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Symposium KK
Microbial Life on Surfaces: Biofilm-Material Interactions: Life at Interfaces
San Francisco, CA, April 25-27, 2011

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Microbial life surfaces has specific consequences for both the microbes, in terms of their physiology, metabolism and gene expression, and for the substrate, as evidenced by the fouling and degradation of a wide array of materials from metals to plastics to medical implants. Understanding the molecular-scale and macro-scale interactions between bacteria and surfaces is thus very important to a wide array of applications -- environmental (subsurface soil remediation, microbial dissolution of minerals, microbial uptake of trace metals, drinking water quality, fate of pathogens in the subsurface) to biomedical (cell-to-cell transfer of genetic material, bacterial infections of implanted devices). The objective of this symposium was to bring together a diverse array of international scientist to explore the biofilm-substrate interface from perspective of the material.

This symposium focused on understanding the interactions between bacteria and surfaces at the molecular and macroscale levels. In order to present a comprehensive set of symposium topics, both fundamental topics on bacterial adhesion measurements and modeling as well as topics that focus on biofilm-materials interactions from the perspective of the industrially relevant, engineered material applications of bacterial adhesion were represented. Symposium KK highlighted research that brings new materials characterization tools to the discipline, both experimental and computational. Papers focused on adhesion, biodegradation, the biophysics of biofilm development, and antifouling and antimicrobial surfaces, while highlighting new techniques and approaches in biofilm research. Biofilm research is an extremely multidisciplinary field, and Symposium KK brought together basic life scientists, physicists, engineers, and clinicians. Presentations were given by academics, members of industry, as well as DoD laboratories (AF and Navy) and will include experts in the field as well as junior faculty, postdocs, and graduate students.

Some topics included:

- Origin of life: small organic molecules can self assemble into larger macromolecular building blocks for life on the surfaces of mica, and this field explores how the surface chemistry of crystalline materials dictates the assembly of organic matter on those surfaces, as well as how organisms influence the growth of inorganic crystals.
- Using organisms to generate and control light (bioluminescence) with no energy lost as heat.
- Exploring the factors needed to design and fabricate microbial fuel cells with higher energy output.
- Current microbial culturing techniques in clinical settings are incapable of detecting the organisms responsible for device-related infections.; detection techniques that rely on microbial chemical (DNA, RNA) signatures are being developed for the medical community.

- The impact of surface structure, super hydrophobicity, and bulk materials properties influence organism attachment and biofilm development.
- Naturally occurring biomaterials tethered to surfaces to provide infection-resistant medical devices.
- The difficulties in modeling microbial attachment to surfaces
- Biofilm-induced degradation of polymeric coatings from multiple complementary approaches, to understand microbial physiology and the chemistry of the microbe-coating and coating-substrate interface.
- The role of extracellular polymeric substances (EPS) in promoting microbial adhesion, protecting the attached organisms from the environment, and the chemical and physical nature of the EPS.

Proceedings will be published in **electronic-only format** as of the Materials Research Society Symposium Proceedings Series.

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14. ABSTRACT

Bacterial communities living on surfaces are called biofilms. Microbial life on a surface has specific consequences for both the microbes (in terms of their physiology, metabolism, and gene expression), and for the substrate, as evidenced by the fouling and degradation of a wide array of materials from metals, to plastics, to medical implants. Understanding the molecular-scale and macroscale interactions between bacteria and surfaces is thus very important to a wide array of applications, from environmental ones such as subsurface soil remediation, microbial dissolution of minerals, microbial uptake of trace metals, drinking water quality, fate of pathogens in the subsurface, to biomedical applications such as cell-to-cell transfer of genetic material, and bacterial infections of implanted devices. This symposium will focus on understanding the interactions between bacteria and surfaces at the molecular and macroscale levels. In order to present a comprehensive set of symposium topics, both fundamental topics on bacterial adhesion measurements and modeling, as well as topics that focus on biofilm-materials interactions from the perspective of the industrially relevant, engineered material applications of bacterial adhesion, will be covered. This symposium will highlight research that brings new materials characterization tools to the discipline, both experimental and computational.

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Symposium KK: Microbial Life on Surfaces---Biofilm-Material Interactions

SYMPOSIUM KK

Microbial Life on Surfaces---Biofilm-Material Interactions

April 25 - 27, 2011

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* Invited paper

TUTORIAL

KK: Imaging Biofilms and Quantifying Struetures

Monday, April 25
1:30 PM - 5:00 PM
Room 2008 (Moseone West)

The goal of the tutorial is to familiarize the partieipants with imaging biofilms grown on various surfaees, teaching novel techniques to analyze biofilm images to quantify biofilm struetures numerieally. The lecture topics inelude: proeedures for biofilm staining and imaging; quantifying biofilm structure to get paramcters sueh as the areal porosity, fractal dimension, average diffusion distanee, and homogeneity index; and interpreting structural parameters extracted from biofilm images.

Instructors:
Haluk Beyenal
Washington State University

SESSION KK1: Nanoseale Investigations of Microbial Properties and Interactions
Chair: Nehal Abu-Lail
Tuesday Morning, April 26, 2011
Nob Hill CD (Marriott)

8:45 AM *KK1.1
Physico-chemical Meehanisms of Initial Mierobial Adhesion to Surfaces and Bond-maturation. Henny C. Van der Mei, Biomedical Engineering, University Medical Center Groningen, Groningen, Netherlands.

Upon initial microbial adhesion to a surface, multiple events occur that are unlikely to be captured in a single mechanism.

Measurements of residence-time dependent desorption in flow displacement systems have shown that the desorption probabilities of initially adhering organisms strongly decrease within seconds to minutes after first contact. Interaction force measurements using Atomic Force Microscopy (AFM) have supported that the bond strength between adhering organisms and substratum surfaces increases within that time span. Surface thermodynamic analyses, application of DLVO-theories and Poisson analysis of retract force-distant curves from AFM indicated that this bond-maturation is due to the progressive involvement of acid-base interactions. Acid-base interactions require close approach between the interacting surfaces, which is firstly achieved by attractive, long-range Lifshitz-Van der Waals forces. Once brought in the close vicinity of a surface, bond -maturation follows as a result of interfacial re-arrangements. Interfacial re-arrangements in the region between an adhering organism and a surface are often associated with changes in the rigidity of the coupling. Quartz Crystal Microbalance with Dissipation (QCM-D) allows monitoring of these interfacial re-arrangements or changes in coupling-rigidity from a change in its dissipation signal, but has been little applied yet to study this aspect of microbial adhesion. Application of physico-chemical mechanisms to explain microbial adhesion to surfaces requires better knowledge of the interfacial re-arrangement occurring immediately after adhesion than hitherto available.

9:15 AM KK1.2

Using Atomic Force Microscopy to Map the Distribution of Protein Molecules on the Surface of Live Microorganisms. Brian H. Lower, School of Environment & Natural Resources, The Ohio State University, Columbus, Ohio.

Antibody-recognition force microscopy (Ig-RFM) is a relatively new technique that uses atomic force microscopy (AFM) to study antibody-antigen interactions, identify proteins, and map the nanoscale distribution of protein molecules in complex biological structures. This is a powerful technique because it permits the study of live cells or isolated biomolecules (e.g., protein) under physiological conditions. Here we describe the use Ig-RFM to probe the cell surface of live bacterial cells using AFM tips that were functionalized with protein-specific antibodies. In doing so we were able to identify specific proteins that were targeted to the external cell surface. We were also able to map the distribution of protein molecules on the cell surface and relative to the substrate on which the bacteria were growing.

9:30 AM KK1.3

Effects of the Temperature and Ionic Strength of Growth Conditions on the Nanoscale Adhesion of *L. monocytogenes* EGDe to Silicon Nitride. Pinar Gordesli and Nehal Abu-Lail; Chemical Engineering and Bioengineering, Washington State University, Pullman, Washington.

The food-borne pathogen *Listeria* monocytogenes is a Gram-positive, facultatively anaerobic and rapidly growing bacterium, with the ability to form persistent biofilms. It is transmitted to animals and humans by contaminated food and can cause listeriosis, a severe disease with high hospitalization and fatality rates. *L.* monocytogenes can adapt to survive and grow in a wide range of environmental conditions allowing this pathogen to overcome the safety barriers in food processing and storage. In this study, the nanoscale adhesion forces of *L. monocytogenes* EGDe to a model surface of silicon nitride were quantified by using atomic force microscopy (AFM) for bacterial cells grown under five different temperatures (10, 20, 30, 37 and 40°C) and five different ionic strengths (0.005, 0.05, 0.1, 0.3 and 0.5M NaCl). Our findings for the cells grown under different temperatures show that the adhesion ability of *L.* monocytogenes EGDe change due to the growth temperature. It was observed that *L. monocytogenes* EGDe adhesion affinity to model inert surfaces achieved its highest values at 30°C followed by those quantified at 37°C. Our nanoscale measurements agree well with studies in the literature that quantified the effects of growth temperature on the ability of the same bacteria to form biofilms. The adhesion affinity of *L.* monocytogenes to silicon nitride surface for cells grown under various ionic strength conditions are currently ongoing. Our results will be used to elucidate some of the fundamental aspects of the survival mechanisms of *L. monocytogenes* EGDe under physical conditions of stress.

10:15 AM *KK1.4

Mineral Surfaces, Amino Acids, and the Origins of Life. Robert M. Hazen[1], Dimitri A. Sverjensky[2,1], Kateryna Klochko[1], Adrian Villegas-Jimenez[1], Namhey Lee[2,1] and Charlene Estrada[2,1]; [1]Geophysical Laboratory, Carnegie Institution, Washington, District of Columbia; [2]Earth & Planetary Sciences, Johns Hopkins University, Baltimore, Maryland.

The chemical origins of life occurred in several steps, each of which increased molecular complexity and patterning of Earth's near-surface environment. The first step, abiotic synthesis of amino acids, sugars, lipids and other essential molecular bio-building blocks, has been well documented through experiments that mimic environments on Earth and in space. However, the second step, which includes selection, concentration and assembly of those molecules into the functional membranes and polymers of life, is less well understood. Our research team investigates how mineral surfaces might have played a role in the critical transition from a dilute, indiscriminate prebiotic soup to micro-environments that were concentrated in molecules poised to foster life. Studies on adsorption of biomolecules onto common mineral surfaces, including competitive molecular adsorption, batch adsorption, molecular stability and decomposition, and potentiometric titration experiments, coupled with extended triple-layer surface complexation and density functional theory modeling, point to at least four plausible roles that such interactions may have played in life's origins. (1) Minerals are able to concentrate molecules from dilute solutions by factors of 1000 or more, thus potentially overcoming the problem of a dilute prebiotic soup. (2) Molecules bound to mineral surfaces may be much more stable than those in solution, thus countering a strong objection to the hypothesis that life's origins occurred at or near a hydrothermal system. (3) Some minerals are able to select and concentrate specific molecules, notably chiral (right- versus left-handed) amino acids, thus providing a possible mechanism for the origins of biological handedness. (4) Finally, mineral surfaces may juxtapose and align molecules to facilitate polymerization and other modes of biomolecular assembly. Our work also underscores the importance of including realistic prebiotic contributions in any origin of life scenario. Any geochemical model for life's origins must thus incorporate such physico-chemical complexities as cycles, gradients, fluxes, and interfaces.

10:45 AM *KK1.5

Molecular Interactions of *Staphylococcus aureus* and Implanted Biomedical Devices. Nadia N. Casillas-Ituarte[1], Supaporn Lamlertthon[2], Eric S. Taylor[1], Alex C. DiBartola[1], Vance G. Fowler[2] and Steven K. Lower[1]; [1]School of Earth Sciences, Ohio State University, Columbus, Ohio; [2]Duke Clinical Research Institute, Duke University, Durham, North Carolina.

