

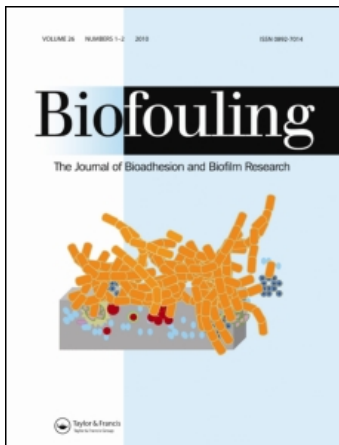
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Engineered antifouling microtopographies: the role of Reynolds number in a model that predicts attachment of zoospores of *Ulva* and cells of *Cobetia marina*

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Engineered antifouling microtopographies: the role of Reynolds number in a model that predicts attachment of zoospores of *Ulva* and cells of *Cobetia marina*

Chelsea M. Magin^a, Christopher J. Long^b, Scott P. Cooper^b, Linnea K. Ista^{c,d}, Gabriel P. López^{e,f} and Anthony B. Brennan^{a,b*}

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A correlation between the attachment density of cells from two phylogenetic groups (prokaryotic Bacteria and eukaryotic Plantae), with surface roughness is reported for the first time. The results represent a paradigm shift in the understanding of cell attachment, which is a critical step in the biofouling process. The model predicts that the attachment densities of zoospores of the green alga, *Ulva*, and cells of the marine bacterium, *Cobetia marina*, scale inversely with surface roughness. The size and motility of the bacterial cells and algal spores were incorporated into the attachment model by multiplying the engineered roughness index (ERI_{II}), which is a representation of surface energy, by the Reynolds number (Re) of the cells. The results showed a negative linear correlation of normalized, transformed attachment density for both organisms with ERI_{II} · Re ($R^2 = 0.77$). These studies demonstrate for the first time that organisms respond in a uniform manner to a model, which incorporates surface energy and the Reynolds number of the organism.

Keywords: biofouling; surface topography; marine bacteria; *Ulva*; *Cobetia marina*; ERI

Introduction

Biofouling, the undesired accumulation of organic molecules and living organisms on a surface (Costerton et al. 1995; Shea et al. 1995; O'Toole et al. 2000), is a significant environmental and economic concern. Biofouling increases drag on vessels by increasing hull roughness, which leads to higher fuel consumption and cost (Townsin 2003; Schultz 2007). The US Naval Sea Systems Command estimates that fuel costs increase 6 to 45% if the hull is fouled, depending on the size of the vessel. Slime layers composed of bacteria and diatoms that develop in the early stages of biofouling (Molino et al. 2009a,b) have been shown to significantly increase hydrodynamic drag and increase fuel consumption (Schultz 2007). Increased fossil fuel consumption is not the only environmental concern raised by biofouling. Hull fouling has been shown to be a primary cause for the introduction and spread of non-indigenous marine species (Otani et al. 2007; Pettengill et al. 2007; Piola and Johnston 2008; Piola et al. 2009; Yamaguchi et al. 2009). The ideal solution to the detrimental effects of biofouling will be a green technology possessing both antifouling (AF)

and fouling-release properties (Genzer and Efimenko 2006; Marmur 2006) to reduce drag and fuel consumption while remaining non-toxic.

Polydimethylsiloxane elastomers (PDMS_e), more commonly known as silicones, are currently marketed as non-biocidal marine coatings. These coatings are known to have fouling-release properties (Chaudhury et al. 2005; Holm et al. 2006; Wendt et al. 2006) due to their low surface energy and low modulus (Brady and Singer 2000; Chaudhury et al. 2005); however, they are not inherently AF (Molino et al. 2009a,b). Non-toxic AF technologies that focus on the manipulation of the surface topography of PDMS_e have been designed to deter attachment of fouling organisms (Carman et al. 2006; Schumacher et al. 2007a,b, 2008; Long et al. 2010). Carman et al. (2006) presented a biomimetically inspired surface topography, Sharklet AFTM, that reduced attachment zoospores of *Ulva* by 86%. *Ulva* is a green alga commonly found in marine biofilms on ships, submarines and other underwater structures. The mature plant of *Ulva* produces motile spores that disperse and colonize surrounding surfaces (Callow and Callow 2000). Evidence suggests that the

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swimming spores are able to select a surface suitable for attachment based on topographical, biological (Joint et al. 2002), chemical, and physicochemical cues (Ederth et al. 2008, 2009; Schilp et al. 2009). An empirical relationship called the engineered roughness index (ERI) has been proposed to quantify topographical “roughness” based on parameters that describe surface energy. The results demonstrate a correlation between the attachment behavior of zoospores of *Ulva* and the ERI (Schumacher et al. 2007b). A predictive attachment model was developed based on the second-generation ERI (ERI_{II}) and correctly predicted spore density on three newly designed surface topographies. Four separate sets of *Ulva* attachment data showed excellent correlation ($R^2 = 0.88$) with the attachment model (Long et al. 2010).

