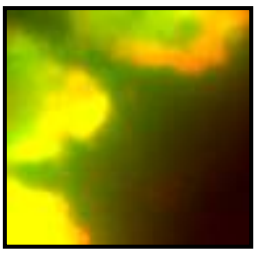
MONTANA STATE UNIVERSITY

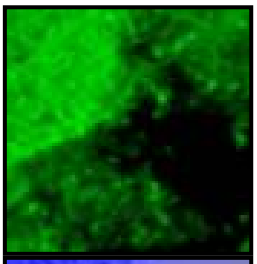
Center for Biofilm Engineering

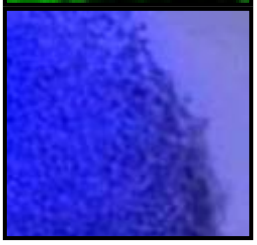
PROCEEDINGS

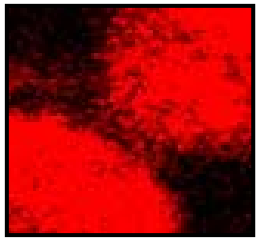
Winter 2006 CBE Technical Advisory Conference

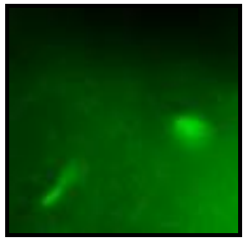
February 2-3, 2006
Montana State University
Bozeman, Montana

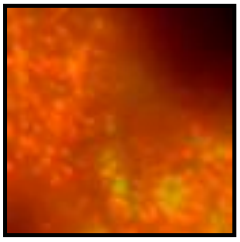


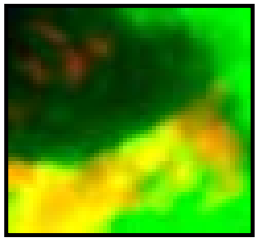












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GENERAL INFORMATION

CBE LEADERSHIP

***Phil Stewart**, CBE Director and Professor, Chemical & Biological Engineering*
***Anne Camper**, Associate Professor, Civil Engineering & Associate Dean for Research, COE*
***Al Cunningham**, Professor, Civil Engineering*
***Brent Peyton,** Associate Professor, Chemical and Biological Engineering*
***Paul Sturman**, CBE Coordinator of Industrial Development*

A BRIEF HISTORY OF THE CBE

The CBE was established in 1990 by a grant from the National Science Foundation's Engineering Research Centers program. The NSF-ERC program was created to increase U.S. industrial competitiveness and to re-invent science and engineering education in U.S. universities. Under the leadership of its founding director, Bill Characklis, the new engineering center also drew support from the state of Montana, Montana State University and industrial partners gathered during its pre-1990 work as the Institute for Biological and Chemical Process Analysis. Since the beginning, CBE researchers have been recognized nationally and internationally for leading-edge biofilm research, and for taking an interdisciplinary approach to the study of microbial growth on surfaces. In the spring of 2001, the CBE's 11-year period of NSF-ERC program support drew to a close. The CBE's continued success is built on the foundation of many years of productive university-industry-government collaboration in pursuit of its vision to be a world leader in fundamental research, science and engineering education, and industrially relevant technology.

MISSION AND GOALS OF THE CBE

The mission of the Center for Biofilm Engineering is to advance the basic knowledge, technology and education required to understand, control and exploit biofilm processes.

The CBE has identified goals in three areas of activity.

In the area of research, the CBE's goal is to do leading-edge fundamental research to elucidate mechanisms at work in bacterial biofilms. The CBE has been a leader in defining the structure and function of biofilms on surfaces, in understanding antimicrobial resistance mechanisms of biofilm, and in identifying the role of signal molecules in controlling bacterial behavior. Researchers at the CBE have demonstrated that biofilms are multicellular attached communities with primitive circulatory systems and a measure of cellular specialization. Understanding these "biofilm basics" presents opportunities for developing more effective strategies to control biofilms in industrial settings.

The second goal of the CBE is to make its research relevant to real systems, where the information can be applied. Industrial concerns shape and focus the research efforts. Technology transfer at the CBE involves not only information, but methods and technology development.

Education is at the heart of the CBE's success. The CBE's third goal is to sustain productive interdisciplinary undergraduate and graduate education programs involving team research on industrially relevant projects.

Industrial Associates Program Benefits

The CBE Industrial Associates Program provides support to help fund industrially relevant research and allows close interaction between industry representatives and CBE researchers and students. Some specific benefits of membership in this program are detailed below.

CBE Technical Advisory Conferences

Twice each year, CBE members convene in Bozeman for a Technical Advisory Conference (TAC)—an exposition of what's new in CBE research and a review of what's happening around the world in biofilm science. The TAC is a great way to keep up on the science as well as to interact with other industry and government representatives and CBE researchers. Meetings are open only to CBE members and invited guests.

Education and Training Workshops

CBE members are entitled to attend either basic or advanced biofilm methods workshops free of charge. Workshops are held the day before TAC meetings in both summer and winter, and feature the latest techniques in growing and assessing biofilms. In addition, the CBE offers specialty workshops (either in our laboratory or at your facility), tailored to your individual company needs. These can range from covering the latest microscopy techniques for your R/D department to assisting the education of your sales force in general biofilm understanding.

Research/Testing Projects

CBE members can fund research and testing projects at a discounted rate. Advantages of directly funding project work at the CBE include complete confidentiality, negotiable intellectual property, and project direction by scientists and engineers at the top of biofilm investigation. In addition, the CBE offers Industrial Associate members no-cost participation in undergraduate research projects of their choosing. This is an opportunity to have a top-notch student work on a problem specific to your needs, at no additional cost.

Product/IP Development Consulting

CBE faculty and staff can assist members in evaluating commercial or product-related ideas as they relate to biofilms or biofilm control. Each member company is entitled to two free days of consulting annually. We can offer confidential feedback on R/D direction, marketing ideas or strategic decisions. Many of our members have found that this benefit alone is worth the annual membership fee.

Regulatory Interactions

Bringing a product to market today frequently requires registration with, or approval of, a regulatory agency. CBE staff maintain close ties with decision makers at FDA, EPA, and other US Government agencies concerned with biofilms. CBE scientists are on the forefront of biofilm methods development and assessment—areas of expertise frequently sought out by the regulatory community. In addition, it's our policy to feature regulatory agency speakers at our TAC meetings whenever possible. If you are seeking insight into the process of registration or approval, the CBE can help.

For More Information

To see if membership is a good fit for your company, please contact Paul Sturman, CBE Industrial Coordinator, at (406) 994-2102, or via email at paul_stu@erc.montana.edu. Or visit us on the web at www.erc.montana.edu.

Presentation and Poster Abstracts
Technical Advisory Conference: February 2-3, 2006

Posters

SPEAKER ABSTRACTS

Keynote Speaker:

W06-S02
On the Emerging Field of Sociomicrobiology—Biofilm Formation and Quorum Sensing in *Pseudomonas aeruginosa*

Peter Greenberg, *Chair and Professor of Microbiology, University of Washington, Seattle, WA*

Pseudomonas aeruginosa is an emerging bacterial pathogen that can cause acute and chronic infections. Quorum sensing controls virulence gene expression in this bacterium, and *P. aeruginosa* can cause chronic biofilm infections. Quorum sensing is a global virulence gene regulator in *P. aeruginosa*. The *P. aeruginosa* quorum sensing system will be described and compared to similar acyl-homoserine lactone signaling systems in other bacteria. A newly discovered mammalian quorum-sensing signal deactivation system will be discussed. The development of *P. aeruginosa* biofilms depends on many factors including quorum sensing. Gene expression patterns in biofilms have revealed information about the special biology of these colonial types of *P. aeruginosa* growth. Recent evidence indicates that innate immune factors can interfere with the ability of *P. aeruginosa* to develop biofilms. For example, lactoferrin blocks the ability of *P. aeruginosa* to settle into colonies on surfaces. Lactoferrin binds iron, and at low iron levels biofilm development is arrested at an early stage. The mechanism of lactoferrin interference in biofilm formation will be discussed.

