Barnacle settlement and the adhesion of protein and diatom microfouling to xerogel films with varying surface energy and water wettability

John A. Finlay[a], Stephanie M. Bennett[b], Lenora H. Brewer[c], Anastasiya Sokolova[b], Gemma Clay[a], Nikhil Gunari[d], Anne E. Meyer[e], Gilbert C. Walker[d], Dean E. Wendt[c], Maureen E. Callow[a], James A. Callow[a] and Michael R. Detty[b]*

[a]*School of Biosciences, The University of Birmingham, Edgbaston, Birmingham B15 2TT, UK;* [b]*Department of Chemistry, University at Buffalo, The State University of New York, Buffalo, New York 14260-3000, USA;* [c]*Biological Sciences Department, California Polytechnic State University, San Luis Obispo, California 93407, USA;* [d]*Department of Chemistry, University of Toronto, Toronto, Ontario, Canada M5S 3H6;* [e]*Industry/University Center for Biosurfaces and Biomaterials Graduate Program, University at Buffalo, The State University of New York, Buffalo, New York 14214, USA*

Previous work has shown that organosilica-based xerogels have the potential to control biofouling. In this study, modifications of chemistry were investigated with respect to their resistance to marine slimes and to settlement of barnacle cyprids. Adhesion force measurements of bovine serum albumin (BSA)-coated atomic force microscopy (AFM) tips to xerogel surfaces prepared from aminopropylsilyl-, fluorocarbonsilyl-, and hydrocarbonsilyl-containing precursors, indicated that adhesion was significantly less on the xerogel surfaces in comparison to a poly(dimethylsiloxane) elastomer (PDMSE) standard. The strength of adhesion of BSA on the xerogels was highest on surfaces with the highest and the lowest critical surface tensions, γ_C and surface energies, γ_S, and duplicated the 'Baier curve'. The attachment to and removal of cells of the diatom *Navicula perminuta* from a similar series of xerogel surfaces were examined. Initial attachment of cells was comparable on all of the xerogel surfaces, but the percentage removal of attached cells by hydrodynamic shear stress increased with γ_C and increased wettability as measured by the static water contact angle, θ_{Ws}, of the xerogel surfaces. The percentage removal of cells of Navicula was linearly correlated with both properties ($R^2 = 0.74$ for percentage removal as a function of θ_{Ws} and $R^2 = 0.69$ for percentage removal as a function of γ_C). Several of the aminopropylsilyl-containing xerogels showed significantly greater removal of *Navicula* compared to a PDMSE standard. Cypris larvae of the barnacle *B. amphitrite* showed preferred settlement on hydrophilic/higher energy surfaces. Settlement was linearly correlated with θ_{Ws} ($R^2 = 0.84$) and γ_C ($R^2 = 0.84$). Hydrophilic xerogels should prove useful as coatings for boats in regions where fouling is dominated by microfouling (protein and diatom slimes).

Keywords: biofouling; microfouling; protein adhesion; diatom adhesion; barnacle settlement; xerogels; fouling release; wettability; critical surface tension; surface energy

Introduction

Upon immersion in seawater, a clean surface undergoes a rapid sequence of events eventually leading to fouling of the surface. A molecular 'conditioning' film first forms consisting of various organic materials such as proteins and carbohydrates (Bakker et al. 2003; Jain and Bhosle 2009). Within hours, bacteria and unicellular algae are likely to colonize and rapidly complete the formation of the primary biofilm, which has also been referred to as 'microfouling' or 'slime' (Callow 2000; Molino et al. 2009a,b). Diatoms are the predominant group of biofilm-forming algae. Diatoms are unicellular algae in which the protoplast is enclosed in a silica case (the frustule) composed of overlapping halves or 'valves'. Adhesion of raphid diatoms is a complex process that is mediated by the secretion of extracellular polymeric substances (EPS), comprising a range of proteoglycans, through one or two slits in the silica cell wall called raphes (see review by Molino and Wetherbee 2008). Contact with the substratum initiates a number of processes that facilitate primary adhesion and gliding motility (Molino and Wetherbee 2008).

Following formation of the primary biofilm, a surface rapidly becomes overgrown by macrofoulers such as barnacles, tubeworms, and macroalgae (Callow 2000). One such macrofouler is the barnacle *Balanus amphitrite* (*Amphibalanus amphitrite*) (Clare and Høeg 2008), which is a common member of coastal fouling communities and has an extremely wide geographical distribution (Otani et al. 2007). Several studies have suggested that cypris larvae of

B. amphitrite prefer to settle on surfaces of high wettability (Rittschof and Costlow 1989; Gerhart et al. 1992; Holm et al. 1997). Cypris larvae of the barnacle *Balanus improvisus*, in contrast, prefer settlement on surfaces of reduced wettability (Dahlström et al. 2004).

There has been a significant effort to develop environmentally benign, non-toxic fouling-release and/or non-fouling coatings for use on marine craft (see reviews by Genzer and Efimenko 2006; Krishnan et al. 2008; Grozea and Walker 2009). Silicone elastomers such as polydimethylsiloxane elastomer (PDMSE) have been the most successful of these coatings, which 'release' accumulated macrofouling under suitable hydrodynamic conditions (Kavanagh et al. 2005; Wendt et al. 2006). Attributes of a silicone fouling-release coating are low-modulus, low-surface energy, and low-microroughness (Wynne et al. 2000; Anderson et al. 2003). However, they are prone to fouling by slimes dominated by diatoms, which are not released at the operating speeds of most vessels and grooming is frequently required to achieve the release of slimes (Tribou and Swain 2010).

