BIOFILM TECHNOLOGIES PATHWAYS TO PRODUCT DEVELOPMENT

February 5–6, 2019

PROCEEDINGS

CENTER FOR BIOFILM ENGINEERING

MONTANA STATE UNIVERSITY

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SESSION 4: Breast Implant Biofilms

SESSION 5: Medical Device Cleaning/Reprocessing

KEYNOTE

Biofilm tolerance favors rapid emergence of antibiotic resistance

Presenter: **Christophe Beloin**[1], Associate Professor
Co-authors: **Masaru Usui**[1,2], **Yutaka Yoshii**[1], **Jean-Marc Ghigo**[1]
Affiliation: [1]Institut Pasteur, Genetics of Biofilm Unit, Department of Microbiology, Paris, France; [2]Laboratory of Food Microbiology and Food Safety, Department of Health and Environmental Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan.

Bacterial infections are a leading cause of morbidity and mortality and the increasing resistance to antibiotics among pathogenic bacteria is a major health concern. This is particularly the case for chronic infections due to the presence of biofilms developing on medical devices or mucosa, for which there is no fully efficient prevention or eradication method. In biofilms, bacteria undergo specific physiological changes and display a characteristic but ill-understood high level of tolerance to both antimicrobial agents and host immune defenses. Whereas enhanced tolerance of biofilms toward antibiotics is a multifactorial process, relapse of infection is mainly explained by the presence, within biofilms, of high levels of so-called persister bacteria that can sustain extremely high concentrations of antibiotics but can regrow as biofilms when treatment is stopped. Persisters are proposed to serve as a potential evolutionary reservoir from which resistance could emerge. While impact of persisters in clinical situations has been largely overlooked, recent studies demonstrated that high-levels of antibiotic tolerance, but not resistance, could be rapidly achieved by exposure of batch planktonic cultures of *E. coli* and other pathogens to cyclic treatments of lethal concentration of antibiotics. Considering the importance of biofilms in chronic infections and the failure of their treatment, there is an urgent need to characterize evolution of persister-associated tolerance and resistance within biofilms. Using laboratory evolution experiments, we exposed biofilms to intermittent exposure of lethal antibiotic concentrations, a situation mimicking clinically relevant situations. We showed that the tolerance and resistance evolutionary path followed by biofilms and planktonic bacteria are different, with biofilms strongly favoring rapid emergence of genetic resistance probably promoted by their intrinsic high level of tolerance. These results reinforce the necessity to develop more antibiofilm innovative strategies to mitigate the emergence of high tolerance and subsequent antibiotic resistance in clinically relevant situations.

SESSION 1: Food-Related Biofilms

Biofilms in beer

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

In 2016, the CBE Standardized Biofilm Methods Lab responded to a Request for Proposals from the Brewer's Association to 1) develop a laboratory method for growing representative biofilms in draught beer lines and 2) test a cleaning procedure that could be used by craft brewers to ensure quality beer at the tap. Working with local breweries and distributors, a growth method was developed and is currently being tested by the National Sanitation Foundation. The results of these studies will be presented.

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Trending topics in food protection: A review from the 2018 food conferences

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

An overview of trending topics discussed at five food conferences last year (plus one in fall of 2017) will be presented. Whole Genome Sequencing, probiotics and the gut microbiome, protective cultures, and bacteriophage are all hot topics in food safety related to the identification or inhibition of pathogens. Soil amendments and water as a source of contamination are also being explored. A highly important and trending topic, which our lab is particularly interested in investigating, is antimicrobial resistance from field to fork. These topics and more will be presented.

Biofilm, sanitizer, and meat safety

Presenter: **Rong Wang**, Microbiologist
Affiliation: US Meat Animal Research Center, ARS, USDA, Clay Center, NE, USA.

