

Montana State University
■ Center for Biofilm Engineering
Bozeman, Montana

Anti-Biofilm Technologies: Pathways to Product Development

Arlington, Virginia
FEBRUARY 1, 2017

presentation

proceedings

Proceedings

Table of Contents

SESSION 1: Medical Device Technologies

- 2** **An extraction free assay for quantifying residual protein and microbial biofilms on working surfaces**
Allan Guan, ORISE Research Fellow, Office of Science & Engineering Laboratories, Center for Devices & Radiological Health, FDA
- 2** **Testing to support safety and effectiveness for medical devices containing antimicrobials**
Brandon Kitchel, Microbiologist, Office of Device Evaluation, Center for Devices & Radiological Health, FDA
- 3** **Antibiotic resistance in biofilms: A review**
Garth James, Associate Research Professor, Chemical & Biological Engineering, MSU, CBE
- 3** **Multimodal preclinical imaging in infectious disease research**
Kevin Francis, Preclinical Imaging Fellow, PerkinElmer
- 3** **Infected megaprotheses: How patients drive science that may help patients**
Nicholas Bernthal, MD, Department of Orthopaedic Surgery, University of California, Los Angeles

1

SESSION 2: Surface Disinfection Technologies

- 3** **Evaluating antimicrobial agents against biofilms**
Phil Stewart, Professor, Chemical & Biological Engineering, MSU, CBE
- 4** **Laboratory attributes of a low-level biofilm claim**
Darla Goeres, Associate Research Professor, Chemical & Biological Engineering, MSU, CBE
- 5** **Changes in a method's variability when used for low-level claims**
Darla Goeres, Associate Research Professor, Chemical & Biological Engineering, MSU, CBE
- 5** **Biofilm method standardization: A regulatory perspective**
Rebecca Pines, Biologist, Microbiology Laboratory Branch, Office of Pesticide Programs, EPA
- 5** **Public health biofilm claims for antimicrobial pesticide products: Pathways to registration**
Alison Clune, Biologist, Antimicrobials Division, Office of Pesticide Programs, EPA
- 6** **European Union perspective on biofilm regulation**
Minna Keinänen-Toivola, Faculty, Satakunta University of Applied Sciences, Rauma, Finland

Presentation Abstracts

SESSION 1: Medical Device Technologies

An extraction free assay for quantifying residual protein and microbial biofilms on working surfaces

Presenter: Allan Guan, ORISE Research Fellow

Co-Author: K. Scott Phillips, Senior Regulatory Research Scientist

Affiliation: Office of Science & Engineering Laboratories, Center for Devices & Radiological Health, FDA, Silver Spring, MD, USA.

Biological contamination of working surfaces in industries such as food processing, water treatment, and medical devices is an important vector of disease transmission in humans. Reservoirs, channels and inaccessible internal surfaces in devices such as surgical tools, endoscopes, heat exchangers, and water filtration membranes are especially challenging to clean and monitor. The buildup of significant bioburden on these surfaces reduces the effectiveness of disinfection and sanitization measures. Recent infectious outbreaks due to biofilm contaminated medical devices have resulted in a number of patient deaths prompting the U.S. Food and Drug Administration (FDA) to issue safety communications for duodenoscopes, bronchoscopes, and heater-cooler units. Foodborne outbreaks of *Listeria monocytogenes* have also been linked with contaminated food-contact surfaces such as deli meat slicers. Investigations have shown that biofilm can persist despite adherence to established sanitation and infection control practices. Therefore validation and monitoring of cleaning is an important quality control process to mitigate these microbial threats. Assays for detecting surface-adherent contamination typically require extraction of biological soil (such as protein or biofilm) from the surface of interest. Reliability is often hampered by extraction efficiency due to physical inaccessibility or poor solubility. In this work, we describe how the o-phthalaldehyde (OPA) protein assay can be modified to measure residual protein on a surface without extraction. The extraction-free assay achieved a limit of detection (LOD) of 1.6 $\mu\text{g}/\text{cm}^2$ for bovine serum albumin (BSA) adsorbed on a surrogate endoscope model. Further application of the assay to quantify protein in *Staphylococcus epidermidis* biofilm on stainless steel coupons achieved a LOD of 9 $\mu\text{g}/\text{cm}^2$. The LOD for protein also satisfies current endoscope reprocessing cleaning benchmarks¹ for protein (<6.4 $\mu\text{g}/\text{cm}^2$). By enabling the detection and quantification of soils in complex or hard-to-reach areas, this method has the potential to improve the margin of safety in industrial cleaning processes.