The release of fouling from a surface can be dependent upon the surface properties such as surface energy or water contact angle and/or can be dependent upon the surface chemistry that interacts with the adhesive holding the fouling to the surface. The critical surface tension, γ_C, measured by comprehensive contact angle analysis is one such surface property that empirically and reproducibly characterizes the first 4–5 Å of the surface (Zisman 1964; Baier and Meyer 1992). There is a zone of minimal bioadhesion for surfaces with values of γ_C between 20 and 30 mN m^{-1} where weak boundary layers are formed, which allows biofouling to be removed by shear forces (Baier et al. 1968; Baier 1984; Baier and Meyer 1992) and many papers have shown that the attachment and adhesion of microfouling is minimal in this range (Dexter 1979; Zhao et al. 2009). Bennett et al. (2010) have shown that the removal of sporelings (young plants) of the green alga, *Ulva linza*, from a series of structurally related organosilica-based xerogels is dependent upon the surface chemistry (hydrocarbon, fluorocarbon, or aminoalkyl).

The adhesion of proteins to surfaces is dictated by the difference in energy between the surface and water at the interface and has little to do with the mechanical properties of the surface (Vogler 2001; Krishnan et al. 2008). Non-polar hydrophobic surfaces, such as PDMSE, have low surface energies while hydrophilic surfaces formed from polymers such as poly(ethylene glycol) (PEG) have much higher surface energies. Consequently, the interfacial energy difference with water is high for hydrophobic surfaces and much lower for hydrophilic surfaces. When amphiphilic biomolecules such as proteins contact a low-energy, hydrophobic surface, significant adsorption on the surface is observed to minimize the interfacial energy. Adsorption of biomolecules on hydrophilic surfaces does not give any significant thermodynamic advantage since the interfacial energy is already low and it has been suggested that these surfaces show resistance to protein adsorption (Vogler 2001; Krishnan et al. 2008).

For diatoms, adhesion strength also appears to be sensitive to surface chemistry rather than to the mechanical properties of the coating and trends are opposite to those observed for macrofoulers including small plants of the green seaweed *Ulva* (eg Krishnan et al. 2006). Diatoms have been found to adhere more strongly to hydrophobic silicone elastomers than to glass (Terlizzi et al. 2000; Holland et al. 2004; Thompson et al. 2008). With the diatom *Amphora*, cells were more easily removed from hydrophilic than from hydrophobic alkanethiol self-assembled monolayers (SAMs) (Finlay et al. 2002) and the attachment strength of *Navicula* increased on SAMs of ethylene glycol with hydrophobic end-group terminations (Schilp et al. 2007). Alkyl chain length in SAMs, which influences lubricity, also contributes to the strength of adhesion of *Navicula* (Bowen et al. 2007).

The cellular mechanism involved in the perception of hydrophilic or hydrophobic surfaces by diatoms has recently been shown to be mediated by a stress response involving nitric oxide (NO) production (Thompson et al. 2008). On hydrophilic acid-washed glass, adhesion strength of diatoms was low and NO production was 4-fold higher than on PDMSE (Silastic® T2) to which cells adhered strongly. The converse was shown for spores of *Ulva*, which adhered less strongly to the PDMSE (Silastic T2) (Thompson et al. 2010).

Organosilica-based xerogel (shortened to 'xerogel' hereafter) surfaces can be tuned to provide different water wettabilities and surface energies (Cho et al. 2002; Tang et al. 2005; Bennett et al. 2010). Sol-gel-derived xerogel materials have been used as both fouling-resistant and fouling-release coatings (Tang et al. 2005; McMaster et al. 2009; Selvaggio et al. 2009; Bennett et al. 2010). Xerogel coatings are economically and environmentally friendly, and can be applied to surfaces by a variety of means including spraying, brushing, dip coating, and spin coating (Ingersoll and Bright 1997; Selvaggio et al. 2009). Several xerogel surfaces have shown both reduced settlement and release of macrofouling organisms (Tang et al. 2005, Bennett et al. 2010).

In the present study, the fouling characteristics of a series of xerogel surfaces modified with fluorocarbon, aminopropyl, and hydrocarbon groups were examined

toward adhesion of bovine serum albumin (BSA), settlement of cypris larvae of the barnacle *B. amphitrite*, and attachment and release of the diatom *Navicula perminuta*. The results with BSA were compared to those of a PDMSE (Silastic T2) standard; the results with *Navicula* were compared to those of PDMSE (Silastic T2) and glass; and the results with *B. amphitrite* were compared to those of PDMSE (Silastic T2), polystyrene, and glass. The fluorocarbon, aminopropyl, and hydrocarbon groups provide a range of surface chemistries, surface energies (as measured by comprehensive contact angle analysis) and water wettabilities (as measured by static water contact angles). Earlier studies have shown that these surfaces are high modulus materials that have similar values of nanoroughness as measured by scanning electron microscopy and atomic force microscopy (Bennett et al. 2010). The current study addresses the questions of (1) whether the various components associated with microfouling, ie proteins and diatoms, adhere less strongly to highly wettable/high surface energy xerogel surfaces compared to hydrophobic/low energy xerogel surfaces, and (2) whether barnacle cyprids prefer to settle on xerogel surfaces with high wettability/high surface energy.

