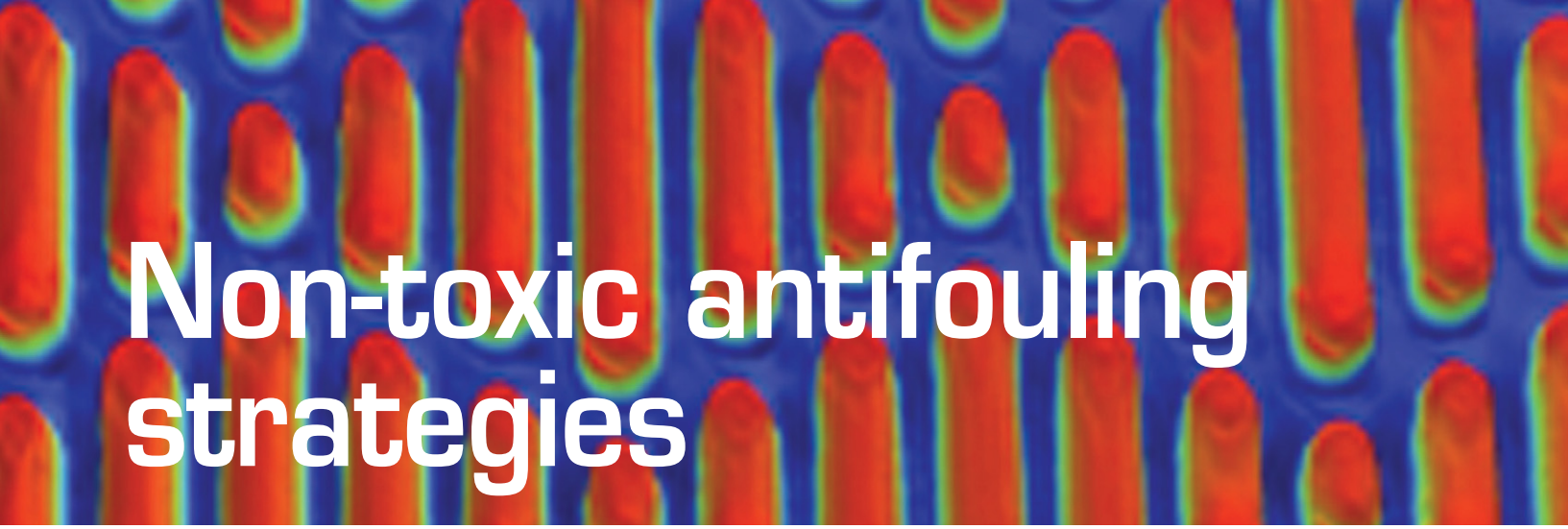

Non-toxic antifouling strategies

The term *fouling* generally refers to an undesirable process in which a surface becomes encrusted with material from the surrounding environment. In the case of *biofouling*, that material consists of organisms and their by-products e.g., extracellular polysaccharides and metabolites. Biofouling limits the performance of devices in numerous applications; however, this review focuses on antifouling biomaterials for marine and biomedical applications. The surface chemistry and physical properties of the substratum are both crucial to preventing the recruitment of biofouling organisms. Natural antifouling surfaces exhibit both chemical and physical attributes. The chemical structure is discussed briefly as it relates to both anti-fouling and fouling-release properties. However, our focus has been to study physical cues as they relate to the initial attachment of fouling organisms.

Chelsea M. Magin[1], Scott P. Cooper[2] & Anthony B. Brennan[1,2,*]

[1] J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville Florida, USA

[2] Department of Materials Science & Engineering, University of Florida, Gainesville Florida, USA

** E-mail: abrennan@mse.ufl.edu*

Biofouling of marine vessels continues to plague sailors as it has for thousands of years[1-7]. The ancient Phoenicians, inventors of the earliest recorded anti-fouling coatings, covered ships with lead sheets[1]. Later in the 17th century metals containing copper were also shown to be effective biofouling deterrents. Metals, such as lead, are effective antifouling agents, but have a negative impact on the environment. Ships are still slowed today by the growth of algae, barnacles, and slime on their hulls due to the absence of a universal, green, antifouling system (Fig. 1). The United States (US) Naval Sea Systems Command estimates that biofouling on ship hulls results in a speed loss of approximately 2% and increases fuel costs 6 to 45% depending on the size of the ship[8]. One source cites total costs associated with biofouling of nearly $1 billion annually[9].