Staphylococcus aureus is responsible for a large percentage of infections associated with implanted biomedical devices. The molecular interactions of this bacterium with a fibronectin-coated probe (model of an implanted device) were analyzed with atomic force microscopy. A group of 100 different isolates of this bacterium were obtained from either patients with an infected cardiac device (invasive group) or healthy carriers (control group). The average binding-force frequency is statistically different ($p = 0.003$) between the two populations, suggesting that a microorganism's "force taxonomy" may provide a fundamental and practical indicator of the pathogen related risk that infections pose to patients with implanted medical devices.

11:15 AM KK1.6

Extracellular DNA Enhances Bacterial Adhesion and Aggregation by Influencing Acid - Base Interactions. Theerthankar Das, Prashant K. Sharma, Bastiaan P. Krom, Henk J. Busscher and Henny C. van der Mei; Biomedical Engineering, W.J. Kolff Institute, University Medical Center Groningen and University of Groningen, Groningen, Netherlands.

Significance and objectives: Bacteria in nature attach to nearly all surfaces and form biofilms with the help of self produced extracellular polymeric substances (EPS). Extracellular DNA (eDNA) present in EPS acts as an adhesive and strengthens the biofilm. In this study we investigated the effect of naturally occurring eDNA on adhesion and aggregation of several bacterial strains and there mechanism of interaction. Methods: Initial bacterial adhesion of Staphylococcus epidermidis 1457, S.

epidermidis 1457 ΔatlE and Streptococcus mutans LT11 to hydrophilic and hydrophobic substrata and surface aggregation in presence and ahsence of eDNA were studied using a parallel plate flow chamber. Physico-chemical surface characteristics of S. epidermidis 1457 and its mutant ΔatlE were determined by contact angle and zeta potential measurements. Adhesion force and bond formation between S. mutans LT11 and substratum or between two S. mutans LT11 in the presence and absence of eDNA was measured by Atomic force microscopy (AFM). Extended DLVO theory was used to calculate total interaction energies of staphylococcal adhesion to the substrata and bacteria in aggregates. All experiments were done in phosphate buffer saline. Results: In the presence of eDNA all bacterial strains showed a higher initial deposition rate and adhered in higher numbers after 60 min to both hydrophilic and hydrophobic substrata when compared to adhesion in the absence of eDNA. On hydrophilic surfaces in the presence of eDNA an increase in the percentage of bacteria in large aggregates was observed compared to aggregates on hydrophobic surfaces. Physico-chemical surface characterization showed that removal of eDNA, from S. epidermidis 1457 surface by DNaseI treatment decreased its hydrophobicity similar to the hydrophobicity of its mutant strain ΔatlE, which lacks production of eDNA. Whereas the zeta potential of the staphylococcal cell surfaces became less negative upon removal of eDNA. Accordingly, favourable total interaction energies in the presence of eDNA became unfavourable in the absence of eDNA due to changes in acid-base interaction energies. However Lifshitz-Van der Waals and electrostatic interaction energies remains attractive and repulsive respectively regardless of eDNA presence. AFM measurements showed significant increases in adhesion force and bond formation between S. mutans LT11 and substratum or between two S. mutans LT11 in presence of eDNA compared to the absence of eDNA. Presence of eDNA molecules on bacterial cell surfaces enhance exchange of electrons between interacting substratum or bacteria and thus increases bond formation. Conclusions: The presence of eDNA on bacterial cell surfaces enhances adhesion kinetics, aggregation, forces of interaction and bond formation due to the involvement of acid-base interactions.

SESSION KK2: Biodegradation I
Chair: Wendy Goodson
Tuesday Morning, April 26, 2011
Nob Hill CD (Marriott)

11:30 AM *KK2.1

Microbial Attack on Polymeric and Carbon Fiber-reinforced Materials and Sensitive Testing Methods. Ji-Dong Gu, Biological Sciences, University of Hong Kong, Hong Kong, Hong Kong.

Biodeterioration of polymeric materials affect a wide range of industries including infrastructure and space. Formation of microbial biofilms on surfaces of polymeric materials being considered as candidate materials for use on the International Space Station has been investigated. The materials included fiber-reinforced polymeric composites, adhesive sealant, polyimide insulation foam, teflon cable insulation, and aliphatic polyurethane coatings. In laboratory simulation experiments, bacterial biofilms formed readily on the surfaces of the materials at a wide range of temperatures and relative humidity. The biofilm population was dominated by Pseudomonas aeruginosa, Ochrobactrum anthropi, Alcaligenes denitrificans, Xanthomonas maltophila, and Vibrio harveyi. Subsequently, degradation of polymeric materials was mostly a result of both fungal and bacterial colonization in sequence, and fungi may have advantages in the early phase of surface colonization than bacteria, especially on relatively resistant polymeric materials. These microorganisms are commonly detected on hardware of spacecraft and in the air-circulation system. Furthermore, degradation of polymeric materials was detected with electrochemical impedance spectroscopy (EIS). The EIS spectra indicated that microbial attack occurred in several steps over time. An initial decrease in impedance was detected due to the transport of water and solutes into the polymeric matrices. A second decrease in impedance occurred as a result of polymer degradation. Our data showed that these materials are susceptible to biofilm formation and subsequent degradation by microorganisms. Plasticizers and additives to the polymeric materials provide the source of carbon and energy for the initial colonization by fungi and bacteria, and bacteria are capable of utilization plasticizers efficiently. Our study suggests that candidate materials for use in space and aviation need to be carefully evaluated for susceptibility to microbial attack.

SESSION KK3: Biodegradation II
Chair: Wendy Goodson
Tuesday Afternoon, April 26, 2011
Nob Hill CD (Marriott)

1:30 PM KK3.1

Real Tme Analysis of Polymer Film Integrity Upon Exposure to Bacteria and Aqueous Media Daniel E. Barlow[1], Justin C. Biffinger[1], Emily R. Petersen[2], Stephen E. Lizewski[1], Jobn N. Russell[1], Pehr E. Peherson[1], Wendy J. Goodson[3] and Peter A. Mirau[3]; [1]Chemistry Department, US Naval Research Laboratory, Washington, District of Columbia; [2]Nova Research, Inc., Alexandria, Virginia; [3]Materials & Manufacturing Directorate, US Air Force Research Laboratory, Wright-Patterson AFB, Ohio.

Polymer coatings are of great importance for protecting and imparting specific properties at the surfaces of man-made structures, but can be affected in many ways by the natural environments they must withstand. We have studied the effects of aqueous media exposure and biofilm formation on polyurethane coatings containing carbon black using in situ ATR-FTIR. Both coated and uncoated germanium ATR prisms were compared. The results show that the polyurethane films are susceptible to water penetration and swelling, and deuterium exchange was also shown to occur within the films upon D_2O exposure. When exposed to Pseudomonas fluorescens in M9 minimal media, the results were consistent with initial settlement of planktonic bacteria which then diminished after depletion of the pyruvate food source. Enhanced biofilm formation was subsequently found to occur on the coated substrates, impacting the film integrity. While ATR-FTIR has been used in the past to study biofilm growth, these results also demonstrate the effectiveness of the method for assessing substrate impact, an often overlooked factor.

1:45 PM KK3.2

Biofilms and Corrosion of 70/30 Cu-Ni Alloy in a Marine Environment. Iwona B. Beech[1,2], Sheelagh Campbell[1], Zakari Macama[1], Beatrice Monica Perez-Ibarra[2], Kathleen Duncan[2] and Jan Sunner[2]; [1]School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, United Kingdom; [2]Botany and Microbiology, University of Oklahoma, Norman, Oklahoma.

Laboratory investigations were carried out to determine whether bacterial biofilms contributed to marine corrosion of 70/30 Cu-Ni alloy. Continuous flow bioreactors operating under two different temperature regimes (24oC and 10oC) were inoculated with biofilm populations, comprising aerobic and anaerobic bacteria, recovered from two systems; one site experiencing corrosion problems and another with no reported failures. Cu-Ni coupons with different surface treatment were exposed in inoculated and control (sterile) reactors fed with filter sterilized natural seawater for 7 months. Upon their recovery from bioreactors, coupons were characterized using light digital microscopy and field emission scanning electron microscopy equipped with EDX. Microbial populations used as inocula, as well as biofilms, subsequently formed on surfaces of coupons exposed in bioreactors, were characterized with PCR and DGGE. Partial 16S rRNA sequences were also obtained. DGGE profiles and sequencing data demonstrated that bacterial populations recovered from the two sites were divers and dissimilar. Abundant biofilms were formed on surfaces of Cu-Ni specimens exposed to these populations. Pitting attack was detected underneath biofilms. Pit density and depth varied significantly between bioreactors and depended on bacterial population and, to some extent, surface treatment of coupons. Pitting morphology on coupons exposed to bacterial populations was similar to that reported in field failures. No appreciable pitting was detected on surfaces of sterile controls, regardless of temperature. The study provides evidence that bacterial biofilms can instigate and accelerate corrosion of 70/30 Cu-Ni alloy and emphasizes that understanding biofilm ecology and metabolic interactions between members of the biofilm

community are essential to achieve control over biocorrosion phenomenon.

2:00 PM KK3.3

Characterization of Pseudomonas Fluorescens Biofilms Using DB-FIB, SEM, (S)TEM and EELS. A. R. Blankemeier[1], W. J. Goodson[2], C. L. Knight[2,3], J. M. Sosa[1], D. E. Huber[1], R. E. Williams[1], H. O. Colijn[1] and H. L. Fraser[1]; [1]Center for the Accelerated Maturation of Materials (CAMM), Department of Materials Science and Engineering, Ohio State University, Columbus, Ohio; [2]Nanostructured and Biological Materials Branch, Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright Patterson Air Force Base, Ohio; [3]UES Inc., Dayton, Ohio.

Biofilms are communities of bacteria that live on substrates. In many cases, biofilms have a detrimental effects on the surfaces to which they adhere. For example, biofilms negatively impact dental enamel, metals, and polymers. One of many challenges in biofilm research is in analyzing the interface between a biofilm and its substrate, to determine how the biofilm adheres to and affects the substrate. This analysis can be challenging due to the limitations of methods such as SEM, confocal microscopy and ultramicrotomy. Indeed, SEM can provide topographical information of the sample's surface but is fundamentally a two dimensional technique. Confocal microscopy is a useful tool for characterizing large areas of the biofilm but lacks the spatial resolution that TEM provides. Traditionally, ultramicrotomy has been the preferred method of sample preparation for TEM analysis of biological systems despite the lack of site-specificity and compromising effect on structure from mechanical sectioning with a knife[1]. Recently, dual-beam focused ion beam (DB-FIB) technology has revolutionized the field of metals, ceramics, semiconductor materials, and TEM sample preparation; however this technique has not been widely applied to soft material characterization largely due to rapid degradation of these materials under ion beam irradiation [2]. In this study, we developed novel processes to reduce ion damage to the biofilm during milling, and successfully used DB-FIB to analyze Pseudomonas fluorescens biofilms and the interface between the biofilm and its substrate, polyurethane paint. An FEI Helios Nanolab 600 DB-FIB was used to create serial section data sets for three-dimensional reconstruction and also to excise and thin foils for (Scanning) Transmission Electron Microscopy ((S)TEM) and Electron Energy Loss Spectroscopy (EELS). Serial sectioning of the biofilm provided novel structural information about surface adherence mechanisms of the bacteria. (S)TEM imaging and EELS characterization was applied to investigate compositional differences across the interface as well as view the internal structures (or inclusions) of the bacteria. [1] Jantou, V., et al., Focused ion beam milling and ultramicrotomy of mineralised ivory dentine for analytical transmission electron microscopy. Micron (2009) [2]] DJ Stokes, F Morrissey, and BH Lich. Journal of Physics: Conference Series 26 (2006) 50-53. A New Approach to Studying Biological and Soft Materials Using Focused Ion Beam Scanning Electron Microscopy (FIB SEM). [3] This work is supported in part by US AFOSR, under STW-21 II program.

