



BIOFILM TECHNOLOGIES
PATHWAYS
TO PRODUCT DEVELOPMENT

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CBE Director's Remarks

CBE's role in regulation and product advancement

Presenters: **Matthew Fields**, Director¹, Professor²

Affiliation: ¹Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

²Microbiology & Immunology, Montana State University, Bozeman, MT, USA.

SESSION 1: Perspectives on Biofilm, Regulation, and Research

Medical biofilms: Insights from the first two decades of the millennium

Presenter: **Robin Patel**, Chair, Division of Clinical Microbiology; Elizabeth P. and Robert E. Allen Professor of Individualized Medicine; Professor of Medicine; Professor of Microbiology; President, American Society for Microbiology

Affiliation: Mayo Clinic, Rochester, NY, USA.

In recent years, there has been an amazing increase in the numbers and types of implanted biomaterials being placed into patients. Unfortunately, use of these devices is sometimes associated with the complication of infection. The development of biofilms on the surfaces of these devices is key to the pathogenesis of associated infection. Examples of device-associated infections include, but are not limited to, periprosthetic joint and other orthopedic device-related infections; intravascular, urinary, peritoneal dialysis and other catheter-associated infections; breast implant and expander infections; ventilator-associated pneumonia; cerebrospinal fluid shunt-associated infections; contact lens-associated keratitis; penile implant infections; vocal prosthesis infections; cochlear implant infections; prosthetic valve endocarditis; cardiovascular implantable electronic device infections; ventricular assist device infections; vascular graft infections; and biliary, urinary, intravascular and other stent-associated infections. Although infection rates are not available for all devices, it is estimated that rates of infection are approximately 1-2% for arthroplasties, 2% for breast implants, 4% for mechanical heart valves, 4% for pacemakers and defibrillators, 10% for ventricular shunts, and 40% for ventricular-assisted devices. In addition to device-associated infections, biofilms can be associated with chronic non-device-related infections, including, but not limited to, pulmonary infections in cystic fibrosis and other diseases associated with bronchiectasis; chronic sinusitis; chronic otitis media; native valve endocarditis; burn and chronic wound infections; diabetic foot infections; and periodontitis. Over the past two decades, biofilm-specific diagnostics have emerged for some of the above-named infection-types, improving their diagnosis. Biofilms render microorganisms relatively resistant to most conventional antibiotics, as well as the host immune system. Over the past two decades, new anti-biofilm treatment strategies have been developed. Additional biofilm-directed diagnostics and anti-biofilm treatment strategies are needed.

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Moving towards meaningful standards for preclinical performance testing of anti-biofilm medical devices and combination products

Presenter: **Scott Phillips**, Regulatory Research Scientist

Affiliation: Office of Medical Products and Tobacco, Center for Devices and Radiological Health, Office of Science and Engineering Laboratories, Division of Biology, Chemistry and Materials Science, US FDA, Silver Spring, MD, USA.

A significant portion of healthcare associated infections (HAIs) are related to medical device use, resulting in tens of thousands of deaths and billions of dollars in expense to the healthcare system in the United States alone each year. One area in need of further development is appropriate standard methods for preclinical performance testing of medical devices pertaining to antimicrobial effectiveness. The outcomes of this testing are most useful when they can be related to a clinical benefit to medical device users. In this talk, I discuss challenges with current *in vitro* performance testing, as well as some possible pathways to successful antimicrobial combination product standards. I will focus on the development and characterization of a library of FDA *ex vivo* tissue-based biofilm test methods, as well as the findings obtained with these methods. I will show that biological tissue stimulates more

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rapid and aggressive colonization, and that biofilm on tissue is more challenging to eradicate than planktonic bacteria or biofilm on abiotic surfaces. In addition to showing why these models and the information on time and space dependent bioburden that they provide is important to discriminate between different anti-biofilm modalities' performance (e.g. contact killing vs. drug eluting) for regulatory science, I will also show how they can reduce the burden for academic and industrial researchers to develop safer, more effective technologies through early stage high throughput screening.

Antimicrobial method development initiatives

Presenter: **Steve Tomasino**, PhD, Senior Scientist

Affiliation: Office of Pesticide Programs, Microbiology Laboratory Branch, US EPA, Fort Meade, MD, USA.

EPA is responsible for regulating hospital disinfectants used in healthcare facilities. The registrant of an antimicrobial product with a public health claim is required to submit efficacy data to EPA in support of the product's registration. An antimicrobial product is considered to make a public health claim if the product bears a claim to control microorganisms that pose a threat to human health. Work related to the registration of antimicrobial pesticides is handled by the Office of Pesticide Programs (OPP) Antimicrobials Division (AD). The Microbiology Laboratory Branch (MLB), under the Biological and Economic Analysis Division of OPP, supports the AD and is charged with the development and standardization of methods for testing the efficacy of antimicrobial products. MLB has been instrumental in advancing the science of antimicrobial product testing, leading multi-laboratory collaborative studies, and providing technical expertise to standard-setting organizations and various agency stakeholder groups. The presentation will provide an update on current test method development initiatives such as evaluating biocides against *Legionella pneumophila* in cooling tower water, determining the bactericidal activity of copper-containing surface products, and assessing the efficacy of antimicrobial wipes. In addition, the status of the technical evaluation of the OECD Quantitative Method for testing bacteria on hard non-porous surfaces will be discussed.