Materials and methods

Chemical reagents

Deionized water was prepared to a specific resistivity of at least 18 MΩ using a Barnstead NANOpure Diamond UV ultrapure water system. 3-Aminopropyltriethoxysilane (AP), 3-methylaminopropyltrimethoxysilane (MAP), 3-dimethylaminopropyltrimethoxysilane (DMAP), 3,3,3-trifluoropropyltrimethoxysilane (TFP), tetraethoxysilane or tetraethyl orthosilicate (TEOS), phenyltriethoxysilane (PH), *n*-propyltrimethoxysilane (C3), *n*-octadecyltrimethoxysilane (C18), and *n*-octyltriethoxysilane (C8) were purchased from Gelest, Inc. and were used as received. 3-Trimethoxysilylpropyltrimethylammonium iodide (TMAP) was prepared by treating DMAP with 6 equivalents of iodomethane in refluxing acetone in the presence of excess potassium carbonate (Bennett et al. 2010). Ethanol was purchased from Quantum Chemical Corp. Hydrochloric acid was obtained from Fisher Scientific Co. Borosilicate glass microscope slides were obtained from Fisher Scientific, Inc.

Sol preparation

The sol/xerogel composition is designated in terms of the molar ratio of Si-containing precursors. Thus, a 1:1 PH/TEOS composition contains 50 mole-% PH and 50 mole-% TEOS. The 1:9 AP/TEOS, 1:9 MAP/TEOS, 1:9 DMAP/TEOS, 1:9 TMAP/TEOS, 1:1 PH/TEOS, 1:1 TFP/TEOS, 1:1 C3/TEOS, 1:1 C8/TEOS, 0.1:0.9:1 C18/C8/TEOS, 1:1:2 TFP/PH/TEOS, 1:1:2 TFP/C3/TEOS, 1:1:2 TFP/C8/TEOS, and 1:1:2 C8/PH/TEOS sols were prepared as previously described (Bennett et al. 2010).

Characterization of sol precursors to xerogels

The molecular weight distributions of the various sol precursors were determined by size-exclusion chromatography (SEC) on a Viscotek GPCmax instrument. Tetrahydrofuran (THF) solutions of sols (20 mg ml^{-1}) were analyzed on a Polymer Laboratories Polypore (300-mm × 7.5-mm) column using THF at a flow rate of 1 ml min^{-1} at 30°C with a refractive index detector. The column set was calibrated with narrow-molecular-weight distribution poly(ethylene oxide) (PEO)standards between MW 162 (log M = 2.21) and MW 915,000 (log M = 5.96).

Xerogel film formation

Xerogel films were formed by spin casting 400 μl of the sol precursor onto 25-mm × 75-mm glass microscope slides. The slides were soaked in piranha solution for 24 h, rinsed with copious quantities of deionized water then soaked in isopropanol for 10 min before being air dried and stored at ambient temperature. A model P6700 spincoater was used at 100 rpm for 10 s to deliver the sol and at 3000 rpm for 30 s to coat.

For barnacle cyprid assays, glass 20-mm × 60-mm Petri dish bottoms (VWR Scientific, Inc.) were soaked in piranha solution for 24 h, rinsed with copious quantities of deionized water, and stored in an oven at 110°C until use. The Petri dish bottoms were cooled to ambient temperature and approximately 1–1.5 ml of the appropriate sol precursor were added and the Petri dish was manipulated until the bottom surface and approximately 5 mm of the side surface were covered. The excess sol precursor was removed with a pipette.

All coated surfaces (glass slides and Petri dishes) were dried at ambient temperature for at least 7 days prior to analysis.

Surface energy analysis

Contact angles were obtained with a contact angle goniometer [Rame-Hart, Model NRL 100] at room temperature using the sessile drop technique for all xerogel formulations. The xerogel films were stored in air prior to characterization and measurements were performed in air as previously described (Baier and Meyer 1992; Tang et al. 2005). Up to 13 different diagnostic liquids were employed, including water,

glycerol, formamide, thiodiglycol, methylene iodide, 1-bromonaphthalene, 1-methylnaphthalene, dicyclohexyl, *n*-hexadecane, *n*-tridecane, *n*-decane, *n*-octane, and *n*-heptane. The liquid/vapor surface tensions of these liquids were determined using data obtained with a ring tensiometer (Cenco-duNuoy). The diagnostic liquids were obtained from varied vendors in as high a purity as possible. The as-received liquid was measured for its surface tension and compared to the theoretical value that is available in published sources. If the surface tension of the as-received liquid was too low, the fluid was further purified (adsorption/column chromatography) and the fractions were tested for the highest liquid/vapor surface tension that could be obtained for that fluid. The liquid/vapor surface tensions used in the calculations are the measured values, and not the theoretical values.

The technique of 'advanced angle' analysis was used (Baier and Meyer 1992). Zisman plots were constructed by plotting the cosine of the average angle measured for each liquid against the liquid/vapor surface tension of the diagnostic liquid (Zisman 1964; Baier and Meyer 1992). A linear least squares analysis was performed to determine the sample's critical surface tension (γ_C) at the $\cos\theta = 1$ axis. The data were also treated as described by Kaelbe (1970) and Nyilas et al. (1977) to give the surface free energy (γ_S) (Baier and Meyer 1992).

