Chemical Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Hydrogels are porous hydrophilic polymeric networks swollen with a solvent, usually water. These soft matters have many uses in modern life including drug delivery, wound dressing, sealants, tissue engineering, industrial

productions, bioreactors and much more. Hydrogels are unique because they have diffusive properties similar to a liquid, but holds spatial integrity as a solid. The common PEG-DA hydrogel has about half the diffusivity of water. That means hydrogels can be used as membranes in pipes, fill in holes, seal cracks, or surround objects all while maintaining its high diffusive properties. This is juxtaposed against a liquid which cannot hold spatial integrity and will simply flow away. Furthermore, a hydrogel is mostly water, and depending on the polymer network used, is often cytocompatible with cells. In summary, hydrogels are diffusive solids. Despite their rising superstar status, not much is known about precision tuning of their diffusive properties. In most literature, the diffusivity of a hydrogel is viewed as a given property that does not change. Hydrogels are generally made by creating a reagent solution of a polymer, water, and a photoinitiator, and then the solution is polymerized with a laser to form a gelly solid. Because our lab creates our own hydrogel, we can alter the ratio of polymer to water in the reagent solution to change the volume fraction of polymers in the resulting polymerized hydrogel. In general, the lower the volume fraction, the less polymers there are to block the diffusion of solutes through the hydrogel. Thus, we can print custom hydrogels to promote or retard diffusion in a particular direction within a hydrogel. Furthermore, it's also possible to combine different pieces of hydrogel together, in the same way individual monomers polymerize. For example, if we implant a hydrogel drug underneath the skin, we can control diffusion of the drug to the skin and to the inner tissue separately.

CBE Poster #752

Date: 07/2019

Title: **Risk factors for chronic biofilm related infection**

Authors: **Philip S. Stewart**[1], Thomas Bjarnsholt[2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Costerton Biofilm Center, University of Copenhagen, Copenhagen, Denmark
Sponsored by: Lundbeck Foundation

The use of implanted medical devices is associated with a small but clinically important risk of foreign body infection. A key question is: Why do some patients develop chronic infection associated with an implanted device, but most do not? The literature on patient-specific risk factors for chronic infections associated with five types of implants was surveyed to glean clues about the etiology of these infections. Important risk factors include immunomodulation/steroid therapy, diabetes, smoking, and renal disease/hemodialysis, findings that support the critical role or compromised innate immunity in determining the vulnerable subpopulation. A model of biofilm-related device infection is presented that posits a requirement for defects in both the ability of innate immune cells to protect a foreign body and a broader systemic innate immune deficiency.

CBE Poster #753

Date: 07/2019
Title: **Investigating the development of multi-kingdom biofilms involved in the microbial defacement of building materials**
Presenter: **Erika J. Espinosa-Ortiz**[1], Postdoctoral Research Associate
Co-authors: Camryn DuBois[2], Paul Sturman[1], Robin Gerlach[1,3]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Mechanical Engineering, Montana State University, Bozeman, MT, USA.
[3]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.

Given favorable conditions of humidity, light and temperature, building materials can provide a suitable environment for microbial proliferation and biofilm formation. Microbial colonization on building components may result in the impairment of the aesthetic quality of the surface (e.g. discoloration), biodeterioration and accelerated weathering of the material affecting its durability, and potential health risks for humans. Commonly detected microorganisms on outdoor building materials include fungi (e.g. black molds), bacteria (actinomycetes and cyanobacteria), microalgae and small animals (e.g. protozoa, rotifers and nematodes). This study aims to investigate the surface colonization and biofilm development of common microorganisms found on building materials, including fungi, algae and cyanobacteria on wood samples. Material biodeterioration — also known as defacement — was evaluated by accelerated tests in an environmental chamber, designed to be operated as a high humidity/partially-wetted system to ensure microbial germination and growth. Wood samples were inoculated with spores of *Aureobasidium pullulans* (a common black mold fungus) and autotrophic microorganisms (*Chlorella vulgaris*, a microalga; a soil isolated cyanobacterium; and *Trentepohlia*, a subaerial alga) and incubated at room temperature (23±1°C) and relative humidity >95%; periodic wetting of the samples with growth medium was performed via a misting system. Microbial proliferation and material defacement were analyzed weekly for eleven weeks. Digital image processing was used as a tool for assessment and analysis of material defacement; this method allows for a less subjective and easily repeatable procedure to determine microbial coverage on material surface compared to simple human visual perception commonly used in standard methods. The potential establishment of co-cultures of various combinations of microbes and their biofilm development on wood samples was further studied under completely submerged conditions (batch experiments performed in Erlenmeyer flasks). Fungi and autotrophic microorganisms were successfully established on the wood panels, in both the environmental chamber and in completely submerged batch systems. The developed systems could be used to test the biodeterioration of building materials under a broad range of operational conditions (relative humidity, temperature, simulation of rain events, light intensities) that might influence microbial proliferation and biofilm development.

CBE Poster #754

Date: 07/2019
Title: **Designing a new biofilm reactor**
Authors: **Madelyn Mettler**[1], Kelli Buckingham-Meyer[1], Darla Goeres[1], Lindsey Lorenz[1], Stephen Pedersen[2], Paul Sturman[1], Diane Walker[1], Bryan Warwood[2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]BioSurface Technologies, Bozeman, MT, USA.
Sponsored by: Montana Board of Research and Commercialization Technology

The design process is a highly collaborative endeavor that requires input from many sides. In partnership with BioSurface Technologies, the Standardized Biofilm Methods Laboratory carried out laboratory testing of new reactor prototypes. This collaboration allowed for an open back-and-forth discussion about feasibility from the manufacturing side and what is necessary from the laboratory side to make a successful reactor. While the initial design decisions were made to mimic a cooling tower, the Industrial Surfaces Biofilm Reactor has broad applications in other industries. The reactor is unique compared to the other biofilm reactors on the market as the coupons are hydrated but not submerged. The applications of the reactor can extend to chemical testing, use in regulatory agencies, paint and coatings industries and, of course, industrial water industries. This poster presents

the design details of the new Industrial Surfaces Biofilm Reactor as well as promising data that shows the reactor's potential to grow a repeatable biofilm.

