Effects of *Escherichia coli* K12 Biofilm on Sensor Thin Film Materials

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***Abstract*— Micro-fabricated sensors enable the study of chemical and physical dynamics in aqueous environments such as rivers, lakes or oceans at low cost. Sensors must work reliably in these environments, which include both biological and chemical challenges. However, sensor thin films have not been studied in detail for aqueous applications, and more specifically how biotic interactions may change sensor material properties. In this study, the long-term effects of biofilm formation on the properties of aluminum (electric conductor) and a-Si_xN_y:H (insulating material) were investigated. Material degradation caused by *Escherichia coli* K12 biofilm growth was determined by electrical sheet resistance measurements (collinear four-point-probe) and Fourier-transform infrared spectroscopy (FTIR) absorption spectra over a time period of 7 weeks. Changes of the surface topography were tested using scanning electron microscopy (SEM) and white light interferometry. Aluminum was found to be heavily degraded at three weeks, whereas a-Si_xN_y:H was inert during the entire investigation period. As differences between thin film sensor materials are evident, more detailed investigations including a broader range of materials should be explored.**

Keywords—Thin films, biofilm, infrared spectroscopy, microbially influence corrosion, SEM

I. Introduction

Biofilms form on many types of substrata, including natural and engineered surfaces. When conditions allow, bacterial cells colonize surfaces and become embedded in extracellular polymeric substance (EPS) [1]. Within minutes of substrate submersion in aqueous environments, microbial growth and EPS production are initiated, leading to the development of biofilm [2]. Biofilms can inhibit the performance of sensors used in studying the dynamics of aqueous environments. Microbial activity leading to deterioration of surfaces containing biofilm is termed bio-corrosion or microbially influenced corrosion (MIC) [3]. For instance, *Pseudomonas aeruginosa*, a bacterial strain that forms corrosive biofilms [4], has been shown to alter surface and material properties of nickel-copper and nickel-zinc thin films [5]. *Escherichia coli* has been shown to accelerate corrosion in marine like environments on aluminum alloys [6], likely due to the secretion of metabolic organic acids [7].

Surface micromachined sensors are based on the deposition of metallic and insulating materials. An inert interface between the active sensor surface and the aqueous media is required for reliable operation [8]. TiN and SiN have been reported to be inert in aqueous media, and still allow fully functional device operation. However, bio-corrosion/degradation of these materials has yet to be determined. Moreover, mechanisms to protect electrically conductive materials from environmental effects, and to improve sensor material reliability awaits investigation. Table 1 summarizes previous work on material and sensor reliability in aqueous environments. The present study examines the effects of biofilm growth on the material properties of two thin film materials. The focus was given to electronic conductivity, surface morphology, and bulk stoichiometric properties, and how they change as a function of exposure to biofilms.

TABLE I. Previous work on material and sensor reliability in aqueous environments

Subject	Methods	Results	Ref
Atomic layer deposition (ALD) post-processing of Al_2O_3 and TiO_2 to encapsulate poly multi-user MEMS processing systems (PolyMUMPs) devices for aqueous environments	Thermal actuation in aqueous media/seawater, and displacement measurements	Performance of MEMS structures enhanced with ALD coating for long term use	[8]
Dissolution kinetics of e-beam, low-pressure chemical vapor deposition (LPCVD) and plasma-enhanced chemical vapor deposition (PECVD) SiO_2 and a-Si_xN_y:H thin films in aqueous media	AFM, ellipsometry, and resistance changes	Combination encapsulation layers demonstrate increased electronic device lifetime	[9]
Biocorrosion of Al 6063 by *E. coli* in seawater	Corrosion rates, and microscopy	*E. coli* accelerates aqueous corrosion rates	[6]
MIC of Ni-Zn and Ni-Cu thin films from *P. aeruginosa*	SEM, polarization, and EIS	*P. aeruginosa* increased corrosion of Ni-Cu alloy but protected Ni-Zn	[5]