Antifouling, in this review, refers to all systems that prevent an organism from attaching to a surface. Historically, the term antifouling was associated only with biocidal compounds. Current antifouling strategies focus on green, non-toxic technologies. Fouling-release describes the force required to remove an organism that is already attached to a surface. These two terms have been used interchangeably in the literature; however they are truly different phenomena.

ISSN:1369 7021 © Elsevier Ltd 2010

Fig. 1 Macrofouling on the hull of a ship increases drag and fuel consumption. Image courtesy of North Florida Shipyards, Jacksonville, FL.

Antifouling paints have been and remain the primary strategy for combating biofouling in the marine industry. Biocides such as tributyltin (TBT) were developed in the middle of the 20th century and were the active components of antifouling paints until recently[1]. Biocidal paints based on TBT have been effective at reducing biofouling[7,10,11]. However, the use of TBT-based paints has been prohibited because they are detrimental to non-target organisms and the surrounding environment[12]. The response to this ban has been the use of copper, zinc, and a variety of organic compounds as the active, antifouling components. The ideal replacement for TBT is an environmentally neutral coating with both antifouling and fouling-release properties[11,13-15].

Biofouling is a major challenge for the biomedical industry as well. Healthcare associated-infections are attributed to biofilms on surfaces such as countertops, doors, beds, surgical tools, or medical devices such as catheters. The Centers for Disease Control and Prevention have reported that these healthcare-associated infections account for an estimated 1.7 million infections and 99,000 deaths annually in the US[16]. Furthermore, these infections accounted for nearly $45 billion of patient costs in 2007[17]. The formation of an atherosclerotic plaque within the arterial wall can be broadly described as a biofouling process[18]. The American Heart Association reported that 16.8 million people in the US were diagnosed with coronary heart disease in 2006. Coronary heart disease is the leading cause of death in the US. The estimated direct and indirect costs of treating this disease total approximately $165.4 billion per year[19].

Biofouling is a very dynamic process, which spans numerous length and time scales (Figs. 2 and 3). Fouling of a new surface in the marine

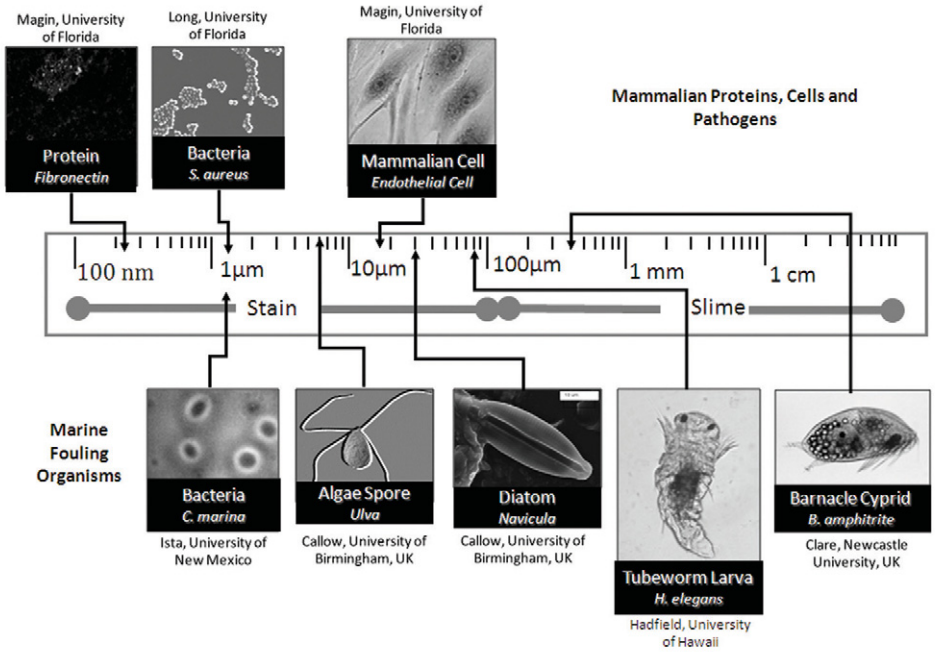

Fig. 2 Schematic demonstrating the hierarchy of fouling organisms. Cells and compounds relevant to biomedical applications are shown above the scale axis. Marine organisms are shown below the scale.