CBE Poster #755

Date: 07/2019
Title: **Fabrication of microfluidic device for investigation of antimicrobial tolerance**
Authors: **Jacob Rotert**[1,2], Tom LeFevre[1,2], James Wilking[1,2], Philip S. Stewart[1,2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: Montana State University Undergraduate Scholars Program, National Science Foundation (DMR-1455247)

The goal of this project is to investigate if bacterial biofilms are protected from disinfectants or antibiotics when they form in recesses or crevices that are sheltered from the main fluid flow path above the biofilm. This research could provide new insight into strategies on how to remove detrimental biofilm from pipe systems or equipment. To investigate this problem, custom microfluidic devices were fabricated that include dead legs perpendicular to the main flow channel. The short and inexpensive fabrication process involves sketching a design in a CAD software, transferring the design to a computer-controlled laser cutter, and cutting three pieces from acrylic sheets. These pieces are then solvent bonded together using a mild solvent along with pressure and heat. Pin connectors for tubing are sealed into ports in the device and microfluidic tubing is attached to allow flow in and out of the device. Flow tests have been performed to visualize the fluid path and exchange of different fluids in the device. In the future, *Staphylococcus aureus* bacteria will be inoculated into the device, allowed to grow for a period, and then treated with bleach or an antibiotic to observe local antimicrobial action and biofilm persistence.

CBE Poster #756

Date: 07/2019
Title: **Structuring microbial biofilms with 3D printing**
Authors: **Reha Abbasi**[1,2], Thomas LeFevre[1,2], Isaak Thornton[1,3], Jason Zeng[1,2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
[3]Mechanical & Industrial Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: National Science Foundation under Grant #1736255 (BuG ReMeDEE)

Most microorganisms, including bacteria, fungi, and archaea, exist in surface-adherent, multicellular communities known as biofilms. These films exhibit enhanced antibiotic resistance and reduced clearance by the immune system and are implicated in a wide variety of medical issues, including hospital-acquired and biomedical implant infections. Biofilm formation and virulence are largely governed by cell-to-cell communication, which is strongly influenced by community structure. However, structure-communication relationships remain poorly understood, and there is a need for technologies that provide well-defined spatial control over microbial structures. Here, we will present a light-based 3D printing platform for structuring bacteria in a biocompatible hydrogel. To explore the impact of spatial location on complex microbial interactions in biofilms, we print biomedically-relevant bacteria like *Pseudomonas aeruginosa* and follow their time-dependent behavior using confocal microscopy. We find that microorganisms in the 3D printed structures exhibit complex and unexpected dynamics. Knowledge gained from these experiments can be used to optimize structure-function relationships and may inform new strategies for biofilm disruption.

CBE Poster #757

Date: 06/2019
Title: **Improved understanding of microbe-mineral interactions using droplet-based microfluidics**
Authors: **Neerja Zambare**[1,2], Nada Naser[1,2], Connie Chang[1,2], Robin Gerlach[1,2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.

Subsurface porous media present an ideal application environment for the precipitation-based technology called Microbially Induced Calcium carbonate Precipitation (MICP). During MICP, precipitation is facilitated by an increase in alkalinity which can result from the ureolytic activity of certain microbes. Such ureolytic biomineralization has been of interest for several subsurface applications such as soil strengthening and stabilization, concrete and limestone remediation, subsurface barrier creation as well as groundwater remediation. Meso-scale flow reactors allow for an understanding of the overall process and how it can be controlled on relevant scales. This is undeniably important from an application standpoint, but meso-scale studies do not offer insight into microbe-mineral interactions at the micro-scale. Phenomena at the micro-scale indeed will determine the success of MICP at the larger scale. For instance, it is hypothesized that precipitation starts at the bacterial cell itself because the negatively charged cell attracts calcium ions. However, this has not been shown definitively at the single cell level, other than with post-mortem scanning electron microscopy images of dried precipitates with cell-sized indentations, which might be prone to artifacts. The main goal of this project was to monitor cell growth and MICP at the single cell level and visualize the precipitation process, including crystal nucleation, aggregate growth and microbe-mineral associations. To investigate such microbe-mineral interactions, we developed microfluidic chips to generate miniature droplets containing single cells of the ureolytic bacterium *Escherichia coli* MJK2, a strain modified to express a green fluorescent protein as well as to carry out ureolysis. The droplets contained dissolved urea, calcium and other nutrients to promote MICP. Bacterial growth as well as calcium carbonate nucleation and growth in stable droplets were recorded via real-time microscopy using Confocal Scanning Laser Microscopy. Calcium carbonate was detected by its ability to auto-fluoresce under UV light, and the precipitate polymorph was identified using Raman Spectromicroscopy. Droplets provide a means to study real-time cell attachment and growth as well as crystal nucleation. These processes are impossible to visualize or quantify in the subsurface, which is where a majority of MICP applications are proposed to occur. Droplet based studies can be used to manipulate cell attachment, thus promoting biofilm formation in porous media. Also, it is important to control which calcium carbonate polymorph forms during MICP because it affects the stability of the precipitate. With the limited potential for sampling during field-scale applications of MICP, it is difficult to control, or even identify calcium carbonate polymorphism. Droplet microfluidics is a novel approach to monitor real-time changes in calcium carbonate crystal phases. The potential to control biofilm formation or polymorphism can be explored readily using droplets by changing the chemical make-up or environmental conditions. The ability to control these factors could aid in maximizing the efficacy of MICP treatments in field applications.