Biofilm claims: Who cares? A commercial perspective

Presenter: **Elaine Black**, Director, Regulatory Affairs

Affiliation: Ecolab, St. Paul, MN, USA.

The deleterious effects of bacterial biofilms span numerous industries, environments and aspects of everyday life. This presentation will look at some of the segments (food safety and public health) that experience biofilm issues using examples both in North America and around the globe. By delving into the question of how much and why a biofilm claim matters to an end user, it will cover the challenges of choosing the right solution for dealing with biofilm issues. The importance of comprehensive, reliable data in addition to application form and application-site expertise will be explored. Key differences in the regulatory landscape with regard to biofilms across the globe will be addressed. Finally, future directions and opportunities will be discussed.

An innovative company's perspective on biofilm regulation

Presenter: **Matt Myntti**, Chief Technology Officer

Affiliation: Next Science, LLC, Jacksonville, FL, USA.

Next Science, LLC, pioneers innovative technologies to address bacterial biofilms. Next Science's experience navigating the challenging and fluid federal regulatory landscape to achieve commercialization likely reflects the experience with similar products and thus our journey may be instructive to other innovators. This presentation will summarize briefly the history of federal regulation of biofilm products by the U.S. Environmental Protection Agency (EPA) and the Federal Food and Drug Administration (FDA), capture where each Agency is now in regulating biofilm innovations, and propose where and how we collectively need to move forward to commercialize life-saving innovations that will benefit society.

SESSION 2: Food-Related Biofilms

Dry biofilms: Challenges of recognition and eradication

Presenter: **Diane Walker**, Research Engineer

Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Over the past few years, researchers have become increasingly interested in biofilm found in low moisture environments, referring to these as dry biofilms or dry surface biofilms. Recent studies have determined that dry biofilms might not be detected by traditional methods of surface testing, yet these biofilms, which may harbor pathogens, can remain on surfaces even after cleaning and disinfecting protocols have been followed. In the food industry, where traditional methods are used for detection, and sanitation procedures are relied upon to prevent contamination, this is especially concerning. This presentation will describe dry biofilms and their environments, the challenges of recognizing their presence, and current methods developed for *in vitro* studies to potentially enhance their eradication.

Evaluation of the effect of chlorine dioxide gas and a liquid probiotic application on hydrated and dehydrated biofilms

Presenter: **Michele Sayles**, PhD, Executive Director

Affiliation: Food Safety & Quality, Diamond Pet Foods, Meta, Missouri, USA.

Biofilms, both hydrated and dehydrated, have the potential to be difficult to eliminate and or control in food manufacturing environments and equipment. These biofilms have the potential to harbor pathogens that then have the potential to cross contaminate food products. Various cleaning and sanitation methods have been studied to evaluate the most effective methods to successfully eliminate and/or control biofilms to help reduce the risk of the potential pathogen cross-contamination. Chlorine dioxide gas is a proven sterilant capable of eliminating all viruses, bacteria, fungi, and spores. It has been used to decontaminate a growing number of food facilities for both contamination response and contamination prevention in order to ensure sterility after renovations, equipment installations and routine plant shutdowns. The use of probiotics as an application in the food manufacturing environment is a relatively new area and the research to date has focused on strains of probiotics that are naturally antagonistic toward pathogens. The primary strategy in applying probiotics into a production environment is twofold: one, to out-compete potential pathogen populations and two, to reduce those pathogenic populations by the antagonistic actions of the probiotics such as the production of antimicrobial metabolic byproducts. One potential use of a probiotic application is to an environment after it has been treated with chlorine dioxide gas as a means to potentially repopulate a clean surface with non-pathogenic bacteria to help establish a healthy microbiome. This presentation will review a study that evaluated the effect of chlorine dioxide gas followed by a liquid probiotic application on hydrated and dehydrated biofilms by using traditional and metagenomic analysis.

Persistent vs. transient *Listeria monocytogenes* in food processing facilities: What makes the difference?

Presenter: **Dumitru Macarisin**, PhD, Research Microbiologist

Affiliation: Center for Food Safety & Applied Nutrition, US FDA, College Park, MD, USA.

The implication of three fruits in recalls and outbreaks of human illnesses, due to contamination by foodborne pathogens, has been on the rise over the last decade. The outbreaks caused by *Listeria monocytogenes* have been particularly puzzling, because the implicated tree fruit commodities (apple and stone fruit) do not support growth of this pathogen. Though the sources and routes of apple and stone fruit contamination by *L. monocytogenes* remain unknown, apple processing facilities have been identified in the past as potential sources of persisting *L. monocytogenes* contamination. In the current work, we sought to understand the composition of microbiota in apple and other tree fruit processing built environments and its association with the occurrence of the foodborne pathogen *L. monocytogenes*. This lecture will deliver novel findings on the occurrence and persistence of *L. monocytogenes* in tree fruit packing environments and will also provide an insight in environmental microbiomes of tree fruit packing facilities and their association with occurrence and persistence of the foodborne pathogen, *L. monocytogenes*.