II. Materials and Methods

A. Sample preparation

Samples of aluminum were deposited using physical vapor deposition (PVD) with thermal evaporation using Modu-Lab PVD. Amorphous silicon nitride with incorporated hydrogen (a-Si_xN_y:H) was deposited using PECVD with a chuck temperature of 100ºC. This low deposition temperature allows a-Si_xN_y:H to

be used as an inert encapsulation layer for most electrically conductive materials, comparable to the use of aluminum herein. Higher deposition temperatures could cause chemical reactions between the material and the substrate [10]. Also, lower deposition temperatures increase the hydrogen concentration in the a-Si_xN_y:H film through the use of NH_3 and SiH_4 reaction gasses. As an extreme example, non-stoichiometric a-Si_xN_y:H films made from PECVD were studied for their ability to be used as an encapsulation layer for sensor applications in aqueous media by studying long term reliability under exposure to biofilm. PECVD a-Si_xN_y:H films were deposited at the Utah Nanofabrication Facility. Samples were cleaved from the wafers and characterized prior to experimentation (see material characterization).

B. Culture Conditions and Biofilm Reactors

Center for disease control (CDC) biofilm reactors (Biosurface Technologies, Bozeman, MT) were used to grow biofilm following standardized methods [11]. Reactors were autoclaved for 30 min. Each reactor contained 350 mL of 1X tryptic soy broth (TSB; BD Bacto) media. Aluminum and a-Si_xN_y:H coated wafers were cut into approximately 1x1 cm samples, and sterilized in a 30% hydrogen peroxide bath for 10 min, followed by 30 min in 70% ethanol. Sample wafers were then glued with a drop of silicone adhesive to polycarbonate sample holders, mounted to CDC reactor rods, and allowed to cure for 24 hours. To ensure sample wafers and rods were thoroughly sterilized, and to guarantee abiotic conditions for control experiments, final sterilization of the CDC reactor rod assemblies involved exposure to UV light (254 nm) for two hours. All steps were performed inside a biosafety cabinet. Samples were prepared in triplicates per time point.

Figure 1. CDC biofilm reactor containing *E. coli* K12 biofilm growing on aluminum and silicon nitride substrate samples.

E.coli K12 was grown in TSB at 37°C while shaking at 125 rpm for 16 h, following standard methods [12]. Subsequently, CDC reactors filled with 350 mL of autoclaved 1X TSB media were inoculated with 2 ml of bacterial enrichment. Inoculated reactors were run for 24 hours in batch mode at 22 °C on a stir plate at 125 rpm, followed by continuous flow operation of a 1:10 dilution of the TSB medium at a flow rate of 2 ml/min for 7 weeks (Figure 1). Abiotic control reactors were included using the same CDC reactor setup filled with either autoclaved deionized water (DI) or 1:10 diluted TSB media both operated in batch mode for 7 weeks. Triplicate sample wafers were collected at the beginning and 3, 5, and 7 weeks of operation. At each time point, sample wafers were removed from the polycarbonate sample holder with a sterile razor blade and transferred into 50 ml Falcon tubes containing 5 ml of 1X phosphate buffer saline solution (PBS). Sample wafers were placed into 50 ml Falcon tubes filled with 4.5g/L Tween 80 in 1X Dulbecco's phosphate-buffered saline (DPBS; Thermo Fisher), 5 ml of lab grade acetone, and 5 ml of isopropanol to remove biofilms. Sample wafers were sonicated at 60 W for 10 min at 22 °C, followed by a deionized water rinse. After biofilm removal, the samples were air-dried and stored in sterile Petri dishes at 22 °C until further analyses.