Biofilm formation is an important strategy supporting bacterial survival under adverse circumstances, which is a major concern for meat safety because biofilm cells are more tolerant to sanitization than planktonic cells of the same species. In addition, the meat processing plants may harbor a wide variety of environmental microorganisms as well as foodborne pathogens, and a large portion of such a mixed microbial community could persist in the plants as multispecies biofilms. The many synergistic and antagonistic interactions would determine the dominant species within the mixed biofilms and consequently, the architecture, activity, as well as sanitizer tolerance of the entire multispecies community. Therefore, mixed biofilm formation by environmental microorganisms with integrated foodborne pathogens could potentially provide an ecological niche for these pathogens to better colonize and obtain a higher survival capability against routine sanitization/cleaning procedures, and as a result, increase the prevalence and contamination rate of these pathogens in commercial plants. We investigated the effect of bacterial coexistence and mixed biofilm formation on sanitizer tolerance of *E. coli* O157:H7 and *Salmonella enterica*, the two major foodborne pathogens of concern in the fresh meat industry. Our research also revealed a potential role of biofilms and their sanitizer tolerance in "High Event Period" contamination incidents at commercial meat plants. Furthermore, we phenotypically and genetically characterized a variety of environmental microorganisms present in meat processing plants and investigated the potential contribution of these background microorganisms to sanitizer tolerance of the pathogens. Our results demonstrated that the environmental microorganisms could develop significant biofilms on contact surfaces, and the biofilm forming ability differed considerably among the samples depending upon the locations where the samples were collected. More importantly, *E. coli* O157:H7 strains that were added into certain mixed biofilm samples obtained significantly enhanced tolerance against common sanitizer treatment, and the mechanisms underlying such protective effect by background microorganism biofilms appeared biofilm structure – and/or species – related as data suggested that certain specific bacterial species present in the environment might render protections to the pathogens in mixed biofilms. These studies that focus on bacterial community dynamics, pathogen integration, and subsequent sanitizer tolerance provide key information on how biofilms in the real world evolve and persist within the meat processing environments. Addressing such knowledge gaps will help identify sources of contamination, determine critical control points, and develop novel strategies to enhance meat quality and safety.

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Biofilm management in food and beverage processing

Presenter: **Erin Mertz**, Area Technical Support Coordinator, Food and Beverage
Affiliation: Ecolab, St. Paul, MN, USA.

Biofilms can cause issues in industrial food and beverage manufacturing. They can be found in drains, hard-to-clean areas of equipment, damaged areas of equipment, and other hard-to-reach areas in the plant environment. One of the most common and most effective biofilm forming organisms, *Pseudomonas*, is a prominent cause of food-spoilage and decay in food and beverage products. *Listeria monocytogenes*, a serious foodborne pathogen, is a known biofilm former which allows it to persist in the environment and cause sporadic outbreaks. In addition, biofilms can harbor other organisms that can cause illness or spoilage. Detecting and removing biofilms is challenging for the food and beverage industry, which could lead to a biofilm being mistaken for antimicrobial resistance. In today's industrial environment, effective cleaning and appropriate use of antimicrobial chemistries have been successful in the management of biofilms. However, there is a need for additional tools.

SESSION 2: Biofilm Detection

Tracking antimicrobial resistance from sink drain biofilms

Presenter: **Amy Mathers**, Associate Professor
Co-authors: Shireen Kotay, Katie Barry, Anna Sheppard, Joanne Carroll
Affiliation: University of Virginia, School of Medicine, Departments of Medicine and Pathology, Charlottesville, VA, USA.

Focusing on the urgent clinical problem of increasing carbapenem resistance in *Enterobacteriaceae* in hospitalized patients, we have been evaluating detection methods in clinical microbiology, environmental microbiology and molecular characterization to understand and halt transmission of carbapenemase genes. With the recent finding of involvement of hospital wastewater premise plumbing as a reservoir for drug resistant organisms, we have expanded our work to model dispersion, refine detection methods and understand complex transmission pathways of carbapenemase producing *Enterobacteriaceae* from hospital wastewater to patients. This has been done within a laboratory sink model as well as within the hospital. The primary thesis of the talk will be to describe our current understanding around the detection and microbial transmission of genetic mechanisms of resistance from the hospital environment to patients and thereby understand the scope and challenges we may face with the spread of antimicrobial resistance among *Enterobacteriaceae* in hospitals as they relate to premise plumbing wastewater biofilms.