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Control of microbial hazards on low moisture processing equipment through non-aqueous cleaning and sanitation

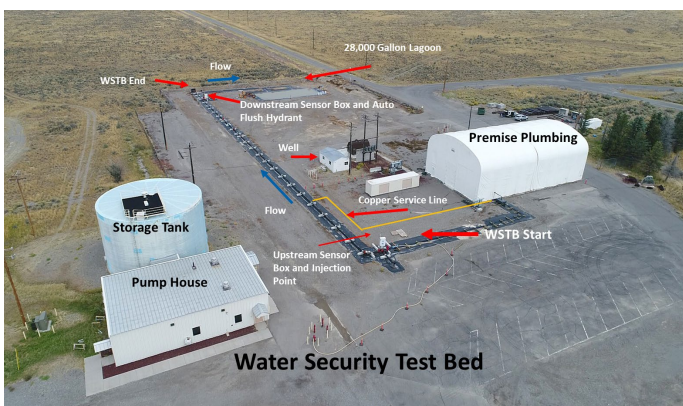
Presenter: Elizabeth Grasso-Kelley, Assistant Professor
Co-Authors: Susanne Keller, Lindsay Halik, Stephen Grove, Nathan Anderson
Affiliation: Department of Food Science and Nutrition, Institute for Food Safety and Health, Illinois Institute of Technology, Bedford Park, IL, USA.

Microbial contamination of low-water activity processing equipment by pathogens, such as *Salmonella*, poses a significant health risk as they may remain viable in the product and processing environment for an extended period of time. Effective cleaning and sanitation procedures are essential for preventing cross-contamination and may be used as a corrective action or preventive control. Due to the nature of low-water activity foods, use of water in these processing environments is discouraged as introduction of moisture may increase microbial risks. Potential cleaning and sanitation methods include physical, thermal, or chemical-based sanitizers. Physical removal includes wiping surfaces or pigging and/or purging internal equipment surfaces, such as piping, with clean material. Studies on purging *Salmonella* contamination in a simulated pilot-scale peanut butter processing line have shown limited efficacy. Results suggest that purging can reduce microbial contamination but is not sufficient to remove all pathogens in this or other low-water activity processing environments. Thermal methods, including circulation of hot oil at ~90°C, was also found to not significantly affect microbial contamination in this same system. Chemical decontamination with a 70% isopropanol based sanitizer with or without added quaternary ammonium compounds has shown promise as a non-aqueous method for significant inactivation of *Salmonella* on contaminated equipment. It is important to understand the hazard(s) as well as the environment to ensure appropriate cleaning and sanitation methods are employed.

Drinking water pipeline and premise plumbing decontamination of *Bacillus globigii*

Presenter: James Goodrich, Senior Science Advisor
Co-authors: Helen Y. Buse, Jeffrey G. Szabo
Affiliation: Center for Environmental Solutions and Emergency Response, US EPA, Cincinnati, OH, USA.

This presentation discusses the efficacy of flushing, disinfection, pipe wall scouring, and relining of drinking water pipes and household appliances contaminated with *Bacillus globigii*, an anthrax surrogate utilizing the full-scale USEPA Water Security Test Bed (WSTB) located near Idaho Falls, ID. The traditional water utility practice of flushing and disinfecting does not appear to be adequate for the removal of the *Bacillus globigii* from the main pipeline wall to insure the safe return to service. Decontamination of the appliances require multiple flushing and disinfection steps and varies by appliance as well. Sampling for *Legionella*, mycobacteria, and free-living amoeba occurrence at the WSTB was initiated with variable results by location and species as well. Future *Legionella* experiments are planned.



SESSION 3: Biofilm Infection

Risk factors for chronic biofilm infections on medical implants

Presenter: Philip S. Stewart¹, Regents Professor
Co-authors: Thomas Bjarnsholt², Professor of Immunology and Microbiology
Affiliation: ¹Chemical & Biological Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.
²Costerton Biofilm Center, University of Copenhagen, Copenhagen, Denmark.

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 conceptual model for the initiation of biofilm-related device infection is presented that posits a tripartite contribution of microbial contamination, compromised cellular immunity in the vicinity of a foreign body, and systemic immune deficiency. An important contribution of this analysis is to shift the focus of preventing biofilm infections on devices away from the use of antibiotics and antimicrobial coatings toward management and strengthening of innate immune function.

Lighting up the lung: Developing optical tools for realtime, point-of-care detection of lung disease in the clinic

Presenter: Bethany Mills, Postdoctoral Research Associate
Co-Authors: Proteus Team (proteus.ac.uk)
Affiliation: Optical Imaging of Microbiological and Immunological Targets at the Point of Care, Center for Inflammation Research, University of Edinburgh, UK.