SESSION 1: Biofilm Control

W06-S03
Enhancing Antibiotic Susceptibility of *Pseudomonas aeruginosa* Biofilms by Quorum Sensing Inhibition

Caol Huff, *PhD Candidate, Chemistry, Center for Biofilm Engineering at Montana State University, Bozeman, MT*

Quorum sensing is a mechanism of cell-to-cell communication used by biofilm-forming bacteria. Because aspects of biofilm behavior appear to be regulated by quorum sensing, these pathways are attractive alternative targets for manipulating and controlling biofilm. Synthetic analogues of signaling molecules possessing the N-acyl-L-homoserine lactone structural motif are currently under construction. This structural component is well conserved among the signaling molecules in biofilm-forming bacteria and it is hoped that these synthetic analogues will weaken biofilm defenses by inhibiting quorum sensing. These synthetic analogues have been used by others to enhance the vulnerability of *Pseudomonas aeruginosa* biofilms to antibiotics. Suitable bioassays for evaluating the synergistic action of these novel quorum sensing inhibitors with antibiotics in *P. aeruginosa* are being developed and will be discussed.

W06-S04
Dormant Cells in *Pseudomonas aeruginosa* Biofilms

Lee Richards, *PhD Candidate, Chemical and Biological Engineering, Center for Biofilm Engineering at Montana State University, Bozeman, MT*

It was hypothesized that *Pseudomonas aeruginosa* biofilms are protected from killing by antimicrobials due to the presence of dormant cells in mature biofilms. A *P. aeruginosa* strain containing a stable, inducible green fluorescent protein was used to visualize and characterize the dormant cell population. After growing the biofilm in the continuous presence of the inducer, most of the cells in the biofilm exhibited green fluorescence. The biofilm was transferred to growth conditions lacking the inducer. As growing cells multiplied, the GFP was diluted, causing these cells to become dimmer. Putative dormant cells retained bright fluorescence even 48 hours after the downshift to non-inducing conditions. The dormant cell population constituted 12% at this time point. By sorting bright and dim cells

in a flow cytometer then plating these samples for colony formation, it was shown that putative dormant cells were still viable. Biofilm cells were also sorted and plated for viability before and after treatment with two different antibiotics. It was found that active cells were much more susceptible to antibiotic treatment than were dormant cells. These data support the hypothesis that *P. aeruginosa* biofilms harbor an inactive, viable cell population that is protected from antibiotic killing.

SESSION 2: Biofilm Structure

W06-S06
Structure and Activity of *Pseudomonas aeruginosa* PAO1 Biofilms

Raaja Raajan Angathevar Veluchamy, *MS Candidate, Environmental Engineering, Center for Biofilm Engineering at Montana State University, Bozeman, MT*

Mathematical models of biofilm play a vital role in understanding biofilm processes in the environment and industry. Modeling has evolved from using simple conceptual models of homogenous biofilms to heterogeneous and stratified biofilms. Most models predict nutrient concentration profiles in the biofilms. However, since these models are based on assumptions about biofilm structure and activity, the predictions are valid only within the domain determined by these assumptions. If the assumptions reflect the conditions in actual biofilms accurately, then the models can realistically predict the behavior of a real biofilm. Nutrient concentration profiles are affected by mass transport outside the biofilm, inside the biofilm, and by the structure of the biofilm. To understand the distribution of biofilm activity, it is necessary to correlate the local nutrient concentration, local mass transport in the biofilm and the biofilm structure. This study shows the correlations among dissolved oxygen concentration, effective diffusivity, and areal porosities in the biofilms of *Pseudomonas aeruginosa* PAO1 grown in a flat plate reactor. We measured three-dimensional distributions of local dissolved oxygen concentration, local relative effective diffusivity and porosity in the biofilm. We have found that the local dissolved oxygen concentrations and relative effective diffusivities correlate weakly with each other or with the areal porosity. However, surface-averaged dissolved oxygen concentration and surface-averaged relative effective diffusivity strongly correlated with each other and with the areal porosity. In our study, surface-averaged dissolved oxygen concentration, surface-averaged relative effective diffusivity and areal porosity decreased towards the bottom of the biofilm, while the coefficients of variation computed for each of these factors increased toward the bottom of the biofilm.

W06-S07
Characterization of *Campylobacter jejuni* Biofilm Formation

Vildan Caner, *CBE Visiting Scientist, Assistant Professor in the Department of Medical Biology, School of Medicine with Pamukkale University, Denizli, Turkey*

It is well known that diseases can be spread by foods. Many of these diseases are infectious and are caused by a variety of foodborne bacteria, viruses and parasites. The CDC estimates that in the United States alone 76 million people suffer foodborne illnesses each year, accounting for 325,000 hospitalizations and more than 5,000 deaths. *Campylobacter jejuni* has emerged in recent years as the primary causative agent of bacterial human foodborne gastroenteritis in industrialized countries. We recognize that biofilms formed by this bacterium are difficult to study in the laboratory. However, many reports about the high incidence of campylobacteriosis in humans and the high prevalence of the bacterium in animals justify such studies. This study addresses the behavior of *C. jejuni* in single species and in mixed culture biofilms. We grew three different biofilms: 1) *C. jejuni* alone, 2) *Pseudomonas aeruginosa* PAO1 alone, and 3) a mixture of *C. jejuni* and *P. aeruginosa* PAO1. The biofilms were grown in flat plate flow reactors operated identically in terms of nutrient concentration and residence time. The structures of the biofilms were quantified from microscopy images of the biofilms, using Image Structure Analyzer (ISA) software. In the future we will compare gene expression profiles of these microorganisms grown in suspended culture, single species biofilms and in mixed culture biofilms, using various molecular techniques such as the real-time quantitative PCR and Western Blotting.

SPEAKER ABSTRACTS

SESSION 3: Phage Interactions with Biofilm

W06-S08
Viruses/Phage: Biofilm Players…but What Else?

Rick Veeh, *Senior Research Associate, Center for Biofilm Engineering at Montana State University, Bozeman, MT*

Viruses exist wherever life is found and probably represent the greatest genetic diversity on Earth. Although humans have studied and employed viruses, including bacteriophage, for over 200 years, we have only been able to image them for about 65 years. Phage have now been shown to play an important role in inducing certain biofilm processes, including lateral gene transfer among bacteria, as well as to have developed adaptive mechanisms of infection that involve degradation of biofilm EPS. Because of the continuing problem of acquired antibiotic resistance in bacteria and because of the inherent host specificity observed in many phage infections, phage therapy is being considered as a possible alternative treatment for control of animal and human bacterial infections. One of the most interesting characteristics that viruses possess is the latency of reproduction that is termed lysogeny. The latter part of the talk will focus on one of the hottest topics in cellular physiology: the role of non-coding RNAs/small interfering RNAs in controlling gene expression. The case will be made that viral processes are similar in kind to other gene-controlling mechanisms, including the expression of oncogenes and perhaps even the evolution of cellular differentiation.