CBE Poster #758

Date: 07/2019
Title: **Thermal stability of urease produced by *Sporosarcina pasteurii***
Authors: **Arda Akyel**[1,2], Anna Martinson[1,2], Adrienne J. Phillips[1,3], Robin Gerlach[1,2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA
[2]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA
[3]Civil Engineering, Montana State University, Bozeman, MT, USA
Sponsored by: MSU Undergraduate Scholars Program, US Department of Energy

Leaking CO_2 sequestration sites and orphaned natural gas and oil wells inhibit society's attempts to reduce global warming. One method to seal subsurface leakage pathways is urease induced calcium carbonate precipitation (UICP), a method of biocement production. The biocement formed through UICP has been shown to decrease

permeability of subsurface leaks in concrete, however, the current method allows calcium carbonate to form while traveling to the fracture. UICP in undesired locations could clog the pathway rather than the fracture. The generation of biocement decreases at low pH conditions with a significant decrease in precipitation at pH conditions lower than 6. Meanwhile, the process of urea hydrolysis increases the pH of its surroundings. Using this information, UICP can be controlled by designing a method to control the pH. UICP solutions at a pH too low for significant amounts of calcium carbonate precipitate could be transferred into deep subsurface fractures. The pH of the UICP solution would increase at a predetermined rate. When the UICP solution reaches the fracture, the pH should be high enough for significant precipitation to begin. While traveling, the buffer solution and pH must be high enough not to denature the urease enzyme, but low enough to significantly slow down the biocement production rate. From this research, ureolytic activity was shown to stop at pH conditions lower than 4.1 from a plant source and at 4.8 for *S. pasteurii* source. With an engineered buffer and a flow rate, UICP would be able to occur only at desired locations.

CBE Poster #759

Date: 07/2019
Title: **Efficacy of treatments on biofilm growth in hydration bladders**
Authors: **Kelsey Meier,** Madelyn Mettler, Kelli Buckingham-Meyer, Lindsey Lorenz, Diane Walker, Darla Goeres, Paul Sturman
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: US Army Combat Capabilities and Development Command (USRDEC)

The purpose of this experiment was to test the biofilm growth potential and biofilm disinfection characteristics of two hydration units. The two units, a reusable unit called Hydramax and a disposable unit called Refuel, were operated under stimulated use conditions by filling and draining the units twice a day. The water used was BAC water, which is dechlorinated, biologically activated (BAC) Bozeman tap water that has gone through two spent granular activated carbon (GAC) filters. The first filter removes residual chlorine and the second increases the microbial burden of the water. This experiment was run for six weeks, with effluent water samples taken twice a week and at the end of each week, two units were destructively sampled. At the end of the six weeks, two units of each type were sampled as controls and the remaining units were treated either with bleach or chlorine dioxide cleaning tablets. It was found that each unit accumulated biofilm at similar rates over the course of the experiment. Differing accumulation of biofilm was noticed when comparing locations in the bladders, bite valves, and tubing. Bleach was a more effective treatment in the Refuel units than in the Hydramax units, however it was not as effective in the bite valves and tubing. The tablets performed similarly in biofilm removal in all locations in both bladder types.

CBE Poster #760

Date: 07/2019
Title: **Characterization of microplastics in precipitation**
Authors: **Bekah Anderson**[1,2], Markus Dieser[1,2], Christine Foreman[1,2]
Affiliation: [1]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: Undergraduate Scholars Program, Montana State University, Bozeman, MT, USA.

The modern lifestyle is plagued by plastic. From disposable bottles to packaging to modern appliances, plastic is nearly impossible to avoid in the West. A rapidly growing area of research is focused on microplastics, which are small plastic particles either directly produced to be small or are derived from the weathering of larger plastic pieces. Extensive research has been conducted on the characterization of marine microplastics, but relatively less is known about freshwater systems and especially transport throughout the water cycle. Due to the ubiquitous nature of plastic, there are likely microplastics in precipitation and they can be found in remote environments. This research project aims to characterize microplastic presence in Montana precipitation, and we believe this project is

the first of its kind. To complete this task, precipitation samples (i.e. rain, snow, hail) were taken, filtered, stained, and observed using epifluorescence microscopy to count and categorize plastic particles. Raman spectroscopy was employed to chemically identify unknown polymers. Thus far, preliminary results have shown plastic particles in precipitation. By the end of this project, we expect to be among the first to officially document microplastics in precipitation. The findings of this research project will further inform the prevalence of microplastics and their role in environmental contamination. As society moves further towards sustainability and environmental responsibility, it is imperative that we actively work to understand and resolve the implications of our plastics use.

CBE Poster #761

Date: 06/2019

Title: **Developing a novel hollow fiber membrane reactor (HfMR) for the cultivation of methane-utilizing biofilms from extreme environments**

Authors: **Ayse Bengisu Kilic**[1,2], Ellen Lauchnor[2,3], Erika J. Espinosa-Ortiz[2]

Affiliation: [1]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
[2]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[3]Civil Engineering, Montana State University, Bozeman, MT, USA.

Sponsored by: National Science Foundation (NSF)

Methane (CH_4) is an abundant trace gas and rich as a carbon and an energy source. Methane is generated from natural and anthropogenic sources and contributes significantly to global warming, second only to CO_2. In order to better understand the dynamics of methane fluxes and contributions to global warming, the potential of microorganisms to contribute to the methane bioconversion needs to be better understood. Methane-utilizing bacteria called methanotrophs use methane as their sole carbon and energy source. In this study, we aim to investigate the development of biofilms and the metabolic activities of both novel methanotrophs isolated from extreme environments and well-known methane-oxidizing bacteria such as *Methylosinus trichosporium* OB3b. We isolated the methane-oxidizing bacterium, *Methylomonas koyamae,* from 4,850 feet below ground surface in an underground laboratory and former gold mine, the Sanford Underground Research Facility (SURF) in Lead, South Dakota. *M. koyamae* utilizes methane as its only carbon source, generates distinct pigments and has an ability to form aggregates. We have characterized the growth and methane-oxidation kinetics of both *M. trichosporium* OB3b and the *M. kyamae* in both, batch conditions and in a novel hollow fiber membrane bioreactor (HfMR) (*Figure.1*). The reactor was designed to study biofilm formation and the methane conversion ability of *M. koyamae* and *M. trichosporium* OB3b. The HfMR was selected to understand the methane fluxes of methanotrophs in a small-scale reactor and solve two major challenges (1) mass transfer inefficiency (gaseous nature of substrate in the liquid culture media) and (2) safety related issues (methane and oxygen). In the HfMR (C), substrate gas (methane(A)) is introduced via a polyvinylidene fluoride (PVDF) membrane (pore size=0.1-0.3 µm) at a pressure of 5 psi, liquid media(B) is provided into the HfMR continuously and excess media removed to waste container(D) simultaneously, with this design, we aim to stimulate biofilm formation on the surface of the membrane. Air is introduced into the headspace of HfMR(C) to assure that enough oxygen is present to sustain methane oxidation. The flow cell was designed using a CAD program and 3-D printed. Glass slides are used as the walls in the reactors to assure visualization inside of the reactor. Microbial activity inside of the reactor will be assessed by obtaining oxygen profiles via oxygen microsensors. We have been successful to identify microbial attachments on the membrane surface with Scanning Electron Microscopy (SEM).