BSA adhesion force measurements using atomic force microscopy

All measurements were obtained using the Molecular Force Probe (Asylum Research, Santa Barbara, CA). Bovine serum albumin (BSA) coated AFM tips (Novascan Technologies, Inc., Ames, IA) were used for all measurements exhibiting an average spring constant of 52 pN nm^{-1}. Cantilevers were calibrated using the thermal noise spectrum method. The resulting force-extension curves were analyzed with custom analysis software (Igor Pro, Wavemetrics). All experiments were carried out in phosphate buffered saline (PBS, pH $\sim$7.4) at room temperature. A small number of force plots were obtained per tip, to minimize the effect of AFM tipcontamination by the sample. A total of 60 force plots were analyzed for each surface type; three surface replicates of each type were examined, each examined by four similar AFM cantilevers at five locations each.

Biofouling assays

Slides coated with polydimethyl siloxane elastomer (PDMSE or Silastic T2) *ca* 500 μm in thickness, provided by Dr AB Brennan, University of Florida (Schumacher et al. 2007), were included in the assays to provide a standard fouling-release coating.

Initial attachment and adhesion strength of diatoms

Cells of *Navicula perminuta* were cultured in 250 ml conical flasks in an illuminated incubator (incident irradiation of 75 mmol m^{-2} s^{-1}) at 18°C in F/2 medium (Guillard and Ryther 1962). After 3 days, the cells were in log phase growth. Cells were washed three times in fresh medium, filtered through 20 μm nylon mesh and diluted to give a suspension with a chlorophyll *a* content of approximately 0.25 μg ml^{-1} (Jeffrey and Humphrey 1975). Six replicate slides of each coating were placed in individual compartments of Quadriperm dishes (E&K Scientific Products, Inc.) to which 10 ml of cell suspension were added. After 2 h at $\sim$20°C on the laboratory bench, the slides were exposed to a submerged wash in seawater to remove cells that had not attached (the immersion process avoided passing the samples through the air-water interface). Three replicates were fixed in 2.5% glutaraldehyde, washed and air-dried. Cells were visualized by autofluorescence of chlorophyll and counted in 30 fields of view (each 0.17 mm^2), 1 mm apart over the central region of each slide, using image analysis software (Imaging Associates Ltd) attached to a Zeiss epifluorescence microscope (Callow et al. 2002). Cell settlement data are expressed as the mean number of cells adhered per mm^2 with 95% confidence limits ($n = 90$).

The three remaining replicates were exposed to a wall shear stress of 52 Pa for 5 min in a water channel that produced fully-developed turbulent flow consistent in characteristics with the flow around the hull of a ship (Schultz et al. 2000, 2003). The density of cells remaining attached was determined as described above and the data expressed as percentage removal; 95% confidence limits were calculated from arcsine-transformed data.

Cyprid settlement assays

Approximately 5 ml of seawater were added to each xerogel-coated Petri dish, which was just enough fluid to cover the entire bottom of the dish and allow the cyprids free range of movement across the surface. A 400-μl drop of seawater containing approximately 30–60 2–4-day-old barnacle cypris larvae was then added to each of the dishes. The PDMSE (Silastic T2) standard was coated on glass microscope slides and could not be completely immersed. To these samples, a 400-μl drop of seawater containing approximately 30–60 2–4-day-old barnacle cypris larvae was added. The larvae were allowed to settle for 48 h at which time the

percentage of barnacles that settled on each slide was determined. The average percentage settlement for each of the experimental coatings was then compared to the glass and polystyrene standards. The concurrent settlement on the PDMSE (Silastic T2) standard was used as a positive control for larval metamorphic competence.

Results

Characterization of the sol precursors and xerogel films

The xerogel surfaces used in this study were cast from sol precursors, which were characterized by size-exclusion chromatography. Sol molecular weights were controlled by hydrolysis times and molecular weight ranges were selected to give sol precursors that gelled immediately upon coating. Values of M_W for the sols varied from 1400 Da for the 1:1TFP/TEOS sol to 4000 Da for the 1:1 C8/TEOS sol. The amine-containing sols for AP, MAP, DMAP, and TMAP gave $M_w > 5000$ Da after hydrolysis for 20 min. Longer hydrolysis times gave particulate material.

Many of the xerogel surfaces were previously characterized (Bennett et al. 2010) by comprehensive advanced contact angle analyses to give values of the critical surface tension (γ_C) (Zisman 1964; Baier and Meyer 1992) and values of the surface free energy (γ_S) were calculated from these data as described by Kaelbe (1970) and modified by Nyilas et al. (1977). Values of γ_C and γ_S for the TMAP/TEOS films used in this study were calculated similarly. Values of γ_C and γ_S for all xerogel surfaces in this study are compiled in Table 1. Static water contact angles, θ_{Ws}, were measured for all xerogel surfaces described in this study and are also compiled in Table 1. Values of θ_{Ws} varied from 35° for the AP/TEOS coating to 105° for the C18/C8/TEOS coating.

