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Altered biofilm formation at plasma bonded surfaces in microchannels studied by attenuated total reflection infrared spectroscopy

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ABSTRACT

This paper is dedicated to Professor P.R. Norton on the occasion of his 75th birthday, in honor of his profound contributions to Surface Science. In this work, we investigate how plasma bonding of a germanium ATR crystal to a microfluidic device can affect biofilm growth and development. Using attenuated total reflection Fourier transform infrared spectroscopy, individual measurements were made at the attachment surface of growing *Pseudomonas fluorescens* biofilms in adjacent flow channels during parallel experiments. Biofilm growth in channels with ATR surfaces exposed to air plasma exhibited a faster accumulation of a biomolecular conditioning layer compared to unexposed channels. As well, in-line microscopy revealed enhanced bulk biofilm growth in plasma-treated channels. Since the surface chemistry the Ge ATR was only partially recovered to its original state during the three day experiments, it is believed that the enhanced biofilm growth was ultimately due effects of plasma exposure. It is proposed that observations are transferable to microfluidic devices with sealing layers from other hard surfaces such as glass, silicon and plastic due to their ability to retain surface functionalization after plasma exposure. Plasma treatment could, therefore, offer a route to faster start up times for bioreactors, but could also result in unexpected artifacts in other studies.

1. Introduction

Growth of surface-adhered bacterial biofilms is a major area of interest in medical, biotechnical and environmental research. Microfluidic bioflow cells, present high surface area environments with strong control over liquid conditions for real-time studies of biofilm growth and contamination. The dominant method for bonding microfluidic devices involves temporary exposure of both microstructured polydimethysiloxane (PDMS) and a planar sealing surface to a plasma gas. This avoids the need for pressure-based sealing, which can cause channel buckling and flow disruption of the elastomeric PDMS, but the role of the altered surface chemistry on biofilm growth is not well-studied. This is probably because it is assumed that the temporary nature of hydroxyl and carbon-oxygen added groups at the PDMS channel wall may not play a strong role [1,2,3]. However, the sealing layer, which is often made from hard, purpose-specific materials, can retain its surface properties after fabrication. Other than planar PDMS, popular sealing materials include optical-quality microscope slides for imaging. Other functional sealing surfaces are also being used to introduce more advanced *in situ* characterization. For example, recent work in our group to introduce novel characterization modalities for passive measurements of biofilms has been achieved using sealing surfaces including electrodes and printed circuit boards for electrochemical measurement and imaging [4,5], nanostructured surfaces for surface enhanced Raman spectroscopy [6] and nanoparticles capable of metal-enhanced fluorescence for pH imaging [7]. Other promising surface-sensitive approaches for *in situ* chemical analysis of microchannel surfaces include surface plasmon resonance and attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) [8,9]. Here we report the use of ATR-FTIR to evaluate the effect of plasma bonding on the growth of biofilms from *Pseudomonas fluorescens* CT07 bacteria at the non-PDMS sealing surface. This approach joins other more widely used techniques for chemical characterization of biofilms, such as chromatography, NMR and colorimetric techniques [10], but has the advantage of applicability to real-time, *in situ* measurements of native hydrated biofilm at the attachment surface without any particular sample preparation or inclusion of foreign molecular species [11]. The ATR element used in this study was from high index of germanium (n = 4), which reduced the depth of penetration of the evanescent electromagnetic field to approximately 600 nm, allowing the study to focus on surface chemistry at the biointerface. A system enabling individual ATR-FTIR measurements in parallel microfluidic channels was exploited to compare the effects of plasma treatment on biofilm growth

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with proper controls in place [12]. In the first part of this work, we sought to determine if plasma-induced chemical changes to the sealing layer could revert back its original state under different experimental conditions. Next we evaluated the effect of the plasma-treated surface on biofilm development at different growth stages. It was found that, indeed, residual chemical changes to the ATR surface could affect the growth of the biofilm, due to enhancement of a biochemical conditioning layer.

