

Montana State University
■ Center for Biofilm Engineering
Bozeman, Montana

Anti-Biofilm Technologies: Pathways to Product Development

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Proceedings

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Presentation Abstracts

SESSION 1: Medical Device Technologies

The central role of biofilm in contamination and colonization of dermal fillers

Presenter: **K. Scott Phillips**, Regulatory Research Scientist, Center for Devices & Radiological Health

Affiliation: U.S Food and Drug Administration, Silver Spring, MD, USA

Background: The use of dermal fillers (DF) to address contour defects resulting from aging, disease, and trauma is increasing exponentially (over 1.7mil. in 2011, >91% in women). Infections are especially a concern for permanent DF and can require surgery, lead to disfiguring necrosis/scarring, or result in bacteremia. Removal of permanent DF can damage tissue and long-term antibiotic therapy can lead to multi-drug resistant infections. Patients suffer financial costs, lost work hours, social stigma and psychological health issues.

Study Question: This work sought to understand how we can make DF use safer.

Methods: Two intervention areas were targeted: skin preparation/injection, and material colonization/biofilm formation. For intervention #1, novel simulated skin and pigskin models were developed to study how to reduce contamination during injection. For intervention #2, a novel flow cell insert was developed to study how chemical and mechanical properties of DF affected *S. aureus* adhesion and 24h biofilm formation.

Results: Injection testing showed that bioburden in DF increased with needle size, but the type of needle (cannula vs. beveled) was not a factor. Threading injection style produced more contamination than serial puncture or fanning. Skin preparation testing compared 70% ethanol, chlorhexidine and povidine iodine wipes, and showed performance differences based on both the chemistry and texture. Interaction studies showed that *S. aureus* adhesion to DF could be reduced by 4 logs by increasing stiffness. However, stiffer materials had discontinuities that formed during injection, creating a microenvironmental niche for bacterial biofilm which may not be accessible to larger ~10µm macrophages. In addition, bacterial adhesion increased with DF hydrophobicity.

Public Health Implications: These results can be used to develop evidence-based guidelines for clinicians on how to reduce potential DF contamination during placement, and show that infection rates might be lowered with development of DF that can reduce adhesion but also self-seal to avoid internal gaps.

Biofilm initiation on medical devices

Presenter: **Philip S. Stewart**, Professor, Chemical & Biological Engineering

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

Infections associated with indwelling medical devices tend to be localized, recurrent, and persistent. Concepts drawn from the fundamental science of biofilm biology, chemistry, and physics are applied to understand and hypothesize how biofilms initiate on a medical device and how an established biofilm persists in the face of host defenses and antimicrobial chemotherapy. One mechanism of biofilm tolerance arises from the failure of a reactive antimicrobial agent to fully penetrate the biofilm. This mechanism is illustrated with experimental data for the penetration of hydrogen peroxide into a catalase-positive biofilm. A second mechanism of biofilm tolerance depends on nutrient or electron acceptor depletion within the biofilm leading to slow growth or dormancy and consequent reduced susceptibility. This mechanism is illustrated by experimental imaging of physiological gradients within

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a mature biofilm. Both of these mechanisms are amenable to analysis by reaction-diffusion theory which predicts that thicker biofilms become progressively more protected. The unsolved paradox is how a biofilm initiates *in vivo* when the biofilm is necessarily so young and thin that the preceding protective mechanisms cannot be effectively implemented. The competing processes of microbial adhesion and tissue integration, which Tony Gristina dubbed 'the race for the surface' in 1987 are revisited. Drawing on seminal animal model studies of biofilm infection, an alternative etiology of *in vivo* biofilm formation and establishment of infection is proposed.

Clinical perspectives on microbial biofilms and medical device infections

Presenter: **Brittany Goldberg, MD**, Medical Officer, Center for Devices & Radiological Health/Office of In Vitro Diagnostics and Radiological Health, Division of Microbiology Devices

Affiliation: U.S Food and Drug Administration, Silver Spring, MD, USA

In the following talk, a clinical perspective on medical device infections will be provided. Four clinical cases will be reviewed to identify specific medical device infections in which biofilms affect clinical management. The medical literature and consensus treatment guidelines will be reviewed for each case, and the potential roles of novel diagnostics will be discussed in the context of the current standard of care. Regulatory considerations through the FDA Pre-Submission program will be briefly discussed.

Animal models and implant associated infection

Presenter: **Tom Schaer, VMD**, Director, Preclinical Research Services, School of Veterinary Medicine

Affiliation: University of Pennsylvania, Philadelphia, PA, USA

This talk will provide an overview of some of the more popular animal models used in anti-infective research. Emphasis will be on models that center on implant associated infections, including the presentation of new data from synovial infection and periprosthetic joint infection (PJI) models. The second half of the discussion will touch on 1) methods to longitudinally monitor local tissue concentrations of anti-infective agents via sampling various tissue compartments and 2) present novel data from a PJI model designed to study efficacy of intervention.

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Regulatory development of an anti-biofilm drug product

Presenter: **Brett Baker**, Founder and CSO

Affiliation: Microbion Corporation, Bozeman, MT, USA

No abstract available.