Adhesion force measurement of BSA on xerogel surfaces

Adhesion force measurements taken using BSA-coated AFM tips showed a prominent disparity between the interactions of BSA (a 'sticky protein') with surfaces of the xerogel samples and PDMSE controls. Values for the maximum adhesion force for eleven samples and the PDMSE standard are given in Table 1. All the 11 xerogel samples show lower adhesion forces (0.22–1.62 nN) compared to the PDMSE (Silastic T2) standard (3.5 nN).

Initial attachment and adhesion strength of diatoms

Diatoms are not motile and reach the substratum surface by sinking through the water column. Thus at the end of the assay, every test sample has approximately the same number of cells in contact with the surface. The gentle washing carried out after the settlement period removed cells that had not attached to the surface. The initial density of attached diatoms was broadly similar on all the surfaces (Figure 1a).

Table 1. Static water contact angles (θ_{Ws}), critical surface tensions (γ_C), and surface energies (γ_S) for xerogel surfaces, BSA adhesion forces, barnacle settlement, and diatom settlement and percentage removal of diatoms for selected xerogel surfaces and glass, polystyrene, and PDMSE (Silastic® T2) standards.

Sample	θ^o_{Ws},	γ_C,[a] mN m^{-1}	γ_S,[a] mN m^{-1}	BSA adhesion force,[b] nN	% Settlement barnacle cyprids[c]	Diatom density,[d] no mm^{-2}	% Diatom removal[e]
Glass	–	–	–	–	61 ± 15	354 ± 18	53 ± 5
PDMSE	–	23.0 ± 0.4	23.0 ± 0.4	3.5 ± 0.7	55 ± 17	450 ± 24	38 ± 5
Polystyrene	–	–	–	–	64 ± 5	–	–
5:45:50 C18/C8/TEOS	105 ± 1	21.9 ± 0.3	24.6 ± 0.9	–	32 ± 8	391 ± 24	24 ± 6
50:50 C3/TEOS	99 ± 1	21.3 ± 0.1	27.5 ± 1.1	0.80 ± 0.11	27 ± 9	396 ± 21	5 ± 3
50:50 C8/TEOS	104 ± 1	21.3 ± 0.1	27.1 ± 0.3	0.86 ± 0.09	17 ± 5	468 ± 20	18 ± 4
50:50 PH/TEOS	74 ± 1	24.5 ± 1.6	32.9 ± 0.5	0.22 ± 0.05	62 ± 5	329 ± 19	29 ± 7
50:50 TFP/TEOS	85 ± 1	18.8 ± 0.1	26.9 ± 0.3	0.61 ± 0.09	32 ± 10	474 ± 26	34 ± 5
25:25:50 TFP/PH/TEOS	84 ± 1	21.0 ± 0.2	26.7 ± 0.3	0.98 ± 0.07	34 ± 8	–	–
25:25:50 TFP/C3/TEOS	92 ± 1	20.3 ± 0.1	24.9 ± 0.6	1.20 ± 0.13	–	–	–
25:25:50TFP/C8/TEOS	100 ± 1	20.4 ± 0.3	24.4 ± 0.3	1.62 ± 0.14	–	–	–
25:25:50 C8/PH/TEOS	94 ± 1	24.5 ± 0.5	30.5 ± 0.6	0.58 ± 0.09	–	–	–
10:90 AP/TEOS	35 ± 1	34.2 ± 0.1	53.3 ± 0.2	0.95 ± 0.22	51 ± 8	548 ± 23	56 ± 5
10:90 MAP/TEOS	57 ± 1	25.2 ± 0.7	47.9 ± 0.2	–	36.5 ± 0.2	544 ± 32	32 ± 7
10:90 DMAP/TEOS	42 ± 1	32.2 ± 2.0	54.7 ± 2.7	0.84 ± 0.11	53 ± 9	565 ± 26	61 ± 5
10:90 TMAP/TEOS	54 ± 1	29.7 ± 0.1	45.5 ± 0.1	0.63 ± 0.10	44 ± 13	490 ± 23	65 ± 4

[a]From Bennett et al. (2010) except for TMAP/TEOS. [b]Average derived from measurements on three surface replicates, each examined by four similar AFM cantilevers in five locations, hence a total of 60 measurements per surface type, ± one SD. [c]Average of three replicate measurements, ± SE. [d]Each value is the mean of 90 counts on three replicate slides, ± 95% confidence limits. [e]Percentage removal under 52 Pa wall shear stress on three replicate slides, ± 95% confidence limits.

The removal of attached cells by hydrodynamic shear from the xerogel surfaces was higher from the hydrophilic surfaces than from the hydrophobic surfaces (Figure 1b). One-way analysis of variance and Tukey tests on arc-sine transformed data showed that removal from three of the surfaces, AP/TEOS, DMAP/TEOS, and TMAP/TEOS, was significantly greater than from the PDMSE (Silastic T2) standard (F 9, 860 = 25.1, $p < 0.05$).

Initial settlement of barnacle cyprids

The settlement of ~30–60 2–4-day old barnacle cypris larvae that were placed on the xerogel, glass, and polystyrene surfaces is shown in Figure 2. The average settlement from a drop assay onto PDMSE (Silastic T2) slides that were settled concurrently are also included as a positive control for larval metamorphic competence. There was a significant difference in the settlement of cypris larvae between coatings (ANOVA, $F = 3.147$, $p = 0.0011$). The average settlement on the C18/C8/TEOS, C8/TEOS, C3/TEOS, TFP/TEOS, and TFP/PH/TEOS xerogel surfaces were significantly lower than settlement on the glass and polystyrene controls (Table 1). The PDMSE (Silastic T2) slides were not included in the statistical analysis due to the different assay type.