W06-S09
Cage-Based MRI Contrast Agents for Diagnosis of Biofilm Infections

Peter Suci, *Assistant Research Professor, Microbiology, Center for Biofilm Engineering at Montana State University, Bozeman, MT*

Engineered nano-platforms have the potential to revolutionize diagnosis and treatment of a variety of disorders. Their essential function is to deliver a large payload of either an imaging agent or a therapeutic agent to specific cell types. There has been an emphasis on using nano-platforms to treat various cancers, and there are obvious parallels between cancers and biofilm infections. The Center for Bioinspired Nanomaterials (CBIN) at MSU is developing a particular type of nano-platform known as the protein cage. A primary appeal of protein cages is the versatility that they offer bioengineers as templates for functional design. We have developed a model system consisting of *Staphylococcus aureus* and a viral protein cage and used it to test our ability to target this bacterium with the cage. Our results indicate that targeting is specific and that sufficient contrast agent can be loaded onto the cells via the cage to image a dense *S. aureus* biofilm using MRI. Data will be presented showing that, using Magnetic Resonance Microscopy (MRM), we can visualize cage loaded with contrast agent as it is introduced into a flow cell colonized by a *Staphylococcus epidermidis* biofilm. Future work will consist of optimizing contrast-agent loading onto the cage and attempting to visualize a *S. aureus* biofilm targeted with the contrast agent-impregnated cage using MRM.

W06-S10
Display Phage Technology: Development of Peptide Aptamers for the Detection and Neutralization of Toxins

Joe Fralick, *Professor, Microbiology, Texas Tech University, Lubbock, TX*

Combinatorial chemistry represents a new branch of biological chemistry that incorporates both molecular biology and immunology disciplines. Its goal is to use high throughput screening of a very large collection of compounds to find the few that have selective activities of interest. The overall approach integrates several drug-discovery disciplines, including synthetic and computational chemistry, analytical methodologies, molecular modeling and high-throughput screening, and has the potential of identifying novel reagents that can be of value to both basic and clinical research. Phage-displayed peptide libraries are one of the tools of combinatorial chemistry.

The methods of phage display technology are based on the general scheme of making a large library, (often involving greater than 109 different peptide ligands) and putting it though repeated iterations of selection and amplification. In selection, those ligands with desired properties (e.g. high affinity) are preferentially separated from the remainder of the library. In amplification, the relatively few selected ligands are copied to form a new generation. In some protocols, mutation or “exon shuffling” provides an increased combinatorial repertoire (evolution) and allows a global search for ligands not included in the initial library. The power of phage display technology

is that it creates a physical linkage between a selectable function (the displayed peptide sequence) and the DNA encoding that function.

Phage-displayed peptide libraries can be used to isolate peptides that bind with high specificity and affinity to virtually any target. The identified peptides can be used as reagents to understand molecular recognition, as minimized mimics for receptors, or as lead molecules in drug design. The latest applications of this technology include but are not exclusive of: i) functional selections of peptide ligands to probe regulatory networks; ii) epitope mapping used in vaccine design and development; iii) mapping of protein-protein contacts; iv) identification of protease substrates; v) receptor antagonists; vi) peptide mimics of non-peptide ligands; and vii) ligand fishing and protein engineering by directed evolution.

In this study we report the use of phage display technology for the isolation and characterization of a combinatorial display phage which can bind cholera toxin with very high affinity/avidity and neutralize its activity as measured by cAMP induction in monolayers of SE480 colorectal adenocarcinoma epithelial cells (ATCC CCL-228) and water accumulation in a mouse ileal loop assay. We further demonstrate that the displayed peptide can inhibit CT in the mouse ileal loop assay.

SESSION 4: Medical Biofilms

W06-S11
Plant-Derived Biofilm Inhibitors and High Throughput 96-Well Screening

Garth James, *Medical Projects Supervisor and* ***Amy Martin,*** *Undergraduate Researcher, Center for Biofilm Engineering at Montana State University, Bozeman, MT*

This research project was a collaborative effort involving the CBE, Sequoia Sciences, Inc., and the Missouri Botanical Garden. The Missouri Botanical Garden supplied two plant collections: a taxonomically diverse collection and a biologically focused collection, comprised of samples collected from the United States and Gabon, Africa. Using a proprietary purification procedure, Sequoia purified approximately 200 to 700 "drug-like compounds" from each plant into approximately 120 chromatographic fractions. The fractions were then screened by CBE for inhibition of *Pseudomonas aeruginosa* biofilms using a high throughput screening assay (HSA). Fractions showing efficacy were subsequently purified by Sequoia and retested by the CBE to identify active compounds. The chemical structures of the active compounds were determined and synthetic versions were produced for further testing. This additional testing was performed by the CBE using rotating disk reactors (RDRs). This presentation focused on refinements and results of the HAS method as well results of testing with the RDRs.

W06-S12
A Laboratory Model of Wound Biofilms

Ellen Swogger, *Laboratory Technician and* ***Pat Secor****, Undergraduate Researcher, Center for Biofilm Engineering at Montana State University, Bozeman, MT*

At the July 2005 CBE Technical Advisory Conference we presented the results of our 50-wound study, performed in collaboration with the Southwest Regional Wound Care Center. These results indicated that biofilm was prevalent in chronic wounds and rare in acute wounds. This finding has led to continued research on the characterization and treatment of chronic wound biofilms.

In one area of this research, treatment strategies are being evaluated using colony biofilms as an in vitro model of wound biofilms. In this model, multi-species biofilms are grown on polycarbonate membranes on top of agar plates. Topical wound treatments can then be applied to the colony biofilm. Thus far, multi-species biofilms consisting of *Enterococcus faecalis* with either *Staphylococcus aureus* or *Pseudomonas aeruginosa* have been tested for repeatability and have shown to be consistent. During the development of this model, it was observed that *P. aeruginosa* inhibits growth of *S. aureus* in the colony biofilm model. Although these two species have been isolated from the same wound, these results suggest that they don't exist as mixed colonies.

The microbial diversity of chronic wounds was also evaluated using molecular strategies. DNA was isolated from chronic wounds and PCR was performed using primers specific for the 16S ribosomal subunit gene. Because all of the PCR products obtained were of the same length (same number of base pairs), they were separated based on their base pair composition using Denaturing Gradient Gel Electrophoresis (DGGE). From the number of bands obtained in the DGGE gel, the number of different species in the wounds was estimated. Overall, the DGGE study indicated that wound

biofilms had more species diversity than indicated from culture-based studies of the wounds. The PCR products used in the DGGE study were also used in a clone study. PCR products were inserted into a cloning vector and transformed into *E. coli*. The *E. coli* were plated on LB plates containing antibiotics to select for clones with the vector. Individual colonies of *E. coli* were then picked and grown in media overnight and the vector was extracted (using a plasma DNA extraction kit). The extracted vector was then sent to Retrogen, Inc. for sequencing and the sequences produced were compared to known sequences using the Basic Local Alignment Search Tool (BLAST). The identity of the organism from which the PCR product originated was determined for various wounds. One of the most interesting findings was the presence of anaerobes in the wound samples. Several attempts to culture anaerobes from wound samples by the clinic have been unsuccessful.