The ERI is a dimensionless ratio based on Wenzel’s roughness factor (r), the depressed surface area fraction ($1 - \Phi_s$), and originally the degrees of freedom of the pattern (df) (Schumacher et al. 2007). Recently, the ERI was extended to include additional topographical designs (Long et al. 2010). The df term was replaced with n , which is the number of unique features in each topography. The ridges topography is comprised of one distinct feature thus an n -value of one. The triangle/pillars topography is composed of two unique features: an equilateral triangle and a round pillar ($n = 2$). ERI_{II} is represented as:

$$\text{ERI}_{\text{II}} = (r * n) / (1 - \Phi_s) \quad (1)$$

Wenzel’s roughness factor (r) is the ratio of the actual surface area to the projected planar surface area (Wenzel 1936). The actual surface area includes the surface area of the feature tops, sides, and depressed surface area between features. The depressed surface fraction ($1 - \Phi_s$) described by Bico et al. (1999, 2002), Quéré (2008) and others is the ratio of the depressed surface area between features and the projected planar surface area. The depressed surface fraction is equivalent to $1 - f_1$, where f_1 is the solid–liquid interface term of the Cassie–Baxter equation for wetting (Cassie and Baxter 1944). The topographies studied were selected to cover a range of ERI_{II} values (Figure 1).

In this study it was hypothesized that the engineered microtopographies in PDMS_e would inhibit attachment of marine bacteria and this inhibition would scale with the ERI_{II} value. The marine bacterium *Cobetia marina*, originally isolated from a marine biofilm (Baumann et al. 1983), was used as a model marine fouling organism. The attachment of *C. marina* to defined solid surfaces has been studied extensively through the use of self-assembled monolayers (SAMs) (Ista et al. 1996, 1999, 2004, 2010) and

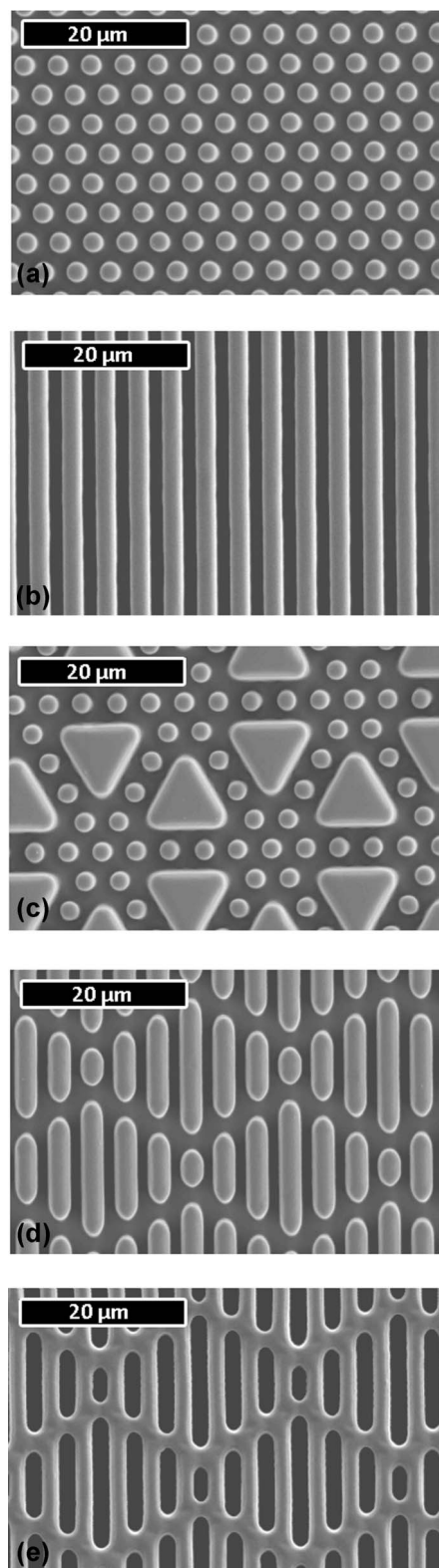


Figure 1. Scanning electron micrographs of (a) pillars, (b) ridges, (c) triangle/pillars, (d) Sharklet AFTM, (e) Recessed Sharklet AFTM surfaces in PDMS_e. The topographies of the Sharklet AFTM and the ridges were positioned so that the features were parallel to the direction of flow when mounted in the flow cell.