The reduced settlement observed on several of the xerogel surfaces did not appear to be due to any adverse physiological effect upon the barnacle larvae; cyprids were active and behaved normally on all coatings for the duration of the test. Earlier studies have also shown that there is no toxicity associated with the xerogel surfaces based on leachate studies (Tang et al. 2005; McMaster et al. 2009).

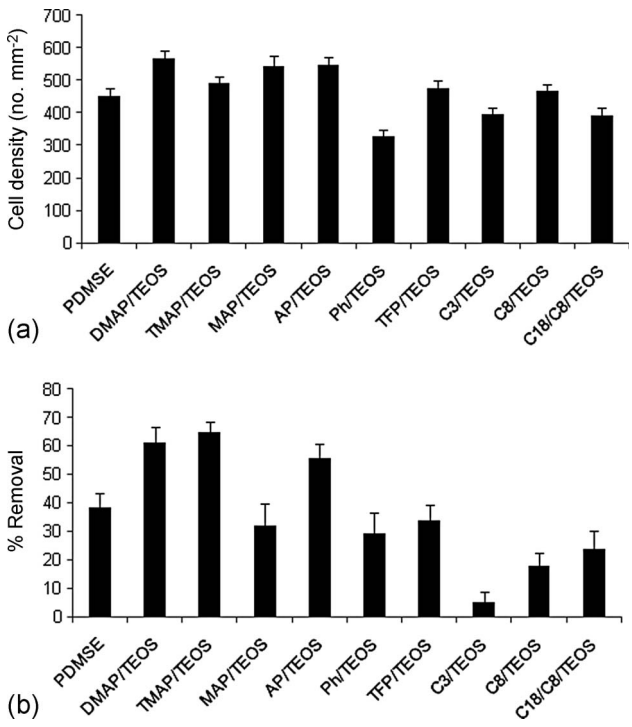

Figure 1. (a) The density of attached cells of *Navicula* on xerogel coatings after gentle washing (initial attachment density) and (b) the percentage removal of cells of *Navicula* under 52 Pa wall shear. Each value is the mean of 90 counts on three replicate slides. Bars = 95% confidence limits.

Discussion

The xerogel surfaces of the present study were robust films with uniform smoothness/roughness, and with values of Young's modulus that indicate the surfaces were much harder than PDMSE (Bennett et al. 2010). The surface topography remains constant in these materials from formulation to formulation allowing trends with respect to fouling and fouling-release to be evaluated in terms of surface energies and chemistries. The values of Young's modulus varied from 0.06 GPa for C8/TEOS to 23.1 GPa for the AP/TEOS films (Bennett et al. 2010). However, because the xerogel coatings were thin (1.0 ± 0.1 μm thick for spin-cast films), the films were unlikely to bend macroscopically in order to release attached fouling and modulus should not impact-release properties, especially of microfouling organisms.

The xerogel surfaces had a range of values of γ_C and γ_S as shown in Table 1. Values of γ_C varied from ~19 mN m^{-1} for the TFP/TEOS film to >30 mN m^{-1} for the AP/TEOS, DMAP/TEOS, and PH/TEOS films. Values of γ_S varied from ~25 mN m^{-1} for the C18/C8/TEOS, C8/TEOS, C3/TEOS, and TFP/TEOS xerogels, to >53 mN m^{-1} for the AP/TEOS and DMAP/TEOS coatings (Bennett et al. 2010). Static

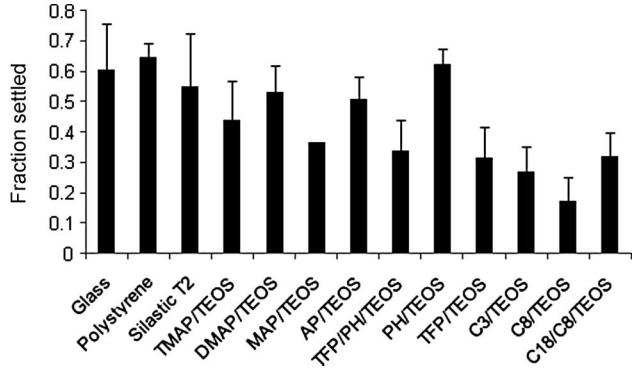

Figure 2. Settlement of barnacle cypris larvae on xerogel coatings applied to glass dishes. Each value is the mean of three replicate measurements. Error bars = the SE of the mean.

water contact angles, θ_{Ws}, varied from 35° for the AP/TEOS coating to 105° for the C18/C8/TEOS coating.

Adhesion force measurements using BSA-coated AFM tips indicated that BSA showed less adhesion to all of the xerogels relative to PDMSE (Silastic T2) controls. Within the xerogel series, the adhesion force was correlated with γ_C and γ_S as shown in Figure 3. The strongest adhesion was observed with both the lowest energy surfaces and the highest energy surfaces. This behavior approximates the 'Baier curve' as shown in Figure 3 (Baier et al. 1968; Baier 1984; Baier and Meyer 1992). The data clearly show a region of minimal BSA adhesion on the xerogel surfaces around 20–25 mN m^{-1} for γ_C and around 30–35 mN m^{-1} for γ_S, which correlate well with the zone of minimal bioadhesion for surfaces with values of γ_C between 20 and 30 mN m^{-1}. In this region, weak boundary layers are formed and fouling (including BSA) can be removed by hydrodynamic shear forces.