C. Materials Characterization

Techniques used to determine changes of the coated aluminum wafers included collinear four-point-probe sheet resistance measurement with a Keithley 2450 SourceMeter, scanning electron microscopy (SEM; Integrated Auger nanoprobe based on Physical Electronics 710), and energy-dispersive x-ray analysis (EDX; Bruker X-Flash 6I10). Wafers coated with a-Si_xN_y:H were analyzed with Fourier transform infrared spectroscopy (FTIR) attenuated total reflectance absorbance (Thermo Fischer Scientific Nicolet iS10 with a Harrick VariGATR ATR attachment) as well as with Filmetrics Profilm 3D white light interferometry.

III. RESULTS

Collinear four-point-probe sheet resistance measurements of the aluminum sample wafers demonstrated a statistically significant increase in the sheet resistance after exposure to biofilm over the 7-week period (one way ANOVA: $F(3, 236)=1185.3$, $P<0.001$; Figure 3). At each time point investigated, an increase in sheet resistance was noticed, likely due to the corrosion of the film. Abiotic control studies show only slight increases in sheet resistance and showed no visual signs of degradation. Due to the high resistance and instability, a-Si_xN_y:H sheet resistance values are not reported.

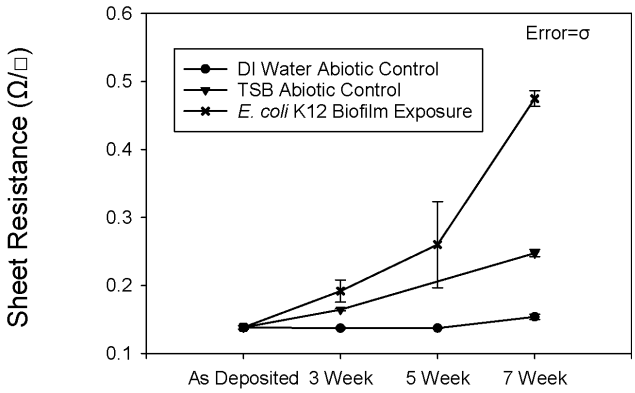

Figure 2. Collinear four-point-probe sheet resistance of aluminum thin film measured at time intervals of 0, 3, 5, and 7 weeks in CDC biofilm reactor.

Apparent differences were observed between aluminum and a-Si_xN_y:H coated samples exposed to *E. coli* biofilm. The original microstructure of the aluminum thin film (i.e., at the

beginning of the experiment) was imaged using SEM (Figure 3a) and shows evidence of hillock formation on the surface [13]. After three weeks of exposure to biofilm, topographical features such as crevices and pits were abundant on the aluminum surface (Figure 3b). By week 7 much of the aluminum thin film was removed (Figure 3d) with only small amounts of aluminum remaining. EDX analysis confirmed the corrosion of aluminum film, showing the exposed underlying silicon substrate within a pit (Figure 3c).

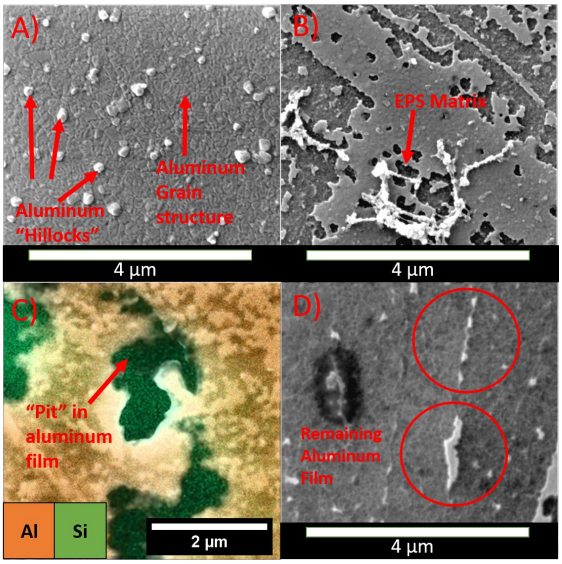

Figure 3. SEM images of aluminum PVD thin film surface a) as deposited showing aluminum hillocks on surface b) after 3 weeks in CDC biofilm reactor c) EDX analysis map of aluminum thin film surface after 3 weeks in biofilm reactor revealing underlying silicon substrate and d) SEM image of aluminum surface after 7 weeks in CDC biofilm reactor.

