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Antimicrobial behavior of novel surfaces generated by electrophoretic deposition and breakdown anodization

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ABSTRACT

Biofilms have devastating impacts on many industries such as increased fuel consumption and damage to surfaces in maritime industries. Ideal biofouling management is inhibition of initial bacterial attachment. The attachment of a model marine bacterium (*Halomonas pacfica* g) was investigated to evaluate the potential of these new novel surfaces to resist initial bacterial adhesion. Novel engineered surfaces were generated via breakdown anodization or electrophoretic deposition, to modify three parameters: hydrophobicity, surface chemistry, and roughness. Mass transfer rates were determined using a parallel plate flow chamber under relevant solution chemistries. The greatest deposition was observed on the superhydrophilic surface, which had micro- and nano-scale hierarchical structures composed of titanium oxide deposited on a titanium plate. Conversely, one of the hydrophobic surfaces with micro-porous films overlaid with polydimethylsiloxane appeared to be most resistant to cell attachment.

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1. Introduction

Fouling by the accumulation of bacteria, algae, and other micro and macroorganisms is problematic for a variety of industries including cosmetics, pharmaceuticals, medical devices, and global marine industries [1,2]; leading to staggering costs. As of 2010, it was estimated that the annual cost due to hull fouling for current US Navy resources ranged from $180–250 million [3]. Those costs are directly related to cleaning and coating costs, as well as frictional drag that increases fuel consumption. The biofouling process on marine surfaces begins with bacterial colonization and biofilms, followed by other microorganisms such as unicellular algae (i.e., diatoms) and eventually invertebrates such as soft corals, sponges, barnacles, and mussels [4]. Prevention of microbial biofilms formation may differ by industry although the underlying problem is the same for all, which is inhibiting the initial bacterial attachment [5].

The ever-present battle to inhibit fouling on ships has been addressed as early as the 1500s, with the use of copper plates on wooden ship hulls to decrease fouling [2,6,7]. By the 1960s antifouling paints, which contained biocide materials such as copper and tributyltin (TBT) prevented cell adherence by leaching toxic substances. However, copper and TBT were found to be toxic to many marine organisms (in addition to the marine bacteria of interest), as it was discovered that the corrosion process of copper leads to the release of cuprous oxide, which interferes with cell division [8,9]. This led the International Maritime Organization to ban TBT in 2001 due to its high toxicity and long half-life of 3 months [10]. Despite copper having lower toxicity level it is currently under review by the US EPA for future restrictions, with the final outcome of that review due in 2015 [11,12].

There are three principal approaches to combat biofouling: direct use of biocides, mechanical detachment, and surface modification to prevent cell attachment. Commercially, antibiotics and cleaning chemicals are commonly deployed as biocides to kill and degrade biofilms [13]. The medical community has attempted to load antibiotics onto medical devices to prevent infections and a recent study demonstrated a novel coating that slowly releases antibiotics as a strategic method to prevent specific microbial adherence [14]. Similarly, disinfecting chemicals are used to non-selectively remove bacteria and biofilms in dental unit waterlines to prevent patients and staff from exposure to these microorganisms [15]. Currently, antifouling technologies used within the marine sectors consist of commercially available paints (29% of the sector) [16]. These paints include a variety of metallic and nonmetallic biocides that are released into the environment; and therefore, the paint has a limited lifetime [7,16]. There are still significant

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environmental concerns regarding the use of such biocides [7,16]; therefore, the pursuit of alternatives is vital. The second principal approach of mechanically detaching attached microorganisms and biofilms is effective but there are concerns of physical damage to surfaces requiring additional maintenance in comparison to other methods [17].

The third principal approach has been addressed by the material science community via the development of novel surfaces in an effort to develop anti-fouling, non-toxic surfaces, through chemical and/or structural modification, that inhibit the initial cell attachment [18–25]. Examples of such treatments include, coating with self-assembled monolayers (SAMs), which can have finely tuned chemical properties: wettability, controlled functional groups, or charge density [22,24,25]. SAMs, with ω-substituted alkane thiolates on gold, have been reported to have increased spore attachment with increased surface hydrophobicity [18]. Many chemical coatings that incorporate such functional groups have been investigated such as siloxane urethane, poly(ethylene glycol) polymers, and combinations of various materials such as patterned poly(ethylene glycol) and fluorinated surfaces [20]. Coating surfaces with fluorinated polymers to create hydrophobic surfaces were found to effectively release attached cells with sufficient shear force (i.e., ship movement or rinsing) [21]. Furthermore, aminopropyl terminated poly(dimethyl siloxane) (PDMS) macromers anchored by a single amine group PDMS were fabricated and shown to reduce biofilm retention. This was attributed to the hydrophobic nature of these surfaces, which may be able to be further improved for antimicrobial properties [23]. Such coatings have been demonstrated on marine vessels; however, the predominate mechanism is foul-release, which was observed to require significant shear to remove established biofilms [7,26] instead of prevention of the initial bacterial attachment.