2. Experimental

Materials and equipment: flow control was achieved by syringe pumps (PHD 2000, Harvard Apparatus, Holliston, MA, USA), which injected liquids from 60 mL syringes (BD Scientific, NJ, USA) to channels via 1.6 mm outer diameter perfluoroalkoxy (PFA) connective tubing (U-1148, IDEX, WA, USA) and syringe tubing connector assemblies (P-200x, P-658, IDEX, WA, USA). Liquids were vacuum degassed before injection to minimize bubble formation on-chip.

Device fabrication materials: the microfluidic channels were fabricated by curing the PDMS polymer solution (Sylgard184, Dow corning, Canada) in the presence of a cross-linking agent at a 10:1 ratio against a master mould (FlowJEM Inc., Toronto, Canada). The mould contained 6 parallel channels with dimensions 0.1 mm (height), 1.5 mm (width) and 2 cm (length). After 8 h at 80 °C, the solidified PDMS was be removed from the mould and prepared for interface with the ATR crystal. The ATR element used was a trapezoidal germanium 24 bounce crystal, with dimensions $50 \times 20 \times 2$ mm and 45° beveled edges. The interface with the ATR crystal was achieved by a compression fitting or by air plasma bonding (SI). Plasma bonding was achieved in a standard plasma cleaner (PCD-001 Harrick Plasma, Ithaca, USA) for 90 s at high power and 650 mTorr. All steps were done in a laminar flow cabinet and so that sterility was maintained at all times. After measurements the MF device and ATR crystal were gently separated by hand and trace amounts of strongly adhered PDMS and biofilm residue were easily removed via the following procedure: 1 h submersion in HCl 1 M, NaOH 1 M and then ultrasound in pure acetone and then in a 70:30% v/v ethanol-water mixture for 30 min each. Finally the crystal was submersed in soap solution and was lightly polishing with a cotton swab.

Biofilm cultivation: the *P. fluorescens* strain CTO7 (gram negative, rod-shaped motile bacterium) was used in this study. The planktonic bacteria were used to create an inoculum solution by shaking in 3 mL of 5 mM growth media for 18 h at 300 rpm in a temperature-controlled (30 °C) incubator oven. Nutrient media was a modified AB type, consisting of 1.51 mM $(NH_4)_2SO_4$, 3.37 mM Na_2HPO_4, 2.20 mM KH_2PO_4, 179 mM NaCl, 0.1 mM $MgCl_2{\cdot}6H_2O$, 0.01 mM $CaCl_2{\cdot}2H_2O$ and 0.001 mM $FeCl_3$. The sole carbon source was 10 mM Na-citrate$\cdot 6H_2O$. Inoculation of the microchannel was conducted for 30 min under a flow rate of 0.2 mL h^{-1}. Following inoculation, growth media was introduced into the channel at flow rate 0.2 mL h^{-1}. All experiments were conducted in a temperature controlled environment at 22.5 ± 0.5 °C.

Data acquisition: the FTIR spectrometer used in this study (Magna 560, Nicolet, USA) was equipped with a liquid nitrogen cooled narrow-band MCT detector and a small internal aperture (1 mm) wheel to focus the probe IR beam within a single channel. The system that controlled the displacement of the ATR-FTIR stage relative to the IR excitation beam is described elsewhere [12]. All spectra were generated from 64 scans with spectral resolution was 4 cm^{-1}. The sample compartment was sealed and purged with a constant flow of dry air with CO_2 removed. Data acquisition and spectral processing were performed by software (OMNIC v5.2, Nicolet, USA and GRAMS/AI v8.0, Thermo Fisher, USA). Optical imaging was achieved by an in-line camera and a variable 2–12x zoom lens with software control (micromanager v1.4 modules, USA). Surface roughness measurements were acquired using atomic force microscopy (DimensionTM 3100, Digital Instruments, Santa Barbara, CA, USA) in 50 μm^2 regions at different locations on the ATR crystal corresponding roughly to the different channel locations. Measurements were conducted before and after application of air plasma over various cycles.