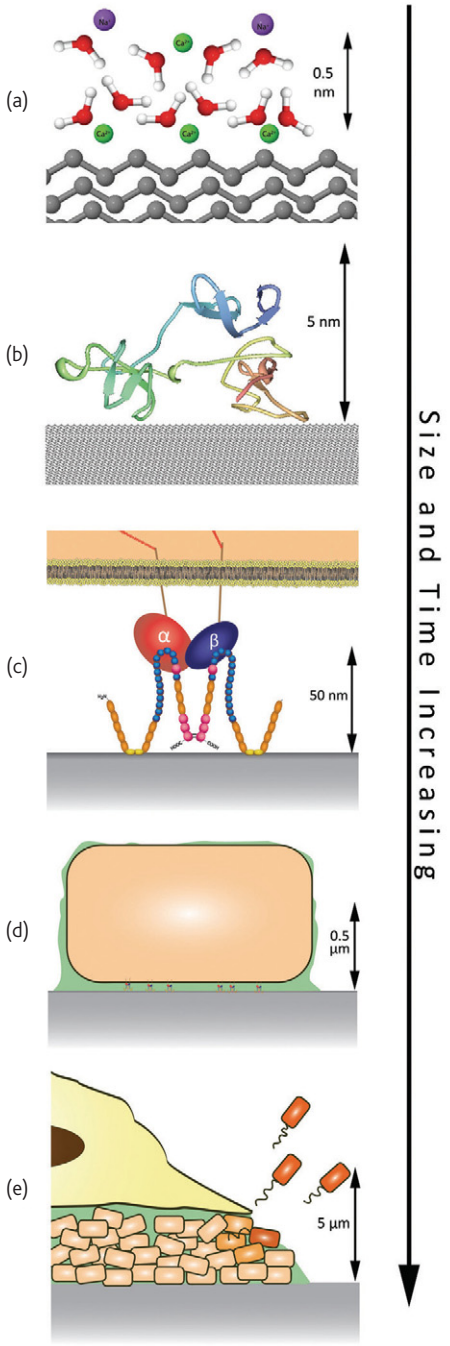

Fig. 3 Schematic of the dynamic biofouling process which takes place over numerous length scales. (a) An electric double layer is established at the surface of a solid such as a linear polymer in less than a second. This electric double layer mediates the adsorption and conformation of proteins. (b) The type-II subunits of fibronectin are shown adsorbed to the surface. These subunits are responsible for binding to gelatin[111]. (c) Fibronectin mediates the binding of a cell to the surface via integrins (shown as α and β subunits) in the cell membrane. The type II domains of fibronectin are shown in yellow. (d) If a bacterial cell is bound to the surface, it undergoes a phenotypic change and excretes an EPS coating. (e) Over time, the cells replicate and continue to build the EPS. The biofilm creates "swarmer" cells, which leave the biofilm to inoculate another surface. Larger cells such as Ulva (in the marine environment) or phagocytes (in the human body) may subsequently interact with the initial biofilm.

environment is typically described as a four phase process: formation of a conditioning layer of organic molecules, primary colonization by micro-organisms such as bacteria and diatoms, unicellular colonization by algal spores, and attachment of multicellular macrofoulers[6,7,20]. Since fouling occurs in an aqueous solution, the properties of the fluid mediate the interaction of the fouling organism and material. Ions and water molecules adsorb to a biomaterial surface to form an electric double layer immediately upon immersion. This electric double layer effectively establishes the charge associated with surface. This electrostatic charge affects the nature of the interaction of proteins and cells with the surface. Antifouling performance scales with both density and sign of the charge[21].