Incisive molecular imaging has the potential to delineate key pathophysiological processes *in vivo in situ*. This talk will outline the work to date and approaches being taken by the Proteus Team in Edinburgh to develop optical molecular imaging for pulmonary pathology. We are interested in directly observing bacteria, immune cells and inflammatory markers through optical endomicroscopy (OEM) imaging platforms, coupled with activatable SmartProbes, which are instilled locally within the distal lung immediately prior to imaging. Upon contact with target enzymes, cells, or bacteria, the SmartProbes switch from “off” to “on” and their fluorescence is detectable using our fiber-based OEM device. We have validated lead-optimized bacterial probes, fibrosis probes, and neutrophil activation probes from bench-top through to phase 0/1 trials within the clinic. Our first in-house built OEM device has also undergone initial clinical validation. We are now extending our trials and developing further SmartProbes and devices, including bespoke optical based imaging/delivery fibers for future multiplexing studies with the ambition of delineating bacterial colonization from pathogenesis within the distal lung.

A regulatory overview of the infection control medical devices

Presenter: Yongqing Chen, Scientific Regulatory Reviewer/Biologist
Co-author: Elizabeth F. Claverie-Williams, MS, CAPT, USPHS-CC, Microbiologist
Affiliation: Center for Device & Radiological Health, US FDA, USA.

The infection control team in CDRH reviews approximately 90 medical devices, primarily in Class I and II, such as patient examination gloves, surgeon gloves, ultrasonic cleaners for medical instruments, surgical masks with an antimicrobial/antiviral agents, surgical drapes and accessories, biological sterilization process indicators, liquid chemical sterilants/high level disinfectants, steam sterilizers, and vascular access flush solutions. Our review tools include FDA guidances, FDA-recognized consensus standards, and well-established scientific procedures, evidence, and/or literature. The infection control team engages with internal and external subject matter experts which aids in quality control of reviews. The infection control expertise ranges from reprocessing, sterilization, toxicology, biocompatibility, microbiology and infection control and prevention.

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Use of the hollow fiber infection model to study emergence of resistance using humanized pharmacokinetic profile of antibiotics

Presenter: Tesfalem Zere¹, ORISE Research Fellow

Co-authors: Narayana Garimella¹, Sarah Aminov², Heather Stone³, Leonard Sacks³, Rodriguez-Chavez, Isaac³, James L. Weaver^{1*}

Affiliation: ¹Division of Applied Regulatory Science (DARS), OCP/OTS, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), 10903 New Hampshire Ave, White Oak, Silver Spring, MD, USA.

²Department of Biochemistry & Molecular & Cellular Biology, Georgetown University Medical Center, 3900 Reservoir Rd., NW, Washington, DC 20057.

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*Corresponding author

Combination therapy is a promising strategy to enhance effectiveness of currently available antibiotics and counter drug resistance. However, reliable *in vitro* systems are needed to investigate the efficacy of clinically relevant drug combinations. In this study, we have evaluated the hollow fiber infection model (HFIM) as a reliable *in vitro* method to quantitatively study emergence of resistance under different treatment regimens. The study was conducted using humanized pharmacokinetic (PK) profiles of ampicillin, ciprofloxacin and fosfomycin and their double or triple simultaneous combination against *Escherichia coli* CFT073 (wild type) and an isogenic hypermutant strain. The dosing regimens were set up to model three daily doses, C_{max} of 10 μ g/mL, for ampicillin and a single daily dose, C_{max} of 100 μ g/mL and 0.16 μ g/mL, for fosfomycin and ciprofloxacin, respectively. In the HFIM, bacteria were exposed to the drugs either as a single or as a simultaneous combination, for ten days. Drug samples and bacterial samples were collected at different time points for PK analysis as well as to account for total and resistant bacterial populations, respectively. Our findings show that combination therapy significantly delayed the emergence of resistant *E. coli* wild type subpopulations. Moreover, no relevant mutations were identified to explain the genetic basis for the emergent resistance. Hence, to enhance conditions for mutation-based resistance in our system and test whether multidrug therapy leads to mutational resistance, an isogenic hypermutable strain of *E. coli* CFT073 was used. Our results showed that, after a significant delay, the hypermutant strain showed resistance to all three drugs (at 3X minimum inhibitory concentration), unlike the wild type. Our preliminary stability assay indicates that the acquired resistance is stable. In conclusion, these findings suggest that strategic combinations of antimicrobials may play a role in controlling the emergence of resistance during treatment. The HFIM system could potentially be used to identify clinically relevant combinations. Further animal and human trials will be needed to confirm this and to evaluate the impact on the host microbiome.

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Busting biofilms—winning the war in wounds

Presenter: Greg Schultz, Director

Affiliation: Institute for Wound Research, Department of Obstetrics & Gynecology, College of Medicine, University of Florida, Gainesville, FL, USA.