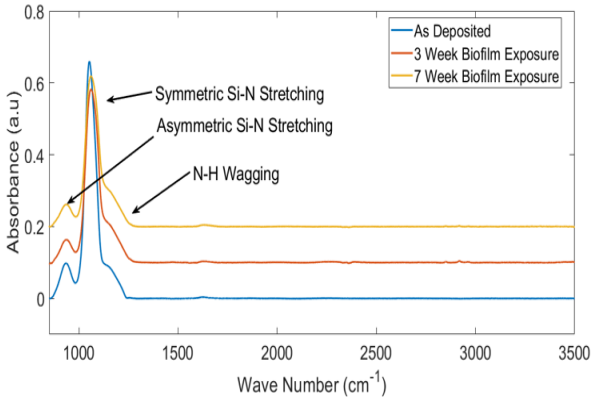

Figure 4. FTIR spectra of a-Si_xN_y:H thin films, measured at timepoints of 0, 3, and 7 weeks.

To study bulk structural changes on the a-Si_xN_y:H samples, FTIR absorption spectra were measured over 500 to 3500 cm^{-1} (Figure 4). Spectra show both symmetric and antisymmetric Si-N stretching modes in all samples, regardless of the duration of exposure to biofilm. Relative absorbances remained the same throughout the specified time intervals and suggested no significant change in the film stoichiometry. These results suggest that a-Si_xN_y:H is a promising material for encapsulating active sensor structures in aqueous media.

White light interferometry data was collected on wafer samples from an as-deposited a-Si_xN_y:H, and 7 weeks biofilm exposed sample (Figure 5). Area maps of sample surfaces were collected and show surface morphology and average roughness values. The as-deposited a-Si_xN_y:H sample had an average roughness of 2.2nm while the 7 week exposed sample had an average roughness of 2.3nm. No visual differences were noticed between samples, further suggesting that a-Si_xN_y:H is a promising encapsulation material to protect metallic thin films used for aqueous sensing.

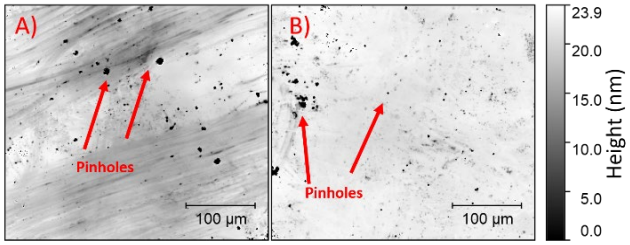

Figure 5. White light interferometry image of A) as deposited a-Si_xN_y:H thin film and B) after exposure to biofilm for 7 weeks.

IV. Conclusion

Biofilm adhesion and growth affects material properties when attached to surfaces and allowed to propagate. In aqueous environments biofilm formation occurs on a wide variety of substrates and can lead to biofouling [14]. Conductive materials are very susceptible to corrosive biofilms and precautions should be made to mitigate damage that can occur due to prolonged exposure in aqueous environments. In the present study, biofilm adhesion and growth was shown to impact aluminum thin films. Aluminum thin films used in aqueous environments showed an apparent increase in sheet resistance, suggesting that they are not reliable for use in aqueous environments by themselves. Therefore, a protective barrier should be considered. Conversely, low deposition temperature a-Si_xN_y:H coatings remained inert throughout the course of the experiment. The results show that this coating has the potential to protect conductive sensor materials from fouling and biological damage. These results suggest that other sensor materials (e.g. gold, polycrystalline silicon, and nickel) should be investigated to determine their susceptibility to microbially influenced corrosion, and the ability of a-Si_xN_y:H to encapsulate the materials to minimize material degradation. Future work should focus on higher resolution time-series investigations to determine when the materials are no longer viable for optimal sensor performance.

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