A layer of proteins adsorbs to a pristine surface within seconds to minutes following immersion[22]. The protein conformation is strongly influenced by both the physical and chemical properties of the surface, including electrostatic charge. Protein conformation defines functionality with respect to cell adhesion[23,24]. This protein layer acts as a conditioning film for the settlement of micro-organisms such as diatoms and bacteria.

A biofilm can be defined as a community of attached micro-organisms connected by an extracellular polysaccharide (EPS) coating. Bacteria undergo multiple developmental stages from planktonic to attached cells. This transformation from the planktonic to attached state induces a phenotypic change that facilitates increased secretion of an EPS coating[25]. The EPS coating is both an adhesive and protective layer that modulates the diffusion of molecules in the biofilm. Consequently, cells in biofilms are more resistant to antibiotics and antibacterial agents[26]. Natural biofilms are composed of several microbial species and their EPS coatings. These cells along with protein and enzyme structures form complex, functional micro-colonies. It was first observed by Zobell and Allen in 1935 that biofilms could stimulate the settlement of secondary macro-organisms[27] such as algal spores[28-30] and larvae of barnacles and tubeworms[31,32]. Reviews on the subject indicate that marine biofilms can also inhibit or have no effect at all on settlement of macro-organisms[33,34]. The interaction between a marine biofilm and secondary colonizers is a complex interplay of surface chemistry, micro-topography, and microbial products i.e., low molecular weight metabolites involved in quorum sensing[34].The diversity of species resulting from various geographic locations creates a broad spectrum of physical, chemical and biological attributes. We have investigated natural structures that are able to resist the adhesion of these complex fouling communities. This review discusses natural surfaces as well as physico-chemical and physical antifouling strategies.

Natural antifouling surfaces

There are natural surfaces that resist biofouling in the marine and the biomedical environments. These natural antifouling surfaces appear to use a combination of chemical and physical structures to inhibit

biofouling. Marine organisms such as sharks, mussels, and crabs have natural antifouling defenses. The endothelium of a healthy artery is another example of a natural antifouling system (Fig. 4). However, it is also recognized that these surfaces will lose their antifouling characteristics due to age, injury or disease.

The skin of the approximately 900 species of Elasmobranchii, which include sharks, skates, and rays is embedded with placoid scales[35]. These scales have a vascular core of dentine surrounded by an acellular "enamel" layer similar to human teeth. For this reason, placoid scales are commonly referred to as dermal denticles. Denticles serve several functions including reduction of mechanical abrasion, reduced hydrodynamic drag[36] and most interestingly protection from ectoparasites[37]. The skin of two members of the porpoise family, i.e., the bottlenose dolphin *Tursiops truncatus* and the killer whale *Orcinus orca*, forms a system of ridges and grooves oriented transversely to the direction of flow. The natural wavelength of the ridges and grooves is 0.3 to 0.4mm with a trough to crest wave height of about 10μm[38]. These topographic features and a mucosal coating secreted by epidermal cells contribute to the antifouling properties of these marine animals.

The microtopographically structured periostraca on shells of the blue mussels *Mytilus galloprovincialis*[39] and *Mytilus edulis*[40] are also effective antifouling surfaces. The grooves and ridges of the periostraca are 1 to 2 μm wide with an average depth of 1.5 μm. The shells of *M. galloprovincialis* significantly reduced settlement of barnacle larvae during a 14 week field exposure trial[39]. Microtopography replicates cast in epoxy resin from the blue mussel *M. edulis*, edible crabs *Cancer pagurus*, the egg-case of the lesser-spotted dogfish *Scyliorhinus canicula*, and the brittle star *Ophiura texturata* reduced fouling for three to four weeks[40]. The short-term performance