2:15 PM KK3.4

Biofilm Degradation of Polyurethane Coatings. Pehr E. Pehrsson[1], John N. Russell[1], Daniel E. Barlow[1], Wendy J. Goodson[2], Caitlyn L. Knight[2], Donald M. Eby[3], Glenn R. Johnson[3] and Steven A. Policastro[1]; [1]Chemistry Division, Naval Research Laboratory, Washington, DC, District of Columbia; [2]Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright-Patterson AFB, Ohio; [3]Materials and Manufacturing Directorate, Air Force Research Laboratory, Tyndall AFB, Ohio.

Polymer coatings are used in a wide variety of militarily relevant applications where biofilms can grow on them. We used a suite of complementary physical and surface analyses to simultaneously examine how Shewanella oneidensis biofilms affected the macroscopic properties of polyurethane-coated Al coupons and clarify the molecular level chemistry underlying these changes. Scanning electron microscopy (SEM), atomic force microscopy (AFM) and profilometry revealed how the coating morphology was affected by the biofilm. Electrochemical impedance spectroscopy provided a macroscopic picture of liquid and ionic diffusion into the coating and how its electrical integrity changed with biofilm exposure. X-ray photoelectron spectroscopy (XPS) and FTIR-ATR provided compositional and chemical bonding information about how the biofilm and other factors altered the coating chemistry. Some samples were also first exposed to a saltfog to determine the synergistic

degradative effects from combined environmental and biological factors. Finally, we clarified how carbon black nanoparticle additives affect biofilm formation and degradation of the coating.

SESSION KK4: Characterization of Microbial LPS and EPS
Chair: Mark Fornalik
Tuesday Afternoon, April 26, 2011
Nob Hill CD (Marriott)

2:30 PM *KK4.1

Characterizing Bacterial Cell Wall Composition Using Cryo-XPS and Multivariate Analysis. Madeleine Ramstedt[1], Ryoma Nakao[2,3], Sun Nyunt Wai[2], Bernt Eric Uhlin[2] and Jean-Francois Boily[1]; [1]Department of Chemistry, Umeå University, Umeå, Sweden; [2]Department of Molecular Biology and Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå Centre for Microbial Research (UCMR), Umeå University, Umeå, Sweden; [3]Department of Bacteriology, National Institute of Infectious Diseases, Tokyo, Japan.

Gram-negative bacteria can alter the composition of the Lipopolysaccharide (LPS) layer of the outer membrane as a response to different growth conditions and external stimuli. These alterations can, for example, promote attachment to surfaces and biofilm formation. The changes occur in the outermost layer of the cell and may consequently influence interactions between bacterial cells and surrounding host tissue, as well as other surfaces. Microscopic analyses, fractionation of bacterial cells or other traditional microbiological assays have previously been used to study these alterations. These methods can, however, be time consuming and do not always give detailed chemical information about the bacterial cell surface. We here present an analytical method that provides chemical information on the outermost portion of bacterial cells with respect to protein, peptidoglycan, lipid and polysaccharide content. The method involves cryo-X-ray Photoelectron Spectroscopy (XPS) analyses of the outermost portion (~10 nm) of intact bacterial cells, followed by a multivariate curve resolution analysis of carbon spectra. It can be used as a tool for characterizing and monitoring variations in the chemical composition of bacterial cell walls or outer membrane vesicle, variations that result from e.g. mutations or external stimuli. The method enables accurate predictions of alterations in polysaccharide content for a range of well characterized Escherichia coli LPS mutants with different surface chemistries. The method may moreover be applied to a wide range of biological samples.

3:30 PM *KK4.2

The Structure of Bilayers Formed by Lipopolysaccharides Isolated from Pseudomonas Aeruginosa PAO1 as Determined by Neutron Scattering. Norbert Kucerka[1], Erzsebet Papp-Szabo[2], Mu-Ping Nieh[4], Thad A. Harroun[3], Jeremy Pencer[5], Sarah R. Schooling[2] and John Katsaras[6,1,3]; [1]Canadian Neutron Beam Centre, National Research Council, Chalk River, Ontario, Canada; [2]Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada; [3]Department of Physics, Brock University, St.Catharines, Ontario, Canada; [4]Institute of Materials Science, University of Connecticut, Storrs, Connecticut; [5]Atomic Energy of Canada Limited, Chalk River, Ontario, Canada; [6]Neutron Scattering Science Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Scattering techniques, in particular neutron and X-ray scattering have played a major role in elucidating the static and dynamic structure of biologically relevant membranes. Importantly, these techniques have evolved to address new sample preparations that better mimic biological membranes. Unlike X-rays, however, neutron scattering lengths are different for isotopes of the same element. This is because neutron scattering is the result of nuclear forces, and the neutron scattering length can experience a dramatic change in magnitude and phase as a result of resonance scattering. Importantly, in the case of hydrogen its scattering length is negative, even at energies far from its resonance energy. In the case of biological material inherently rich in hydrogen, dramatic changes in scattering amplitudes can therefore be achieved through the substitution of

hydrogen for its isotope deuterium (positive scattering length), selectively tuning the sample's contrast. The asymmetric outer membrane of Gram-negative bacteria contains lipopolysaccharides (LPSs) which contribute significantly to the bacterium's surface properties and play a crucial role in regulating membrane permeability. Neutron diffraction studies performed on aligned, self-assembled bilayers of Na-, Ca-, and Mg-salt forms of LPS isolated from Pseudomonas aeruginosa PAO1, have discovered that water penetrates Ca2+-LPS bilayers to a lesser extent than either Na+- or Mg2+-LPS bilayers. It seems that Ca2+ alters the structure of smooth LPS bilayers in such a manner that the end result is a "compacting" of the inner/outer core region of LPS bilayers. This differential water penetration could have implications as to how small molecules permeate the outer membrane of Gram-negative bacteria and, possibly, how non lamellar phases are formed.

4:00 PM KK4.3
Computer Modeling of the LPS Membrane of Pseudomonas Aeruginosa. Roberto Lins, Thereza Soares, Frederico Pontes and Agrinaldo Nascimento Junior; Department of Fundamental Chemistry - DQF, UFPE, Recife, Brazil.

Lipopolysaccharides (LPSs) are the major constituent of the outer membrane of Gram-negative bacteria, and are believed to play a key role in processes that govern microbial metal binding, surface adhesion, and microbe-mediated oxidation/reduction reactions. It is also a major causative agent of nosocomial illness, eliciting both chronic and acute infections in burn, immunocompromised, and cystic fibrosis. Overall bacterial survival is ensured via phenotypic variation in the LPS (number of acyl chains per LPS molecule), such as the presence of inner and outer core and relative expression of O-antigen A- and B-bands. Availability of metal ions in the environment, pH and ionic strength may also alter membrane structural dynamics and stability influencing adhesion, surface charge, immunogenicity and biofilm formation. A complete understanding of biofilm formation mechanisms in different media by Gram-negative bacteria will require the characterization of the structure, dynamics, aggregation and interactive properties of the LPS. Using our previously developed atomistic model for the LPS membrane of Pseudomonas aeruginosa, molecular dynamics simulations and electrostatic calculations have been carried out to characterize the effects of the presence of B-band, number of acyl chains and availability of metal ions in the environment on the structural dynamics and surface charge of the LPS membrane. The results are compared to experimental data and a parallel is drawn between membrane stability, molecular shape, adhesion and endotoxicity.

4:15 PM *KK4.4
The Perfect Slime - Biofilm Matrix Materinl. Hans-Curt Flemming and Jost Wingender; Biofilm Centre, University of Duisburg-Essen, Essen, Germany.

The microorganisms in biofilms live in a self-produced matrix of hydrated extracellular polymeric substances (EPS) that form their immediate environment. EPS are mainly polysaccharides, proteins, nucleic acids and lipids; they provide the mechanical stability of biofilms, mediate their adhesion to surfaces and form a cohesive, three-dimensional polymer network that interconnects and transiently immobilizes biofilm cells. In addition, the biofilm matrix acts as an external digestive system by keeping extracellular enzymes close to the cells, enabling them to metabolize dissolved, colloidal and solid biopolymers. Here we describe the functions, properties and constituents of the EPS matrix that make biofilms the most successful forms of life on earth.

4:45 PM KK4.5
Gel-like Exocellular Material Produced by an Anaerobic Bacterial Strain Growing Using Fe(III) as an Electron Acceptor. Milva Pepi, Marcella Ruta, Simone Gasperini and Silvano E. Focardi; Department of Environmental Sciences, University of Siena, Siena, Italy.

Bacteria are unicellular microorganisms ubiquitous in the environment and able to adapt to extreme conditions, producing biomaterials in order to allow the colonization of a particular ecologic niche, where much more complex organisms as the

eukaryotic cells, do not adapt as easily as well. Bacteria could represent an important source for new biomaterials production, in terms of innovation and particular adaptability of this kind of materials. Gels produced by bacteria can give opportunities in order to collect heavy metals and metalloids from solutions, allowing the recovery of these toxic elements from polluted sites. An extreme environment with the presence of organic contaminants and heavy metals deep polluted sediments selected for anaerobic Fe(III)-respiring bacteria, able to use citrate as carbon and energy source. A bacterial strain showing these features was isolated from these polluted sediments and characterized in terms of the capability to grow and to produce a gel-like exocellular material. The bacterial strain was named OR9 and was able to grow in anaerobic conditions, in a medium containing Na-acetate as carbon and energy source, and Fe(III)-citrate as electron acceptor. A turbidity was observed during growth of the bacterial strain in anaerobic conditions, followed by the gel formation and the clarification of the solution. The gel precipitates at the bottom of the vial, originating a mineral along the incubations. In this case the metalloid arsenic was added at concentrations of 1 and 5 mM. The anaerobic bacterial strain was isolated from a site highly polluted by arsenic. For this reason the addition of arsenic, both as As(III) and As(V) could have done a possible compatibility with the bacterial strain. The abatement of arsenic as As(III) and As(V) was obtained in the bacterial culture forming the gel. A characterization of the gel of the OR9 strain is in progress in order to detect its nature and mechanisms of the metalloid precipitation. Characterization of the gels produced by bacteria could represents an important base of study in order to characterize the active sites, determinant in the arsenic chelation and in its removal from solution. The characterization of the gel and the determination of the mechanism of arsenic precipitation are in progress. These characteristics of bacteria could represent sources of innovation, providing new molecules of high industrial interest. Again the exploitation of natural polymers as exopolysaccharides, could offer an interesting base for potential heavy metals and metalloids removal from contaminated sites or for production of new material.

SESSION KK5: Poster Session: Microbial Life on Surfaces
Tuesday Evening, April 26, 2011
8:00 PM
Exhibition Hall (Moscone West)

KK5.1

The Effects of Sodium and Calcium Binding on the Structure of the LPS Membrane of Pseudomonas Aeruginosa. Agrinaldo J. Nascimento and Roberto D. Lins; Química Fundamental, Universidade Federal de Pernmbuco - UFPE, Recife, Pernambuco, Brazil.

