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Microscale patterned surfaces reduce bacterial fouling-microscopic and theoretical analysis

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ABSTRACT

Microscale patterned surfaces have been shown to control the arrangement of bacteria attached to surfaces. This study was conducted to examine the effect of patterned topographies on bacterial fouling using *Enterobacter cloacae* as the test model. *E. cloacae* is an opportunistic pathogen involved frequently in nosocomial infections. It is an important model organism to be studied in the context of healthcare associated infections (HAI) and polydimethylsiloxane (PDMS) based urinary catheter fouling. Patterned surfaces, such as Sharklet^{TM}, have shown the promise of being a benign surface treatment for prevention of catheter associated urinary tract infections (CAUTI). To the best of our knowledge, inhibition of fouling by *E. cloacae* has not been demonstrated on microscale patterned PDMS surfaces. In this study, the Sharklet^{TM} and smooth PDMS surfaces were used as controls. All pattern surfaces had statistically significantly lower percentage area coverage compared to the smooth PDMS control. A cross type feature (C-1-PDMS), demonstrated the most significant reduction in percent area coverage, 89% ($p < 0.01$, $\alpha = 0.05$), compared to the smooth PDMS control and all other patterned test surfaces. Additionally, theoretical calculations show that C-1-PDMS is the only surface predicted to hold the thermodynamically stable Cassie state, which occurs due to trapping air pockets at the liquid–solid interface. Combined the results provide new insights for designing environmentally benign, novel, microscale patterned surfaces for restricting bacterial fouling.

1. Introduction

Bacterial biofilm formation forms the basis for other types of biofouling, such as algal spore and barnacle larvae settlement [1]. The human health implications of bacterial pathogenic biofilms are well documented [2–4]. Previous research [5–8] has addressed the application of antifouling surface designs employing low critical surface energy as a potential solution. Reported findings show that biofouling retention strength to surfaces generally follows a decreasing trend with critical surface tension (used as surface energy) [9]. This relationship was also observed in aquatic biofouling systems; however, lowering surface energy alone is not sufficient to reduce biofouling [10]. An additional example for the use of surface modifications to control biofouling, is the use of patterned surfaces, which were reported to have efficacy for reducing marine biofouling in controlled laboratory settings [11–17]. Encouraged by the earlier studies that reported the possibility of benign patterned surfaces for bacterial fouling control [18–20], the objective of this study was to assess the effect of patterned surfaces on fouling by *Enterobacter cloacae*, a bacterium responsible for HAIs in the context of CAUTI [21], which to our knowledge has not been studied before. Another objective of this study is to analyze data from the bacterial fouling of patterned surfaces obtained in this study, to understand how patterned surfaces are capable of reducing bacterial fouling suggested previously [22] by taking in to account wetting theory, namely nonwetting state (Cassie–Baxter [23]) to wetting state (Wenzel [24]) transitions observed on patterned surfaces.

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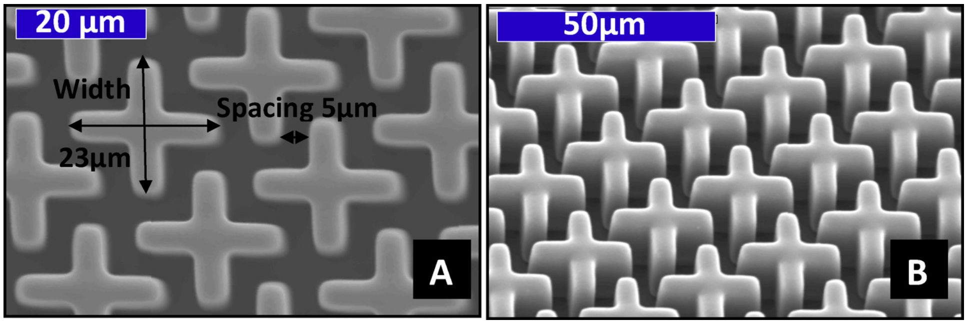

Fig. 1. SEM images of the cross patterned surface, C-1-PDMS, (A) top view, (B) perspective view on PDMS. Nomenclature is defined in Table 1.