1.AAMI TIR30/Ed.2, A compendium of processes, materials, test methods, and acceptance criteria for cleaning reusable medical devices

Contact information: kenneth.phillips@fda.hhs.gov

Testing to support safety and effectiveness for medical devices containing antimicrobials

Presenter: Brandon Kitchel, Microbiologist/Lead Reviewer

Affiliation: Office of Device Evaluation, Center for Devices & Radiological Health, FDA, Silver Spring, MD, USA.

On September 21–22, 2016, the FDA held an advisory panel meeting to discuss the classification of wound dressing products containing antimicrobial agents. These products range from solid wound dressings to wound gels/creams and washes. Mr. Brandon Kitchel will be presenting information from this panel meeting regarding the types of performance testing that are typically presented for review of

Proceedings

these products in premarket submissions (e.g., USP<51> and AATCC Test Method 100). Additionally, this talk will address the types of data that typically support a product's safety and effectiveness which may help in understanding the benefits and risks of device use including biocompatibility and antimicrobial performance testing for wound dressings that contain antimicrobials as preservatives.

Antibiotic resistance in biofilms: A review

Presenter: Garth James, Associate Research Professor, Chemical & Biological Engineering

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

Growth within a biofilm can reduce the susceptibility of bacteria to antimicrobial agents by a variety of mechanisms involving phenotypic and genotypic changes to the bacteria. The term "biofilm tolerance" has been used to refer to phenotypic changes in biofilm bacteria that reduce antimicrobial susceptibility as a means of distinguishing that phenomenon from those involving heritable genetic changes ("classical antimicrobial resistance"). We have found that ciprofloxacin tolerance of *Pseudomonas aeruginosa* biofilms grown in a drip-flow reactor involves genes associated with oxygen limitation and transition to stationary phase. Biofilm growth can also promote the acquisition of classical antimicrobial resistance through horizontal transfer of existing resistance genes as well as de-novo mutations. Enhanced mutation rates in biofilms have been linked to oxidative stress. Thus, biofilm growth and associated physiological stresses can result in both phenotypic biofilm tolerance and genotypic biofilm resistance.

Multimodal preclinical imaging in infectious disease research

Presenter: Kevin Francis, Preclinical Imaging Fellow

Affiliation: PerkinElmer, Waltham, MA, USA.

Since its inception more than two decades ago, non-invasive optical imaging has revolutionized how disease events are longitudinally and spatially monitored in real-time in small animals. Optical reporters, luciferases and fluorescent proteins, are engineered into cells (e.g., to track bacteria) or directly into animals (e.g., to monitor host responses) to enable the generation of light that can be visualized through the tissues of a live animal. This technique is equally applicable to imaging of fluorescent dyes and particles, allowing fluorescently tagged biological events (e.g., tracking of antibodies and peptides) to be monitored both independently and in combination with genetically tagged events. This presentation will focus on studies developed to monitor and track bacteria in mice and rats during infection, with a particular focus on bacterial biofilm related diseases.

Infected megaprotheses: How patients drive science that may help patients

Presenter: Nicholas Bernthal, MD

Affiliation: Department of Orthopaedic Surgery, University of California, Los Angeles, CA, USA.