Bacterial Lipopolysaccharide (LPS) molecules consist of a lipid A - an endotoxin, a nonrepeating "core"oligosaccharide, and the O-antigen, a long variable polysaccharide chain. LPS is credited as the major factor of virulence in humans and other mammals. It acts as a weak non-specific antigen, which is poorly neutralized by antibodies. Unlike its planctonic counterpart, Gram-negative bacteria when forming biolfims can cause sceptic shock, fever and even lead to death. These microorganisms have a great metail ions sorption capacity in their cell walls. Such characteristic is very important to explain phenotypical variation, mobility and bioavailability of metals in environment. Metal ions and their complexes have been reported to bind to the negatively charged phosphoryl and carboxyl groups in the LPS. The availability of metal ions is highly dependent on the local environment. In turn, ionic coordination number, solvation shell and net charge are expected to influence the packing, stability, adhesion and permeability properties of these membranes. In addition, the pH has been reported to have a significant impact the adhesion of LPS to different materials. Therefore, the elucidation of the interactions between LPS and different metal ions is expected to shed light into problems such as antibiotic resistance and material adhesion. In this work, we have performed quantum calculations and molecular dynamics simulations of the LPS membrane of Pseudomonas aeruginosa in the presence of several concentrations of Na^+ and Ca^{2+} ions. While both ions are abundant ions in physiological media, Ca^{2+} ions are commonly found in the LPS of soil-living bacteria. On the other hand, these microorganisms are exposed to high concentration of Na^+ ions in infecting tissues. Changes in the pKa values for the phosphoryl and carboxyl groups were monitored as a function of the ratio Ca^{2+}/Na^+ ions in the LPS membrane. Differences

in the LPS lateral diffusion and acyl chain order parameters suggest that metal ion and pH can dramatically affect membrane dynamics, surface charge and stability.

KK5.3

Biodegradable Star Polymers for Antimicrobial Applications. Daniel J. Coady[1], James L. Hedrick[1], Kazuki Fukushima[1] and Yi-Yan Yang[2]; [1]IBM, San Jose, California; [2]IBN, The Nanos, Singapore.

Biocompatible and biodegradable antimicrobial materials are becoming increasingly important due to the rise in antibiotic resistant bacteria. Previously, our efforts have utilized the self-assembly and aggregation of amphiphilic poly(carbonate) block copolymers with pendent tetraalkylammonium groups for such applications. In an effort to simplify the self-assembly process we have synthesized analogous amphiphilic block-star polymers to eliminate the need for aggregation and create more consistent size distributions. These advancements are envisioned to mimic natural antibiotic proteins for potential use as polymeric drugs.

KK5.4

Rechargeable Antimicrobial and Biofilm-controlling Biomaterials. Yuyu Sun, Biomedical Engineering, The University of South Dakota, Sioux Falls, South Dakota.

Despite major medical advances, infectious diseases continue to be the third leading cause of death in the United States and the leading cause worldwide. The use of antimicrobial devices can be a potentially effective approach to reduce such risks. However, most of the currently available antimicrobial devices are only effective for a short period of time (days), and are not suitable for long-term applications. Novel rechargeable antimicrobial and biofilm-controlling biomaterials are developed to fight disease. The new biomaterials act as "rechargeable batteries" that bind and then slowly release various antimicrobial agents to prevent microbial colonization and biofilm formation. Extended use consumes antimicrobials and reduces disease-fighting activities. However, the consumed antimicrobials can be repeatedly recharged to extend antimicrobial duration for long-term use. In recharging, antimicrobials can be changed/rotated to enhance antimicrobial potency and reduce the risk of microbial resistance. At the time when the infections are cleared, the remaining drugs in the biomaterials can be "quenched" to stop the therapy when no further drug release is need. If needed, the biomaterials can be recharged again to re-initiate drug release. The new biomaterials are attractive novel drug carriers for multiple medical/dental applications in which the devices are readily accessible for recharging. Representative examples include long-term central venous catheters, dentures, tubing in dental unit waterlines and ventilators, etc. The new biomaterials can also be used for the antimicrobial treatment of high-touch, high-risk surfaces in healthcare settings and other related fields to reduce the risk of cross-contamination and cross-infection.

KK5.5

Potential of Amoxicillin based Chitosan Nanoparticle against Escherichia Coli Biofilm. Vivek Pandey[1], Sandeep Singh[1], Preetam Varma[1,2], Himanshu Pandey[2], Vikas Pruthi[3], Ravi P. Tewari[1] and Vishnu Agarwal[1]; [1]Motilal Nehru National Institute of Technology, Allahabad, India, Allahabad, India; [2]Sam Higginbottom Institutes of Agriculture, Technology & Sciences, Allahabad, India., Allahabad, India; [3]Department of Biotechnology, Indian Institute of Technology, Roorkee, India, Roorkee, India.

Escherichia coli is a gram negative bacilli generally reside in lower intestine of endotherms. The harmless strains are part of the normal flora of the human gut, and can benefit their hosts by producing vitamin K2 and by preventing the establishment of pathogenic bacteria within the intestine. The pathogenic form of E. coli strains can cause serious food poisoning in humans including gastroenteritis, urinary tract infections, and neonatal meningitis. In some cases, virulent strains can cause for haemolytic-uremic syndrome (HUS), peritonitis, mastitis, septicemia and gram-negative pneumonia. E. coli causes infections mostly in its biofilm mode of growth which is characterized by production of exopolysaccharide (EPS) and enhanced

antibiotic resistance. The resistance of hiofilm residing cell against the drug generally developed due to penetration barrier, expression of drug resistant phenotypes or reaction of drug with EPS components. To check the efficacy of commonly used antibiotic amoxicillin against E. coli in absence of reaction of drug molecule with EPS components, in the present work we analyzed effect of chitosan based nanopartiele mediated drug delivery system as a potent biofilm inhibitor. The E. coli biofilm was developed on polypropylene pieces at 37C for 48h using CDC biofilm reactor. The chitosan nanoparticles were synthesized by ionotropic gelation method. Amoxicillin with minimum inhibitory concentration (MIC90) of 2.5 μg/ml was used to inhibit the biofilms either directly or encapsulated within chitosan nanoparticle. It was found that the biofilm inhibition was about 35% more in case of drug releasing chitosan nanoparticle. The study demonstrated that a significant amount of drug reacts with EPS components and hence the effective dosage available to sessile community is less than the amount added.

KK5.6

Effect of Nano-and-Micro Crystalline Diamond Surfaces in the Size of Bacteria. Adriana Collazo[2], Olga Medina[1], Jose Nocua[1], Ramon Gomez-Moreno[5], Daniel Montano[1], Javier Avalos[3,4], Concepcion Rodriguez[5] and Gerardo Morell[1,3]; [1]Department of Physics, University of Puerto Rico, San Juan, Puerto Rico; [2]Department of Biology, University of Puerto Rico, San Juan, Puerto Rico; [3]Institute for Functional Nanomaterials, University of Puerto Rico, San Juan, Puerto Rico; [4]Department of Physics, University of Puerto Rico at Bayamón, Bayamón, Puerto Rico; [5]Department of Biology, University of Puerto Rico at Bayamón, Bayamón, Puerto Rico.

The following work shows the changes in the bacterial division of the P. aeruginosa on nanocrystalline diamond (NCD) surfaces and compares it with microcrystalline diamond (MCD), stainless steel AISI 304 (SS), silver (Ag), polyethylene (Poly) and copper (Cu), with the purpose of comparing their antibacterial efficiency with NCD's. The results show that the inhibitory properties of NCD become perceivable just after 13 hours of bacterial transference. NCD was shown to be a good bactericidal surface, overmatched only by copper. The polyethylene, silver, stainless steel and MCD were found to be less inhibiting than NCD. Valuable properties of NCD as the high resistance to oxidation and corrosion, the extreme mechanical hardness and the biological compatibility with blood and tissue makes it more useful than copper. In order to study the bactericidal properties of each surface and their effect on the bacterial size, different characterization techniques were employed, such as scanning electron microscopy (SEM), atomic force microscopy (AFM), the measurement of the contact angle and the evaluation of the colonization factors via a statistical analysis of the bacterial count. These techniques helped to establish a correlation between the bacteria size (NCD: 1.82μm y MCD: 3.02μm), the roughness, the hydrophobicity/hydrophilicity and the colonization susceptibility of the given materials.

KK5.7

Functionalized Single Wall Carbon Nanotubes as an Antimicrobial Agent for Pseudomonas Aeruginosa and Staphylococcus Aureus. D. M. Hernandez-Lugo[1,2,3], O. Medina[3,4], J. E. Nocua[3,4], M. Rivera[4], A. Colon[6], A. Collazo[6], D. Montano[4], A. Borrero[7], R. Rivera[7], J. Avalos[3,5], G. Morell[3,4] and B. R. Weiner[1,3]; [1]Department of Chemistry, University of Puerto Rico, Rio Piedras Campus, San Juan; [2]Center for Advance Nanoscale Materials, NASA, University of Puerto Rico, Rio Piedras Campus, San Juan; [3]Institute of Functional Nanomaterials, University of Puerto Rico, Rio Piedras Campus, San Juan; [4]Department of Physics, University of Puerto Rico, Rio Piedras Campus, San Juan; [5]Department of Physics, University of Puerto Rico, Bayamon Campus, Bayamon; [6]Department of Biology, University of Puerto Rico, Rio Piedras Campus, San Juan; [7]Department of Biology, University of Puerto Rico, Bayamon Campus, Bayamon.

Carbon nanotubes (CNTs) have emerged as a novel and promising class of nanomaterials with unique optical, electrical, mechanical, and thermal properties. Several studies have demonstrated that single-walled CNTs (SWCNTs) in suspensions have strong antimicrobial activities to bacterial cells. As part of this study we analyze the antimicrobial activity of these CNTs in relation to their surface group. Functionalized SWCNTs (-COOH) and NO-functionalized SWCNTs have been used in order to determine their inhibitory efficiency. As part of this study we found that functionalized SWCNTs have a higher

antimicrobial activity when compared to NO-functionalized SWCNTs. The antimicrobial activity of CNTs was examined by looking at the growth curve using 640nm wavelength. Functionalized and NO-functionalized single-wall carbon nanotubes were characterized by using scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Raman Spectroscopy, X-ray Photoelectron Spectroscopy (XPS) and IR.

KK5.8
Abstract Withdrawn

KK5.9
Biofilm Elimination and Detachment Using Photocatalytic TiO2 Surfaces. Yanling Cai[1], Hakan Engqvist[2], Maria Stroemme[1] and Ken Welch[1]; [1]Nanotechnology and Functional Materials, Uppsala University, Uppsala, Sweden; [2]Division for Materials Science, Uppsala University, Uppsala, Sweden.

Biofilms are a prevalent mode of microbial life found in nature. Bacteria in biofilms are 10-1000 times more resistant to antibiotics than when in planktonic form, and in many cases are developing resistances to existing antibiotics; as such, there is a growing requirement for new strategies in biofilm elimination. Dental plaque is an example of a biofilm that often results in dental diseases such as caries. Furthermore, dental plaque is often associated with restorative dentistry materials, which often enhance and increase the accumulation of bacteria. The aim of the present work was to perform an in vitro evaluation a novel dental adhesive containing photocatalytic TiO2 nanoparticles for on-demand biofilm elimination and detachment through ultraviolet (UV-A) irradiation. The dental adhesive was prepared by adding 20 wt% TiO2 nanoparticles to a light cured resin matrix of HEMA and bis-GMA polymers. Spontaneous hydroxylapatite formation on the surface of the adhesive samples upon storage in simulated body fluid indicated good bioactive properties, and suggests that the material should better integrate with the adjacent tooth tissue. The nanoparticle-containing adhesive was shown to be photocatalytic by the degradation of rhodamine-B dye under UV-A irradiation. Biofilm elimination and detachment testing was accomplished by irradiating the biofilm-coated surface of the adhesive with UV-A light. Detachment of the biofilm was assessed after the UV treatment by measuring the amount of biofilm remaining on the surface after the samples were subjected to an ultrasound treatment. It was found that UV-A irradiation led to a significant increase in detachment of biofilm from the adhesive surface compared to the non-irradiated adhesive surface. Results also showed that a dose of approximately 6 J/cm2 led to a 1 log reduction in the concentration of viable bacteria in a biofilm that was grown on the surface of the adhesives. As much as 7 log reduction in bacteria was achieved with a total UV-A dose of 45 J/cm2.