has been reported to influence secondary colonization (Shea et al. 1995). The present report shows the correlation between the ERI_{II} value and attachment of *C. marina*. Additionally, a single equation is presented that relates attachment densities of spores of *Ulva* and cells of *C. marina* to the ERI_{II} multiplied by the estimated Reynolds number for the individual organisms.

Materials and methods

Materials

The base material used for engineered topographical modification was a platinum-catalyzed PDMS_e (Silastic[®] T-2; Dow Corning Corporation). The elastomer was prepared by hand mixing 10 parts resin and 1 part curing agent by weight for 5 min. The mixture was degassed under vacuum (28–30 in Hg) for 30 min, removed from the vacuum chamber, and poured into negative topographical molds to cure for 24 h at ~22°C.

Pattern designs

The patterns studied included Sharklet AFTM, recessed Sharklet AFTM, ridges, pillars, and triangle/pillars (Schumacher et al. 2007b). Sharklet AFTM previously described (Carman et al. 2006, Schumacher et al. 2007b) consists of 2 μm wide ribs of various lengths (4, 8, 12, and 16 μm) that are combined by feature length in the following order: 4, 8, 12, 16, 12, 8, and 4 μm at a feature spacing of 2 μm to form a diamond. This diamond of protruding features was the repeat unit for the arrayed pattern. The spacing between each diamond unit was 2 μm. This pattern was inspired by and is similar to the skin of a shark (Beichert et al. 2000) in terms of feature arrangement. Recessed Sharklet AFTM is the negative of Sharklet AFTM. It has the same arrangement of features indented into the surface instead of protruding out from the surface. Ridges and pillars were designed with an analogous feature spacing of 2 μm. Ridges were continuous in length and separated by 2 μm. The pillars were hexagonally packed so the distance between any two adjacent pillars was 2 μm. The triangle/pillars pattern is a multi-feature pattern formed by replacing a set of six hexagonally packed 2 μm pillars with a 10 μm equilateral triangle. This triangle placement maintained a 2 μm feature spacing between the edges of the triangle and the pillars.

Pattern fabrication

Pattern designs were transferred to photoresist-coated silicon wafers using standard photolithographic

techniques described previously (Schumacher et al. 2008). The patterned wafers were deep reactive ion etched using the Bosch process to a depth of approximately 3 μm to create negative molds of the engineered topographies. Wafers were subsequently stripped of photoresist with an O₂ plasma etch. Hexamethyldisilazane was vapor deposited onto the processed wafers to methylate the surfaces and prevent adhesion of PDMS_e during the replication process.

Topographical replication

Engineered topographies were transferred to PDMS_e by replication of the patterned and etched silicon wafer. The resulting engineered topographies contained features that protrude from or are indented into the surface at a height of approximately 3 μm. Pattern fidelity and feature height were evaluated with light and scanning electron microscopy.

Sample preparation

Samples were provided as smooth PDMS_e surrounding two 13 mm × 13 mm squares of engineered topographies of interest adhered to glass coverslips (60 mm × 24 mm, No. 2 thickness). Engineered topographies were attached to glass coverslips using a two-step process previously described (Carman et al. 2006). Topographies (smooth, Sharklet AFTM, recessed Sharklet AFTM, ridges, pillars, and triangles/pillars) were randomly assigned to one of two positions on each coverslip. The topographies of Sharklet AFTM and ridges were positioned on coverslips so that features were parallel to the direction of flow when mounted in the flow cell. The resulting coverslip was approximately 0.8 mm thick and contained an adhered PDMS_e film with two 13 mm × 13 mm square areas containing topography bordered on all sides by smooth (no topography) areas.