As stated previously, diatoms are not motile and reach the substratum surface by sinking through the water column. The density of attached cells after gentle washing was approximately the same on all of the xerogel surfaces as shown in Figure 1a, indicating initial adhesion was similar for all surfaces. In contrast, the ease of removal of adhered cells was highly correlated with θ_{Ws} and γ_C as shown in Figure 4. Diatom removal was greater on xerogel surfaces with lower values of θ_{Ws} ($m = -0.65$, $R^2 = 0.74$, Figure 4a) and higher values of γ_C ($m = 3.13$, $R^2 = 0.69$, Figure 4b). Previous studies using model surfaces of hydroxy terminated alkanethiol SAMs (Finlay et al. 2002) or ethylene glycol SAMs with different terminations (Schilp et al. 2007) have shown that diatoms attach more strongly to hydrophobic surfaces and furthermore that this relationship holds for several diatom species (Holland et al. 2004) and concurs with observations that diatom slimes accumulate on hydrophobic panels immersed in the sea (Terlizzi et al. 2000; Molino et al. 2009a; Tribou and Swain 2010).

Coatings using hydroxyl-terminated alkanethiol SAMs varied the ratio of hydroxydecyl to decyl alkylgroups on the alkanethiols (Finlay et al. 2002). The xerogel surfaces of this study presented alkane, fluoroalkyl, and aminoalkyl functionality to the diatoms. The aminoalkyl functionality included

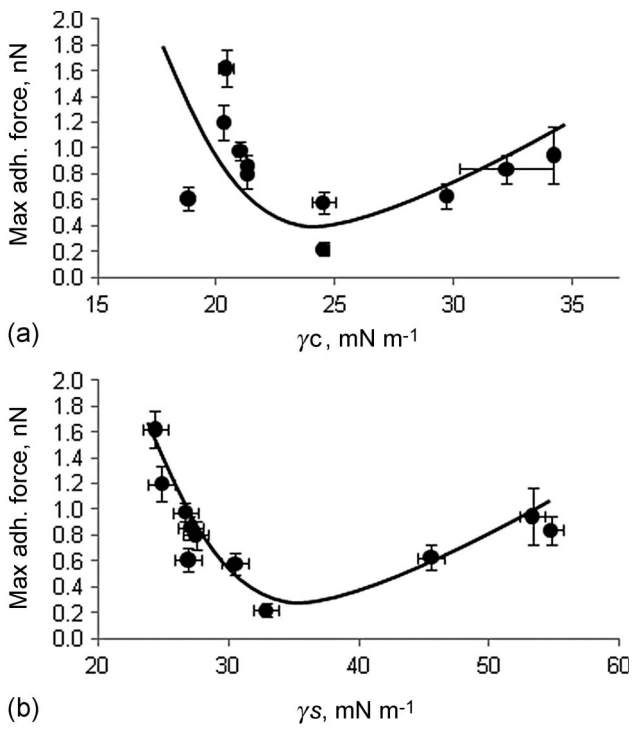

Figure 3. Maximum adhesion force of BSA-coated AFM tips on xerogel surfaces plotted as a function of (a) xerogel critical surface tension and γ_C, (b) xerogel surface energy, γ_S. Each value is the mean of five replicate measurements. Error bars = ±one SD for both the maximum adhesion force (vertical bars) and γ_C or γ_S (horizontal bars). The lines have no mathematical significance, but approximate 'the Baier curve.'

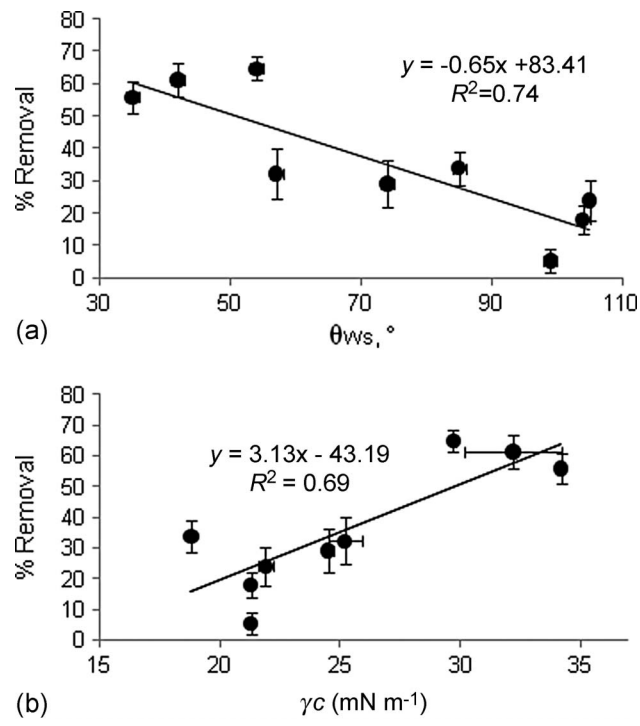

Figure 4. The percentage removal of cells of *Navicula* from xerogel surfaces under 52 Pa wall shear plotted as a function of (a) static water contact angle (θ_{Ws}) and (b) critical surface tension (γ_C) for the sample surfaces. Each value is the mean of 90 counts on three replicate slides. Vertical bars = 95% confidence limits in percentage removal; horizontal bars = ±one SD for (a) static water contact angle (θ_{Ws}) or (b) critical surface tension (γ_C).