KK5.10
Interaction of Alcohols and Ions with Biofilms Using Microrheology. Anderson Sunda Meya[1], Jasmine Jones[1], Kamirah Demouchet[1], Fook C. Cheong[2], Simone Duarte[3] and David Grier[2]; [1]Department of Physics, Xavier University of Louisiana, New Orleans, Louisiana; [2]Department of Physics & Center for Soft Matter Research, New York University, New York, New York; [3]Department of Basic Science and Craniofacial, New York University College of Dentistry, New York, New York.

Dental Biofilms, the extracellular matrix formed by the growth of polysaccharides from sucrose, provide the optimal environment enabling the pathogenesis of the bacteria, Streptococcus mutans (S. mutans). The presence of S. mutans is primarily associated with dental caries or tooth decay, the most virulent disease today. The objective of this research is to understand the effects of various ions and alcohols on the viscoelastic properties, the mechanical properties, and the intermolecular interactions of the dental biofilms. A holographic microrheological study allows a non-invasive method to accurately measure the physical properties of the dental biofilm in three-dimensions with nanometer resolution by measuring precisely probes particles' three-dimensional trajectories. Applying this technique to biofilms, in particular, shows promise for high-throughput combinatorial screening of candidate therapeutic or remedial agents.

KK5.11

Use of Antimicrobial Peptides and Proteins for Prevention of Biofilm Formation. Caitlin Knight[2,1], Matthew Dickerson[2,1], Lawrence Brott[1] and Wendy Goodson[1]; [1]Nanostructured and Biological Materials Branch, Materials and Manufacturing Directorate, Air Force Research Laboratory, Air Force Research Laboratory, Wright-Patterson AFB, Ohio; [2]UES Inc., Dayton, Ohio.

Biofilms are communities of bacteria that colonize surfaces. They are problematic in both medical and industrial settings where biofilms cause chronic infections on medical implants and foul pipelines and storage tanks. Their ability to colonize many different substrates and to resist to biocidal treatment makes freeing biofilms from surfaces a challenging and costly task. Regular maintenance intervals require pipelines to be shut down and application of biocidal agents may be costly or have negative environmental impacts. Thus, preventing initial biofilm formation is a much more economical solution. We propose developing coatings that will help prevent initial attachment and maturation of biofilms by covalently attaching AMPs (antimicrobial peptides) and enzymes, such as DNase, to surfaces. Part of the innate host defense system, antimicrobial peptides have evolved over millions of years to provide protection against a wide range of microbes, including Gram positive and Gram negative bacteria, and fungi. AMPs are small (1-10 kDa), cationic, amphipathic peptides that are resistant to degradation. Unlike many antibiotics whose efficacy requires cells to be metabolically active, AMPs act by forming pores in the cell membrane, causing leakage of cytosol. Using silane chemistry and the heterobifunctional crosslinker PMPI (N-[p-Maleimidophenyl]isocyanate) the antimicrobial peptide Cecropin A was attached to glass surfaces and successfully decreased the number of cells attached to the surface after 24 hours.

KK5.12

Bacterial Effects on $CaCO_3$ Crystallization. Jenny A. Cappuccio[2], Veronica Pillar[1] and Caroline Ajo-Franklin[1]; [1]Materials Science Division, Lawrence Berkeley National Lab, Berkeley, California; [2]Earth Sciences Division, Lawrence Berkeley National Lab, Berkeley, California.

Geologic carbon dioxide sequestration, the underground storage of carbon dioxide, will be an essential component of global climate change mitigation. Carbonate minerals are a promising form of stable CO_2 storage, but their formation occurs on a geologic timescale, not than human timescale. Many microbes can influence the precipitation of carbonate minerals; however the mechanisms of such mineralization are largely unknown. Hypothesized mechanisms include metabolic processes altering pH and supersaturation, as well as interactions with cell surface molecule , i.e. extracellular polymeric substances (EPS), cell membrane, and protein surface layers (S-layers), that may alter mineral nucleation. This work investigates these mechanisms by allowing calcium carbonate ($CaCO_3$) to form in abiotic or microbial solutions of Escherichia coli (E. coli) or Synechocystis sp. PCC 6803 (Syn. sp. 6803) with varying calcium ion concentrations, via the ammonium carbonate diffusion method. Both the resulting $CaCO_3$ and bacteria was imaged using optical microscopy. Surprisingly, formation of crystalline $CaCO_3$ was accelerated in the presence of both species. This rate acceleration also occurred for metabolically inactive bacteria, suggesting metabolic change was not the operating mechanism under these conditions. Calcium carbonate crystals increased in number as cell density increased. Scanning electron microscopy and fluorescent microscopy show that both species of bacteria cluster on the edges and crevices of the crystals, further supporting this idea. Bacterial surface charge was assessed using zeta potential measurements and correlated to biomineralization experiments. From these results, we postulate that the charged bacterial surfaces attract Ca^{2+} ions, serving as nucleation sites for $CaCO_3$, thereby accelerating crystal formation. These observations provide substantive evidence for a non-specific nucleation mechanism, and stress the importance of microbes, even dead ones, on the rate of formation of carbonate minerals. This work also indicates that additional microbial engineering could optimize these interactions and be used to implement the sub-surface sequestration of CO_2 as stable mineral carbonates on an accelerated timescale.

SESSION KK6: Antimicrobials and Antifouling Coatings
Chair: Brian Lower
Wednesday Morning, April 27, 2011
Nob Hill CD (Marriott)

8:15 AM *KK6.1

Starved Bacterial Biofilms and the Possible Origin of Life between Mica Sheets. Helen Greenwood Hansma, Department of Physics, University of California at Santa Barbara, Santa Barbara, California.

Biofilms of *Pseudomonas aeruginosa* bacteria respond differently to nutrient limitation than bacteria grown in liquid culture. While bacteria in liquid culture become round when starved, *P. aeruginosa* in biofilms become elongated when starved (Steinberger, et al., 2002, Microbial Ecol. 43:416). In both cases, the response to starvation serves to maximize the bacterial surface area that is available for nutrient uptake. This research on biofilms was done by Atomic Force Microscopy, which also led indirectly to the hypothesis that life might have originated between mica sheets (Hansma, 2010, J. Theor. Biol. 266:175). The spaces between mica sheets may have served as cells or compartments within which life could originate and evolve before free-living cells existed. The mica lattice spacing of 0.5 nm is comparable to the periodicities of biological macromolecules such as single-stranded nucleic acids, carbohydrates, and proteins. The potassium ions that hold mica sheets together may be the original source of the high potassium ion concentration in the cytoplasms of cells. Error tolerance is also extremely high in the Mica Hypothesis for the origin of life. Error tolerance is a major requirement for the origin of life, because almost everything is likely to go wrong. With a million mica sheets per millimeter of thickness, mica provides the potential for a huge redundancy in prebiotic molecules of all types.

8:45 AM *KK6.2

Microbial-nanomaterial Interactions. Hilary Lappin-Scott[1] and Sara K. Burton[2]; [1]Centre for Nanohealth, Swansea University, Swansea, Wales, United Kingdom; [2]Biosciences, University of Exeter, Exeter, Devon, United Kingdom.

Various nanomaterials (both manufactured and naturally produced) reach natural environments, for example from waste waters containing healthcare products and pharmaceuticals or be taken into the human body as nanomaterials used for targeted drug delivery. Consequently, they will be in contact with interacting microbial communities carrying out essential functions in these habitats. Given this, little is known of the ecotoxicity of various nanomaterials on microorganisms and specifically whether they disrupt such processes. However there is some evidence that nanoparticles disrupt lipid bi-layers and affect some genetic and transcriptional processes. Our work includes how to effectively monitor nanoparticle-microbial interactions, including the importance of standardisation of methodologies; and to understand nanoparticle- microbial uptake and toxicity and the control of deleterious microbial growth on surfaces using nanomaterials. The novel characteristics of nanoparticles within newly developed materials will be explored.

9:15 AM KK6.3

Bio-inspired Bactericidal Macromolecular Coatings. Thomas Blin[2], Viswas Purohit[2], Xavier Laloyaux[1], Alain M. Jonas[1] and Karine Glinel[1]; [1]Institute of Condensed Matter and Nanosciences (Bio- & Soft-Matter), Université catholique de Louvain, Louvain-la-Neuve, Belgium; [2]Laboratoire Polymères, Biopolymères, Surfaces, CNRS - Université de Rouen, Mont Saint Aignan, France.

The formation of biofilms on material surfaces is a persisting problem inducing many damages in industrial equipments such as the clogging and the corrosion of pipelines or the reduction of heat transfer. More dramatically, the biofilms serve as a reservoir for the development of pathogens. Therefore, there is a great interest to fabricate materials preventing the bacterial attachment which is the first step of the biofilm formation. The most efficient approach to prevent bacterial adhesion is to

immobilize a bactericidal substance on material surface. Different routes based on silver derivatives, antibiotics or poly (ammonium) salts have been developed in this way. However, they are not completely satisfying regarding their efficiency, their environmental impact or their role in the emergence of multi-resisting pathogens. Beside these synthetic approaches, there is a fascinating strategy developed by living organisms such as frogs which secrete a thin skin mucus containing antibacterial peptides to protect themselves against bacterial attachment. Compared to conventional bactericidal substances, these peptides offer the advantages to act at very low concentrations, to have a broad spectrum of antibacterial activities and to have a very low propensity to promote pathogen resistance. Here we explore the fabrication of coatings inspired from the frog skin and based on biocompatible macromolecular layers functionalized by an antibacterial peptide. For this, polysaccharide layers or poly(ethylene glycol) derived brushes were grafted onto substrates by "grafting to" and "grafting from" techniques, respectively. Then magainin-I peptide produced by a claw frog was immobilized by one of its extremities onto the hydroxyl groups of the polymer layers through a heterolinker, keeping its accessibility and its activity against bacteria. The antibacterial properties of the coatings were evidenced against various gram+ and gram- micro-organisms such as L. ivanovii, P. aeruginosa and E. coli. This strategy was also adapted to coat superparamagnetic microparticles in order to prepare killing magnetic particles which can be used to disinfect sensitive aqueous solutions or to achieve localized antibacterial action. Moreover, smart coatings switching their surface properties from bactericidal to cell-repellent with temperature were prepared by grafting magainin-I peptide onto temperature responsive brushes showing a collapse temperature in the physiological range. These non conventional approaches could be advantageously adapted to coat various materials or items used in medicine or food industries. References: (1) X. Laloyaux et al. Adv. Mater. 2010, in press. (2) K. Glinel et al. Bioconj. Chem. 2009, 20, 71. (3) A. M Jonas et al. Macromolecules 2007, 40, 4403.

9:30 AM KK6.4

The Role of the pH Conditions of Growth on the Bioadhesion of Individual and Lawns of Pathogenic L. *monocytogenes* Cells. Nehal Abu-Lail and Bong-Jae Park; Gene and Linda Voiland School of Chemical Engineering and Bioengineering, Washington State University, Pullman, Washington.

The work of adhesion that governs the interactions between pathogenic *Listeria* monocytogenes and silicon nitride in water was probed for individual cells using atomic force microscopy and for lawns of cells using contact angle measurements combined with a thermodynamic-based harmonic mean model. The work of adhesion was probed for cells cultured under variable pH conditions of growth that ranged from pH 5 to pH 9. Our results indicated that *L.* monocytogenes cells survived and adapted well to the chemical stresses applied. For all pH conditions investigated, a transition was observed in the generation time, physiochemical properties, biopolymer grafting density and bioadhesion for cells cultured in media adjusted to pH 7 of growth. In media with pH 7, the generation time for the bacterial cells was lowest, the specific growth rate constant was highest, the cells were the most polar, cells displayed the highest grafting density of surface biopolymers and the highest bioadhesion to silicon nitride in water represented in terms of the work of adhesion. When compared, the work of adhesion values quantified between silicon nitride and lawns of *L.* monocytogenes cells were linearly correlated with the work of adhesion values quantified between silicon nitride and individual *L.* monocytogenes cells.