Infection is the most common cause of failure of endoprosthetic reconstructions of bony deficits after tumor resection surgery. In developing a preclinical model that allows non-invasive, real time longitudinal tracking of both bacteria and host response, we have established a tool that lets us understand our patients' infections, assess antimicrobials, and begin to harness the host immune response to combat these infections. Epidemiologic data from our patients guides our preclinical experimental design, and our resultant data is driving clinical trials. We have shown how novel antimicrobials, drug delivery mechanisms, and host modification can combat implant infections and improve outcomes for our patients.

Proceedings

SESSION 2: Surface Disinfection Technologies

Evaluating antimicrobial agents against biofilms

Presenter: Phil Stewart, Professor, Chemical & Biological Engineering

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

A meta-analysis of published data relating to antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms was performed based on the premise of a dose-response relationship. Kill data, quantified as a log reduction, from biofilms treated with a wide variety of antimicrobials such as chlorine, essential oils, peracetic acid, quaternary ammonium compounds, and antibiotics were included. In general, dose response relationships were only apparent within the data set of a single investigation, suggesting that the specific test method used is a critical determinant of the outcome. Factors of particular importance were the surface area to volume ratio and the initial viable cell density. When these factors are taken into account, the single tube disinfection method listed in the EPA-HQ-OPP-2016-0357 docket titled “Two Proposed Test Methods and Guidance for Antimicrobial Efficacy Testing” yields results similar to those from other published biofilm methods. These recommendations are offered for those working in research and development related to biofilm control: 1) in addition to lab-specific methods, use a standard biofilm method, 2) include benchmark agents as comparators, 3) report key test method parameters, and 4) measure a dose response. This meta-analysis demonstrated the challenges in evaluating claims of antimicrobial efficacy against biofilms. These challenges have real-world impact because they currently limit investment in innovative chemistries and confound selection of optimal agents from the existing stable of antimicrobials. Quantitative characterization of biofilm test methods is needed to address these difficulties.

Laboratory attributes of a low-level biofilm claim

Presenter: Darla Goeres, Associate Research Professor, Chemical & Biological Engineering

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

The impetus for the development and validation of standardized methods is often market driven. The stakeholders in this case, researchers from government agencies such as the Centers for Disease Control and Prevention, the World Health Organization or NASA, regulatory agencies, academia or industry, identify a need that is not being met by the current tools available on the market. Researchers respond by creating new processes or formulations to meet this need while concurrently, methods are developed which validate the new process or product's utility to solve the problem. In the 1990's, biofilm was identified and accepted as the key factor explaining why biocides were not effective in controlling the microbial contamination of many industrial and engineered systems. In response, anti-biofilm formulations and processes, along with the methods to validate them, were or are in the process of being developed and validated.

In the fall of 2016, the US EPA released a docket that proposed a process for companies to register products with a “kills biofilm” claim. The docket states that the claim would be added to an existing product that is registered with a hospital level disinfectant claim. The “add on” biofilm claim must be supported with a 6-log reduction in *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilm grown in the CDC biofilm reactor and treated according to the Single Tube Method. A logical next step would be the development of products and methods for a low level (2 – 4 log reduction) biofilm claim. This presentation will explore potential methods that would support a low-level “kills biofilm” claim.

Proceedings

Changes in a method's variability when used for low-level claims

Presenter: Darla Goeres, Associate Research Professor, Chemical & Biological Engineering

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

The reproducibility of any antimicrobial test method is a function of the efficacy of the products being tested. When used to test highly effective products, the reproducibility of the test method will be at its best. That is, pass/fail evaluations of products will be reproducible across many independent tests. When the same method is used to test moderately efficacious products against a low-level claim, the reproducibility of the test may be much worse. The reproducibility vs efficacy relationship can be quantified for planktonic, dried surface, and biofilm test methods that have been standardized over the last 30 years. Consequences for assessing low-level claims using these methods will be considered.