3. Results and discussion

After the Ge ATR was exposed to air plasma, water droplets were observed to wet the surface more efficiently, while the portions of the surface that were protected from the plasma remained hydrophobic (Fig. 1). The changes to the ATR wetting properties appeared not to be related to surface roughness, which remained constant (300 +/− 95 nm) before and after plasma exposure for different experimental cycles. Based on an analysis of the FTIR spectra obtained from the plasma exposed surface, two classes of surface contaminants were observed. The first were germanium oxide groups, which likely formed due to interaction with reactive oxygen species in the plasma. Primarily, these included broad increase to spectral density between 800 and 600 cm^{-1}, and also a smaller peak at 840 cm^{-1}. These are consistent with different oxygenated germanium species, Ge-O-Ge, GeO, GeO_2 (referred to generally as GeO_x) with various orientations with respect to the ATR crystal surface, as expected from the amorphous crystal surface used here [13–17]. Experience shows that exposure of the Ge ATR crystal to plasma gas is important to achieve good plasma bonding. This is likely because GeO_x groups are necessary for the strong bonding with the activated PDMS. Secondly, organic and silonol groups were detected based on absorption bands for OH (3450 and 3270 cm^{-1}) bands; C=O and COOH (structured peak near 1700 cm^{-1}); Si–O–Ge, Si–O–Si and C–O groups (1040 to 1120 cm^{-1}) and CH_2 and CH_3, including Si–CH_3, (2850 to 2950 and a broad peak 1400 cm^{-1}) [18]. Significant variability in their intensity correlated to cleanliness of the plasma chamber, presence of the corresponding PDMS device in the plasma chamber and time of plasma exposure. Therefore, it is likely that origin of these groups was likely from fragments of oxygenated PDMS chains from residue in the plasma bonding chamber and from the PDMS device, which was often exposed to plasma at the same time as the Ge ATR crystal. As plasma bonding of microfluidic devices is among the most heavily repeated step in a typical microfluidics laboratory, decommissioning the chamber for rigorous cleaning is rare. Therefore, we conducted plasma bonding under such typical conditions.

The results presented in Fig. 1 were stable in air for days. Next the response of plasma induced chemical groups CH_2, C=O/COO^- and GeO_x were monitored by ATR-FTIR during different experimental conditions. Of the six parallel flow channels, four were plasma bonded and two channels were held against the Ge ATR crystal solely by pressure clamping. The clamping system was actually applied to all six channels in order to ensure that the plasma exposure was only difference in assembly methods between channels 1–4 (plasma) and channels 5,6 (no plasma). See supporting information for more details about the system and device interface with the ATR accessory. Experimental conditions were as follows. A constant flow of the AB nutrient solution under sterile conditions was injected into channels 1 and 2 for the entire experiment. For (plasma treated) channels 3 and 4 and (non-plasma treated) channels 5 and 6, first a liquid inoculum was injected for 30 min followed by flow of the AB nutrient solution until the duration of the experiment. All time-varying results from consecutive single beam spectra were acquired starting immediately following the first application of a pure nutrient solution in channels 3–6 as a background (t = 0). In all cases, the in-line optical microscope system was used to verify that no temporarily surface attached bubbles were present during this study. This was important, due to recent demonstration of a significant influence that bubbles can have on biofilm growth rate and possibly the microchannel surface chemistry [19]. After t = 0, a slow continuous reduction in spectral density in the positions of CH_2, CH_3 (Fig. 2a) and CO (Fig. 2b) was recorded. This indicated that some of the contaminant groups could be washed away under flow conditions. Control experiments in sterile channels showed similar results (SI). In the case of GeO_x (Fig. 2c) a continuous reduction in the spectral density near the major peak at