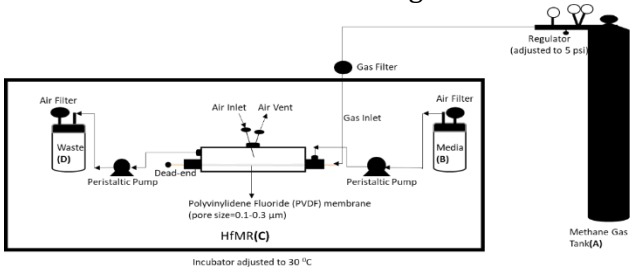


Figure 1: Hollow fiber membrane reactor (HfMR) schematic

CBE Poster #762

Date: 06/2019
Title: **Micro-fabricated impedance spectroscopy sensor to monitor biofilm growth**
Authors: **Matthew McGlennen**[1,2], Markus Dieser[1], Christine Foreman[1,3], Stephan Warnat[1,2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Mechanical & Industrial Engineering, Montana State University, Bozeman, MT, USA.
[3]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.

Electrochemical Impedance Spectroscopy (EIS) is a label-free, non-invasive, electrical measurement method capable of detecting biogeochemical signals correlating to cellular activity. Biofilm detection and viability can be monitored directly with changes in dielectric properties by using EIS. However, EIS has not been extensively used to study biofilm growth and formation for in situ applications. Dielectric measurements to determine biofilm physiology require precise and sensitive sensors since small changes in natural electrochemical biofilm composition are anticipated. Micro-fabricated sensors take advantage of their high sensitivity and low cost to overcome the challenges associated with biofilm research when used as impedance sensors, and have the potential to replace expensive and time consuming research methods. In addition, sensor fabrication is based on matured semiconductor technologies and guarantee precise and repeatable devices. Overall, the goal of this project is to develop and test a micro-fabricated impedance spectroscopy platform to detect, quantify, and analyze biofilm physiology in aqueous environments. Fluorescence microscopy and other biological analyses will be coupled to electrochemical models and will be used to predict physiological changes in biofilm in environmental and laboratory based investigations. In addition, material degradation and reliability will be investigated to optimize sensitivity and prolong sensor lifespan resulting in a robust *in situ* biofilm research tool.

CBE Poster #763

Date: 07/2019
Title: **Interactions in cooperative and competitive biofilm consortia**
Authors: **Martina Du**[1,2], Jacob Fenton[1,2], Ross Carlson[1,2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: Montana INBRE

Bacterial productivity is often reliant on cooperation, competition, and trading of essential nutrients. Most natural communities are very complex so studying these important multispecies interactions using defined cocultures can help decode the ecological functions of metabolite exchanges in helpful biofuel production systems or harmful medical infections. The cooperative coculture model involves a synthetic coculture comprised of two *E. coli* strains that have complementary gene knockouts to force an obligatory symbiosis. The coculture consists of an arginine-secreting *E. coli* strain that cannot catabolize lactose; and a lactose-catabolizing *E. coli* strain that cannot synthesis the essential amino acid, arginine. Collectively, the two strains can grow via cross feeding. Strain abundance and ratios were studied using three different lactose positive strains that secreted varying amounts of acetate which altered the economics of metabolite exchange. The competitive model involves a pathogenic strain of *S. aureus* growing as a monoculture biofilm then invading it with a liquid culture of probiotic *E. coli* Nissle to study the competitive interactions between the bacteria. *E. coli* Nissle is being used because it excretes an antimicrobial peptide which gives it potential to be competitive and displace the pathogenic biofilm. The biofilms being invaded will be inoculated after varying periods of growth to determine at which points the invader could be successful in displacing the biofilm at different stages of growth.

CBE Poster #764

Date: 06/2019
Title: **A simulated and modeled assessment of the impact of hydrological parameters on terrestrial subsurface microbial communities**
Authors: **KaeLee Massey**[1], Sara Altenburg[1], Heidi Smith[1,2], Matthew Fields[1,2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Microbiology and Immunology, Montana State University, Bozeman, MT, USA.
Sponsored by: ENIGMA

An essential and understudied ecosystem that supports microbial life is the terrestrial subsurface. The amount and quality of groundwater contained within the subsurface is critical for human activities such as farming, irrigation of crops, and industrial practices. However, as a result of human activity contamination events have altered these habitats which impacts microbial processes and community compositions. Understanding how microorganisms are able to adapt and remediate perturbed conditions is critical for human health since as microbial metabolisms within these subsurface environments to help sustain life above ground (*e.g*., global elemental cycles). The use of porous media will promote detailed measurements of key mass transport variables (*e.g.,* advection, dispersion, biotransformation, and mass flux) for a packed bed reactor with low fluid velocities. The focus of this project is to simulate *in situ* conditions of groundwater flow through shallow subsurface (porous media) environments. Ultimately, this will facilitate the ability to take detailed measurements of key mass transport variables at the mesoscale and correlate these variables to the larger field scale of the terrestrial subsurface.