Bacteria

Chemostat culture

A logarithmic chemostat culture of *C. marina* ATCC 25374 (American Type Culture Collection, Manassas, VA) strain (Baumann et al. 1983; Arahall et al. 2002) was established in modified basal medium (200 mM NaCl, 50 mM MgSO₄ · 7H₂O, 10 mM KCl, 10 mM CaCl₂ · 2H₂O, 19 mM NH₄Cl, 0.33 mM K₂HPO₄, 0.1 mM FeSO₄ · 7H₂O, 5 mM Tris-HCl (pH 7.5), and 2 mM glycerol) as described previously (Ista et al. 1996). The chemostat was maintained at a flow rate of 1 ml min⁻¹ with constant stirring resulting in a cellular concentration of 5 × 10⁷ cells ml⁻¹.

Stationary phase culture

A stationary phase culture of *C. marina* was grown in 1 l of the modified basal medium described above. The inoculated culture grew for 21 h at 25°C while shaking at 400 RPM.

C. marina attachment assay

Two bacterial attachment assays were performed with *C. marina*, one using the chemostat culture and one using the stationary phase culture described above. Samples were sterilized by immersion in ethanol for 20 min, soaked in artificial seawater for 1 h and then placed into a laminar flow cell apparatus (Ista et al. 1996) that was mounted on the stage of an optical microscope (Zeiss Axioscope 40). The sample coverslip forms the top plate of the flow chamber to minimize gravitational effects on attachment. The flow cell was then connected to the culture vessel through tubing and a peristaltic pump (Ista et al. 1996, 2004). The cells were introduced into the flow cell at a rate of $2 \times 10^{-5} \text{ l s}^{-1}$ for 2 h. Under these conditions for a flat surface, the Reynolds number was $\sim 2 \times 10^{-3}$, indicating laminar flow and the surface shear rate was $6.6 \times 10^2 \text{ s}^{-1}$ (Ista et al. 2004). Bacterial attachment was monitored through a camera attached to a phase contrast microscope. Ten random images were taken of each topography at 15 min intervals. Images were acquired with Axiovision software and processed with ImageJ software (Rasband 1997–2009). Each image was first converted to 8 bit format and then processed using the Fast Fourier Transform (FFT) bandpass filter associated with ImageJ to eliminate background unevenness. The cell densities were determined through direct counting using the ImageJ cell counter plug-in. The attached cells in 10 randomly selected fields of view were counted at each time point. The average number of cells per square millimeter (cells mm^{-2}) was calculated for each replicate. Three replicates were analyzed for each topography. The position of each topography was varied in the flow stream.

Statistical methods

Cell counts are reported as the mean number of cells mm^{-2} from 10 counts on each of three replicate topographies ($n = 30$) with 95% confidence intervals. The cell count data were transformed using a natural logarithm. Statistical differences between surfaces were evaluated with the transformed data using one-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons.

The transformed mean number of cells mm^{-2} for each engineered topography was plotted vs time for

kinetic analysis. The transformed data were also plotted against the calculated ERI_{II} to determine if any correlations existed between attachment of cells of *C. marina* and the ERI_{II} . Regression analysis was performed to evaluate the strength of the correlation.

Results and discussion

Only slight variations in attachment densities were measured over the entire assay period. This includes samples exposed to both logarithmic growth phase and stationary growth phase of *C. marina* (Figure 2). Cells of *C. marina* were calculated to attach at a mean density of $683 \pm 79 \text{ cells mm}^{-2}$ and $413 \pm 51 \text{ cells mm}^{-2}$ on the smooth PDMS_e at the 120 min time point for the logarithmic growth phase and stationary growth phase, respectively. The cell density for both growth phases was reduced significantly (Figure 3) on all topographies relative to the smooth surface at 120 min. The logarithmic growth phase cell density mm^{-2} was lower on the triangle/pillars (1.7 ± 2.6), Sharklet AFTM (0.1 ± 0.2), and recessed Sharklet AFTM (1.0 ± 1.2) topographies compared to the cell density on pillars (14.2 ± 10.2) and ridges (15.8 ± 7). The attachment density of *C. marina* cells in the stationary growth phase followed the same order, ie, a lower attachment density was measured on triangle/pillars (55 ± 0.3) and Sharklet AFTM (36 ± 0.2) patterns than on pillars (126 ± 0.3) and ridges (166 ± 0.1).

Nearly all of the attached cells were single or in small clusters. Cells appeared to attach in clusters around pillars on the pillar pattern. The majority of cells were attached within the $\sim 2 \mu\text{m}$ wide channels of the ridge patterns. Nearly all cells settled next to the edges of the triangle or clustered around the pillars of the triangle/pillar patterns. Cells did not attach to the tops of the $10 \mu\text{m}$ triangles. Cells were attached either between the features of the Sharklet AFTM pattern or around the edges of the diamond repeat units. On the recessed Sharklet AFTM pattern cells attached within the $\sim 2 \mu\text{m}$ wide depressions.