SESSION 2: Surface Disinfection Technologies

Assessment of biofilm on dry hospital surfaces: How this informed development of model test systems

Presenter: **Karen Vickery**, Associate Professor, Medicine and Health Sciences; Scientific Director, Surgical Infection Research Group

Affiliation: Macquarie University, New South Wales, Australia

Healthcare associated infections cause increased patient morbidity and mortality. Bacteria responsible for these infections contaminate the patient environment and act as a source of infection for subsequent patients. Infection control procedures including hand hygiene, environmental cleaning, and disinfection aim to reduce the risk of pathogen transmission. However, despite these measures multiple

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antibiotic resistant organisms (MRO) continue to be isolated from hospital environments. We surmised that persistence of MROs in the environment is due to their incorporation into biofilms contaminating dry hospital surfaces, which renders them tolerant to desiccation, normal hospital cleaning, and disinfection procedures.

Environmental surfaces of a decommissioned intensive care unit were destructively sampled and the presence of MROs incorporated into biofilms was determined using a combination of culture, PCR, Fluorescent In Situ Hybridisation, next generation sequencing, confocal laser scanning microscopy, and scanning electron microscopy (SEM). Based on these results a model test system was developed that visually resembled clinical dry surface biofilms.

Biofilm: Real world problems, solutions and regulations—An industry perspective

Presenter: **Elaine Black**, Principal Regulatory Specialist

Affiliation: Ecolab, Saint Paul, MN, USA

Biofilm formation has wide ranging implications in fields ranging from industrial processes like oil drilling, paper production and food processing to health-related fields like medicine and dentistry. This presentation will chart the issues caused by biofilms that some industries tackle every day. This will include a brief look at industrial non-public health issues and a more in-depth discussion of both economically important and public-health related biofilms in the food and healthcare industries. The challenges of biofilm detection and mitigation will be highlighted with the use of case studies from these fields. An industry perspective of standard biofilm methodologies and performance standards for EPA regulation of biofilm control will also be presented.

Use of the Single Tube Method to evaluate the efficacy of disinfectants against *Pseudomonas* biofilm: 2015 Collaborative study

Presenter: **Rebecca Pines**, Biologist, Microbiology Laboratory Branch

Affiliation: Office of Pesticide Programs, U.S. Environmental Protection Agency, Fort Meade, MD, USA

The U.S. EPA is considering the use of the ASTM Single Tube Method (ASTM E2871-13) as an efficacy method to support the registration of antimicrobial products with biofilm claims. In 2014, the EPA's Office of Pesticide Programs Microbiology Laboratory Branch (MLB) launched a collaborative study to assess the method's performance. The 2014 study yielded unexpected levels of variability in log reduction values for the high efficacy treatments. Discussions with the collaborators revealed that the variability may have been the result of inadvertent contact and splashing of the carrier-associated inoculum onto the inner walls of the reaction tube during carrier deposition. To prevent/mitigate this problem, a splashguard was developed and employed in subsequent collaborative testing. In late 2015, MLB organized a second collaborative study to reassess the method's performance with the splashguard, utilizing a combination of filtration and direct plating for inoculum recovery. In this study, laboratory-grade sodium hypochlorite and a quaternary ammonium-based product, each with a high and low level of presumed efficacy, were tested against a *Pseudomonas aeruginosa* biofilm. The preliminary findings and observations from this study will be presented.

Using statistical confidence and power to assess performance standards of antimicrobial test methods

Presenter: **Al Parker**, CBE Bio-statistician; Assistant Research Professor, Mathematics

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

A performance standard (PS) for an antimicrobial test method defines an acceptable outcome for an antimicrobial product being tested. The PS also specifies the number of tests that must be performed, the number of laboratories required to conduct the tests, and the test microbes used. The specifications

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set by the PS can be evaluated by two desirable and quantifiable statistical characteristics: (1) the confidence level of the PS, which is the percentage of ineffective products that the PS correctly fails and (2) the power of the PS, which is the percentage of excellent products with high efficacy that the PS correctly passes. This approach was recently applied to re-set the PS for the use-dilution method, a dried surface test required by US EPA for registering liquid antimicrobials applied to inanimate surfaces.

A regulatory model harmonized with the product development pathway

Presenter: **Marc Rindal**, Regulatory Microbiologist, Antimicrobials Division

Affiliation: Office of Pesticide Programs, U.S. Environmental Protection Agency, Fort Meade, MD, USA

The purpose of this talk is to provide an update to EPA's current and proposed registration pathway for products making antibiofilm efficacy claims. The Agency registers antimicrobial pesticides which includes those products making efficacy claims against biofilm. The EPA's framework for registering antibiofilm products will be presented here for discussion. The registration process for similar products making public health claims will also be presented. Performance standards and the role of test parameters will be explored focusing on the specific data requirements and their relationship to test methodology and label claims.

Lighting the way to long-lasting biofilm remediation—Photochemistry meets biology

Presenter: **Chuck Pettigrew**, Principal Scientist

Affiliation: Procter & Gamble, Mason, OH, USA

We have developed a system for the photocatalyzed generation of aqueous chlorine dioxide, a well-known biocide. Photocatalysis allows for in situ generation of a sustained dosing level without build-up of concentrations capable of out-gassing. To better understand the potential of the photoClO₂ system for disrupting biofilms, we partnered with the Center for Biofilm Engineering at the Montana State University. Exciting initial results suggest that ClO₂ can disrupt biofilms, resulting in partial removal of the biofilm from the test surface. Future tests will seek to better understand the ability of photoClO₂ to remove and/or kill biofilms.

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