Special Presentation

W06-S13
The Two-Photon "Capture" Microscope: A Slime Guy's Fervent Dream

Bill Costerton, *Director, Center for Biofilms, School of Dentistry, University of Southern California, Los Angeles, CA*

As we accumulate more and more evidence that bacteria in natural and pathogenic ecosystems vary profoundly from cells of the same organism growing as planktonic cells in liquid cultures, we begin to move toward the study of real bacteria in their actual environments. This movement is accelerated by recent revelations that biofilm cells from natural and pathogenic environments often fail to grow and produce colonies when they are removed from these ecosystems and spread on the surfaces of agar plates.

Just as we resolve to study bacteria in their real environments, we have serendipitously developed many methods for direct examination that indicate the viability and the species identity of individual cells seen by modern microscopy. We can also assess the expression of specific genes by introducing reporter constructs into mixed bacterial biofilms, and measuring the color of specific chromophores. So we have been able to examine biofilms under the confocal microscope, but we have not heretofore been able to identify previously uncultivated organisms, or to determine their physiological activities or their genomic makeup.

Two simultaneous developments in widely separated fields have shown us the way out of the dilemma posed by the collapse of methods used in microbiology for the past 160 years, and may enable us to enter the 21st century with a modern armamentarium. First, Zeiss has modified its PALM microdissection system for use with its 2-photon confocal scanning laser microscope, so that as few as 8 bacterial cells can be examined in a detailed "Z stack" and then excised and catapulted out of a frozen section for recovery and DNA extraction. The capability for detailed examination means that we can determine whether the cells to be recovered are truly clonal (sister cells), and whether the part of the section to be excised has any contaminating cellular material from any other source. This 2-photon confocal microscope with PALM microdissection capability can recover as few as 8 or as many as 40 clonal bacterial cells from areas of special interest, like the parts of biofilms that obviously elicit strong inflammatory reactions from surrounding tissues. The second development is the new capability of the Multiple Displacement Amplification (MDA) technique, developed by Roger Lasken of the Center for Genomic Sciences (Allegheny Health System), to produce enough DNA to allow full genomic characterization of the recovered clone.

The amplified DNA can be subjected to an exhaustive analysis of the full genome, or specific primers can simply be used to determine the 16 S rRNA sequence of the recovered clone so that this sequence can be compared with known genomic databases; both previously cultivated and uncultivated organisms can be identified. When this 16 S rRNA sequence is known, FISH probes can be constructed to identify cells of the clonal species in other biofilms. We can construct FISH probes to identify organisms that have never been grown, we can supply DNA for gene expression arrays that include most or all of the genes used in real environments, and we can conduct genomic analysis of clonal variants that have developed in specific locations in chronic infections. Parallel work on eukaryotic cells leads us to anticipate that we can recover as few as 800 clonal bacterial cells and amplify enough mRNA to conduct *in situ* analyses of the expression of specific genes.

FDA Presentation

W06-S14
FDA Regulatory Outlook: How to Get a Submission Approved by the U.S. FDA

V.M. Hitchins, *A.D. Gantt, Jr., and J. M. Morris,* *Division of Biologics, Office of Science and Engineering Laboratories, Center for Devices and Radiological Health, U.S. FDA, Rockville, MD*

The U.S. Food and Drug Administration (FDA) is responsible for ensuring that foods, drugs for both humans and animals, medical devices, and transplanted tissues are safe and effective. In addition, FDA ensures that blood used for transfusions and equipment that emit radiation are safe. The Agency uses state-of-the-art science to make decisions regarding the safety and effectiveness of products, considering the total product lifecycle from premarket development to postmarket monitoring and surveillance of product safety.

From a regulatory perspective, the addition of an active agent to a device has been considered a combination product. FDA's Office of Combination Product (OCP) was created to assign an FDA Center to have primary jurisdiction for the review of the product, based on the primary mode of action. The speaker will briefly review the goal and function of the OCP as well as the different regulatory approaches and challenges faced with combination products such as devices with antimicrobial agents.

The speaker will discuss how sponsors of submissions can use guidance documents and voluntary national and international consensus standards to help assess and assure the safety and effectiveness of FDA-regulated products.

Infection associated with devices is a very important adverse event and a major public health problem in the U.S. With the emergence of antibiotic-resistant microorganisms and the high costs to treat infections, many companies are interested in developing and marketing new technologies which allow the attachment or incorporation of active agents such as biocides and antibiotics on many medical devices to inhibit or reduce the incidence of infections associated with devices. The FDA is faced with new challenges from both a regulatory as well as a scientific perspective in ensuring that these new products will be safe and effective to protect the public health.

Session 5: The Biofilm Matrix

W06-S15
The Biofilm Extracellular Matrix

Phil Stewart, *Director, Center for Biofilm Engineering at Montana State University, Bozeman*

The aggregation of microbial cells in biofilms and the complex multicellular interactions that ensue depend on the production of a cohesive extracellular matrix. Bacteria and fungi that build biofilms secrete extracellular polymeric substances (EPS) that form a highly hydrated slime in which cells are embedded and held in dense agglomerations. This presentation addresses five topics pertaining to the EPS: 1) progress in imaging the normally invisible watery gel that constitutes the biofilm matrix, 2) the biochemical composition and multiplicity of polymers that likely contribute to matrix structure, 3) diffusive and convective transport properties of hydrogels, 4) models of matrix cohesion and the need to better understand interactions between matrix polymers, and 5) potential strategies for targeting the matrix and as means of biofilm control.

W06-S17
The Role of Staphylococcal Extracellular Polymers in Biofilm Formation and Immune Evasion

Michael Otto, *Principal Investigator, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institute of Health, Hamilton, MT*

Staphylococcus aureus and *S. epidermidis* are the most important pathogens involved in nosocomial infections. As colonizers of human epithelia, and as pathogens during infection, their persistence relies on efficient mechanisms to evade human host defenses. Biofilm formation is one general mechanism by which staphylococci evade attacks by host defenses, but the molecular details are unknown. Furthermore, in contrast to *S. aureus*, which has several elaborate techniques to subvert attacks by innate host defenses, our knowledge of immune evasion strategies in *S. epidermidis* is limited.

To understand biofilm resistance, we characterized gene expression in *S. epidermidis* biofilms. According to our results, an *S. epidermidis* biofilm is in a quiescent state with reduced metabolic activity. This explains resistance to antibacterial agents that primarily target dividing cells with an active

metabolism, such as inhibitors of protein and cell wall synthesis. Notably however, changes of gene expression in the biofilm also comprised up-regulation of specific resistance genes.

One operon that was up-regulated in the biofilm, *cap*, codes for the synthesis of poly-gamma-glutamic acid (PGA). *Bacillus anthracis* is the only human pathogen in which a PGA capsule has been described to protect from phagocytosis. We could show that PGA in *S. epidermidis*, although produced at a very low level, efficiently protects from neutrophil phagocytosis and human antimicrobial peptides. Importantly, a cap mutant revealed dramatically reduced virulence in an animal model of catheter infection, indicating that PGA is a key virulence factor of *S. epidermidis*. PGA is produced by a series of coagulase-negative staphylococci, but not by *S. aureus*, suggesting that it may be a substitute for immune evasion strategies only present in *S. aureus*.

Another exopolymer present in several biofilm-forming pathogens, polysaccharide intercellular adhesin (PIA), has long been recognized as an important virulence factor and structural component of the biofilm matrix. We could demonstrate that in addition, PIA serves as a specific molecule that protects from mechanisms of innate host defense. Importantly, the introduction of positive charges in the N-acetyl-glucosamine polymer PIA via deacetylation by the IcaB enzyme is a crucial prerequisite for all functions of PIA, including biofilm formation and immune evasion.