9:45 AM KK6.5

Nanostructured Mesoporous Silicon as an Effective Carrier For Extended Antibacterial Therapy. Mengjia Wang[1], Phil Hartman[2], Armando Loni[3], Leigh Canham[3] and Jeffery L. Coffer[1]; [1]Chemistry, Texas Christian University, Fort Worth, Texas; [2]Biology, Texas Christian University, Fort Worth, Texas; [3]Intrinsiq Materials Ltd, Malvern, Wors, United Kingdom.

Surface modification of devices to permit incorporation and subsequent sustained release of antimicrobials is an active strategy in biofilm control. One nanostructured candidate capable of acting as a time-release surface is mesoporous silicon (PSi), a porous form of the key elemental semiconductor. Porous silicon possesses the useful properties of a simple fabrication procedure, significant control over pore size and surface chemistry, a large surface area, and in vivo biocompatibility. For drug delivery, the porous character of the matrix offers the potential to ideally improve the delivery of

poorly soluble agents for an extended duration in a tailored manner. In this work, nanostructured particles of porous silicon are demonstrated to act as an effective carrier for the sustained delivery of antibacterial agents with an enhanced inhibitory activity. Methods are described for the incorporation of significant amounts of the established antibacterial compound triclosan (Irgasan) into mesoporous silicon of varying porosities. Such materials were characterized by a combination of scanning electron microscopy (SEM), energy dispersive x-ray analysis (EDX), x-ray diffraction (XRD), thermal gravimetric analysis (TGA), and antimicrobial assays. Assessment of antibacterial activity was carried out versus the bacterium Staphylococcus aureus as a function of time with concomitant assessment of triclosan release; significant, sustained inhibition of bacterial growth was demonstrated in the triclosan-containing porous Si for time intervals greater than 100 days. Significantly, enhanced dissolution (relative to room temperature equilibrium solubility) of the triclosan was observed for the initial 15 days of drug release, inferring some amorphatization or nanostructuring by the porous Si matrix.

10:30 AM KK6.6

Assessment of Marine Biofilm Attachment and Growth for Antifouling Surfaces under Static and Controlled Hydrodynamic Conditions. Maria Salta[1], Julian A. Wharton[1], Paul Stoodley[1], Simon P. Dennington[1], Robert Wood[1] and Keith R. Stokes[2,1]; [1]nCATS, School of Engineering Sciences, University of Southampton, Southampton, Hampshire, United Kingdom; [2]Physical Sciences Department, Dstl, Salisbury, Wiltshire, United Kingdom.

Marine biofouling is the accumulation of organisms on underwater surfaces, causing increased ship hydrodynamic drag, which results in higher fuel consumption and decreased speed and range. Biofilms constitute a major component of the overall biofouling and may lead to a 14 % increase in ship fuel costs. Past solutions to antifouling (AF) have used toxic coatings which have subsequently been shown to severely affect marine life. The prohibited use of these antifoulants has led to the search for bio-inspired AF strategies. Current approaches towards the production of alternative coatings include the incorporation of natural AF compounds into paints. Significant effort is being directed towards more environmentally benign strategies, however, ultimately we believe that a combination of surface texturing and chemistry will lead to the most effective antifouling performance. Screening assays for novel AF compounds are often separated into two categories; toxicity and AF assays. Increasingly there is evidence that active compounds affect organisms at non-toxic concentrations, hence, the necessity for more insightful AF testing directly on surfaces for both static and hydrodynamic conditions. Our study assessed natural product (NP) antifouling performance of an isolated compound from a terrestrial source (a derivative of quinone) against biofilm organisms which included the marine bacteria, *Cobetia marina*, *Marinobacter hydrocarbonoclasticus* and the bioluminescent bacterium *Vibrio harveyi* and the diatom *Amphora coffeaeformis*. Novel bioassay protocols were developed to test the *in-situ* AF efficacy of the NP on coated surfaces. This was assessed by quantifying biofilm growth and adhesion kinetics using a multidetection microplate reader utilising viability staining and natural bioluminescence. Additionally, flow cells and microfluidic channels have been uniquely adapted permitting the AF performance of coatings and NPs to be explored in terms of biofilm attachment and growth for controlled hydrodynamic regimes. These bioassays were corroborated using a suit of microscopy techniques, including atomic force and confocal laser scanning microscopy, in order to compare biofilm structures in the presence and absence of the NP. The NP showed a marked AF efficacy against *C. marina* and *M. hydrocarbonoclasticus* attachment at very low concentrations (10 $\mu g\ mL^{-1}$) with a clear impact on biofilm morphology on NP-containing surfaces. By directly assessing the surface AF effect on biofilm formation, greater insights on NP activity have be obtained (i.e. toxicity, microtopography and/or contact effects), as well as better understanding of the NP kinetics within the coating system and how its interaction with the biofilm.

10:45 AM KK6.7

Preparation, Characterization and In Vitro Antibacterial Activity of Fluoridated Hydroxyapatite Nanothick Coatings for Biomedical Applications. Xiang Ge[1], Yang Leng[1], Chongyun Bao[2], Sherry L. Xu[3], Renke Wang[2] and Fuzeng Ren[1]; [1]Department of Mechanical Engineering, The Hong Kong University of Science and Technology, Hong Kong, Hong Kong; [2]West China College of Stomatology, Sichuan University, Chengdu, China; [3]Department of Biology, The Hong Kong University of Science and Technology, Hong Kong, Hong Kong.

Introduction: Percutaneous type of orthopedic and dental implants requires not only a good adhesion with bone, but also the ability to form good attachment and seal with connective tissues and skins. Currently, the skin-seal of such implants still remains as a problem to be resolved. Electrochemical deposition method can be used to modify the surfaces of metallic implants with coatings in order to improve the antibacterial activity and skin seal of the implants. With a carefully control of electrochemical parameters, we successfully deposited a nanothick and dense coating of fluoridated calcium phosphate on titanium substrate. After heat treatment, the fluoridated calcium phosphate transformed to fluoridated hydroxyapatite (FHA). The FHA nanothick coating was systematically characterized by various techniques to obtain comprehensive properties of the coating. The in vitro antibacterial activity evaluation of samples was conducted with a film attachment method against S.aureus, E.coli and P.gingivalis. Materials and Methods: Titanium plates were used as substrates. The electrolyte was mixed with three kinds of aqueous solutions (0.042 M $Ca(NO_3)_2$, 0.025 M $NH_4H_2PO_4$ and 0.01M NaF) scquentially. The titanium plates were cathodically treated in an electrochemical cell which contained three electrodes: a titanium plate as the cathode, a platinum plate as the anode and a saturated calomel electrode as the reference electrode. The electrochemical deposition process was conducted at a constant current density within acidic environment at room temperature for 6 minutes. Then, the specimens were thermally treated at 600°C for 3 hours in a humid air atmosphere. Dissolution behavior of coatings was examined by immersing each type of specimens into an solution (mixing 0.1M Tris and HCl, 30mL, pH=7.3) at 37°C. Nanoscratching tests were conducted by a nanoindentation system. The zeta potential of coatings was tested with an electro kinetic analyzer. The in vitro antibacterial activity of FHA and HA coating was tested against S.aureus, E.coli and P.gingivalis; while the acid etched pure titanium plate was selected as control. Results and Conclusions: A nanothick (~200 nm) coating of FHA was deposited on acid etched pure titanium substrates with an electrochemical deposition method followed by a heat treatment. The dissolution test results indicate the importance of crystalline structure on chemical stability and also the positive role of F¬- ions in apatite structure stability. The Lc of the FHA coating was 147% higher than that of the HA coating with the same thickness level. The zeta potential of FHA is 13.9% less negative than that of HA. The survivability of bacteria on the FHA coating was much less than that on HA coating and pure titanium substrates, which indicates that FHA nanothick coating has potential clinical applications for inhibiting percutaneous orthopedic and dental implants associated bacterial infections.

11:00 AM KK6.8

Anti-biofilm Betaine Medical Deviee Surfaees after 90 Day Serum Exposure. Sarah Guedez, Heather Lapp, Raisa Fabre, Victoria E. Wagner and Christopher R. Loose; Semprus BioSciences, Cambridge, Massachusetts.

Indwelling catheters put patients at risk for infections which often result in significant morbidity and mortality. Biofilms associated with such device infections are often recalcitrant to currently available therapeutics. Traditional prevention strategies have largely focused on applying leaching antimicrobial coatings to devices with variable clinical success, and drawbacks include short-term duration, limited spectrum of activity, potential toxicity and generation of drug-resistant strains. We examined the performance of a potentially superior approach by using highly water-coordinating, nonfouling betaine polymers as inert coatings to prevent bacterial attachment and subsequent biofilm formation in a blood product environment. This study demonstrates the long-term antimicrobial activity of betaine-modified Carbothane®/ $BaSO_4$ using a modified flow biofilm reactor system (mCDC). Polyurethane catheter substrates (Carbothane®/ $BaSO_4$, 14-French rods) were modified using betaine, zwitterionic structures. To mimic the clinical setting, we subjected betaine-modified materials to serum, a complex media, for periods up to 90 days prior to biofilm challenge. Escherichia coli ATCC 25922 was used as the challenge microbe. Briefly, samples of control and betaine-modified rods exposed to 50% fetal bovine serum for 1, 30, 60, or 90 days were tested for antimicrobial/antibiofilm activity using the mCDC system. Samples were incubated with a bacterial suspension of 1e6 cfu/ml in 1xPBS in the mCDC reactor (batch mode) for 2 hours at 37°C with agitation. Thereafter, the rods were transferred to a fresh reactor and exposed to modified M63 media under flow at 8 ml/min. Biofilm growth was monitored by plate counts and macroscopic visualization of biofilm surface coverage after 24 hours. Log reduction (LR) differences were calculated on surface modified rods and polyurethane controls. Betaine surface modified rods maintained performance over the 90 days of serum exposure with a mean LR of 1.94 ($p<0.0001$). Previous work has demonstrated that such betaine structures show superior resistance to thrombus formation in blood flow-loop studies after serum exposure,

giving the potential for dual antimicrobial and antithrombotic performance.

11:15 AM KK6.9

Structural Dynamics of Pseudomonas Aeruginosa Lipid A as a Function of the Number of Acyl Chains.

Frederico J. de Santana Pontes, Thereza A. Soares and Roberto D. Lins; UFPE, Recife, Brazil.

Lipopolysaccharides (LPS), found in the outer membrane of Gram-negative bacteria, perform an important role in the structural integrity of the microbe as well as protect the membrane from certain kinds of chemical attack through complexation, uptake of ions and efflux mechanisms. LPS are comprised of three parts: the O antigen, the oligosaccharide core and the lipid A. The latter exhibits long fatty acid chains, which binds the sugar moiety into the bacterial membrane. The lipid A domain is the main responsible for the toxicity of Gram-negative bacteria. Changes in the number of acyl chains affects directly the toxicity levels, adhesion and permeability of the LPS. The understanding at molecular level of these processes are indispensable for future applications of Gram-negative bacteria in relevant tasks such as decontamination of soil and water, adhesion to solid surfaces and antibiotic resistance, for example. Previously, an atomistic model for LPS membranes of Pseudomonas aeruginosa was developed (Lins and Straatsma, Biophys. J., 2001), validated (Soares and Straatsma, Mol. Simulation, 2008) and successfully applied to structural studies (Soares et al, J. Braz. Chem. Soc., 2008) and metal uptake processes (Lins et al, Biomacromolecules, 2008). In the present work, we have used computer simulations to investigate the influence of phenotypical variations of the acyl chains on the flexibility, electrostatic potential and charge distribution of the lipid A of P. aeruginosa. Dependence of lipid A lateral diffusion, order parameter, molecular shape and diglucosamine tilt angle for symmetrical tetraacyl, pentaacyl and symmetrical hexaacyl lipid A membranes are characterized. These results are compared against experimental data and insights into the relationship between the number of acyl chains and endotoxicity are drawn.