The feature dimensions of the topographies used in each study were measured and the average heights, widths and spacings were used to calculate the ERI_{II} value. Due to slight variations in feature height, the value of the ERI_{II} for each pattern varied between the two bacterial attachment assays (Supplementary Tables 1 and 2) [Supplementary material is available *via* a multimedia link on the online article webpage]. Recessed Sharklet AFTM is a newly designed topography with a higher ERI_{II} value (24) than Sharklet AFTM that was included in the logarithmic growth phase attachment assay to further the ERI_{II} series.

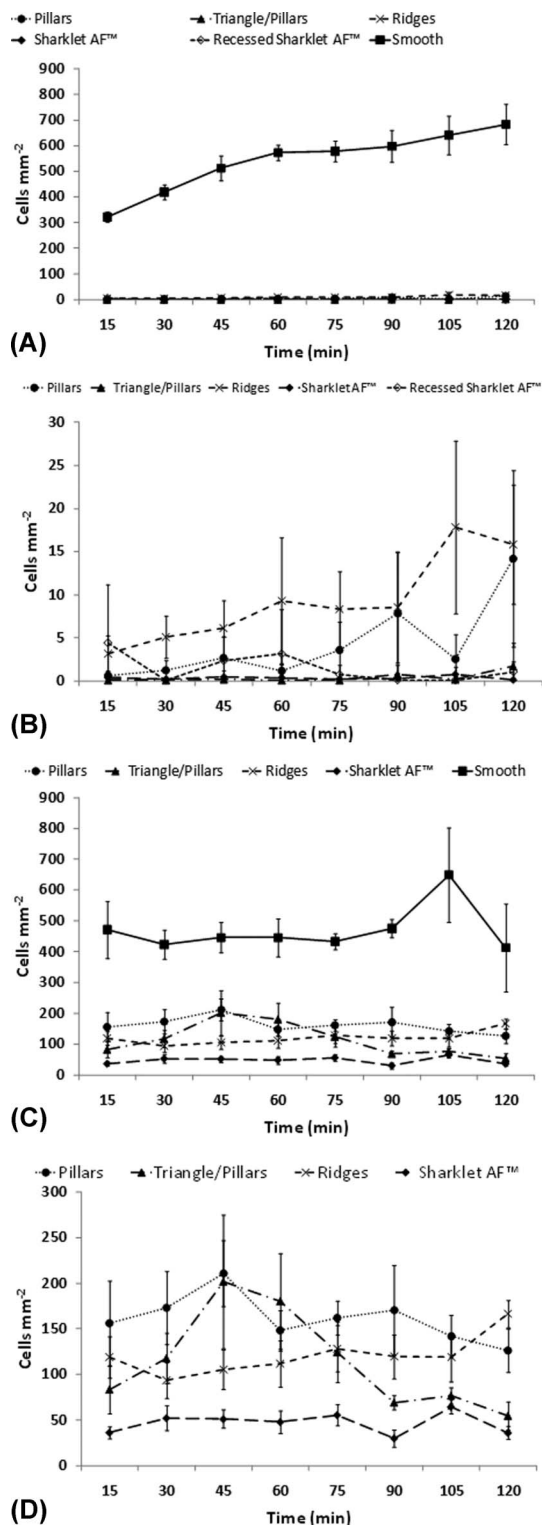


Figure 2. *C. marina* attachment vs time for (A) chemostat culture (logarithmic growth phase), all surfaces; (B) chemostat culture (logarithmic growth phase), topographically modified surfaces only; (C) overnight culture (stationary growth phase), all surfaces; and (D) overnight culture (stationary growth phase), topographically modified surfaces only. Error bars represent 95% confidence intervals.

The natural logarithm of the normalized mean cell density measured on each surface at 120 min was plotted against ERI_{II} of each pattern (Figure 4). A linear regression model was fitted to each set of data. An inverse linear relationship existed between mean cell density and ERI_{II} for bacterial attachment in both the stationary ($R^2 = 0.84$, $p = 0.047$) and logarithmic ($R^2 = 0.40$, $p = 0.038$) growth phases.