Current research has also focused on the prevention of the initial bacterial attachment as an alternative treatment, and it has been demonstrated that a broad range of factors play a role in cell attachment including wettability/hydrophobicity, surface chemistry, surface roughness and topography, surface elastic modulus, and color [18,19,21,27–31]. Wettability has been shown to be of great importance in both field and laboratory assays; and thus, a factor that must be addressed when developing antifouling surfaces [18,27]. Surface roughness and topography must be considered when engineering surfaces since they have been shown to alter attachment of bacteria [28,29]. The elastic modulus of the surface has also been proven to be key in addressing bioadhesion and must be contemplated in selecting antifouling surfaces [30]. This study was designed to investigate the attachment of a model marine organism to novel surfaces developed previously by a hybrid method employing breakdown anodization (BDA) [32] and electrophoretic deposition (EPD) [33] and to evaluate the ability of these surface modifications minimize the initial cell attachment. It is important to note that biofilm formation in any industry intersects at a critical step—the initial stage of individual cell adhesion. This emphasizes the need to understand the initial stage of attachment. The three parameters that were modified in the generation of these surfaces included hydrophobicity, surface chemistry, and roughness. Observing initial bacterial adhesion is crucial in evaluating the surfaces' ability to minimize or prevent future biofilm formation.

2. Materials and methods

2.1. Bacterial cell preparation

The model marine bacterium selected for this study, *Halomonas pacifica* ATCC 27,122 was obtained from the American Type Culture Collection (ATCC) (Rockville, MD) due to its fouling properties [34]. This bacterium was labeled with enhanced green fluorescent protein (EGFP) and gentamicin resistance via electroporation for visualization and is referred to as *H. pacifica* g [35]. *H. pacifica* g is a non-motile rod-shaped gram-negative microorganism and is grown in sterile artificial seawater (38.5 g/L sea salts, Sigma–Aldrich, Buchs SG, Switzerland), supplemented with bacteriological peptone (5 g/L, Sigma), and yeast extract (1 g/L, Sigma) at 30 °C in the presences of gentamycin sulfate antibiotic (30 mg/L, OmniPur, Gibbstown, NJ) [36]. The equivalent spherical radius of *H. pacifica* g cell radius was previously reported to be 0.97 μm [35]. Cultures were grown for 16 h at which time they were harvested for surface deposition experiments and characterization [37]. Cells were harvested by centrifugation (accuSpin 3R centrifuge, Fisher Scientific, Pittsburgh, PA) for 15 min at 3689 g and further rinsing the pelleted cells twice with KCl solutions to remove traces of growth medium. The analyte (KCl) solutions were prepared with reagent-grade salt (laboratory grade, Fisher Sci.) and DI Water (Millipore, Billerica, MA) at unadjusted pH (5.6–5.8). Two ionic strengths (IS) of the electrolyte solution, 10 mM KCl and 100 mM KCl where used which correspond to the IS of freshwater and 5% seawater [38].

2.2. Bacterial cell characterization

To analyze the relative hydrophobicity of *H. pacifica* g cells, a semiquantitative microbial adhesion to hydrocarbons (MATH) test was employed [39]. The relative hydrophobicity of the organism in each of these solutions is reported as the percent of total cells that partition into the model hydrocarbon (dodecane) [40]. Specifically, test tubes were set up to have 4 mL of the cell suspension (in the respective analyte salt solutions: 10 mM KCl or 100 mM KCl) with 1 mL of *n*-dodecane (laboratory grade, Fisher Sci.). Test tubes were vortexed (AutoTouch Mixer Model 231, Fisher Sci.) for 2 min followed by a rest period of 15 min to allow phase separation and the final absorbance reading after the rest period was compared to the initial absorbance acquired after harvesting. The optical density of the cells in the aqueous phase was measured using a spectrophotometer at 546 nm (BioSpec-mini, Shimadzu Corp., Kyoto, Japan).

The zeta potential of the bacterial cells was measured using a ZetaPALS analyzer (Brookhaven Instruments Corp., Holtsville, NY) at 25 °C with harvested cells suspended in 10 mM and 100 mM KCl solution. This test was repeated at least three times with freshly harvested cells and the measured electrophoretic mobility values were converted to zeta potential using the Smoluchowski equation [41].

2.3. Preparation and characterization of engineered surfaces

Quartz surfaces have previously been used to observe deposition behavior under electrostatically unfavorable [42] and favorable conditions [35] (when coated with positively charged amininosilane). This study employs unmodified quartz microscope slides (electron microscopy diatome quartz microscope slide, 3″ × 1″, Fisher Sci.) cut to fit (9 × 20 mm) within the flow chamber. Prior to the deposition experiments, the quartz substrates were cleaned by sonicating in 2% Extran solution (EMD Chemicals Inc., Darmstadt, Germany) for 15 min at room temperature, followed by a thorough rinse with deionized water. Next, the quartz was sonicated in 2% RBS 35 detergent solution (Pierce Biotechnology, Rockford, IL) for 15 min at 50 °C, followed by rinsing with deionized water. Finally, quartz samples were submerged in Nochromix (Godax Laboratoreies, Inc., Cabin John, MD) solution overnight and rinsed with deionized water the following day and allowed to air dry prior to use in transport studies.

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