SESSION KK7: Biofilm Growth and Detection I
Chair: Roberto Lins
Wednesday Morning, April 27, 2011
Nob Hill CD (Marriott)

11:30 AM *KK7.1

Detection of Biofilms, on Biomaterials in Infected Patients, Using a New DNA-based System. Bill Costerton, Center for GenomicSciences, Pittsburgh, Pennsylvania.

When bacteria have formed a biofilm on the biomaterials comprising a medical device, like a prosthetic joint, the organisms are very difficult to detect using traditional culture methods. We have examined aspirates, associated tissues, and the surfaces of orthopedic implants that have been obtained pre-operatively and inter-operatively from 50 patients suspected of having device-related infections on the basis of clinical indications. These specimens have been examined by routine culture methods, and by the new DNA-based IBIS method, as well as by fluorescence in situ hybridization (FISH) and deep 16 S rRNA pyrosequencing. Cultures only detected bacteria in +/- 20% of these cases, which were all scheduled for surgery to replace the prosthesis, while the IBIS sytem detected bacteria in 100% of cases. The IBIS system detected the same organism found by culture, in all of the cases that were culture positive. When specimens were examined using species or genus specific -FISH probes, the cells that reacted with the probes were of the same genus as indicated by the IBIS in 60% of cases and of the same species in 35% of cases. Pyrosequencing confirmed the IBIS diagnosis in 82% of cases. This demonstration that modern DNA-based methods can detect the presence of bacterial biofilms, with much more sensitivity and accuracy than traditional culture methods, allows surgeons to select the 2-step replacement procedure that is more suitable for the replacement of infected hardware, and to use antibiotics that are effective because the IBIS detects the species involved and also detects the presence of antibiotic resistance plasmids. We also observed that the FISH technique allowed us to "map" the

infecting biofilms, in situ in tissues and on the surfaces of biomaterials, and thus to determine which surfaces of which materials had been receptive to bacterial adhesion and biofilm formation. This study will facilitate the collection of accurate microbiological data, in clinical trials of all devices containing biomaterials, by replacing culture methods that detect biofilms only very poorly, with accurate and sensitive DNA-based methods.

SESSION KK8: Biofilm Growth and Detection II
Chair: Roberto Lins
Wednesday Afternoon, April 27, 2011
Nob Hill CD (Marriott)

1:30 PM *KK8.1

Studying Extracellular Electron Transfer in Microbial Fuel Cells at the Single Cell and Biofilm Level. Bradley Ringeisen[1], Justin Biffinger[1], Lisa Fitzgerald[1], Ricky Ray[3], Brenda Little[3], Xiaocheng Jiang[2], Jinsong Hu[2] and Charles Lieber[2]; [1]Chemistry, Naval Research Laboratory, Washington, District of Columbia; [2]Chemistry, Harvard University, Boston, Massachusetts; [3]Naval Research Laboratory, Stennis Space Center, Mississippi.

Electrochemically active biofilms are beneficial to energy harvesting applications as direct cell-electrode contact has been found to increase the power densities of microbial fuel cells (MFCs). We have studied Shewanella oneidensis MR-1, a model electrochemically active bacterium often used in MFC experiments, attachment to nano- and macro-scale electrodes under different oxygen conditions. In macroscopic MFCs using carbon electrodes, wild type MR-1 appears to preferentially form biofilms under air exposure, while strict anaerobic conditions limit cell attachment. We find significant current generation under air exposure, even though oxygen diffusion to the electrode would result in electron scavenging and limited power production. MR-1 cells on the outside of the biofilm most likely metabolize the dissolved oxygen, preventing oxygen exposure to the cells deeper into the biofilm and closer to the electrode. We have also used genetic deletion mutants of MR-1 and a nanoelectrode platform to further elucidate the extracellular electron transfer mechanism. Studies show that MR-1 mainly uses a self-mediated electron transfer mechanism when attaching to indium tin oxide/gold nanoelectrodes, while nanofilament or pili mediated electron transfer plays a role in macroscopic carbon electrode MFCs.

2:00 PM KK8.2

The Interplay between Substrate, Medium, and Biofilm in Biofilm-substrate Interactions. Wendy J. Goodson[1], Caitlin L. Knight[1,2], Michelle L. Kay[1,2], Pamela F. Lloyd[6,2], Andrew R. Blankemeier[3], Ryan W. Schlosser[1], Daniel E. Barlow[4], Pehr E. Pehrsson[4], John N. Russell[4], Donald M. Eby[5], Glenn R. Johnson[5] and Hamesh L. Fraser[3]; [1]Nanostructured and Biological Materials Branch, Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright-Patterson AFB, Ohio; [2]UES, Inc., Dayton, Ohio; [3]Center for Accelerated Maturation of Materials, Department of Materials Science and Engineering, The Ohio State University, Columbus, Ohio; [4]Surface Chemistry Branch, code 6170, Naval Research Laboratory, Washington, District of Columbia; [5]Microbiology and Applied Biochemistry, Materials and Manufacturing Directorate, Air Force Research Laboratory, Tyndall AFB, Florida; [6]Laser Hardened Materials Branch, Materials and Manufacturing Directorate, AFRL, Wright-Patterson AFB, Ohio.

Microbial biofilms are communities of bacteria that live on a wide range of substrates. They are a nuisance in industrial settings, as they often impact the material they colonize and can be extraordinarily difficult to remove. In the military, biofilms are particularly problematic in fuel distribution systems, where they can affect paint, metal, and the fuel itself. To influence the development of mitigation strategies, we are investigating the interplay between biofilm, substrate, and medium to understand how each contributes to biofilm formation and its effect on the substrate. Using a suite of microscopic and

spectroscopic characterization tools, we investigated Pseudomonas fluorescens biofilm formation on conductive polyurethane paint. Through conventional plating techniques and confocal microscopy, we quantitated biofilm growth and survival on the paint over a month-long exposure to P. fluorescens in M9 minimal medium with and without pyruvate. The presence of pyruvate in the medium enhanced biofilm growth initially, but was not necessary for biofilm growth on the paint over 28 days, indicating that the biofilm may utilize the paint as a nutrient source. The presence of pigment (carbon black particles) in the paint did not enhance biofilm growth, suggesting that the biofilm most likely used the polyurethane, not the carbon black, as a nutrient source. SEM of the biofilms showed that P. fluorescens reacted to growth under these conditions by producing inclusions, whose chemical composition is under spectroscopic analysis. In addition to investigating how biofilm growth was influenced by the substrate and medium, we are also examining how the biofilm affects the substrate. Using SEM and in situ total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy, we are characterizing morphological and chemical changes in the substrate as a result of biofilm colonization.

2:15 PM *KK8.3

Controlling Carbonate Mineral Precipitation by Biofilms for Environmental and Industrial Benefit. Robin Gerlach, Center for Biofilm Engineering, Chemical and Biological Engineering, Montana State University, Bozeman, Montana; Chemical and Biological Engineering, Montana State University, Bozeman, Montana.

Biofilms have been shown to have positive and negative impacts on human health, industrial production processes, and the environment. The observed resistance of biofilm communities to antimicrobials and environmental stress factors has significant potential for benefit in the areas of aquifer decontamination, enhancement of soil stability, enhanced secondary oil recovery, abatement of saltwater intrusion, filtration, and geologic carbon sequestration. Understanding and controlling biofilms and their influence on transport and reaction in porous media is important in order to optimize the efficacy of beneficial technologies and minimize their negative effects. This presentation will focus on the potential benefit of using biofilm-induced carbonate mineral formation for the development of beneficial biofilm technologies, with emphasis on environmental restoration and carbon sequestration technologies. We have demonstrated that biofilm-mediated ureolysis can be used to induce carbonate mineral formation, which in turn influences the processes indicated in deep geological carbon sequestration (such as mineral-trapping, solubility trapping, formation trapping, and leakage reduction) and calcium carbonate mediated co-precipitation of environmental contaminants. Batch and flow experiments at atmospheric and high pressures (> 74 bar) combined with time-lapse microscopy and reactive transport modelling have been used to improve our understanding of biofilm-mineral-fluid- surface interactions on the micro- and macro-scale. This work has been supported by the Zero Emissions Research Technology (ZERT) fund (U.S. DOE, Award No. DE-FC26-04NT42262), the US DOE EPSCoR program (Grant No. DE-FG02-08ER46527), U.S. DOE, Office of Science (BER) (Grant No. DE-FG-02-09ER64758), and the National Science Foundation (NSF Award No.: DMS-0934696).

2:45 PM KK8.4

Characteristics of Surface Adsorbed Bacterial Luminescence. Satoshi Sasaki, School of Bioscience and Biotechnology, Tokyo University of Technology, Hachioji, Tokyo, Japan.

Luminescent bacterium is known to convert chemical energy into light. The bacteria produce autoinducer and they respond to this molecule to switch on luciferase structural operon. Luminescence is therefore controlled by the cell-population density. When we regard bacterial cells as enzyme bags, substrates such as oxygen or autoinducer diffuse into the bags through the semipermeable cell wall and finally catalyzed by the enzyme. Oscillation in the product concentration is often observed in systems where a semipermeable membrane separates the substrate and the enzyme. Such behavior is simulated using a reaction-diffusion model. We have reported several characteristics of the luminescence from bacterial suspension. For example, in batch culture higher initial bacteria density resulted in earlier luminescence starting time. Apart from such linear character, we have reported an oscillation in the luminescence intensity both spatially and temporally. We came to an idea that bacteria group do not behave like the sum of single cells. Nonlinearity of the bacterial luminescence might then be a key to understand the phenomena. In our previous experiment, we separated bacteria in small groups that showed similar

character such as motility or adsorption activity. For example, bacteria separated according to their motility using a microfluidic device were proved to show different bioluminescent intensities per cell. On an agar plate, luminescence intensity from actively dividing cells were less than that from matured cells. Apart from such basic study to understand the oscillation in bacterial luminescence, reactors were designed to realize stable bacterial luminescence. In a PDMS cell, only the parts of the suspension that faced the wall were illuminated. This result suggested that the geometrical symmetry of oxygen supply to the suspension helped maintain spatial stability without convection of the bacterial luminescence. Here we report the luminescence behavior of bacteria that adsorbed on the materials surface. In this work, we show experimentally that the bacteria adsorb on the inner surfaces of terephthalate (PET) or polystylene (PS) polyethylene bottle, and that they start to emit light when a liquid broth is added. We also show that the luminescence from the suspension oscillates. Experimentally, using a self-made luminescence detector, measurement of luminescence intensity from well-stirred bacterial suspensions in the bottles of different materials (PVC, PET or PS) was performed. After the observation of oscillation in luminescence, the bottles were washed three times using 20 mL of the same broth, followed by the luminescence measurement. Such washing and measurement were repeated, and oscillation was observed repeatedly. Chemical condition of adsorption on the surface was investigated using surface analysis methods such as AFM or FTIR, and materials character on the cell-adsorption and on the oscillation mode will be discussed.