Wound healing is a complex biological process that progresses through a sequence of phases (hemostasis, inflammation, repair, and remodeling) that are regulated primarily by cytokines, growth factors, proteases and extracellular matrix components. Chronic wounds do not progress through these phases, and typically become “stuck” in an inflammatory condition that is characterized by elevated levels of pro-inflammatory cytokines, proteases, and reactive oxygen species (ROS), which impair healing by degrading extracellular matrix molecules, growth factors and receptors that are essential for wound cell migration, proliferation and scar formation. Recent evidence suggests that bacterial biofilms contribute to the chronic inflammation in a high percentage of chronic wounds. Bacterial biofilms are very difficult to eradicate with oral antibiotics or topical antiseptics, which have led to the principles of Biofilm-Based Wound Care that expand the previous concept of Wound Bed Preparation that emphasize removing biofilms by debridement followed by use of dressings or treatments that prevent reformation of biofilms by killing planktonic bacteria that regenerate biofilms. After inflammation and proteases are reduced in wound beds, advanced wound treatments that enhance healing include exogenous growth factors, collagen/basement membrane dressings that trap proteases and release growth factors, topical protease inhibitors

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(doxycycline), bioengineered skin substitutes and vacuum assisted closure. These concepts are formalized in the STEP-DOWN THEN STEP-UP concept of biofilm based wound care. Using a pig skin wound explant biofilm model, commercially available antimicrobial wound dressings and topical treatments generally are able to prevent formation of biofilms by killing planktonic bacteria. However, the ability of antimicrobial wound dressings and topical treatments to kill mature bacterial biofilms vary widely. A new concentrated nonionic surfactant product with very low cytotoxicity was able to prevent formation of biofilms and eliminate mature biofilms after 2 to 3 days of daily application and wiping. Visualization of bacterial biofilms in chronic skin wounds at the point-of-care (POC) is a major challenge for clinicians. A rapid, inexpensive, simple, POC technology that utilizes a cationic membrane combined with a cationic dye was developed that localizes biofilm matrix on wound beds. Recent clinical results demonstrate the “biofilm wound map” technology localizes biofilm on chronic wound beds and predicts subsequent development of wound slough and wound healing.

Development and characterization of complex wound biofilm models

Presenter: **Petra Kohler Riedi**, Senior Research Specialist

Co-authors: Joe Stoffel, Brittany Hadj Romdhane

Affiliation: 3M Corporate Research Laboratory, St. Paul, MN, USA.

Microbial biofilms are associated with wound chronicity and often exist as complex communities of multiple microbial species. Biofilms of multiple bacterial species have been reported in clinical wound samples, as have microbial biofilms containing both bacterial and fungal species. The goal of our work was to develop and characterize biofilm models containing bacterial and fungal species to mimic the multispecies biofilms found *in vivo*, and to evaluate the performance of antimicrobial wound care products in these complex model systems. We adapted a multispecies bacterial biofilm model combining *Staphylococcus*, *Enterococcus*, and *Pseudomonas*, and substituted the fungal pathogen *Candida albicans* for *Enterococcus*. The antimicrobial effectiveness of several topical antimicrobial products was evaluated in the multibacterial and multikingdom biofilm models. In a multibacterial biofilm model, *Staphylococcus* was highly susceptible to killing by several antimicrobial products whereas significantly fewer *Staphylococci* were killed by the same products in the multikingdom model. Subsequently, several other methodologies for growing multikingdom biofilms in formats amenable to testing the antimicrobial performance of wound dressings were developed and characterized.

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SESSION 4: Oral Biofilm

***In vitro* models of oral biofilms for evaluating antimicrobial susceptibility**

Presenter: **Garth James**, Associate Research Professor

Affiliation: Chemical & Biological Engineering; Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

The Medical Biofilms Laboratory at the Center for Biofilm Engineering has adapted standard methods, such as the drip-flow reactor (DFR, ASTM E-2647) and the CDC biofilm reactor (CDC-BR, ASTM E-2562) to evaluate antimicrobial efficacy against oral biofilms. These biofilms have included single- and dual-species biofilms, defined polymicrobial biofilms, and undefined polymicrobial biofilms derived from saliva. Antimicrobial agents evaluated included chlorhexidine gluconate, commercially available mouth rinses, and sodium hypochlorite. This presentation will provide an overview of how these methods were adapted to simulate aspects of supragingival caries-forming biofilms, subgingival biofilms associated with gingivitis and periodontitis, and biofilms infecting root canals.

Targeting oral biofilms using nanotechnology

Presenter: Hyun (Michel) Koo, Professor and Director

Affiliation: Center for Innovation & Precision Dentistry, Biofilm Research Labs, School of Dental Medicine, University of Pennsylvania, Philadelphia, PA, USA.

This presentation focuses on catalytic iron oxide nanoparticle technology to design pH-responsive therapeutic approaches targeting the oral biofilm microenvironment and to develop small-scale microrobots for automated biofilm disruption and removal. Using *in vitro*, *ex vivo* and *in vivo* biofilm models, we demonstrate how pH-activated nanoparticles can degrade the biofilm matrix and kill the embedded bacteria, and help prevent biofilm-associated oral diseases without deleterious effects on the host tissue and the resident microbiota. Current limitations, challenges and future directions of this nanotechnology will be also discussed.