The lowest mean cell densities for the logarithmic growth phase of *C. marina* correlated with the highest ERI_{II} values, eg triangle/pillars (5.8), Sharklet AFTM (19), and Recessed Sharklet AFTM (24), but were not different statistically. The second lowest cell densities were measured on ridges and pillars (ERI_{II} values of 5.5 and 3.2, respectively). Cell densities measured in the stationary phase attachment assay followed a similar trend. Triangle/pillars and Sharklet AFTM with the highest ERI_{II} values (6.1 and 13, respectively) had the lowest cell densities. Ridges and pillars had the

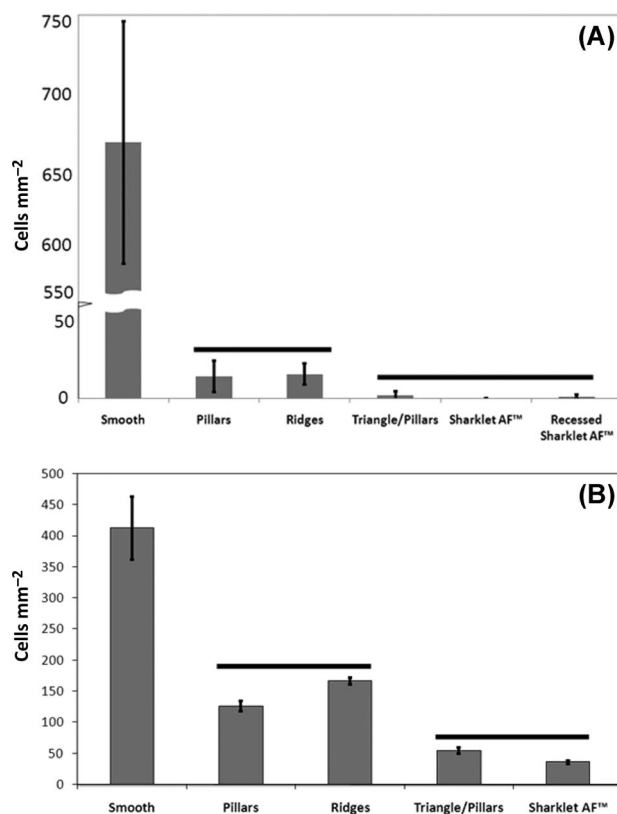


Figure 3. *C. marina* attachment data on PDMS surfaces represented as mean cell density (cells mm⁻²) \pm 95% confidence interval ($n = 30$). (A) surfaces exposed to *C. marina* cells in the logarithmic growth phase established in a chemostat culture for 120 min; (B) surfaces exposed to *C. marina* cells in the stationary growth phase established through an overnight culture for 120 min. Solid horizontal bars = statistically different groups (ANOVA $p = 0.05$, Tukey test $p = 0.05$).

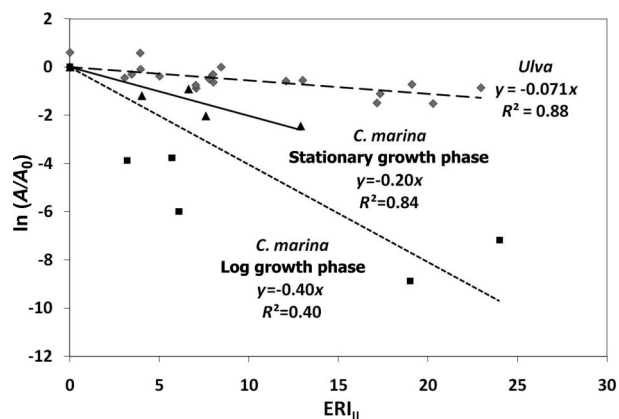


Figure 4. The attachment model shows the correlation between *C. marina* attachment normalized to attachment on a smooth surface and the engineered roughness index (ERI_{II}) at 120 min for the stationary and logarithmic growth phases. Plotted is the calculated ERI_{II} for each pattern tested vs the natural logarithm of the experimental mean bacterial cell density at the 120 min time point minus the mean bacterial cell density on a smooth surface at the same time point ($\ln(A/A_0)$) ($n = 30$). Data for attachment of zoospores of *Ulva*, taken from Long et al. (2010) are also plotted.

next highest ERI_{II} values (5.9 and 3.2) and the second lowest attachment densities.