CBE Poster #765

Date: 07/2019
Title: **Thermally induced calcite precipitation as a novel fracture sealing technique**
Authors: **Abby Thane**[1], Joe Eldring[1,2], Ryanne Daily[1,3], Randy Heibert[1,4], Robin Gerlach[1,3], Adrienne Phillips[1,5]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Mechanical & Industrial Engineering, Montana State University, Bozeman, MT, USA.
[3]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
[4]Montana Emergent Technologies, Butte, MT, USA.
[5]Civil Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: U.S. Department of Energy (DE-FE0026513) *Any opinions, findings, conclusions or recommendations expressed herein are those of the authors and do not necessarily reflect the views of the Department of Energy (DOE).*

Thermal degradation of urea is often used in selective catalytic reduction (SCR) aimed at reducing NO_x emissions in exhaust gases of diesel engines or power plants. In the work presented, thermally degraded urea in the presence of calcium ions, is shown to produce calcium carbonate. This process, known as Thermally-Induced Calcite Precipitation (TICP) is investigated for subsurface applications such as geothermal fracture sealing where temperatures and pressures increase with depth. Previous studies successfully utilized a similar process known as Microbially-Induced Calcite Precipitation (MICP) to seal fractures in subsurface wellbore environments. However, the application range of this technology is limited in high temperature environments found in deeper wells. In this study, two shale cores with artificial fractures were loaded into stainless steel columns and sealed using TICP under simulated subsurface temperature conditions (130°C) and chemistry (in produced water collected from the Bakken oil field). The permeability of both fractured cores was reduced by approximately 2.5 orders of magnitude. Field Emission Scanning Electron Microscopy (FE-SEM) and stereomicroscopy showed crystal formations within and around the fractures. These crystals were shown through X-ray Powder Diffraction Spectroscopy (XRD) to be different morphologies of calcium carbonate including aragonite, vaterite, and calcite. Results suggest that TICP has the potential to be used successfully to seal fractures in high temperature environments.

CBE Poster #766

Date: 06/2019
Title: **Sodium bicarbonate amendment for enhanced astaxanthin production from *Haematococcus pluvialis***
Authors: **Berrak Erturk**[1,2] Christian Lewis[1,2] Brent M. Peyton[1,2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: Church and Dwight Co.

Haematococcus pluvialis is a freshwater green microalga which is widely considered to be the best natural source for the high value keto-carotenoid, astaxanthin. The aim of this study was to quantify effects of bicarbonate addition and determine improved conditions for growth and astaxanthin production. To achieve this experimental design *H. pluvialis* was cultivated in two stages called the "green" and "red" stage. It is well known that *H. pluvialis* stores astaxanthin inside lipid bodies as esters. Previous studies have shown that the addition of high concentration bicarbonate salts near nitrogen depletion immediately ceased cellular replication and resulted in high lipid content. Combining these observations, we hypothesized that a bicarbonate amendment near nitrogen depletion would also enhance the production of high value compounds found in microalgae. In this study, sodium bicarbonate amendment at the "green" stage was also used to provide additional carbon source for this organism to obtain optimal growth and higher astaxanthin concentrations. *H. pluvialis* (UTEX 2505) was cultured in stirred batch reactors containing MES-Volvox medium continuously shaken at 120 rpm with a 12:12 light dark cycle. 50mM of sodium bicarbonate was found to be the best concentration and was compared with control conditions. According to the results the astaxanthin accumulation rate went up from 0.13 mg L^{-1} day^{-1} to 0.64 mg L^{-1} day^{-1} with an astaxanthin concentration of 1.56 ± 0.01 mg L^{-1} and 3.95 ± 1.25 mg L^{-1} respectively. Whereas an addition of lower concentration of 2.5 mM sodium bicarbonate at the "green" stage increased the final astaxanthin accumulation rate up to 0.71 mg L^{-1} day^{-1} with an astaxanthin concentration of 6.70 ± 0.89 mg L^{-1}. 2.5 mM sodium bicarbonate amended cultures reached at a cell concentration of 2.92±0.11 E+06 cells/ml whereas the control condition stayed at 1.91± 0.13 E+06 cells/ml which indicated that 2.5 mM of sodium bicarbonate increased cell growth. On the contrary 50mM ceased growth altogether, but resulted in higher astaxanthin accumulation inside the cell. The only concern with the sodium bicarbonate amendment at the "green" stage was the pH increase resulting in ammonia lost which was present as a nitrogen source in the medium. Ongoing experiments will maintain the pH around 7 to attempt higher astaxanthin production rates.

CBE Poster #767

Date: 07/2019
Title: **Approaching microbial interaction studies with hydrogel particle communities**
Authors: **Shawna Pratt**[1,2], Petria Russell[1,2], Jake Fredrickson[1,2], Connie Chang[1,2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
Sponsored by: National Science Foundation EPSCOR

Coupling drop-based microfluidics and biocompatible hydrogels allows for the physical isolation of microbes in chemically permeable microspheres. Here, we are developing hydrogel particle cell encapsulation and incubation as a high-throughput method to study cell to cell interactions and perform single cell resolution long term culturing and assays. In this work, cells are inoculated into hydrogel (alginate) precursor solutions. The solutions flow through a microfluidic device where they are mixed and form monodisperse droplets at kilohertz rates. The droplets are made in sizes between 10 μm and 200 μm. In droplets, the precursor solutions crosslink, forming solid alginate spheres with single embedded microbes. The nature of alginate allows the particles to be simultaneously permeable to aqueous solutions and physically constraining to microbes. After gelation, the particles are introduced to a microfluidic incubation device, where thousands are arranged in a 2-D array. Media and other aqueous solutions flow through the incubation device and around the particles, carrying nutrient, as well as chemical signals from the encapsulated cells, between particles in the array. Currently, we are working to optimize cell growth in hydrogels, hydrogel material properties, and the design of the culturing device. By successfully developing these methods, we hope to provide an adaptable platform that facilitates diverse microbial research,

namely: semi isolated culturing of 'unculturable' or slow growing microorganisms that may rely on a syntrophic relationships for growth; studies of multispecies interactions associated with traits such as improved growth and virulence; and studies of microbial response to constant and oscillating chemical signals and stresses.