Different techniques to visualize, quantify, and investigate biofilms

Presenter: **Matthew W. Fields**, Director, Center for Biofilm Engineering; Professor, Microbiology & Immunology
Co-authors: **Heidi J. Smith**, Manager, CBE Bio-Imaging Facility; Assistant Research Professor, Microbiology and Immunology
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

The diversity of traditional and advanced methods and instrumentation within the CBE (including the Bio-Imaging Facility) as well as other MSU capabilities allows for a wide range of sample types (*e.g.*, macromolecular composition, cryosections, hydrated biofilms, from 2D to 3D, disturbed to intact, exopolymer matrices, and surface) to be quantified, characterized, and imaged. The CBE Bio-Imaging Facility houses light/epifluorescent microscopes, stereoscopes, confocal microscopes, a laser micro-

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dissection microscope, an optical coherence tomography system, and confocal Raman microscopes. Each quantification and imaging technique has advantages (and disadvantages); it is also important to understand the optical and analytical constraints of each technique and/or platform when designing an experiment. This talk offers information on different methods/techniques and imaging capabilities within the CBE/MSU. Information on sample requirements (e.g. penetration depth, sample preparation, and sample state) will be summarized for instrumentation within the CBE and MSU. Currently, we are adding to these capabilities by integrating new techniques that target active microbial populations and identify specific compounds of interest (*e.g*., BONCAT and stable isotope probing). Microscopy and imaging technology are rapidly evolving fields and the future of imaging within the CBE is focused on developing correlative microscopy techniques that will establish inter-connectivity between individual analysis platforms.

Electrochemical sensing of quorum sensing molecules and virulence factors

Presenter: **Edgar D. Goluch,** Associate Professor and Entrepreneur
Affiliation: Department of Chemical Engineering, Northeastern University, Boston, MA, USA; QSM Diagnostics, Inc., Boston, MA, USA.

Real-time measurement of molecules secreted by bacterial cells provides important insights about the species that are present in a sample as well as what the cells are doing. My group has been using electrochemical sensors to study the production of siderophores by *Pseudomonas aeruginosa* in various engineered environments for the last nine years. Electrochemical sensors allow us to study the concentration and production rate of these electroactive molecules in real time at concentrations as low as 100 nM. Functionalizing the electrochemical sensors with aptamers, which selectively and specifically bind to target molecules, allows for the measurement of virtually any molecule of interest. By tuning the electrical potentials at which these sensors operate, it is possible to detect the molecules directly in biological fluids without any sample processing. I founded a startup company based on this technology, QSM Diagnostics, Inc., to identify and monitor the activity of bacterial species in specimens using unique secreted quorum sensing molecules and virulence factors.

SESSION 3: Antimicrobials and Regulation

Global regulatory impediments and their effect on trade

Presenter: **Adrian Krygsman,** Director, Product Registration
Affiliation: Troy Corporation, Florham Park, NJ, USA.

Regulation for chemical products continues to evolve and become more resource intensive. With a global economy, companies look at regulatory requirements globally trying to maximize their resources in the hope of "registered once, registered everywhere." Unfortunately, this goal is not possible with substantial differences existing depending on the region that require continual oversight and expenditures. Emerging regulations in the Far East, for example, follow the European model as an initial template with specific country specific differences. There are numerous examples of regulatory hurdles that must be addressed depending on region and country. The Globally Harmonized System (GHS), Registration, Evaluation and Assessment of Chemicals (REACH), Biocidal Products Regulation (BPR), Korea BPR, Korea REACH, EPA Registration Review Program, Canadian Re-evaluation Program, and most recently developments with Chinese raw materials (USTR 301 program) and key active ingredient shortages are just a few of the most prominent obstacles to global trade. One size simply does not fit all. Past regulatory approaches meeting the most conservative country or region no longer work requiring endogenous company resources to ensure compliance. Local representation is a necessity, as well as local regulatory expertise, in order to ensure business is not affected. Ultimately as some issues (USTR 301) illustrate it's important to ensure that multiple corporate functions address these issues. Procurement and regulatory now need to work more closely than ever to ensure there is not total

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dependence on one region or country for raw materials. These ever-changing hurdles also now require a strategic sales plan in order to maximize sales and ensure the market supports company resources.

Biofilm claims and product development

Presenter: **Dan Klein**, Senior Manager, R&D Microbiology
Affiliation: STERIS Corp., Mentor, OH, USA.