In conclusion, immune evasion in staphylococci, especially in *S. epidermidis*, is mediated by protective exopolymers. They are predominantly produced in biofilms, thus contributing to biofilm resistance. The biosynthesis of the polymers is an attractive target for antimicrobial therapy. Additionally, the polymers are being investigated as antigens for vaccine development.

W06-S18
Exotoxin Expression during Biofilm Growth of *Pseudomonas aeruginosa*

Ailyn Lenz, *PhD Candidate, Microbiology, Montana State University, Bozeman, MT*

Pseudomonas aeruginosa is an opportunistic pathogen able to form biofilms on the pulmonary tissue of cystic fibrosis patients. These biofilm-associated bacteria are characterized by their ability to secrete a wide array of virulence factors that inhibit the immune response and contribute to the chronic nature of the pulmonary infections. Calcium concentration influences virulence-factor production, including enzymes and toxins secreted by the Type I, II and III secretion systems. Here, we used microarrays to identify factors expressed during biofilm-associated growth at low (0 mM) and high (2 mM) Ca2+ concentrations. The results were then compared to planktonically cultivated cells. We identified genes for exotoxins exoS, exoT, and exoY, as having increased expression during early biofilm formation. Quantitative RT-PCR was used to verify the expression of these genes in continuous-flow biofilms over time. At low [Ca2+], the expression of exoS and exoT showed ten-fold increased expression in biofilms compared to planktonic cells during the first day of growth. ExoY had 100-fold increased expression. As the biofilms developed, the expression of these genes decreased, and by the third day of growth had similar expression level to planktonic cells. When the biofilms were exposed to calcium, exotoxin expression in biofilms had levels comparable to that of planktonic cells. The results suggest that biofilm matrix material may chelate available calcium, causing increased expression of exotoxin genes during the early stages of biofilm development. Exotoxins may be responsible for the lack of neutrophil polarity and chemotaxis that we observed during exposure of the neutrophils to *P. aeruginosa* PAO1 biofilms. Therefore, we are currently using similar QRT-PCR studies to quantify the exotoxin gene expression response of *P. aeruginosa* biofilm following exposure to human neutrophils.

W06-S19
Shear Strain Response through Cation Cross-Linkage of the EPS

Mike Sutton, *Undergraduate Researcher, Mechanical Engineering, Center for Biofilm Engineering at Montana State University, Bozeman, MT*

The goal of this project is to investigate the response of biofilm's material properties when exposed to various chemicals. The hypothesis is that the cations created when a salt is put into solution will bind with the EPS of the biofilm and increase the biofilm's material strength. The biofilm used in this experiment is *Pseudomonas aeruginosa*. The *Pseudomonas aeruginosa* is grown on 25mm membranes in a stationary incubator. After the FRD1 has been incubated on a membrane for 24 hours, a treatment is applied. The membrane is soaked in a known concentration of particular salt solution for one hour

prior to testing. The solutions tested so far have been: $FeCl_2$, $FeCl_3$, $AlCl_3$, and $CaCl_2$, at .2 and .02 Molar concentrations. A rheometer is used to perform the testing of the samples.

The type of test performed on the rheometer is called a creep test. In this test, a constant 15 Pa shear stress is applied to the biofilm sample for five minutes. At the end of the five minutes the load is released. Since biofilm is viscoelastic (having both solid and liquid properties), when the load is released the biofilm wants to return to its original position. This stage of the test is known as the recovery. After 5 minutes of recovery, the test is complete.

Comparing the strain of the untreated samples versus the strain of the treated samples shows how the treatment of the biofilm affects its material properties. Also, this data allows for calculations to be made that can determine the constitutive properties of the biofilm and how treatment affects these values.

The results from this project so far show a clear increase in the material strength of the treated biofilm. Equation fitting is currently being run on the data. We hope these equations will provide insight on how the treatments affect the change in constitutive constants.

W06-P360
A Model for the Removal of *Pseudomonas aeruginosa* Biofilm in a Cystic Fibrosis Lung

E. Martin, *E. Pulcini, and H. Reinemer-Berg, Center for Biofilm Engineering at Montana State University, Bozeman, MT*

Pseudomonas aeruginosa is a common pathogen of cystic fibrosis patients. When it colonizes the cystic fibrosis patient's lung, it often causes chronic pneumonia. It creates a large mass of cells on the tissue of the lungs and secretes a large amount of extracellular polymeric substances (EPS) that cement the cells into a biofilm state. When *Pseudomonas aeruginosa* forms a biofilm, it becomes resistant to almost all known antibiotics. A cystic fibrosis patient does not have the ability to break up the mucous in the lungs by coughing; therefore the bacterial biofilm develops at a much more rapid pace. As a result of this rapid development, chronic life-threatening bacterial pneumonia can develop. This research uses the science of molecular mimicry to prevent *Pseudomonas aeruginosa* biofilms from attaching to the surface and forming biofilm. Once the cells are in a planktonic phase, they become susceptible to most commonly known antibiotics. Once the bacterial cells are vulnerable to the antibiotics, cystic fibrosis patients can be treated more easily, and the chronic cycle of pneumonia can be broken.

W06-P360
Influence of Carbon Source, Iron Minerals and Electron Shuttling Compounds on the Bacterial Reduction of Chromate

E. Field, *L. Jennings, A. Cunningham, B. Peyton, and R. Gerlach., Center for Biofilm Engineering at, Montana State University, Bozeman, MT*

Natural subsurface soil systems can contain a variety of compounds, including organic electron shuttling compounds and iron minerals. Both iron minerals and electron shuttling compounds are known to influence the reduction of oxidized environmental contaminants such as reducible heavy metals and organics. In order to properly design environmental restoration processes, a more complete understanding has to be gained of the combined influence of electron-shuttling compounds, iron minerals, and supplemental electron donors (carbon sources). This research describes the influence of electron-shuttling compounds such as anthraquinone-2,6-disulfonate (AQDS), 2-hydroxy-1,4-naphtoquinone (HNQ), and humic substances, different iron mineral phases, and a number of carbon sources on the reduction of Cr(VI) by a Gram-positive fermenting bacterium (*Cellulomonas sp.* strain ES6). Cr(VI) reduction by strain ES6 was drastically enhanced in the presence of the electron-shuttling compounds HNQ and AQDS. Natural humic substances slightly enhance the rate of Cr(VI) reduction. Depending on the experimental conditions, the presence of Fe(III) minerals can result in an increase or decrease in Cr(VI) reduction rates. If the bacteria are allowed to reduce surface-associated Fe(III) previous to Cr(VI) reduction, Cr(VI) reduction rates are drastically increased. If Cr(VI) and Fe(III) minerals are added simultaneously, no statistically significant change in Cr(VI) reduction rates are observed. Among the carbon sources tested, molasses showed the highest Cr(VI) reduction rates. The rates with molasses as supplemental carbon and electron source were almost as fast as rates observed in the presence of less complex carbon sources (sucrose, lactate, etc.) and AQDS. Since we have shown previously that the type of carbon source and presence of electron-shuttling compound can significantly influence growth and activity of *Cellulomonas sp.*, these results indicate that the proper choice of carbon source and organic matter capable of electron shuttling has the potential to significantly improve existing subsurface remediation scenarios.

W06-P361
A 3D Computer Model Analysis of Four Hypothetical Biofilm Detachment Mechanisms

J. D. Chambless, *and P. S. Stewart, Center for Biofilm Engineering at Montana State University, Bozeman, MT*

Four hypothetical mechanisms of detachment were incorporated into a three-dimensional computer model of biofilm growth and development. The model integrated processes of substrate utilization, diffusion, growth, cell migration, and detachment in a cellular automata framework. The purpose of this investigation was to characterize each of the mechanisms with respect to four criteria: the resulting biofilm structure, the existence of a steady state, the propensity for sloughing events, and the behavior during starvation. The four detachment mechanisms analyzed represented various physical and biological influences hypothesized to affect biofilm detachment.