CBE Poster #768

Date: 07/2019
Title: **High-throughput antibiotic resistance profiling of single cells using drop-based microfluidics**
Authors: **Carter Hoffman**[1,2], Geoffrey Zath[1,2], Michael Franklin[1,3], Connie Chang[1,2]
Affiliation: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2]Department of Chemical & Biological Engineering, Montana State University, Bozeman, MT, USA.
[3]Department of Microbiology and Immunology, Montana State University, Bozeman, MT, USA.
Sponsored by: National Institutes of Health

Communities of microbes exhibit heterogeneity, arising not only between different species, but also within the genetic and physiological states of the individual species which constitute the community. Bacterial infections can be comprised of such communities, where heterogeneity plays a significant role in the development of antibiotic resistance. For example, complex biofilm communities are often found within the pulmonary tissue of cystic fibrosis patients, and the chronicity of these infections is generally the result of a minority subpopulation of antibiotic-resistant microbes. Probing such microbial subgroups for a molecular basis of antibiotic resistance furthers the understanding of resistance mechanisms, and may aid in identifying novel antibiotic targets. However, rapidly collecting statistically significant sample sizes requires high-throughput techniques, while comprehensively identifying genetic resistance motifs requires sequencing at the single-cell level. Current methods of studying antibiotic susceptibility typically rely on the use of well plates. These methods are limited to quantifying the minimum concentration of a drug which inhibits microbial growth (minimum inhibitory concentration, MIC), and are unable to provide single-cell information. We overcome these limitations through the use of high-throughput, single-cell microfluidics, which enables co-encapsulation of individual microbial cells with unique, barcoded antibiotic solutions from a 96-well plate, into picoliter-sized water-in-oil emulsion drops. Microbial growth within drops can be monitored by increased green fluorescent protein (GFP) expression, and changes to this growth under antibiotic stress can yield a distribution of antibiotic susceptibilities for the population, with single-cell resolution. This not only yields MIC values, discerned by drops which lack microbial growth, but detects resistant outliers, discerned by drops with unusually high growth. Furthermore, we can isolate mutants of interest by sorting based on the fluorescence intensities of carrier drops, a technique similar to conventional fluorescence activated cell sorting (FACS). Finally, by sequencing these isolates, genes contributing to antibiotic resistance can be identified. This work will greatly increase the throughput of antibiotic susceptibility testing, and the understanding of microbial antibiotic resistance mechanisms.

CBE Poster #769

Date: 07/2019
Title: **3D Printing of Human Sinuses**
Authors: **Lora Frische**[1,2], Matthew Fields[1], Jim Wilking[1,2]
Affiliation: [1] Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
[2] Chemical & Biological Engineering Department, Montana State University, Bozeman, MT, USA.
Sponsored by: National Science Foundation

Twelve percent of people in the United States suffer from debilitating, chronic sinus infections. These infections result in 13 billion dollars spent annually on productivity costs alone. Recent studies show that biofilms likely contribute to the chronic nature of these infections. The paranasal sinuses are a complex series of channels, cavities and membranes, which gives biofilms many places to thrive, and also makes the sinuses difficult to study. The understanding of these infections is lacking, which leaves patients with limited, inadequate treatment options. A new, tangible understanding of this complex system is possible with 3D printing. The model is developed through image analysis and editing of a CT scan from a patient. The shape and size of paranasal sinuses differ

greatly from person to person, meaning that no two sinus infections are alike. This opens a door to personalized medicine, helping medical professionals to identify causes and origins of sinus infections, as well as investigate both mechanical and chemical treatments.

Montana Biofilm S&T Meeting
AGENDA: July 16–18, 2019

Monday
July 15

6:00–8:30 pm
Registration and welcome reception
Larkspur Foyer, Hilton Garden Inn Bozeman

Tuesday
July 16

7:30–8:00 am
Registration and continental breakfast
Larkspur Foyer, Hilton Garden Inn

8:00–8:10
Introductory remarks
Larkspur Ballroom
Matthew Fields, CBE Director
Paul Sturman, CBE Industrial Coordinator
Laura Wahlen, Research Scientist, Baxter Healthcare

SESSION 1: Biofilm Methods

8:10–8:40
Proposed standard method for antimicrobial urinary catheters: ruggedness test results
Darla Goeres, Associate Research Professor, Chemical and Biological Eng., MSU; PI, Standardized Biofilm Methods Laboratory, CBE

8:40–9:10
Monitoring hand hygiene and its effects on hospital-associated infections (HAI)
Al Parker, Biostatistician, CBE; Asst. Research Professor, Mathematical Sciences, MSU

9:10–9:40
Product-specific method development to assess microbial contamination
Chris Jones, Director of RandD, Sharklet Technologies

9:40–10:10 Networking Break

10:10–10:40
Interlaboratory study results for the drip flow reactor
Diane Walker, Research Engineer, CBE

10:40–11:10
Understanding your production facility's microbiome using 16S metagenomics
Michele Sayles, Executive Director, Food Safety and Quality, Diamond Pet Foods

11:10–11:35
CBE Address
Matthew Fields

Poster Pitches

11:35–12:00
Presented by CBE researchers

12:00–1:00 pm
Lunch, Hilton Garden Inn

SESSION 2: Wound Biofilms

1:00–1:30
Architecture and phylogenetic structure of chronic wound biofilms
Garth James, Associate Research Professor, Chemical and Biological Eng., MSU; PI, Medical Biofilms Laboratory, CBE

1:30–2:00
Integrating symptom science with innovative molecular measures: Focus on understanding the trajectory of healing vs. non-healing in chronic venous leg ulcers
Joyce Stechmiller, Associate Professor, Behavioral Nursing Science, University of Florida

2:00–2:30
Chronic wounds are chronic infections caused by biofilm
Randall Wolcott, MD, Medical Director, Southwest Regional Wound Center

CBE Open House: Poster session and lab demonstrations

3:00–5:00
3rd Floor Barnard Hall, MSU
Schedule available onsite

Wednesday
July 17

7:30–8:00 am
Registration and continental breakfast
Larkspur Foyer, Hilton Garden Inn

SESSION 3: Alternative Biocides

8:00–8:30
Consumer Product Preservation: Sustaining product microbial quality in a dynamic environment
Chuck Pettigrew, Principal Scientist, Procter and Gamble

8:30–9:00
Modern solutions for product protection
Ed Rolls, Global Account Director, Cosmetic Ingredients Division, Symrise

9:00–9:30
Alternatives for preservation
Julie Vaughn Biege, Global Business Development Director, Industrial Products;
Rosanna Stokes, Business Development Manager, Consumer Products, Emerald Kalama Chemicals

Presentation ToC | Presentation Abstracts | Poster ToC | Poster Abstracts | Agenda | NASA Session | Things to Do in Bozeman

9:30–10:00
Biocidal-free future in EU. Wood protection and coating. Why and how?
Berit Lindegaard, Product Mgr., Danish Technological Institute

10:00–10:30 Networking Break

10:30–11:00
HCPA Microbiology Preservative Subcommittee (MPS) supporting and enhancing the microbiological quality of consumer, household, and industrial products
Tony Rook, RandD Associate Director, Microbiology, The Sherwin-Williams Co.