As biofilm data and claims continue to evolve as a customer requirement, Research & Development organizations are doing more to evaluate the activity of disinfectants against biofilm as well as the ability of medical devices to mitigate and remediate biofilm formation. This can be challenging with the evolution of the regulatory environment and optimization and development of new test methods requiring diligence throughout the product development process. This presentation will outline experiences and lessons related to the development of products and claims for biofilm prevention and remediation. The focus will be on EPA registered disinfectants as well as FDA regulated medical devices including historical data to establish biofilm prevention strategies in a washer/disinfector, and biofilm issues encountered during product development as well as the process of generating test data to achieve a biofilm claim on an EPA registered disinfectant.

Antimicrobial pesticides: Regulatory update and methods development initiatives

Presenter: **Steve Tomasino**, Senior Scientist
Affiliation: US EPA, Office of Pesticide Programs, Fort Meade, MD, USA.

Abstract not available.

SESSION 4: Breast Implant Biofilms

Controversies in biofilms and breast implants

Presenter: **Roger N. Wixtrom**, DABT, President
Affiliation: LSCI, Springfield, VA, USA.

An estimated 300,000 breast augmentations and 85,000 post-mastectomy breast reconstructions using breast implants were performed in the United States in 2017. In addition to well-documented quality-of-life benefits observed for both cosmetic and reconstruction patients, there are also recognized adverse events associated with these devices, which in at least three instances have been potentially linked to the presence of biofilms. These include the more frequent complications of infection and capsular contracture, as well as the quite uncommon occurrence of breast implant-associated anaplastic large cell lymphoma (BIA-ALCL). In regard to surgical procedures, the human breast is termed a "clean-contaminated" site, owing to the natural presence of bacteria within the breast. Indeed, studies have suggested that the normal microflora of the breast is even more diverse than that of the skin. For these particular adverse events, significant controversies remain with respect to the source of the bacteria, the role of biofilms, optimal prophylaxis, impact of device surface texturing of various sorts, and appropriate biofilm test conditions. The challenges experienced in attempting to gain resolution of these issues can provide useful illustrations potentially pertinent to other long-term implantable medical devices.

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Influence of breast implant textures on bacterial attachment and biofilm formation

Presenter: **Garth James**[1], Associate Research Professor, Chemical & Biological Engineering, MSU; PI, Medical Biofilms Laboratory, CBE
Co-authors: Laura Boegli[1], John Hancock[2], Lisa Bowersock[1], Albert Parker[1], and Brian M. Kinney[3].
Affiliations: [1]Center for Biofilm Engineering, Montana State University, Bozeman, MT; [2]Establishment Labs, Santa Barbara, CA; [3]University of Southern California, Beverly Hills, CA, USA.

Breast implants with textured surfaces have become popular due to better maintenance of position and other aesthetic properties. Clinical results indicate textured implants also have a lower association with capsular contracture, although recently, associations have been made between breast implant-associated anaplastic large cell lymphoma and textured implants. Surface textures have been generated using techniques such as lost-salt, imprint stamping, and molding. Classification schemes have been proposed that include macrotexture plus, macrotexture, microtexture, mesotexture, and nanotexture. The foreign body response to textured implants results in a less dense capsule and a more disorganized collagen fiber alignment than smooth implants. Deeper textures have been shown to have more tissue in-growth than shallower textures. Texture also influences the responses of fibroblasts and macrophages on surfaces. In addition to these host-biomaterial interactions, surface texture also influences bacterial attachment and biofilm formation. We evaluated bacterial attachment and biofilm formation on the outer surface material of implants with various surface areas and roughness, including Natrelle® (Smooth), SmoothSilk®/SilkSurface® (Silk), VelvetSurface® (Velvet), Siltex®, and Biocell®. The roughness and surface area of each material was assessed using non-contact profilometry. Bacterial attachment (2 hours) and biofilm formation (24 hours) were evaluated for *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Ralstonia pickettii* over nine independent experiments using a CDC biofilm reactor and viable plate counts (VPC) as well as confocal scanning laser microscopy (CSLM). VPC of the textured implants were compared relative to the Smooth implant. Surface areas increased with roughness and were similar among the three least rough implants (Smooth, Silk, and Velvet) and among the roughest implants (Siltex and Biocell). Overall, VPC indicated there was significantly more bacterial attachment and biofilm formation on the Siltex and Biocell implants than the Silk or Velvet implants; although there were differences between species and time points. CSLM confirmed the formation of thicker biofilms on the implants with rougher surface textures. Overall, this study indicated that implant surfaces with rougher texture, resulting in more surface area, harbored greater biofilm loads than those with smoother surfaces.