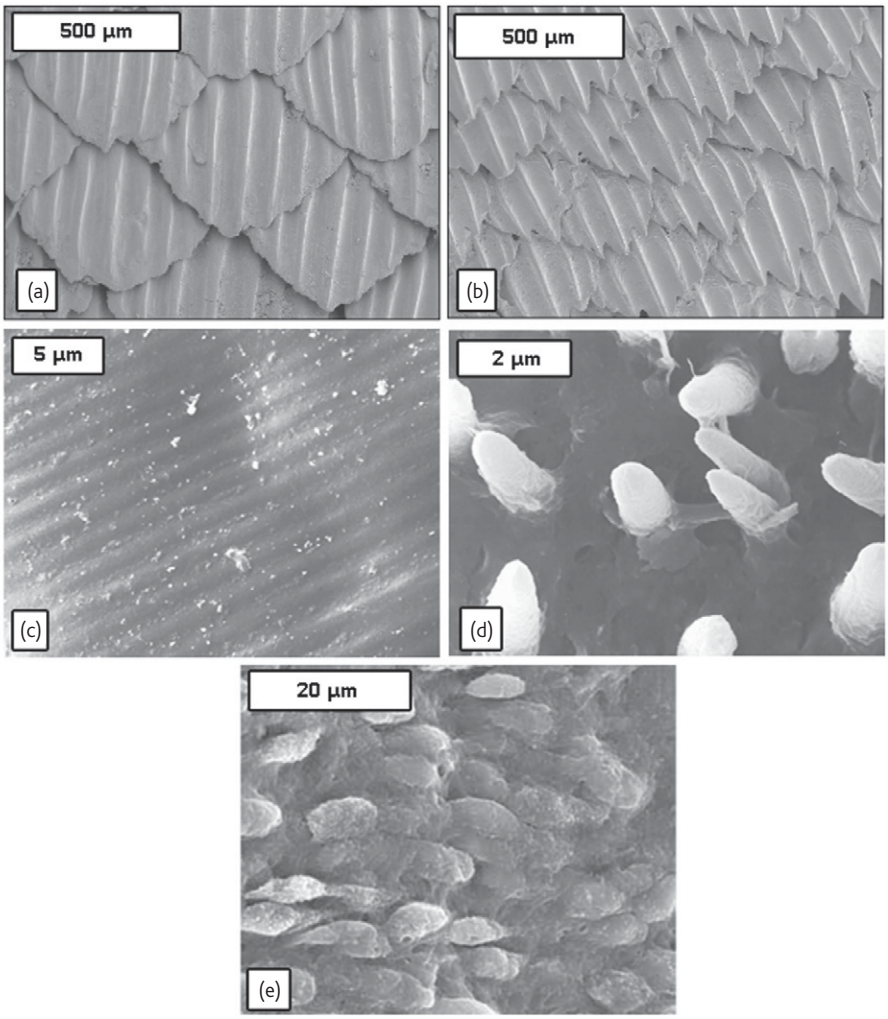

Fig. 4 Scanning electron micrographs of natural textured surfaces: a) Spinner shark skin, b) Galapagos shark skin, c) Mussel shell (M. edulis) and d) Crab shell (C. pagurus) reprinted from[40] with permission from the publisher Taylor & Francis Group (http://www.informaworld.com), e) Porcine pulmonary artery reprinted from[83] with permission from Elsevier.

implies that natural antifouling is a combination of chemistry and microtopography.

The inner surface of a blood vessel is another natural surface that resists the constant presence of fouling proteins and cells. The endothelium consists of a continuous monolayer of endothelial cells with a cobblestone-like morphology and a distinct topography (Fig. 4). Endothelial cells express a negatively charged glycoprotein coat that repels platelets and leukocytes. These cells also secrete bioactive substances that inhibit thrombosis and smooth muscle cell proliferation[41,42]. This combination of chemistry and microtopography creates an ideal anti-thrombogenic, i.e., antifouling, surface.

Physico-chemical antifouling strategies

Surface chemistry is a significant factor in the formation, stability, and release of adhesion of fouling organisms to surfaces. The work by Baier in the late 1960s demonstrated a correlation between relative adhesion of fouling organisms and the energy of the surface[43]. The Baier curve (Fig. 5), as this relationship is known, has been confirmed in several marine and biomedical environments[15,43]. A key characteristic of the Baier curve is that minimal fouling is typically achieved at a critical surface tension of 22-24 mN/m. This surface tension, often referred to as surface energy, is approximately equal to the dispersive component for water. In an aqueous system, water must rewet the system when proteins and cells are removed. For solids with a surface energy of ~22mN/m, the thermodynamic "cost" for water to re-wet the surface is minimized.