11:00–11:30
The design, synthesis, and evaluation of new classes of antimicrobials for the control of biofilms
Tom Livinghouse, Professor, Chemistry and Biochemistry, MSU

11:30–11:50
Presentation of CBE awards
Matthew Fields

11:50–1:00 pm
Lunch, Hilton Garden Inn

SESSION 4: Young Investigators

1:00–1:30
Oral biofilm-stimulated human gingival epithelium differentially regulates inflammatory responses in co-cultured immune cells
Jason Brown, Research Assistant, University of Glasgow Dental School

1:30–2:00
Investigation of synovial fluid induced *Staphylococcus aureus* aggregate development and its impact on surface attachment and biofilm formation
Matthew Pestrak, Graduate Research Associate, Microbial Infection and Immunity, The Ohio State University

2:00–2:30
Evolution of *Pseudomonas aeruginosa* in a chronic burn wound model
Erin Gloag, Postdoctoral Researcher, Microbial Infection and Immunity, The Ohio State University

2:30–3:00
CBE-NBIC scientific and collaborative opportunities
Jeremy Webb, Professor, Microbiology, University of Southampton; Co-Director, National Biofilms Innovation Center

3:30-5:00
Business Meeting
Hilton Garden Inn

Dinner
6:00 pm
Hart Ranch, Gallatin Gateway

Thursday July 18

7:30–8:00 am
Registration and continental breakfast
Larkspur Foyer, Hilton Garden Inn

SESSION 5: Biofilms and Host Response

8:00–8:30
Mechanics of biofilm infection
Phil Stewart, Professor, Chemical and Biological Engineering, MSU, CBE

8:30–9:00
Early recruitment of neutrophils Prevents *Staphylococcus aureus* biofilm formation
Brian Pettygrove, PhD Student, Microbiology and Immunology, MSU, CBE

9:00–9:30
***Pseudomonas aeruginosa* biofilms and adaptations during chronic infections**
Dan Wozniak, Professor, Microbial Infection and Immunity, The Ohio State University

9:30–10:00
Breaking down the immunobiology of implant fibrosis/foreign body response
Joshua Doloff, Asst. Professor, Biomedical Engineering, Materials Sci. and Engineering, Johns Hopkins University School of Medicine

10:00–10:30 Networking Break

SESSION 6: Biofilms in Space

10:30–11:00
Development of nanoengineered materials for organisms (NEMO) to resist biofilm formation in space
Kasthuri Venkateswaran, Senior Research Scientist, California Institute of Technology, JPL

11:00–11:30
Management of biofilms in the operation of the ISS water recovery and management system
Layne Carter, ISS Water Subsystem Manager, NASA

11:30–12:00
Design considerations for mitigating biofilm growth on the ISS and future missions
Mononita Nur, Aerospace Engineer, NASA

12:00–12:10
Meeting wrap up

***1:30-4:30**
NASA Session
Join our NASA speakers and attendees for a brainstorming session on maintaining wastewater systems for extended space flight
Larkspur Ballroom A, Hilton Garden Inn

NASA ECLSS, AES & SLPSRA Joint Biofilm Workshop

At the 2019 Montana Biofilm Science and Technology Meeting
Montana State University, Bozeman, Montana, July 18, 2019

Workshop Description

NASA and the CBE are hosting a Biofilm workshop on July 18, 2019, to invite attendees to provide ideas to help the Agency not only in maintaining wastewater systems and understanding the microbiology of the built environment for extended spaceflight, but also in developing future research directions for its microgravity Physical Sciences and Space Biology Programs. Long duration missions provide significant challenges, particularly to the crew environment. Of specific concerns are the Water Processing Assembly (WPA), where recycled water can become susceptible to microorganism contamination, and biofouling and biocorrosion of surfaces and environmental subsystems. Workshop participants are invited to provide guidance to mitigate contamination issues related to the WPA. Workshop participants are also invited to provide research ideas by describing studies of biofilm formations that make use of the environment aboard the International Space Station. Ideas are also welcomed from researchers who are not able to attend the workshop in person.

Workshop Participants

Keynote Speaker: TBD

Panel Chair: Robert McLean, Texas State University

Panel Co-Chair : **Luis Zea**, University of Colorado,

Panel Co-Chair: **David Tomko**, NASA Head Quarters

Kasthuri Venkateswaran, California Institute of Technology

Kevin Y. Sato, NASA Headquarters

Mononita Nur, NASA Marshall Space Flight Center

Donald L. Carter, NASA Marshall Space Flight Center

Sridhar Gorti, NASA Marshall Space Flight Center

Walter Schneider, NASA Marshall Space Flight Center

To submit ideas for Biofilm mitigation or ISS research, visit (http://tinyurl.com/NASA-RFI-NNH19ZTT002L)

Image credit: Energia/Petr Andreychuk, Biofilm in Russian Condensate Plumbing

Image credit: NASA/ S130-E-012312

Looking for great ideas for biofilm mitigation in exploration, as well as biofilm research on the International Space Station

Discover Montana's Great Outdoors!

Hike the College 'M' Trail

Distance: 3 miles RT
Time: 2 hrs (including drive-time)

See that big white M on the southern face of the Bridger Mountains? You can hike to it. Round trip will take about 60 to 75 minutes. The panoramic view is beautiful. **Pro tip**: Two trails will take you to the M. The most direct (and strenuous) trail is to the right.