Mechanical factors that may contribute to the involvement of implant surface texture on the pathogenesis of breast implant associated complications

Presenter: **Hainsworth Shin**, Biomedical Engineer
Affiliation: Center for Device & Radiological Health, US FDA, USA.

The foreign body capsule (FBC) at the soft tissue-implant interface serves as the body's defense against implant materials too large for inflammatory cell-mediated uptake/removal. This defense mechanism, while imparting a degree of biocompatibility and sustaining implant integrity, represents a chronically inflamed state with the potential to interfere with the success of implants or promote pathogenesis. Breast implants (BIs) are soft tissue implants associated with the formation of a FBC which is largely believed to impact the host tissue response. As with all soft tissue implants, the biomaterial surface of the BI is a key determinant of how the body reacts to it. Surface texturing has been implicated in BI-related pathologies such as capsular contracture and lymphomagenesis, i.e., anaplastic large cell lymphoma (ALCL). In 2011 FDA released a safety report recognizing a potential link between breast implants (BIs) and incidence of a rare form of lymphoma, BIA-ALCL, in women. In 2017, FDA released another safety communication recognizing a correlative link between BIA-ALCL and BI surface texturing. One theory for the pathogenesis of BIA-ALCL receiving attention is that the increased surface area of textured BIs evokes a hyper-inflammatory environment by allowing for greater adhesion of

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bacterial colonies in comparison with smooth-surfaced implants. The ensuing chronic inflammatory state and/or constant state of lymphocyte recruitment and activation due to persistent presence of bacterial antigens (or superantigens) in the setting of a genetic predisposition leads to cell transformation and lymphomagenesis. However, some aspects of the pathobiology are difficult to explain or inconsistent with this theory, suggesting the possibility for a multi-factorial etiology. Notably, the link between surface texturing and BIA-ALCL hints that mechanical factors in the cellular physiology/pathobiology of the tissue-implant interface may be involved. For example, by increasing implant contact area with host tissues, surface texturing promotes tissue ingrowth, which influences implant micromotion. The micromotion associated with textured implants may introduce a mechanical force environment that plays a role in the onset of BIA-ALCL by physically enhancing bacterial growth, influencing immune cell activity in the tissue-implant interface, or both. In this talk, I will discuss our research on the potential for mechanical factors such as interstitial fluid shear stresses, hydrodynamic pressures, and matrix deformation/strains in the cellular mechanoenvironment of the BI interface to play a role. Disclaimer: The views, talking points, findings, and/or conclusions presented in this abstract/talk have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any agency determination or policy. The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services.

Breast implant surface texture impacts tissue response

Presenter: **TracyAnn Perry**, Vice President, Science & Research
Affiliation: Establishment Labs, Costa Rica