aminopropyl and methylaminopropyl groups, which are capable of functioning as hydrogen bond donors or acceptors, dimethylaminopropyl groups, which are capable of functioning only as a hydrogen bond acceptor, and trimethylpropyl ammonium functionality that is permanently positively charged and that functions neither as a hydrogen bond donor nor acceptor. The relationship that diatoms adhere more strongly to hydrophobic surfaces and are more easily removed from hydrophilic surfaces is robust, not only over a range of materials (PDMSE, SAMs, and xerogels), but also over range of surface chemistries.

Cypris larvae of *B. amphitrite* showed reduced settlement on hydrophobic coatings relative to more hydrophilic coatings (Figure 2). The settlement of the barnacle cyprids was highly correlated with θ_{Ws} and γ_C, as shown in Figure 5. Cyprid settlement was reduced on xerogel surfaces with higher values of θ_{Ws} ($m = -0.39$, $R^2 = 0.84$, Figure 5a) and lower values of γ_C ($m = 2.01$, $R^2 = 0.84$, Figure 5b). This behavior is in contrast to that observed for the settlement of

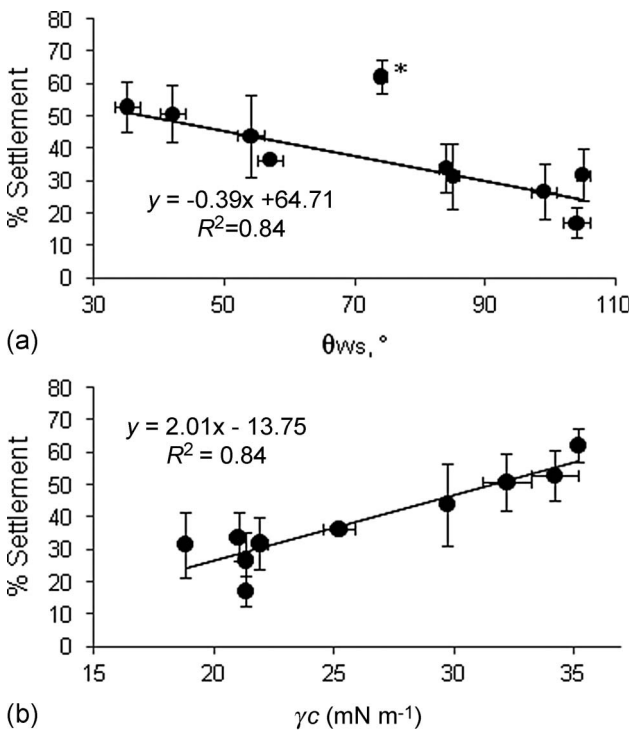

Figure 5. The mean settlement of cypris larvae of *B. amphitrite* on xerogel coatings plotted as a function of (a) static water contact angle (θ_{Ws}) and (b) critical surface tension (γ_C) for the sample surfaces. Each value is the mean of three replicate surfaces. Vertical bars = the SE of the mean for settlement; horizontal bars = $\pm$one SD for (a) static water contact angle (θ_{Ws}) or (b) critical surface tension (γ_C). In (a), the asterisked value was not included in the calculation of slope and R^2. When included, $m = 0.39$ and $R^2 = 0.53$.

zoospores of the macrofouling alga *Ulva linza* on xerogel surfaces where high settlement was associated with low surface energy and higher values of θ_{Ws} (Bennett et al. 2010).

Overall, xerogel surfaces have a high potential as fouling-release or easy-clean materials that can be tailored to perform in locales that favor either macrofouling or microfouling. Xerogel materials offer uniform smoothness/roughness and can be tuned (Cho et al. 2002; Tang et al. 2005; Bennett et al. 2010) to provide surfaces of different water wettability and critical surface tension/surface energy. In earlier studies, several xerogel surfaces were comparable to PDMSE (Silastic T2) with respect to removal of *Ulva* sporelings (young plants) and the ease of removal increased as wettability/surface energy decreased (Bennett et al. 2010). Ideally, a xerogel surface can be designed to provide foul release for both macrofoulers (*Ulva*, barnacles) and microfoulers, which follow opposite trends with respect to adhesion as a function of wettability/surface energy. A surface of intermediate wettability/surface energy might be selected that, while not ideal for either microfoulers or macrofoulers, would provide some release of both classes of fouling. Several of the xerogel surfaces used in this study (AP/TEOS, DMAP/TEOS, TMAP/TEOS) displayed greater removal of diatoms under shear than the PDMSE control while all of the xerogel surfaces of this study showed lower adhesion of the protein BSA than the PDMSE (Silastic T2) standard. The settlement of barnacle cyprids on five of the xerogel surfaces of this study (C18/C8/TEOS, C8/TEOS, C3/TEOS, TFP/TEOS, and TFP/PH/TEOS) were reduced relative to glass and polystyrene controls and the settlement on the C8/TEOS xerogel was significantly less than the PDMSE (Silastic T2) standard ($p < 0.05$ using student T test).

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