The first detachment mechanism was based on the height of the biofilm. The probability of a cell detaching was made proportional to the square of its height above the substratum. This mechanism reflects, in an empirical fashion, the influence of fluid shear. The second mechanism made biofilm cell detachment a result of substrate limitation. When the local concentration of substrate around a biofilm cell dropped below a particular level for an extended period of time, the cell detached from the biofilm. The third mechanism addressed erosion. The detachment probability of a cell was made inversely proportional to the number of neighboring cells. Only cells that were located at the biofilm-bulk fluid interface were able to detach. The final mechanism combined the height and neighbor mechanisms. The different detachment mechanisms demonstrated diverse characteristics regarding structure, sloughing, and the existence of a steady state. The height dependent mechanism produced flat biofilms that lacked sloughing events. A clear steady state was achieved for every case run with this mechanism. Detachment based on substrate limitation produced significant sloughing events in almost every case. The resulting biofilm structures included distinct, hollow clusters separated by channels. This mechanism, like the height mechanism, also produced clear steady states of biofilm growth. The neighbor mechanism produced neither a non-zero steady state nor sloughing events. The behavior of this mechanism was highly dependent on the specified detachment constant. If the constant was too high, the biofilm decayed completely, and if it was too low, the biofilm achieved unrestricted growth. The structures of the biofilms produced by this mechanism were similar to those of the substrate limitation mechanism, but did not include cluster hollowing. The final mechanism combined the neighbors and height mechanisms to create an amalgamation of their individual behaviors. This scenario created streaming biofilm structures that were unlike those of any other mechanism. Only experiments that were dominated by height detachment reached a steady state. Starvation conditions affected each of the mechanisms differently. The height mechanism provided a slow, exponential decay during starvation, while the substrate limitation mechanism gave a quick and complete removal of all biofilm cells. The biofilms with the combined detachment decayed in two phases, eventually removing all cells.

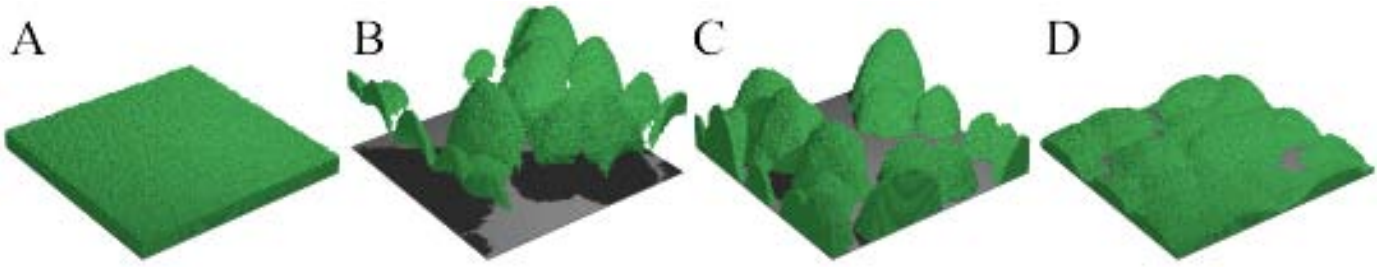

Figure 1. Images showing the structures resulting from the implementation of each detachment mechanism: (A) Height, (B) Substrate Limitation, (C) Neighbors, (D) Combined height and neighbors.

W06-P362
Characterization of the Growth of *Mycobacterium avium* in Biofilms

A. Karmacharya, *S. Broadaway, A. Camper, B. Pyle, and T. Ford, Center for Biofilm Engineering at Montana State University, Bozeman, MT*

Mycobacterium avium is an opportunistic intracellular pathogen, primarily in immunocompromised people. It is widely distributed in the environment and has been isolated from water, soil and air. The main objective of the present study was to characterize the growth of *M. avium* in dual species biofilms of *M. avium* and *Pseudomonas aeruginosa*. The bacteria were grown on stainless steel coupons in a batch mode in autoclaved tap water. Weekly analysis of the coupons showed that *M. avium* density was consistently higher in the monospecies than in the dual species experiment, although the difference was relatively small. Although both the organisms were inoculated to the same optical density, in the biofilm *P. aeruginosa* density was about one log higher than the *M. avium* density, which could be attributed to the difference in their growth rates. Future research will include the observation of the growth of *M. avium* under different environmental conditions.

W06-P363
Anti-Biofilm Properties of Chitosan-Coated Surfaces

R.P. Carlson, *R. Taffs, W. Davison, and P.S. Stewart, Center for Biofilm Engineering, at Montana State University, Bozeman, MT*

The surface-associated process of microbial colonization and biofilm formation is a persistent and ubiquitous problem facing a broad range of disciplines. Examples include tenacious medical infections related to biofilm formation on implanted medical devices as well as microbial fouling of municipal water distribution systems. Common biofilm control strategies like the use of antibiotics or oxidizing chemicals are typically limited in their efficacy at inhibiting or removing biofilms.

We have investigated a chitosan coating system for retarding or preventing the formation of biofilms. The system does not utilize the common strategy of impregnating a polymer with an antimicrobial agent; instead it utilizes a biological polymer as both the coating material and the actual anti-biofilm agent. The thin, flexible coating is highly effective at retarding or preventing the formation of *Staphylococcus epidermidis, Klebsiella pneumoniae, Staphylococcus aureus* and *Candida albicans* biofilms under medically relevant conditions. For instance, *S. epidermidis* surface counts (CFU/cm^2) are reduced on average 300,000-fold on the chitosan coating as compared to a control. More traditional coatings impregnated with antimicrobial agents like chlorhexidine were found to reduce *S. epidermidis* surface counts by less than 10-fold. The findings suggest this coating has potential for applications on surfaces susceptible to biofilm formation, like implantable medical devices.

W06-P364
Spatial Patterns of DNA Replication, Protein Synthesis, and Oxygen Concentration within Bacterial Biofilms Reveal Active and Inactive Regions

S. Abdul Rani *and P.S. Stewart, Center for Biofilm Engineering at Montana State University, Bozeman, MT*

Biofilms harbor both active and inactive cells, and it is a challenge to characterize the spatial and population heterogeneity of specific activities within a biofilm. Spatial patterns of DNA replication and protein synthetic activity were imaged by techniques developed using staphylococcal systems. The first technique measures DNA synthetic activity by pulse-labeling with the thymidine analog 5-bromo-2-deoxyuridine (BrdU) followed by immunofluorescent detection of brominated DNA. The second technique makes use of an inducible green fluorescent protein construct that can be used to detect the capacity for *de novo* protein synthesis. These techniques were applied to biofilms grown in three different reactor systems. In all cases, measurements revealed that, even in simple single-species biofilms, complex spatial distributions of anabolic activity occur. In a colony biofilm system, two distinct regions of DNA synthetic activity were observed: one close to the nutrient interface and another adjacent to the air interface. A similar pattern was measured by GFP induction. The dimension of the growth zone at the air interface ranged from 16 to 38 microns. When pure oxygen was introduced, a wider zone of active DNA replication (46 microns) and GFP synthesis (58 microns) was measured at the gas interface. Stratified patterns of activity were also observed in biofilms developed in two continuous flow reactors. While biofilms harbor regions of active anabolism, the techniques also demonstrate that these biofilms contain regions of complete inactivity. Such inactive zones may contribute to the special ecology of

biofilms and tolerance to antimicrobial agents. The techniques, particularly BrdU labeling, are generic and may find application to many microbial biofilm systems.