One way of systematically varying surface energy without altering the bulk material is through self-assembled monolayer (SAMs). In an extensive study, Whitesides and co-workers tested the ability of a wide range of SAM chemistries to resist protein adsorption. The authors conclude that SAMs, which are hydrophilic, electrically neutral, and contain hydrogen bond acceptors, are most effective at resisting protein adhesion. Zwitterionic structures have both positive and negative domains, but remain electrically neutral overall. It has been demonstrated that zwitterionic compounds similar to phosphorylcholine such as sulfobetaine resisted protein adsorption when the surface density and chain length of the SAMS were carefully controlled[44,45].

Even though surface energies for poly(ethylene glycol)(PEG) and its oligomers typically fall above the zone of low cell adhesion defined by Baier, it is widely recognized that these materials exhibit resistance to protein adsorption and biofouling [44,46-48]. The mechanism for protein resistance for high molecular weight PEG is well explained by steric repulsion[49]. Andrade and de Gennes postulated that during protein adsorption water must be removed from the PEG structure. This dehydration is thermodynamically unfavorable because it leads to confinement of polymer chains which previously had high conformational entropy. Even though the model system of oligo(ethylene glycol) SAMs tested by Whitesides restricted conformational freedom of end groups into densely packed films, these surfaces also showed protein repellent properties. Grunze and others have proposed that the chain conformation and packing of SAMs affect the penetration of water into the SAM surface and are also important determinants of resistance to protein adsorption[47,50].

The surface chemistry of SAMs is strongly influenced by their physical structure. Ethylene-glycol terminated SAMs have been shown to be especially fouling-resistant in numerous studies. *Ulva*

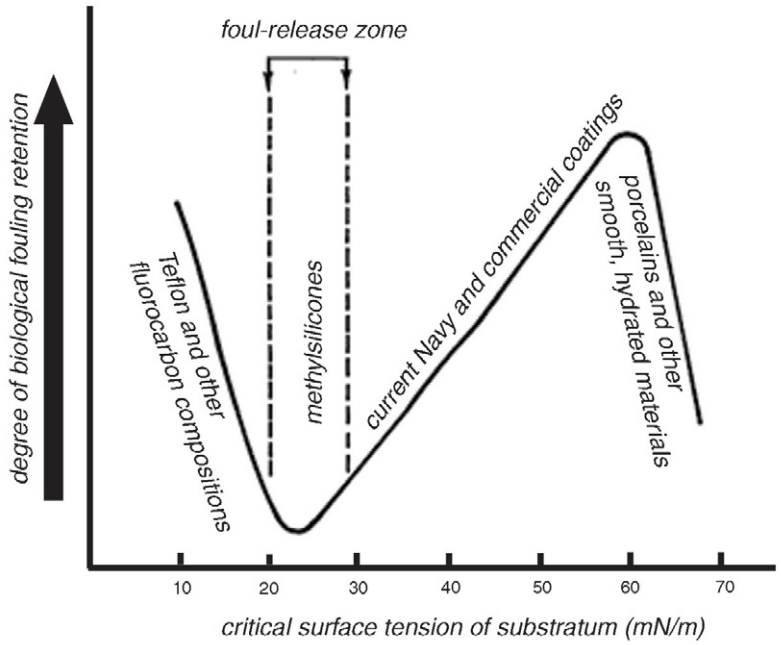

Fig. 5 The Baier curve demonstrates the relative amount of biofouling versus critical surface tension of the substrate. Reprinted from[43] with kind permission from Springer Science + Business Media.

zoospore attachment to SAMs systematically increased with decreasing wettability and correlated with adsorption of the protein fibrinogen[48]. Experiments have shown that higher numbers of *Ulva* spores attach to hydrophobic SAMs versus hydrophilic ones in static assays[51]. However, the attachment strength of *Ulva* spores is greater on hydrophilic SAMs[52]. The mechanism for delay of *Ulva* attachment by PEG-based surfaces is not fully understood. However, like resistance to protein adsorption, infiltration of water into the SAM surfaces may create a hydration energy that prevents effective interaction of the adhesive used by Ulva with the surface[48].