Oral biofilm models for testing mechanical disruption on structure and community

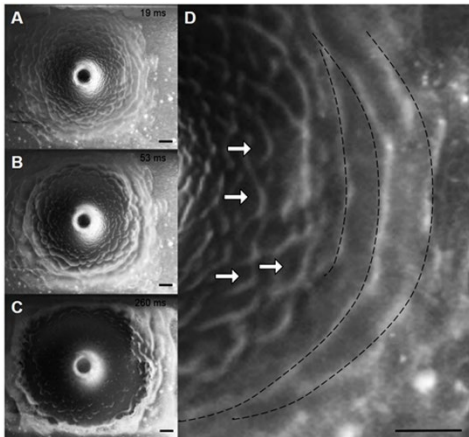
Presenter: Paul Stoodley¹, Professor

Co-authors: Yalda Khosravi², Raja Kandukuri², Sergey Borisov², Dirk de Beer², Arjun Chennu², Lledó Prades², Cristian Piciooreanu², Nick Cogan², Stefania Fabbri², Micelle Starke², Marilyn Ward²

Affiliation: ¹Infectious Disease Institute (IDI), Dept. Microbial Infection and Immunity, Dept. Orthopaedics, Dept. Microbiology, The Ohio State University, Columbus, OH, USA.

²National Centre for Advanced Tribology at Southampton (nCATS) and National Biofilm Innovation Centre (NBIC), Mechanical Engineering, University of Southampton, UK.

High velocity microsprays and air jets have been shown to liquefy biofilms grown from *Streptococcus mutans*, a cariogenic oral pathogen (Figure 1), causing them to detach from, as well as flow over surfaces. This disruption induces turbulence within the biofilm, greatly increasing the rapid delivery of particles and dentifrices into the biofilm, which are otherwise limited by diffusion over time scales normally associated with brushing and mouth washing. We hypothesized that such disruption may also disrupt the anoxic microenvironment that naturally develops within dental biofilms (due to the mass transfer limitation of dissolved oxygen into the biofilm) and allows the establishment of anaerobic periopathogens such as *Porphyromonas gingivalis*. However, in most lab model mixed community biofilms grown from human plaque and saliva are grown under an anoxic headspace which is assumed to be required for the establishment of such oxygen sensitive anaerobes. We established a simple well plate model in which simulated human plaque biofilms were grown from pooled human saliva and plaque under an oxic environment on hydroxyl apatite discs and an oxygen planar optode to measure the influence of a high velocity microspray on the oxygen concentration at the base of the biofilm. Biofilms were grown for 4 days and the relative change in abundance of six representative species were quantified by quantitative real time PCR (qRT-PCR). Obligate anaerobes were able to establish in the biofilm after 24 hours and remained in the community for the 4 days. After 66 hours the biofilm was anoxic at the base, however, after shooting with a microspray, biofilm was cleared from a circular area with a diameter of approximately 1 cm dia. The dissolved oxygen at the base of the remaining biofilm was increased to almost 100% of saturation. Thus, mechanical disruption by



Ripple formation in biofilms induced by a high shear jet impingement of air ejected at a velocity of 42 m/s from a 1 mm diameter nozzle positioned perpendicularly 5 mm from a S. mutans the biofilm at A) 19, B) 53 and C) 230 ms.

high velocity microsprays and jets may not only physically remove biofilm and increase the delivery of antimicrobial dentifrices but also disrupt the pathogenic microenvironment (anoxic and acidic) thus tipping the balance from a pathogenic to a commensal community.

SESSION 5: Reusable Medical Devices

Evaluating performance criteria for the cleanliness of reusable medical devices

Presenter: **Darla Goeres**, Associate Research Professor

Affiliation: Chemical & Biological Engineering, Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Biofilm is often defined as a self-organized cooperative community of cells embedded in an extracellular matrix. In the laboratory and literature, careful distinction is made between individual cells suspended in an aqueous environment and cell aggregates or biofilm. Seldom is the same careful distinction made between individual cells on a surface compared to aggregated clumps of cells. It is not uncommon to find both referred to as biofilm, especially because one of the most widely used methods to assess biofilm density is the viable cell count, a technique that cannot differentiate between individual cells on a surface and biofilm clumps. In this interactive presentation, the audience will be shown confocal images of surface associated cells and asked to vote on whether they consider the cells a biofilm, or not. Similar to a choose-your-own adventure, we will then consider the survey results in the context of assessing the cleanliness of reusable medical devices, where the goal is to set an acceptance level for the cells and matrix left behind.

Quality control of endoscope reprocessing: Three-hospital clinical study using rapid, point-of-reprocessing methods to detect protein and biofilm

Presenter: **Sang Won Lee**^{1,2,3}, PhD Student

Co-authors: Michael Wong¹, Anant Agrawal¹, Allan Guan¹, Shervin Abdollahi¹, Yi Wang¹, Ralph J Basile⁴, Kaumudi Kulkarni⁴, Miranda Gavette⁴, Jahan Azizi⁴, Jerri Tripp⁵, Elena Campbell⁵, Marc Bloom⁵, Norton Elson⁵, Mohamed Labib⁶, Dacheng Ren^{2,3,7,8}, K. Scott Phillips^{1*}

Affiliation: ¹Division of Biology, Chemistry and Materials Science, Center for Devices and Radiological Health, Office of Science and Engineering Laboratories, United States Food and Drug Administration, Silver Spring, MD, USA.

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⁷Department of Civil and Environmental Engineering, Syracuse University, Syracuse, NY, USA.

⁸Department of Biology, Syracuse University, Syracuse, NY, USA.