3:30 PM KK8.5

Electrochemical Sensing of Aerobic Marine Bacterial Biofilms and the Influence of Nitric Oxide Attachment Control. Julian A. Wharton[1], Stephane Werwinski[1], Robert Wood[1], M. D. Iglesias-Rodriguez[2] and Keith R. Stokes[3,1]; [1]National Centre for Advanced Tribology at Southampton (nCATS), University of Southampton, Southampton, Hants., United Kingdom; [2]Ocean Biogeochemistry and Ecosystems, National Oceanography Centre, University of Southampton, Southampton, Hants., United Kingdom; [3]Physical Sciences Department, Dstl, Salisbury, Wiltshire, United Kingdom.

Suitable in situ techniques capable of sensing for the presence of a biofilm on metallic surfaces are becoming increasingly necessary, especially in order to maintain seawater pipe system performance. This study has investigated the detection of aerobic marine bacterial biofilms using electrochemical impedance spectroscopy by monitoring the interfacial response of Pseudoalteromonas sp. NCIMB 2021 attachment and growth in order to identify characteristic events on a 0.2 mm diameter gold electrode surface. Uniquely, the applicability of surface charge density has been proven to be valuable in determining biofilm attachment and cell enumeration over 72 h duration on a gold surface within a modified continuous culture flow cell (a controlled low laminar flow regime with a Reynolds number close to 1). In addition, the potential for biofilm disruption has been evaluated using 500 nM of the nitric oxide (NO) donor sodium nitroprusside (NO is important for the regulation of a number of diverse biological processes). Ex-situ confocal microscopy studies were performed to confirm biofilm coverage, thickness, and morphology, plus the determination and quantification of the NO biofilm dispersal effects. Overall, the capability of the sensor to electrochemically detect the presence of initial bacterial biofilm formation and extent has been established and shown to have potential for real-time biofilm monitoring.

3:45 PM KK8.6

Bacterial Biofilm Physical Properties from the Inside. Olivier Galy[1], Christophe Beloin[2], Patricia Latour-Lambert[2], Jean-Marc Ghigo[2] and Nelly Henry[1]; [1]Physicochimie Curie (UMR168), Institut Curie - CNRS - UPMC, Paris, Ile de France, France; [2]Unite de genetique des biofilms, Institut Pasteur, Paris, Ile de France, France.

Bacterial biofilms are communities of bacteria associated with biological or artificial surfaces where they form a complex 3D living material. Whereas bacterial biofilms are innocuous in most natural environments, their formation in medical and industrial settings has a major socio-economic impact — they are implicated for instance in nosocomial infections, product contamination, energy losses and bio-fouling. Many molecular factors associated with bacterial adhesion and biofilm development have been described using extensive molecular genetics analyses, significantly improving our knowledge of these sessile bacterial organisations. Yet, much remain to be done and new approaches complementary to the ongoing

molecular studies will certainly help gaining new insights and finding new strategies to fight against these bacterial structures. We address in our research group the question of the reciprocal influence of biofilm physical properties and biochemical molecular events. Up to now, no relationship has been established between the measured physical parameters and biofilm molecular properties. Previous studies in the field did not take into account the high heterogeneity of the bacterial architecture, providing averaged values, which probably strongly weakened the ability of the obtained data to report relevant biological functioning of the biofilm. Here, I will show the first step of this investigation, i.e. the development of appropriate methodologies to measure the relevant parameters describing the biofilm physical properties. We propose here an original approach based on the use of magnetic colloids to probe biofilm biophysics locally from the inside. The principle consists inserting exogenous magnetic micro-colloids into the 3-dimensional bacterial material either from the initial steps of the colonization or during the biofilm growth at various steps of the development. Thanks to their magnetic properties, these objects will be remotely actuated using adapted magnetic device. I will describe here the detail of the experiment and show the first measurements of the local rheological parameters of an E. coli biofilm. I will pay special attention to the effect of biofilm growth shear stress on the values of the material visco-elastic parameters and on their spatial distribution. As well, I will consider the possible dependence of the biofilm physical properties on individual cell molecular properties. Eventually, I will conclude by giving the 3D physical picture of a bacterial biofilm emerging from these findings.

SESSION KK9: Influence of Surface Properties on Growth
Chair: Wendy Goodson
Wednesday Afternoon, April 27, 2011
Nob Hill CD (Marriott)

4:00 PM KK9.1

Effect of Dental Polymer Degree of Conversion on Oral Biofilms. Alison Kraigsley, Sheng Lin-Gibson and Nancy J. Lin; Polymers Division, National Institute of Standards and Technology, Gaithersburg, Maryland.

Polymeric composites are the primary materials used for dental restorations ("fillings"), replacing traditional amalgam due to their esthetic qualities and lack of mercury. However, failure at the tooth-composite interface typically occurs within 3-10 yrs, often due to secondary caries (recurrent tooth decay). *Streptococcus mutans* (*S. mutans*) attaches to surfaces in communities of cells ("biofilms") and is known to have a significant role in the development of caries, yet the effects of composite properties on biofilm growth are not known. Polymeric degree of conversion (DC), a critical property of dental composites, varies clinically and affects many material properties, including surface chemistry and soluble leachables. The objective of this research was to determine the effect of DC on *S. mutans* biofilms and to isolate the individual contributions of surface methacrylates and leachables, both of which change with DC. Polymer disks of 50:50 (by mass) bisphenol A glycerolate dimethacrylate (bisGMA) and triethylene glycol dimethacrylate (TEGDMA) were photopolymerized between untreated glass slides for 7 to 60 s per side, resulting in a DC range of 50 % to 76 % as quantified using near infrared spectroscopy. *S. mutans* biofilms were grown on polymer disks for 4 h and 24 h in medium containing 1 % sucrose by mass. Crystal violet (CV) was used to measure overall biomass, and methylthiazolyldiphenyl-tetrazolium bromide (MTT) was used to quantify metabolic activity. The contributions of pendant methacrylate groups on the material surface and leachables released from the disks were decoupled. Pendant surface methacrylates were studied by evaluating biofilms on coverslips functionalized with varying amounts of methacryloxypropyldimethoxysilane. Surface methacrylates were measured using X-ray photoelectron spectroscopy. Effects from leachables were determined by leaching polymer disks in medium then evaluating biofilms grown in that leached medium in the absence of disks. Relative amounts of leachables were characterized using absorbance. Results from biofilms cultured on polymer disks with varying DC values demonstrated that metabolic activity decreased as DC decreased. Biomass, however, did not change as a function of DC, indicating a true decrease in metabolic activity (not a reduction in overall biofilm biomass). When surface chemistry and leachables were evaluated separately for their effects on biofilm metabolic activity, surface chemistry had no significant effect on biofilm metabolic activity or biomass, whereas leachables from low DC polymers had a negative effect on metabolic activity similar to that seen in biofilms cultured directly

on low DC polymers. Thus, low DC decreases *S. mutans* biofilm metabolic activity, likely due to an increased amount of leachables. Additional studies are needed to further identify individual leachable components responsible for this effect. Work was supported by NIDCR/NIST Interagency Agreement Y1-DE-7005-01.

4:15 PM KK9.2

Initial Oral Biofilm Formation on Microstructured Titanium Surfaces on Healthy and Periodontitis Patients. Argelia Almaguer-Flores[1], Miryam Martínez-Hernández[1], Laurie Ann Ximénez-Fyvie[1] and René Olivares-Navarrete[2]; [1]Laboratorio de Genética Molecular, Universidad Nacional Autónoma de México, Facultad de Odontología, México, Mexico; [2]Biomedical Engineering, Georgia Institute of Technology, Atlanta, Georgia.

Biofilm formation on implant surfaces has a strong influence on healing and long-term outcome of dental implants. It has been shown that this formation can show different affinities to titanium surfaces, depending of the substrate characteristics, like roughness and hydrophilicity. The aim of this study was to analyze initial oral biofilm composition on titanium (Ti) samples with different surface properties, in patients with periodontal health and chronic periodontitis. To achieve this, four Ti-disk surfaces: (PT [Ra<0.2mm]), acid-etched (A [Ra<0.8mm]), sand-blasted/acid-etched (SLA [Ra=4mm]), and hydrophilic SLA (modSLA), were placed in a removable device on 20 patients (n=10 periodontally healthy, n=10 chronic periodontitis), for 48 hours. Samples were analyzed using a checkerboard DNA-DNA hybridization technique. The mean levels (counts $x10^5$) and proportions (%DNA probe count) of 40 species were obtained for each clinical group. Significant differences were determined using Kruskal-Wallis and the Mann-Whitney tests. In the results it could be observed that all the experimental surfaces supported biofilm formation. *Neisseria mucosa*, *Eikenella corrodens* and *Parvimonas micra*, were the most frequently detected species on all substrates, especially in samples from healthy patients. In contrast, *Streptococcus oralis* and *Prevotella intermedia* were found in higher levels on the substrates in periodontitis subjects. The influence of surface roughness was observed with some species like *E. corrodens* and *S. oralis*, which were present in greater numbers on SLA and modSLA than on PT or A from all patients. Species of *Streptococcus* and *Actinomyces* were found in higher proportions on the hydrophilic modSLA surface. This study showed that all substrates supported initial biofilm formation. Substrates placed in healthy patients had a similar microbial composition. In contrast, substrates in periodontitis subjects presented more variability in the adhesion of the same strain depending on the surface properties.

4:30 PM KK9.3

Bacterial Attachment to Superhydrophobic Post Array Surfaces. Benjamin Hatton and Joanna Aizenberg; Wyss Institute, Harvard University, Cambridge, Massachusetts.

Bacterial surface attachment represents the first stage of biofilm growth. Experimental investigation of the ability of pseudomonas aeruginosa, bacillus subtilis and escherichia coli bacteria to attach to superhydrophobic post array surfaces has been made as a function of the post size (ie; diameter). Sample surfaces were exposed to continuous streams of bacterial growth solutions, and then checked for viable surface contamination. Superhydrophobic surfaces consisting of ordered arrays of high aspect ratio posts and interconnected walls, having different feature sizes, were made by a photolithographic etching process and then replicated in PDMS and epoxy by soft lithography molding. It was found that the extent of surface contamination was dependent on the diameter of the post features, presumably because of the restricted area available for bacterial attachment. Post diameters on the order of 1.5 μm or smaller were found to prevent bacterial attachment entirely, and therefore these post array surfaces remained sterile (by definition), even after extensive bacterial exposure. We believe such results could be applied to the design of the surfaces for biomedical tools and catheters as a means of preventing bacterial contamination.

4:45 PM KK9.4

Micro- and Nano-patterned Surfaces for the Control of Bacterial Behavior. Ronn S. Friedlander[1] and Joanna Aizenberg[2]; [1]Harvard-MIT Division of Health Sciences and Technology, MIT, Cambridge, Massachusetts; [2]School of

Engineering and Applied Sciences, Harvard University, Cambridge, Massachusetts.

Bacterial biofilms have immense medical, ecological and commercial significance. Biofilm growth has thus far proven difficult to control, particularly on indwelling medical devices. We investigate the effects of surface geometry on bacterial proliferation and biofilm formation. Previous work has shown that the spacing of micropost arrays can affect bacterial orientation on surfaces in a scale-dependent manner. Through micropatterning of various materials including epoxy (EPO-TEK UVO114; Epoxy Technology), polydimethylsiloxane and poly (2-hydroxyethyl methacrylate), we are able to observe the behavior of several species of adherent bacteria in a finely controlled milieu. Bacteria investigated include Staphylococcus, Corynebacterium, Escherichia coli and Pseudomonas aeruginosa. These investigations are carried out at both the solid-liquid and solid-air interface. Micropost array and honeycomb patterns are used to determine whether and how topography on a relevant scale affects the proliferation, colony morphology and interspecific competition among bacterial species. We find that colony shape and structure is altered on micropatterned surfaces. Furthermore, this effect is more pronounced in species that match the size of the surface features of the substrates.