Bacterial biofilms have been implicated with breast implant complications including capsular contracture and breast implant associated anaplastic large cell lymphoma. The actual mechanisms for both are unclear and are almost certainly multifactorial. Capsular contracture is more frequently associated with smooth implants than macrotextured implants. Several other risk factors are associated with this high incidence including contamination of the implant surface with *Staphylococcus epidermidis* biofilm, age of the implant, submuscular vs. subglandular placement of the implant, and other clinical complications such as seroma or hematoma. Characterizing surface texture is key to predicting and optimizing performance of the breast implant in the patient. A number of different methodologies exist for characterizing the physical properties of a surface texture. The data presented here used scanning electron microscopy (to image surface topography), X-ray computed tomography imaging (to calculate surface area) and non-contact profilometry (to calculate surface roughness) to characterize the surface architecture and properties of a number of breast implant surfaces. Surface areas from the front of the shells ranged from 85 to 551 mm^2 (8% to 602% higher than the surface area of a flat surface). Textures were grouped into 4 categories based on surface area measurements: smooth (80–100 mm^2), microtexture (100–200 mm^2), macrotexture (200–300 mm^2), and macrotexture-plus (>300 mm^2) (Atlan *et al.*, 2018). Non-contact profilometry was used to calculate average surface roughness. Textures were grouped into three categories: smooth (<10μm), microtexture (10-50μm), or macrotexture (>50μm) based on ISO 14607:2018 specification. Preclinical tissue response was evaluated histologically at 6 weeks. Increasing capsule disruption, tissue ingrowth, and tissue adherence were seen with each category of increasing surface texture complexity. Biofilm formation was assessed for *S. epidermidis* using a CDC biofilm reactor, viable plate counts and confocal scanning laser microscopy. Implant surfaces with a rougher texture and higher surface area, harbored greater biofilm loads than those with smoother surfaces. Improved understanding of the surface texture properties of breast implants is critical to optimizing and predicting the performance of the breast implant in the patient. Indeed the data presented here suggest that structural modification of the surface texture can markedly alter the pathophysiology of the foreign body response as has been shown with the innovative Motiva

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SmoothSilk®/SilkSurface® implants reporting capsular contracture rates of <1% at 6 years (Chacon *et al.*, 2018).

SESSION 5: Medical Device Cleaning/Reprocessing

Reusable medical devices: Understanding the challenges and presenting a path forward

Presenter: **Paul Sturman**, CBE Industrial Coordinator
Co-authors: Darla Goeres
Affiliation: Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA.

Reusable medical devices present a challenge in that they must be adequately cleaned between patient uses. Biofilm contributes to this challenge because it may be difficult to remove, and it may harbor pathogenic organisms that can cause hospital acquired infection. Many devices cannot be fully sterilized between uses due to size, access to all surfaces within the device, or because of sensitive electronics. Furthermore, devices may be constructed of materials that are not compatible with aggressive antimicrobial treatment. Stakeholder communities (patients, clinicians, regulatory authorities, and device manufacturers) recognize the need for performance standards that are both achievable and protective of human health. To achieve this end, these standards must recognize the importance of biofilm as a potentially harmful agent. This session seeks to identify the challenges of cleaning reusable medical devices through focusing on commonly reprocessed devices (endoscopes) as well as less portable operating room devices (heater/cooler devices), as well as other devices. Presentations from industry, academia and regulatory authorities will address specific devices as well as the challenge of reusable device cleaning in general.

Overview of biofilms in flexible endoscopes

Presenter: **Kaumudi Kulkarni**, Manager of Research and Development
Affiliation: Healthmark Ind., Fraser, MI, USA.

Flexible endoscopes are complex medical devices with long, narrow interconnected channels. Flexible endoscopes are associated with more documented cases of healthcare-acquired infections than any other type of reusable medical device. The internal channels of endoscopes are exposed to cyclical wet and dry phases during usage and reprocessing and thus form buildup biofilms that are hard to remove. Presence of biofilms in scopes prevent complete decontamination of scopes, thereby posing a disease transmission risk. Implementation of microbiological surveillance of endoscope reprocessing coupled with better extraction techniques is crucial to detect bacterial growth in endoscopes and prevent cross contamination in patients after endoscopic procedures.

Fluorescence microscopy-based SOP for detecting cellular contamination of endoscopes

Presenter: **Scott Phillips**, Regulatory Research Scientist
Affiliation: Center for Device & Radiological Health, US FDA, Silver Spring, MD, USA.

Gram-negative outbreaks associated with the use of reprocessed endoscopes have occurred around the world about once a year on average. A number of recent studies are finding organisms and even biofilm on regularly cleaned and high-level disinfected endoscopes. The gold standard surveillance method in hospitals is sampling and culturing scopes. However, there is no standard protocol for endoscope manufacturers to analyze parts for further investigation into mechanisms of failure. While there are some analytical (spectroscopic) methods that can be applied to studying parts taken out of service, most

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of them do not conclusively identify bacterial cells, leading to uncertainty in investigations focused on identifying contamination risk. Microscopy can identify cells but needs a robust and simple approach. In this work, we show a simple DAPI staining procedure that is inexpensive, rapid, high signal to noise, high staining efficiency, and capable of detecting several key organisms. We also show that our method is capable of detecting cells embedded within a build-up biofilm following treatment with three high level disinfectants (glutaraldehyde, ortho-phthalaldehyde and peracetic acid) and show images from testing of clinically used endoscope parts that have been identified by culture as having Gram-negative contamination. The method could be a useful tool to help study and identify root causes of endoscope contamination.