Biofilm method standardization: A regulatory perspective

Presenter: Rebecca Pines, Biologist

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

To support the registration of antimicrobial products with a public health claim, manufacturers are required to submit product performance data to the United States Environmental Protection Agency (EPA) using established testing guidelines and standardized methods suitable to support the specific label claim. Prior to implementing an efficacy method for a new label claim, such as treatment of biofilm on an environmental surface, the EPA followed a process to gather stakeholder input, develop standard operating procedures (SOPs), determine method performance through inter-laboratory collaborative studies, and perform statistical analyses. The result of this process was the generation of two SOPs to assess a product's performance against both *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms. Adapted from ASTM standard methods, EPA's SOPs have been extensively evaluated in several inter-laboratory studies. The resulting data led to the development of the EPA's "Draft Guidance to Assess the Efficacy of Antimicrobial Pesticide Products Intended to Control Public Health Biofilms on Hard, Non-Porous Surfaces." To gather further stakeholder input, the guidance document, method SOPs, and data used to support the performance of the methods were posted in a docket on regulations.gov for public comment. Comments will be reviewed by the EPA and the guidance document and SOPs will be updated prior to being finalized and used for regulatory purposes.

5

Public health biofilm claims for antimicrobial pesticide products: Pathways to registration

Presenter: Alison Clune, Biologist

Affiliation: Antimicrobials Division, Office of Pesticide Programs, EPA, Fort Meade, MD, USA.

The Environmental Protection Agency's (EPA) proposed approach to regulating antimicrobial claims against public health biofilms reflects the current approach to regulating public health claims against planktonic organisms. EPA regulates antimicrobial pesticides, including those intended for use against biofilms, under FIFRA. FIFRA requires that the Agency review data substantiating public health efficacy claims. Therefore, label claims and test methods are interdependent. Many efficacy claims can be supported by the established test methods in the OCSPP 810 Guidelines. In contrast, test methods supporting novel efficacy claims should be reviewed by EPA prior to testing. For public health biofilm claims, EPA's published draft guidance for public health biofilm claims proposes efficacy claims that can be supported by the recommended test methods without modification. Registrants seeking additional claims against public health biofilms can develop test methods that support the desired label claims. Acceptable novel methods generally build on established methods. EPA anticipates that registrants will propose methods to support biofilm claims against additional organisms, use sites, and surfaces, and for biofilm matrix removal. Suggestions for substantiating novel biofilm claims will be discussed,

Proceedings

however EPA strongly encourages applicants to arrange a pre-submission meeting to discuss their specific proposal.

European Union perspective on biofilm regulation

Presenter: Minna Keinänen-Toivola, Faculty

Affiliation: Satakunta University of Applied Sciences, Rauma, Finland.

Annually over 4 million people are estimated to acquire a HealthCare Associated Infection (HCAI), according to the European Centre for Disease prevention and Control (ECDC). Not only does this have an impact on public health, but it also brings with it high healthcare costs. A potential and promising weapon against bacterial growth and, possibly, the development of multi-drug resistant bacteria has been found in AntiMicrobial (nano-)Coatings (AMC). In coatings fortified with an active ingredient, the ingredient is responsible for the reduction and even elimination of the micro-organisms on coated surfaces. In EU, under over sixty universities, research institutes and companies from twenty-nine European countries in the AMiCI consortium since 2016 (Anti-Microbial Coating Innovations to prevent infectious disease, <http://www.amici-consortium.eu/>) will jointly study the impact of applying antimicrobial (nano-) coatings on decreasing the spread of infections. The beneficial aspects of these measures will be assessed in the context of potential environmental adverse effects, as well as development of bacterial resistance. It is the first time that this pressing issue is addressed on such a scale. As a practical example, research results on copper as an antimicrobial material in different facilities in Finland will be presented.

Acknowledgments:

AMiCI consortium is supported by COST (European Cooperation in Science and Technology).