The regression analysis for cell attachment to the surfaces established a statistically significant trend for both the logarithmic growth phase and stationary growth phase. These trends were analyzed in terms of the attachment model as Equations (2) and (3). The variable A is the attachment on a surface with a given ERI_{II} value and A_0 is the attachment on a smooth surface ($ERI_{II} = 0$).

$$\ln\left(\frac{A}{A_0}\right) = -0.40 * ERI_{II} \quad (2)$$

$$\ln\left(\frac{A}{A_0}\right) = -0.20 * ERI_{II} \quad (3)$$

Adhesion of marine bacteria to surfaces has been attributed to many factors, viz. the substratum composition (Ista et al. 2004, Ekblad et al. 2008), surface chemistry (Ista et al. 1996, 1999; Poolman et al. 2004; Cordero et al. 2009), substratum mechanical properties (Ekblad et al. 2008; Cordero et al. 2009), and surface roughness (Kerr and Cowling 2003). Substratum composition, substratum mechanical properties and surface chemistry were kept constant in this study by using PDMSe for all surfaces. Surface roughness was systematically studied by using the ERI_{II} algorithm to describe engineered microtopographies.

Plots of *C. marina* cell attachment over time indicated that cells in both the logarithmic and stationary growth phase reacted to surface topography nearly instantaneously (Figure 2). Bacterial attachment density did not change significantly from the first time point (15 min) to the last (120 min). This response was due to physical, not chemical, changes in the substratum. These results are consistent with the work of Kerr and Cowling (2003) in which the effect of surface roughness on bacterial adhesion was observed to be almost instantaneous.

Cell densities measured at the last time point indicated that all topographies showed a statistically significant reduction in the density of attached cells in both growth phases relative to the smooth surface (Figure 3). A fairly strong inverse linear relationship existed between the natural logarithm of the mean cell density and ERI_{II} value for both bacterial attachment assays (Figure 4). These relationships, however, did not follow the same regression line. Attachment of cells established in a chemostat culture (logarithmic growth phase) correlated with a line with a steeper slope ($m = -0.40$) than the cells in the stationary growth phase ($m = -0.20$). The slopes of these lines were both different from the slope of the regression line for attachment of spores of *Ulva* vs ERI_{II} value ($m = -0.071$) from four separate studies reported in previous work (Long et al. 2010).

It is possible that the slope of the line in the attachment model (m) encompasses the organism's sensitivity to a surface (Equation 4).

$$\ln\left(\frac{A}{A_0}\right) = -m * ERI_{II} \quad (4)$$

This sensitivity could be related to a number of factors including the size and shape (Kerr and Cowling 2003; Carman et al. 2006), motility (Shea et al. 1995), and surface chemistry (Shea et al. 1991; Ista et al. 2004; Poolman et al. 2004; Cordero et al. 2009; Ista et al. 2010) of the attaching organism and/or conditioning of the surface by the culture medium and secreted products (Jain and Bhosle 2009). It has been observed that in late stationary phase, cells of *C. marina* tend to shrink to approximately $1 \mu\text{m}$ in diameter and take on a more rounded shape. The same cells in the logarithmic growth phase are rod-shaped, approximately $2 \mu\text{m}$ in length and often joined in pairs. This size and shape difference may influence bacterial attachment. The results from this study also showed stationary phase cells adhered to hydrophobic topographic surfaces in higher numbers than logarithmic-phase cells. The higher densities of bacteria attached to surfaces exposed to the stationary phase culture could

be due to the fact that there were simply more bacteria cells in the overnight culture than the chemostat culture. A standard overnight culture has approximately 10^9 cells ml^{-1} whereas the chemostat is maintained at approximately 10^7 cells ml^{-1} . The influence of size and shape of the organisms moving in a fluid can be described by the Reynolds number.

The Reynolds number is the ratio of inertial forces to viscous forces in fluid flow. Viscous forces dominate inertial forces in conditions such as those experienced by cells of *C. marina* and spores of *Ulva* while moving through water (Purcell 1977; Berg 1983; Dusenbery 2009). Therefore bacteria and spores operate at low Reynolds number. Alternatively, the Reynolds number can be considered to represent the scale separation in the flow. That is to say the bacteria and algal spores are not large compared to the smallest scales in the flow at which viscosity dissipates kinetic energy. The *Ulva* spore is a flagellated cell capable of propelling itself through the water. A recent report proposed that the flagellar motion of the swimming unicellular alga, *Chlamydomonas*, is coupled to its hydrodynamic environment (Polin et al. 2009). The bacterial cells in this study were not flagellated. The Reynolds numbers for the swimming *Ulva* spore and the cells of *C. marina* in both growth phases were calculated using the following equation:

$$Re = \frac{\rho VL}{\mu} \quad (5)$$

where ρ and μ are the density and viscosity of the fluid used in the assay, V is the velocity of the organism relative to that of the fluid, and L is the characteristic length of the organism (see Supplementary Information for full calculation) [Supplementary material is available via a multimedia link on the online article webpage]. The characteristic length is a dimension relevant to the geometry of the flow; in this case it is the diameter of the body moving through the fluid. The characteristic length, ie the diameter of the spore body at its widest point, and the velocity of the *Ulva* spore were taken to be $5 \mu\text{m}$ and $150 \mu\text{m s}^{-1}$, respectively (Callow et al. 2002; Heydt et al. 2009). The characteristic length of *C. marina* varies with growth phase. It is $2 \mu\text{m}$ for the logarithmic growth phase and $1 \mu\text{m}$ for the stationary growth phase. The velocity of the bacterial cells relative to the fluid near the wall was estimated to be 20% of the average fluid velocity in the flow cell. When the bacterial cells enter the boundary layer near the wall, the velocity will be considerably less than the average bulk velocity due to the no-slip boundary condition. The flow velocity of the fluid, which is a key component of the Reynolds number, has been demonstrated to affect the attachment density of

spores of *Ulva* (Granhag et al. 2007). The size and motion of the organisms were incorporated into the slope of the attachment model by multiplying the ERI_{II} value by the Reynolds number of the organism.

$$m = m' Re \quad (6)$$

$$\ln\left(\frac{A}{A_0}\right) = -(m' Re) * \text{ERI}_{\text{II}} \quad (7)$$

The incorporation of the Reynolds number into the attachment model allowed four separate spore attachment assays (Schumacher et al. 2007, 2008; Long et al. 2010) to be combined with both *C. marina* attachment assays into a single data set that yields a regression with high correlation to $\text{ERI}_{\text{II}} \cdot Re$ ($R^2 = 0.77$) (Figure 5).

Further work is planned to investigate the role of the slope of the attachment model line as an indicator of the sensitivity of an organism to a surface. This work could include attachment of different species or organisms such as new or mutant strains of bacteria to elucidate which factors contribute to the sensing of topographically modified surfaces. It could also include altering the flow conditions to change the organism's Reynolds number and investigate the role of the hydrodynamic environment on biological attachment.

Microtopographies created in non-toxic materials such as PDMS are a green alternative to biocidal methods for reducing biofouling. The attachment model has been demonstrated to correlate the ERI_{II} value multiplied by the Reynolds number for the organism to the attachment density of cells from two kingdoms (Bacteria and Plantae). An inverse linear

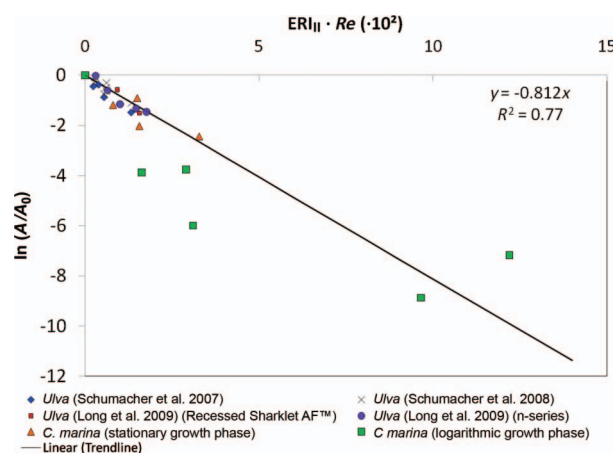


Figure 5. The attachment model shows the correlation between attachment of zoospores of *Ulva* and cells of *C. marina* normalized to attachment on a smooth surface and the engineered roughness index (ERI_{II}) multiplied by the Reynolds number of the organism.

relationship exists between mean cell densities for the two different organisms and $ERI_{II} \cdot Re$. Plots of the attachment of *C. marina* over time indicate that both logarithmic phase and stationary phase cells reacted to surface topography within 15 min of exposure to the surface. At the 120 min time point, all topographically modified surfaces showed a statistically significant reduction in attachment compared to smooth surfaces for both bacterial attachment assays. The incorporation of the Reynolds number into the attachment model created a regression model with a high correlation of attachment for spores of *Ulva* and cells of *C. marina* in both growth phases to $ERI_{II} \cdot Re$.

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