Bear Spray Cannisters Available for Loan

Hike Palisade Falls

Distance: 1 mile RT
Time: 2.5 hrs (including drive-time)

Nestled in the Gallatin Forest near the Hyalite Reservoir is Palisade Falls. As a bonus, the drive through Hyalite Canyon is gorgeous. The half-mile hiking trail is paved, making it one of the few hikes that is truly handicapped accessible. The payoff is a beautiful 80-foot-tall waterfall.

Hike Lava Lake

Distance: 6 miles RT
Time: 4.5 hrs (including drive-time)

You'll enjoy views of the stunning Spanish Peaks and other mountains as you hike to beautiful Lava Lake. It's a heavily traveled trail that folks in these parts love. It's a rocky path, so hiking shoes are preferable to sneakers as you ascend 1,600 feet to the majestic lake.

Guided Fly Fishing (float or wade)

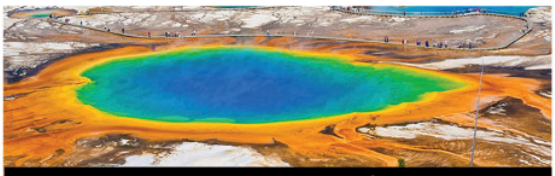
Time Commitment: 6-8 hrs (not including drive-time)
Cost: $550 total for 2 people (includes food & non-alcoholic beverages, waders, boots, flies, rods and reels, and terminal tackle)

People travel to Bozeman from all around the world because of the world-class fly-fishing this beautiful region offers. Even first-time anglers will enjoy fishing any one of the rivers that run through it. Not sure you could even cast a line? No sweat. The guides from the award-winning Fins & Feathers will have you fishing in no time.

Visit Yellowstone National Park

Time: 10-12 hrs (including round-trip drive-time)
Cost: $35 per vehicle for 7 days of park entry from date of purchase
Website: www.nps.gov/yell

Consider driving to Yellowstone through beautiful **Paradise Valley** to Gardiner, Montana (1h 15m). You'll enter the park at the famed **Roosevelt Arch**. You'll soon be at the **Boiling River** where you can relax in geothermal waters. A few miles up the road you'll arrive at **Mammoth Hot Springs** where you'll see the famous hydrothermal structures. From there, wind southward to **Old Faithful** (1h 45m), the world's most famous geyser, which shoots off every 90 minutes or so. While in **Geyser Basin**, stop off at **Grand Prismatic Spring**. On your return, consider exiting the park to the town of **West Yellowstone**. You'll traverse different terrain than you did on the way in, and see striking mountains as you drive alongside the Gallatin River. Be careful! Watch for big horn sheep on the road as you drive the 20-mile stretch through the canyon between **Big Sky** and **Bozeman** (West Yellowstone to Bozeman (1h 45m).


Grand Prismatic Spring


Mammoth Hot Springs


Old Faithful


Bison in the Lamar Valley

Presentation ToC | Presentation Abstracts | Poster ToC | Poster Abstracts | Agenda | NASA Session | Things to Do in Bozeman

Things to Do (and Eat!) around Bozeman!

Nova Cafe

Backcountry Burger Bar

Copper Whiskey Bar & Grill

Breakfast (Main Street area)

Western Cafe No-frills hearty food prepared in the spirit of a diner served with authentic western charm. Plus, the waitress just might call you "Hon." #homeybonus

Cateye Cafe They loosely enforce a semi-serious list of dos and don'ts. But the food is terrific and there's a $0.50 discount if you wear your cateye glasses.

Nova Cafe So dang good. If there's a line, don't worry ... it will be worth the wait. Do it.

Lunch (Main Street area)

The Co-op Think Whole Foods, but hyper local. This grocery store with a healthful hot-food bar and salads is so good they built two of them within a mile of each other.

Mackenzie River Pizza There's a reason locals and tourists alike enjoy this Main Street mainstay. Great pizza, and so much more.

Backcountry Burger Offering tasty beef and bison burgers, craft beer, and locally fermented kombucha on tap.

Dinner (Main Street area)

Plonk This eatery offers a good wine list and tasty food. Think: Duck-fat poached scallops and lamb tartar.

Open Range Worth the hefty price it commands. Should you declare its pork chop to be the world's best, I wouldn't argue. Plus, craft cocktails.

Copper It's easy to overlook Copper, especially since located below street level. Good food. Reasonable prices. Terrific atmosphere.

There are dining treasures throughout this region of the Treasure State. **Sir Scott's Oasis in Manhattan, Montana**, is a locally owned, no-frills steakhouse that's worth the 25-minute drive west from downtown Bozeman. **Montana's Rib and Chop House in Livingston, Montana**, is a 30-minute drive east that takes you alongside a historic railroad. Plus, you'll be in beautiful Livingston. If it were me, I'd enjoy a pre-dinner cocktail at the historic Murray Hotel and take a 5-minute walk to the Chop House. Notice that stiff breeze? It's near omnipresent in Livingston. Now imagine that breeze when it's -20 degrees!

Bozeman Hot Springs

Distance from Downtown Bozeman: 15-minute drive
Cost: $10.50 per adult
Website: bozemanhotsprings.com

Bozeman Hot Springs uses geothermic-heated waters in each of its 12 pools. Temperatures range from a chilly 57 degrees to 106 degrees. The waters come from an underground well, and feature naturally occurring therapeutic minerals such as sodium and silica.

Montana Grizzly Encounter

Distance from Downtown Bozeman: 15-minute drive
Cost: $8 per adult
Website: grizzlyencounter.org

Founded in 2004, Montana Grizzly Encounter is a nonprofit rescue and education sanctuary. The facility features five bears, four of which "were born in unfortunate captive situations and could never be released into the wild." The fifth, named Bella, was orphaned in Alaska.

Gallatin History Museum

Distance: Downtown
Cost: $7.50
Website: gallatinhistorymuseum.org
Learn about the rich, wild, and wealthy history of the region.

Museum of the Rockies at MSU

Distance: 6-minute drive
Cost: $14.50 for 2-day pass
Website: museumoftherockies.org
Dinosaurs, a planetarium, and world-class traveling exhibits.