Every year, there are more than 75 million endoscopies performed in the United States, helping healthcare providers perform hundreds of essential procedures ranging from colonoscopy to treatment of pancreatic cancer. FDA facilitates patient access to best-in-the-world endoscopic technologies and ensures that endoscopes can be safely used. An ongoing challenge related to endoscope use and duodenoscopes in particular has been antibiotic resistant bacterial infection outbreaks. Research has shown that biofilm can build up on the internal and external surfaces of endoscopes over time, making current cleaning and disinfection procedures (called “reprocessing”) less effective. To address this problem, the FDA has proactively taken measures with several warnings to manufacturers and healthcare facilities. The FDA required manufacturers to study endoscopes being used in the clinic, and the results showed that about 2-5% of endoscopes do in fact have bacterial contamination. This talk will discuss collaborative research done by FDA, industry and academic partners that can quantify the amount of protein on endoscopes after reprocessing. These tools can detect and quantify contamination at levels that are lower than most current approaches. The tools are quick and inexpensive for hospitals to use. We call them FDA QC-ER, or “FDA Quicker” for short, because the tools can help hospitals maintain the quality (quality control) of their endoscope reprocessing (ER). In this talk, we will introduce FDA QC-ER and review results from testing in three hospitals and what we learned about factors that contribute to endoscope cleanliness. Quality control of endoscope reprocessing can help hospitals to prevent future infection outbreaks.

Medical devices containing antimicrobials– A regulatory perspective

Presenter: **Ramesh Panguluri**, Microbiologist/Team Lead

Affiliation: Disinfection, Reprocessing and Personal Protection Equipment Devices Team, Center for Device & Radiological Health, US FDA

Abstract not available.



February 4-5, 2020

Hyatt Regency Crystal City Hotel, Arlington, VA
Draft AGENDA

1/29/2020 9:58 AM

Tuesday February 4

7:30-8:00 am
Registration Regency Foyer
Meeting Regency F

8:00-8:15 am
Introductory remarks
Matthew Fields, CBE Director
Paul Sturman, CBE Industrial
Coordinator

8:15-8:35 am
**CBE's role in regulation and
product advancement**
Matthew Fields, CBE Director

SESSION 1 Perspectives on Biofilm, Regulation, and Research

8:35-9:20 am
**Medical biofilms: Insights from
the first two decades of the
millennium**
Robin Patel, Chair, Division of
Clinical Microbiology, Professor,
Microbiology & Medicine, Mayo
Clinic

9:20-9:55 am
**Moving towards meaningful
standards for preclinical
performance testing of anti-
biofilm medical devices and
combination products**
Scott Phillips, Regulatory Research
Scientist, Center for Device &
Radiological Health, US FDA

9:55-10:25 am
Break

10:25-11:00 am
**Antimicrobial method
development initiatives**
Steve Tomasino, Senior Scientist,
Office of Pesticide Programs,
US EPA

11:00-11:30 am
**Biofilm claims, who cares?
A commercial perspective**
Elaine Black, Senior Regulatory
Manager, Ecolab

11:30-12:00 pm
**An innovative company's
perspective on biofilm regulation**
Matt Myntti, Chief Technology
Officer, Next Science

12:00-1:00 pm
Lunch, Regency E

SESSION 2 Food-Related Biofilms

1:00-1:35 pm
**Dry biofilms: Challenges of
recognition and eradication**
Diane Walker, Research Engineer,
CBE

1:35-2:10 pm
**Evaluation of the effect of chlorine
dioxide gas and a liquid probiotic
application on hydrated and
dehydrated biofilms**
Michele Sayles, Executive Director,
Food Safety & Quality, Diamond
Pet Foods

2:10-2:45 pm
**Persistent vs. transient listeria
monocytogenes in food
processing facilities: What makes
the difference?**
Dumitru Macarasin, Research
Microbiologist, Center for Food
Safety & Applied Nutrition,
US FDA

2:45-3:15 pm
Break

3:15-3:50 pm
**Control of microbial hazards on
low moisture processing
equipment through non-aqueous
cleaning and sanitation**
Elizabeth Grasso-Kelley, Assistant
Professor, Food Science &
Nutrition, Illinois Institute of
Technology

3:50-4:25 pm
**Drinking water pipeline and
premise plumbing
decontamination of *Bacillus
globigii***
James Goodrich, Senior Science
Advisor, Wide Area &
Infrastructure Decontamination
Branch, US EPA

5:00 pm
Networking Reception
Chesapeake View

Wednesday February 5

7:30-8:00 am
Registration Regency Foyer
Meeting Regency F

SESSION 3 Biofilm Infection

8:00-8:35 am
**Risk factors for chronic biofilm-
related infection of implanted
medical devices**
Phil Stewart, Regents Professor,
Chemical & Biological
Engineering, MSU, CBE

8:35-9:10 am
**Optical imaging of distal lung
infection**
Bethany Mills, Postdoctoral
Researcher, Optical Imaging
PROTEUS Hub, University of
Edinburgh Queens Medical
Research Institute

9:10-9:45 am
**A regulatory overview of the
infection control medical devices**
Yongqing Chen, Scientific
Regulatory Reviewer/Biologist,
Center for Device & Radiological
Health, US FDA