W06-P365
Drip Flow Biofilm Reactor: Growing a Repeatable Biofilm Under Low Shear

J. Whitaker, N. Beck, *D. Goeres, D. Walker, M. Hamilton and B. Warwood, Center for Biofilm Engineering at Montana State University, Bozeman, MT*

The Center for Biofilm Engineering at Montana State University and a Montana-based company, BioSurface Technologies Inc. (BST), have collaborated to develop a bioreactor capable of growing biofilms with high cell density under low fluid shear conditions. The reactor, originally developed by Dr. Phil Stewart's research team, is called the Drip Flow Biofilm Reactor (DFR). Currently, BST manufactures reactors that are operated under high and medium shear. The DFR met the need for a reactor that grows biofilm under low shear conditions and close to the liquid-air interface. The Standardized Biofilm Methods Research Group developed a standard operating procedure (SOP) for growing a repeatable biofilm in the DFR using *Pseudomonas aeruginosa*. The design of the reactor, as well as the SOP, underwent many changes to optimize the repeatability. Ruggedness testing was performed by varying four operational factors around the SOP conditions; temperature (22°C), reactor angle (10°), flow rate (0.8 mL/min/channel) and nutrient concentration (270 mg/L Tryptic Soy Broth (TSB)) during continuous flow phase.

Results from ruggedness testing showed that temperature had the greatest effect on the visual appearance of the biofilm. Flow rate and temperature had a more significant effect on the log density than nutrient concentration and reactor angle when independent variables were compared. All four experimental conditions were relatively easy to control. Nutrient concentrations above 300 mg/L TSB caused a decrease in log density. Operating the DFR at the SOP resulted in a mean log density of 9.3 cfu/cm^2 with a repeatability standard deviation of 0.32. These results demonstrate that the DFR and its associated SOP are repeatable and rugged to small changes in the operational factors.

W06-P366
Measuring Viable Cell Loss Resulting from Coupon Manipulation During Disinfectant Testing

A. Hilyard, *K. Buckingham-Meyer, D. Goeres, and M. Hamilton, Center for Biofilm Engineering at Montana State University, Bozeman, MT*

A laboratory disinfectant efficacy test against biofilm bacteria begins by growing a biofilm in a well-controlled biofilm reactor that contains removable growth surfaces (coupons). Disinfectant efficacy is determined by comparing the number of viable cells remaining on a disinfectant-treated coupon to the number on an untreated (control) coupon. The typical testing protocol requires that each coupon is subjected to a variety of manipulations; e.g., treating, neutralizing, removing, and disaggregating. If, due to these manipulations, a significantly larger fraction of viable bacteria is lost from the treated coupon than from the control coupon, the viable bacteria count for the treated coupon would be artificially small, thereby biasing the efficacy measure. For this reason, the observed efficacy is a measure not only of the disinfectant's killing power, but also includes the disinfectant's propensity to remove viable bacteria from the coupon surface. The purpose of this investigation was to determine the extent to which viable bacteria are lost from control coupon surfaces due to laboratory manipulation for each of three different laboratory test systems.

Three different systems were tested to simulate high, low, and no shear environments for glass coupons. The three systems used were the CDC reactor (high shear), drip flow reactor (low shear), and the static biofilm reactor (no shear). The bacterium used for these experiments was *Pseudomonas aeruginosa* (ATCC 15442). Five experiments were done in duplicate for each system. Six coupons were sampled during each experiment from all three reactors. Two coupons were scraped right away, two coupons were rinsed, treated, then scraped; the final two coupons were rinsed, treated, neutralized, then scraped. For the coupons that were rinsed and treated, the rinse and treatment water were also sampled. For the coupons that were rinsed, treated, and neutralized, the rinse water and treatment/neutralizer water were sampled. All samples were homogenized, diluted, and plated for viable cells.

These experiments showed that, for the drip flow reactor, greater than 75% of the viable cells remained on the coupon surface after the coupon was rinsed, treated, and neutralized. In contrast, fewer than 16%

of the viable cells remained on the surface of coupons from the static biofilm reactor after being rinsed, treated, and neutralized. The percent of viable cells on the coupons from the CDC reactor were between these two values. For coupons from the static biofilm reactor, the greatest percent of washed-off cells was found in the treatment/neutralizer water. These experiments demonstrate the importance of including wash-off measurements as well as surface counts when performing biofilm disinfectant tests.

W06-P367
Mechanisms of Uranium Mobilization in Oilfield Sea-water Injection Pipelines

M. VanEngelen *and B. Peyton, Center for Biofilm Engineering at Montana State University, Bozeman, MT*

The accumulation of uranium in oilfield sea-water injection pipelines represents both a human health and environmental liability. It is believed that the accumulation of uranium is a result of the reduction of soluble U(VI) species to insoluble U(IV) species, a process carried out by the metabolic activity of sulfate reducing bacteria (SRB). Methods of accumulation prevention, principally through U(IV) re-oxidation mechanisms, are currently under investigation. Recent experiments using CSTR bioreactors demonstrated the potentially positive role of nitrate on the U(IV) re-oxidation processes. The results of these preliminary experiments showed that the use of appropriate chemical additives could provide methods of successful mobilization of uranium species in oilfield sea-water injection pipelines. Further investigations include the construction of a CSTR-in-series bioreactor system, one aimed at better simulating flow through a pipeline. In addition, a variety of chemical additives, including nitrate, nitrite, and iron, and chemical additive combinations will be screened for their U(IV) re-oxidizing capability.

W06-P368
Influence of the Quorum Sensing Signal Autoinducer-2 on *Mycobacterium avium* Biofilm Formation

H. Geier *and T. Ford, Microbiology, Montana State University, Bozeman, MT*

Mycobacterium avium is an opportunistic pathogen often found to colonize water distribution systems by forming highly complex communities called biofilms. Quorum sensing, the cell-density dependent regulation of gene expression, has many times been shown to be an important factor in the regulation of biofilm formation and maturation in different bacterial species. The current study was performed to test the influence of the signaling molecule autoinducer-2 (AI-2) on *M. avium* biofilm formation.

M. avium was grown in Middlebrook 7H9 medium with OADC enrichment at 37°C. After seven days, the culture was centrifuged for 20 minutes at 8000 rpm and the pellet was resuspended in water. This aqueous culture was then used to inoculate the microtiter plates (150 µl per well) in the presence of different concentrations of chemically synthesized AI-2, resulting in final concentrations of 2.5, 0.25, 0.025 and 0.0025 mM, respectively. The negative control contained water instead of the AI-2 solution. After fourteen days, the biofilms were quantified by crystal violet-staining. The cell density in the supernatant was measured by viable counts on Middlebrook 7H10 agar. The structure of the biofilms was observed using confocal laser scanning microscopy.

Biofilms accumulated more biomass with increasing AI-2 concentration, culminating in the doubling of the biomass in the presence of 2.5 mM AI-2. However, the cell density of the supernatant decreased with increasing AI-2 concentration. Microscopic observation revealed significant differences in biofilm structures depending on the AI-2 concentration, with complexity of the biofilm structure increasing with the AI-2 concentration.