Growth of *Mycobacterium chimaera* in heater-coolers

Presenter: **Joseph O. Falkinham, III**, Professor of Microbiology, Fellow, Royal Society for Public Health
Affiliation: Department of Biological Sciences, Virginia Tech, Blacksburg, VA, USA.

Nontuberculous mycobacteria (NTM) infections in cardiac-surgery patients, caused by either *Mycobacterium chimaera* or *Mycobacterium abscessus*, have been traced NTM-laden aerosols produced by Sorin 3T heater-cooler units of cardiopulmonary bypass equipment. As NTM are resistant to disinfectants and preferentially form biofilms, a protocol to disinfect the water reservoir(s) of heater-coolers to 1) reduce NTM numbers, and 2) disrupt surface biofilms of heater-coolers was developed. The objective was to prevent potential NTM aerosolization from the water reservoir(s) and, further, to reduce the possibility of re-inoculation of the heater-cooler reservoir(s) from NTM biofilms after disinfection. Delayed reappearance of NTM in heater-cooler water samples is an indication of biofilm killing. A laboratory-scale CDC bioreactor and different heater-coolers were inoculated with *M. chimaera* or *M. abscessus* to measure the ability of different biofilm-disruption steps coupled with Clorox® disinfection to reduce NTM colony-forming units (CFU) in water and to delay the re-appearance of NTM after disinfection. Following inoculation of heater-coolers with NTM, only 0.1 % of the inoculum was recovered from the water. The majority of NTM cells adhered to pipe, pump, and reservoir surfaces, thus creating an additional (i.e., biofilm) challenge to disinfection. A combination of an enzyme-detergent cleaning agent (Prolystica®, Steris) and Clorox® were equivalent to Clorox® alone in reducing *M. chimaera* CFU (6-logs) in heater-cooler water reservoir samples. *M. chimaera* re-appeared within three weeks following Clorox®-only disinfection. In contrast, re-appearance of *M. chimaera* in the heater-cooler's water reservoir was delayed up to 12-weeks by the combination of enzyme-detergent cleaning agent Prolystica® and Clorox® exposure. A combination of an enzyme-detergent and Clorox® was an effective disinfection treatment and significantly delayed the reappearance of *M. chimaera* in heater-cooler water reservoirs. The 12-week delayed reappearance of *M. chimaera* in the water reservoirs greatly reduces the demand for personnel to repeat cleaning and disinfection cycles.

Ultrasound device disinfection: A patient safety risk

Presenter: **Marcia Ryder**, Research Scientist
Affiliation: Ryder Science, Inc., Brentwood, TN, USA.

Ultrasound procedures have dramatically increased in acute care settings and in recent years expanded into alternate healthcare facilities i.e. outpatient ambulatory settings and medical offices. With expansion comes increased risk. Patients undergoing ultrasound procedures are at risk for cross-contamination and infection when proper reprocessing is not completed, resulting in increased antibiotic prescription, and even death. Numerous ultrasound specific guidelines have been developed globally and within the United States. Despite this, a high degree of non-compliance with disinfection recommendations and policies threatens patient safety. Examination of effectiveness of ultrasound

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equipment is limited and performed with traditional culturing techniques. The presence and impact of biofilm on bacterial transfer with or without performance of recommended disinfection procedures is a gap in the protection of patients in the face of an expanding technology that is a critical tool in modern medicine.