9:45-10:20 am
Break

10:20-10:55 am

Use of the hollow fiber infection model to study emergence of resistance using humanized pharmacokinetic profile of antibiotics

Tesfalem Zere, ORISE Research Fellow, Center for Drug Evaluation & Research, US FDA

10:55-11:30 am

Busting biofilms—winning the war in wounds

Greg Schultz, Professor, Obstetrics & Gynecology, College of Medicine, University of Florida

11:30-12:00 pm

Development and characterization of complex wound biofilm models

Petra Kohler-Riedi, Senior Research Specialist, 3M

12:00-1:00 pm

Lunch, Regency E

SESSION 4 Oral Biofilm

1:00-1:35 pm

In vitro models of oral biofilms for evaluating antimicrobial susceptibility

Garth James, Associate Research Professor, Chemical & Biological Engineering, MSU; PI, Medical Biofilms Laboratory, CBE

1:35-2:10 pm

Targeting oral biofilms using nanotechnology

Hyun (Michel) Koo, Professor, Orthodontics; Director, Center for Innovation & Precision Dentistry, School of Dental Medicine, University of Pennsylvania

2:10-2:45 pm

Oral biofilm models for testing mechanical disruption on structure and community

Paul Stoodley, Professor, Microbial Infection and Immunity, Ohio State University

2:45-3:15

Break

SESSION 5 Reusable Medical Devices

3:15-3:45 pm

Evaluating performance criteria for the cleanliness of reusable medical devices

Darla Goeres, Associate Research Professor, Chemical & Biological Eng., MSU; PI, Standardized Biofilm Methods Laboratory, CBE

3:45-4:15 pm

Quality control of endoscope reprocessing: Three-hospital clinical study using rapid, point-of-reprocessing methods to detect protein and biofilm

Sang Won Lee, PhD Student, Biomedical & Chemical Engineering, Syracuse University

4:15-4:45 pm

The FDA perspective on products intended to resist or inhibit microbial infection

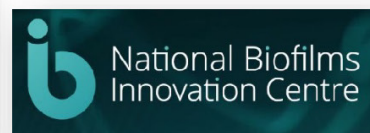
Ramesh Panguluri, Microbiologist/Team Lead, Disinfection, Reprocessing and Personal Protection Equipment Devices Team, Center for Device & Radiological Health, US FDA

Save the Date!

2020 Montana Biofilm Meeting

July 14-16, 2020

Bozeman, MT



CBE gratefully acknowledges Elsevier and the National Biofilms Innovation Centre for sponsoring the 2020 Biofilm Technologies: Pathways to Product Development meeting.



Selected Dining Options Nearby



SOCi Urban Italian Kitchen

Authentic Italian fare can be found inside the Renaissance Arlington Capital View Hotel. This stylish restaurant provides a fresh, modern take on the rustic spirit of Old World Italy.
2-minute walk from Hyatt Regency



Ted's Montana Grill

Where important ingredients, including American bison, are used to create classic American dishes.
8-minute walk from Hyatt Regency



Clark Street Grill

From Yelp Review: This is a little diner. Pretty good for what it is- nothing fancy, basic food. I had a perfectly acceptable burger and fries for lunch.
2-minute walk from Hyatt Regency



Ruth's Chris Steak House

Sizzling steaks and spectacular 11th-story view. Richly remodeled and elegantly redesigned, this Arlington steak house has been expertly tailored to elevate any occasion.
8-minute walk from Hyatt Regency



Fresh Kitchen

Fresh Kitchen offers comfort food with a DC twist by partnering with local produce growers, dairy farms, and specialty vendors to bring you farm-to-table meals.
3-minute walk from Hyatt Regency



Taj of India

Taj of India offers a calm and casual atmosphere that is good for dining with friends, family members, and co-workers.
10-minute walk from Hyatt Regency



Legal Sea Foods

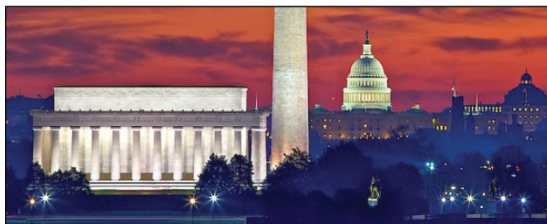
For all those who love seafood, the world really can be your oyster. Choose from among the 40 varieties of fresh fish and shellfish we serve with simple, New England preparations.
7-minute walk from Hyatt Regency



The Portofino Restaurant

The Portofino is a family owned and operated Italian restaurant that's offered fine-dining experiences in Arlington since 1970.
11-minute walk from Hyatt Regency

Getting to Downtown D.C.



If you decide to head into downtown DC, of course Ubers, Lyfts, and taxis can get you there. If you'd prefer to take the subway, the Crystal City Metro Station is a 16-minute walk. There are lots of subway options for getting into the city. Here's one: Take the "Yellow" subway to Gallery Place/Chinatown Station (15 minutes). Great restaurants (American fare, Chinese, Thai, etc.) are nearby. Plus, you're a 20-minute walk to the White House (to the west), the National Mall/monuments/museums (to the south), and the U.S. Capitol (to the southeast).

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