2. Theory

The presence of patterning on a surface leads to the incorporation of air pockets on immersion in to a liquid or in the presence of a liquid droplet on a surface. The stable state of a droplet suspended over air pockets on such patterned surfaces without complete interfacial adhesion of the liquid to the solid surface is defined as the Cassie state [25]. The stable equilibrium state with complete interfacial adhesion following the expulsion of the trapped air is defined as the Wenzel state [24]. The nature of the stability of these trapped air pockets has been subject to theoretical analysis in the literature. A thermodynamic feasibility condition for underwater stability was derived based on a Gibbs free energy analysis of the composite interface [26].

$$r > r_{\min} = \left(\frac{-1}{\cos\theta_Y}\right) + \left(f_0\left(1 + \frac{r_f}{\cos\theta_Y}\right)\right) \quad (1)$$

where r is the Wenzel roughness of the topographical surface, r_f is the Wenzel roughness of the wetted fraction of the surface, f_0 is the wetted fraction of the surface, θ_Y is Young's equilibrium contact angle for a smooth surface of the same material and $r_{\min}$ is the minimum roughness value for which the submerged topographical surface will sustain the Cassie state.

If the thermodynamic criterion is met Eq. (1), then another approach computes the equilibrium condition on the basis of the Laplace pressure across the air water interface, assuming that the interface is pinned at the edge of the microstructural features.

Eq. (2) takes into account these wetting parameters to predict the pressure required for a Cassie state to Wenzel state transition [27,28].

$$P_C = \left(\frac{\gamma_{LV} L \cos(\pi/2 - \theta_Y + \psi)}{A_C - A}\right) \quad (2)$$

where P_C is the difference in pressure across the air–liquid interface, γ_{LV} is the liquid–vapor interfacial tension, L is the perimeter of the triple phase contact line, $\cos(\pi/2 - \theta_Y + \psi)$ is the resolution component of the interfacial tension in the normal direction with ψ being 90° for all test samples considered here and $(A_C - A)$ is the area over which pressures acts to force the liquid in to capillary. Eqs. (1) and (2) were then used to determine the robustness of the Cassie state for the topographies being considered in this study for additional analysis and interpretation of the results.

3. Experimental

3.1. Test materials and surface fabrication

Polydimethylsiloxane elastomer (PDMS) sheets were chosen on the basis of widespread use in catheters and other medical implant surfaces [29]. This material is well suited to the fabrication procedure employed to produce the microscale patterned surfaces. A standard photolithography process to prepare the PDMS patterned surfaces was used as previously described [30]. The Sharklet^{TM} samples (raised pillars) were made using silicon wafer molds provided by Dr. Liwen Jin. The patterns selected for testing comprises 2 hexagonal repeating units (HC-7-PDMS and 11-H-PDMS), 2 cross type repeating (C-1-PDMS and C-5-PDMS) units and 1 sinusoidal ridge type repeating unit (Sharklet^{TM} pattern on PDMS) as a control capable of reducing bacterial fouling and the smooth surface (Smooth PDMS) as a control incapable of reducing bacterial fouling without any surface texturing or treatment. The hexagonal repeating units were chosen as baseline simple and highly symmetric patterns (each being inverse of the other) for testing in the similar Wenzel roughness range. The 2 cross type patterns are two size scale versions of the same pattern to compare across both the lower and upper limits of the feature sizes being considered in the study. The intersecting ridges of the cross pattern allows of greater mechanical stability of the features allowing for an exploration of greater range of Wenzel roughness. The Sharklet^{TM} pattern is the sinusoidal repeating unit that was shown to be capable of reducing bacterial fouling previously and was included as a control sample.

3.2. Test organism

E. cloacae (ATCC 700258) was used in this study, since *E. cloacae* strains are a normal inhabitant of the human gastrointenstinal tract [21] and can be frequently isolated from soil and water [31,32]. *E. cloacae* are opportunistic pathogens and have been shown to be a frequent cause of nosocomial infections and infections in immune compromised patients [21].

3.3. Characterization

The samples tested were characterized using scanning electron microscopy (SEM) (JEOL 6400 and JEOL 5700, JEOL Inc., Peabody, MA, USA) (Figs. 1 and 2), profilometry (Wyko NT 1000 Profiler, Veeco Instruments, Tuscon, AZ, USA), and goniometry (Rame-Hart Instrument Co., Netcong) (Table 2). Characteristic measures such as recessed area fraction and Wenzel roughness factor, r, is a measure of the roughness of patterned surfaces is the ratio of total surface to the unit planar area of measurement [24], were calculated from the carefully measured feature width, spacing and height, given in Table 1.

3.4. E. cloacae culture and bacterial fouling

An overnight culture of *E. cloacae* was grown in 20 ml of Tryptic Soy Broth (TSB) (BD Difco, Sparks, MD) at 25 °C. The various test surfaces were attached firmly onto glass slides by applying a vacuum. The slides were sterilized by soaking them in a 70% ethanol solution for 10 min, rinsed with sterile filtered water, and dried in a sterile petri dish. Then, the slides were aseptically placed into a slide