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Biofilm Technologies: Pathways to Product Development

February 5–6, 2019Hilton Crystal City Hotel, Arlington, VA
PROCEEDINGS

3/19/2019 12:06 PM

Tuesday February 5

7:30-8:00 am
Registration & Cont. Breakfast, Commonwealth Foyer
Meeting, Williamsburg/Yorktown Ballroom

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

Keynote

8:15-8:50 am
Biofilm tolerance favors rapid emergence of antibiotic resistance
Christophe Beloin, Associate Professor; Co-Director, Institut Pasteur Microbiology Course, Institut Pasteur

SESSION 1
Food-Related Biofilms

8:50-9:25 am
Biofilms in beer
Diane Walker, Research Engineer, CBE

9:25-10:00 am
Trending topics in food protection: A review from the 2018 food conferences
Diane Walker

10:00–10:30 am
Break

10:30-11:05 am
Biofilms, sanitizers, and meat safety
Rong Wang, Research Microbiologist, Meat Safety & Quality Research, US Meat Animal Research Center

11:05-11:40 am
Biofilm management in food and beverage processing
Erin Mertz, Area Technical Support Coordinator, Food & Beverage, Ecolab

11:40-12:00 pm
Discussion Session

12:00–1:00 pm
Lunch, Richmond/Roanoke

SESSION 2
Biofilm Detection

1:00-1:30 pm
Tracking antimicrobial resistance from sink drain biofilms
Amy Mathers, MD, Associate Director, Clinical Microbiology, University of Virginia

1:30-2:00 pm
Different techniques to visualize, quantify, and investigate biofilms
Matthew Fields

2:00-2:30 pm
Electrochemical sensing of quorum sensing molecules and virulence factors
Edgar Goluch, Associate Professor, Chemical Engineering, Northeastern University

2:30-3:00 pm
Break
Sponsored by Decon7 Systems

SESSION 3
Antimicrobials and Regulation

3:00-3:30 pm
Global regulatory impediments and their effect on trade
Adrian Krygsman, Director, Troy Corporation

3:30-4:00 pm
Biofilm claims and product development
Dan Klein, Senior Manager, R&D Microbiology, STERIS Corporation

4:00-4:30 pm
Antimicrobial pesticides: Regulatory update and methods development initiatives
Steve Tomasino, Senior Scientist, Office of Pesticide Programs, US EPA

Wednesday February 6

7:30-8:00 am
Continental Breakfast, Richmond/ Roanoke
Meeting, Williamsburg/Yorktown Ballroom

SESSION 4
Breast Implant Biofilms

8:00-8:05 am
Session Introduction
Garth James, Associate Research Professor, Chemical & Biological Engineering, MSU; PI, Medical Biofilms Laboratory, CBE

8:05–8:40 am
Controversies in biofilm and breast implants
Roger Wixtrom, President, LSCI

8:40–9:10 am
Influence of breast implant textures on bacterial attachment and biofilm formation
Garth James, Associate Research Professor, Chemical & Biological Engineering, MSU; PI, Medical Biofilms Laboratory, CBE

9:10-9:45 am
Mechanical factors that may contribute to the involvement of implant surface texture on the pathogenesis of breast implant associated complications
Hainsworth Shin, Fellow, Biomedical Engineer, Center for Device & Radiological Health, US FDA

9:45-10:15 am
Breast implant surface texture impacts tissue response
TracyAnn Perry, Vice President, Science & Research, Establishment Labs

10:15-10:45 am
Break

SESSION 5
Medical Device Cleaning/Reprocessing

10:45-11:00 am
Reusable medical devices: Understanding the challenges and presenting a path forward
Paul Sturman

11:00-11:35 am
Overview of biofilms in flexible endoscopes
Kaumudi Kulkarni, R&D Manager, Healthmark Industries Company

11:35-12:10 pm
Fluorescence microscopy-based SOP for detecting cellular contamination of endoscopes
Scott Phillips, Regulatory Research Scientist, Center for Device & Radiological Health, US FDA

12:10 pm–1:10 pm
Lunch, Richmond/Roanoke

1:10-1:45 pm
Growth of *Mycobacterium chimaera* in heater-coolers
Joe Falkinham, Professor, Microbiology, Virginia Technological Institute

1:45-2:15 pm
Ultrasound device disinfection: A patient safety risk
Marcia Ryder, Research Scientist, Ryder Science, Inc.

2:15-2:45 pm
Break

2:45-3:45 pm
Round Table Discussion

Save the Date!
2019 Montana Biofilm Meeting
July 16–18, 2019
Bozeman, MT