AI-2 does not appear to have a metabolic effect on the growth of mycobacterial cells, as has been shown for other selected bacteria. However, this study does suggest that AI-2 plays a role in *M. avium* biofilm formation by regulating the complexity of the biofilm architecture and by inducing the switch from the planktonic to the biofilm mode of growth.

W06-P369
Comparison of Disinfection Behavior on Biofilm with Chlorine and Silver: Microscope Approaches

J. Kim, *B. Pitts, P. Stewart, A. Camper, and J. Yoon, Center for Biofilm Engineering at Montana State University, Bozeman, MT*

The systematic understanding of how various disinfectants are involved in controlling biofilm growth is essential in order to establish the effective

strategy for biofilm control since many successful disinfectants in a planktonic phase encounter the lack of disinfection performance in a heterogeneous biofilm phase.

Two contrasting disinfectants (chlorine and silver ion) were selected to examine the disinfection behavior on biofilm in a comparative way. Chlorine is moderately reactive and is easily involved in chlorination reaction with the various components of cells. Silver has no oxidizing capacity, but is involved in rendering the various enzymes in cell membranes inactive by binding.

Three measuring methods—plate counts (cell reproduction), measurement of respiratory activity with CTC stain, and BacLight live/dead (SYTO9/PI, the membrane integrity)—were selected to examine the disinfection behavior of chlorine and silver ion on biofilm, along with the confocal scanning laser microscopy (CLSM) and epifluorescence microscopy. PA01 biofilms were grown on glass coupons in a CDC biofilm reactor. After one-day growth in batch and 2–3 days growth in flow system, coupons were removed from the CDC reactor, rinsed and then soaked in the 10 mg/L of silver or chlorine solution. For each sampling, coupons for CLSM were removed from each disinfectant solution and soaked in the neutralizing solution, rinsed and stained with CTC/DAPI or SYTO9/PI. At the same time, coupons for colony counting and epifluorescence microscopy were collected in 10 mL of PBS solution in glass vials and were suspended via 10 sec of sonication and 2 min of vortexing. One mL of each suspended solution was used for colony counting and the rest of the solution was filtered and stained for epifluorescence microscopy. Images were collected via epifluorescence microscope and (CSLM) and analyzed using MetaMorph and Imaris software.

The results show that silver appears to be more effective in affecting the respiratory activity in comparison with chlorine, based on equal culturability. However, silver maintained better membrane integrity than chlorine, as displayed by BacLight live/dead staining. This observation is plausible from the different oxidative reactivity between silver and chlorine, further providing the explanation for the superiority of silver ion for biofilm disinfection.

W06-P370

Biofilms Summer School 2006: Three Workshops by the Structure-Function Research Group

H. Beyenal, *Center for Biofilm Engineering at Montana State University, Bozeman, MT*

The Structure-Function Research Group at the CBE is conducting three back-to-back biofilm workshops in the summer of 2006. The first, scheduled for July 24–25, is "Biofilm Structure: Quantification and Image Analysis." The goal of the workshop is to provide participants with the knowledge needed for imaging biofilms using light microscopy and Confocal Scanning Laser Microscopy (CSLM), to interpret biofilm images, and to quantify the information extracted from the images. The second workshop is "Fundamentals of Biofilm Research," scheduled for July 26–28. The goal of the workshop is to familiarize the participants with the fundamentals of biofilm processes, and with the procedures used to grow biofilms under well-defined conditions. The third workshop—"Microsensors: Manufacture and Application,"—will be held July 31–August 4. Microsensors (pH, dissolved oxygen, ion selective microelectrodes, etc.) are becoming indispensable tools for studying biofilms and small biological samples. The goal of this workshop is to provide the participants with the knowledge necessary to design, manufacture, and apply such microsensors. Lab sessions and lectures will illustrate the electrochemical principles of the measurements, explain how microsensors are manufactured, and demonstrate how they are applied to study biofilms. All of the workshops will be held at MSU's Center for Biofilm Engineering. Instructors will be Drs. Zbigniew Lewandowski and Haluk Beyenal.

POSTER ABSTRACTS

W06-P371
Evaluation of Novel Antimicrobial Compounds Aganocides™ for Potential Applications in Treatment and Prevention of Biofilm in Catheter-Associated Urinary Tract Infections

L. Bickle, *G. James, and E. Pulcini, Center for Biofilm Engineering at Montana State University, Bozeman, MT*

Urinary catheters are frequently used in medical practice, but routine catheter insertion can introduce bacteria, such as *Escherichia coli*, from the urethra into the bladder. Once introduced, bacteria can attach and colonize the lumen of the catheter, resulting in a biofilm and urinary tract infection that responds poorly to antibiotic therapy. For long-term catheter patients, an infection may cause blockage in the catheter, even serious kidney or blood infections. Sterile saline is currently used to prevent biofilm formation; however infection remains a problem and many infected catheters must be replaced. NovaCal Pharmaceuticals, Inc. (NovaCal) recently developed a promising alternative to sterile saline in the form of antimicrobial rinse solutions. An in vitro test system was developed to evaluate the efficacy of NovaCal's antimicrobial rinses as an alternative to sterile saline or catheter replacement.

The test system involves 5 catheters aseptically installed with a sterile Artificial Urine Media flow at 0.75 mls/minute. Each catheter is inoculated with a urease positive strain of *Escherichia coli* overnight culture and allowed an attachment period of 2 hours. The media flow is resumed and the biofilm is grown for 3 days. Four test solutions and a sterile saline control are pumped (2mls/minute) via valves from the bag-end of the catheter, through and out the bladder-end of the catheter. The total treatment time is 40 minutes, including 10 minutes to pump and 30 minutes of stationary disinfection. The catheters are then sampled by scraping sections of tubing and plating on agar for viable cell counts. The catheters are also imaged using confocal and scanning electron microscopy. Using the in-vitro system, NovaCal's test solutions showed a significant reduction in viable cell counts, which were verified visually by microscopy.

W06-P372
Current Research in Soils and Water

S. Clark, J. Faulwetter, M. Rahman, *Anne Camper, Otto Stein and Mark Burr, Center for Biofilm Engineering at Montana State University, Bozeman, MT*

Salmonella typhimurium **Virulence Factors in Drinking Water Biofilms:** *Salmonella typhimurium* has been isolated from drinking water distribution system biofilms and may be implicated in waterborne outbreaks of salmonellosis. While many studies have been conducted into the pathogenesis of this bacterium, effective molecular biology tools now make it possible to identify virulence factors involved in pathogenesis and may allow researchers to predict behavioral patterns of *Salmonella* associated with biofilms in low nutrient systems as well as in planktonic stages of growth. One such tool we currently employ is the use of microarrays to identify transcriptomic patterns of an organism under different growth conditions.

Microbial Community Comparisons Between Soils and Rhizosphere: Constructed wetlands are widely used for wastewater treatment, but little is known about the rhizospehere microbial ecology in these systems. Current research goals include identifying the active microbial community in relation to the total community via DGGE profiling and investigating the bulk substratum community versus the rhizosphere community, particularly whether the rhizosphere community is plant-specific.

Corrosion and Nitrification in Water Distribution Systems: Corrosion and nitrification are two major problems in water distribution systems. Both nitrification and corrosion are influenced by microbial activity. In addition, some heterotrophic species are responsible for problems associated with nitrification. The mechanism of bio-corrosion is not well understood and heterotrophic nitrifies are not yet well identified in these systems. In this project we will try to identify the main species of microorganisms responsible for corrosion and heterotrophic nitrification. We used some modified CDC reactors to simulate a domestic water supply system and molecular technique such as PCR and DGGE for microbial community analysis.