Bowen, *et al*. tested the effect of SAM chain length on the settlement and release of zoospores of *Ulva* and cells of the diatom *Navicula perminuta*. This study showed that chain length affected release more than settlement. Alkane chains greater than 12 carbons long corresponded to higher release of these organisms under flow. This fouling-release behavior is associated with greater rigidity of the alkane chain and subsequently higher lubricity[53].

Attachment of a medically relevant bacterium (*Staphylococcus epidermidis*) and a marine bacterium (*Cobetia marina*) was reduced up to 99.7% by surfaces coated with hexa(ethylene glycol)-terminated SAMs[54]. The response of *C. marina* to surface energy was opposite of that predicted by the Baier curve, i.e., attachment density increased with decreasing surface energy. Attachment of *Ulva* showed the same relationship only when the cosine of the advancing water contact angle was greater than zero ($\cos\theta_{AW} \geq 0$)[55].

Hydrogels – crosslinked polymer networks that swell in the presence of water – have also been investigated for antifouling applications. Rasmussen et al. demonstrated that hydrogel surfaces of alginate, chitosan, and polyvinyl alcohol substituted with stilbazolium groups (PVA-SbQ) inhibited settlement of *Balanus amphitrite*[56]. This group also showed that the PVA-SbQ surface inhibited adhesion of the marine bacterium *Pseudomonas* sp. NCIMB2021[57]. Hydrogels based on 2-hydroxyethyl methacrylate (HEMA) reduced fouling in two algal colonization bioassays and with the addition of benzalkonium chloride remained visually clean in field testing for up to 12 weeks[58]. Crosslinked poly(ethylene glycol) diacrylate surfaces were evaluated as fouling-resistant membrane coatings. Surfaces that were more hydrophilic based on contact angle measurements exhibited less protein adsorption[59]. The antifouling character of these surfaces is representative of high surface energy regime of the Baier curve.

Amphiphilic surfaces and heterogenous surfaces formed by patterning or mixing chemistries are other examples of nontoxic polymer coating designs that have shown antifouling properties[60]. Self-assembled and nano-structured polymer thin films were also reviewed in the context of antifouling[61]. Another class of chemical deterrents to biofouling includes naturally occurring biomolecules. For instance, it has been proposed that enzymes could break down the EPS of attached cells[62,63] or catalyze the production of repellent compounds[64,65]. However, it remains difficult to identify a single enzyme which is effective universally. Numerous chemicals have been isolated from natural sources and several reviews discuss specific strategies in detail[66-69]. Dalsin, *et al.*[70] have provided an extensive review of bioinspired polymers. These chemistries attempt to mimic the complex biopolymers which naturally resist fouling, such as the adhesive pad of the mussel.

It is clear that protein adsorption and subsequent biofouling are strongly influenced by surface chemistry. Correlations have been observed between protein adsorption and biofouling in both the marine and biomedical environments. Resistance to protein adsorption could be used as an inexpensive way to screen new materials for antifouling properties. A single chemistry has not yet emerged as a universal antifouling strategy. However, a variety of surface chemistries have shown promise as fouling-release coatings. A combination of chemical and physical antifouling strategies is therefore necessary to produce an optimal coating.

Physical antifouling strategies

It has been recognized that cells respond to substratum topography since 1914 when Harrison observed that fibroblasts found in the embryonic nervous tissue of frogs elongated when cultured on spider silk[71]. This phenomenon was later termed "contact guidance" by Paul Weiss after obtaining similar results when growing nerve cells on glass fibers[72]. Recently, techniques developed in the microelectronics industry, such as photolithography and electron beam lithography, have been used by several research groups including our own to create molds for producing micro- and nano-scaled topographies with various shapes and spatial arrangements[73-75]. Microtopography, in the marine environment, has been shown to deter biofouling on mollusk shells[39,40] and affect attachment of barnacles[76,77] and bacteria[78].

Nearly eight years ago our group designed engineered microtopographies composed of pillars or ridges with various heights

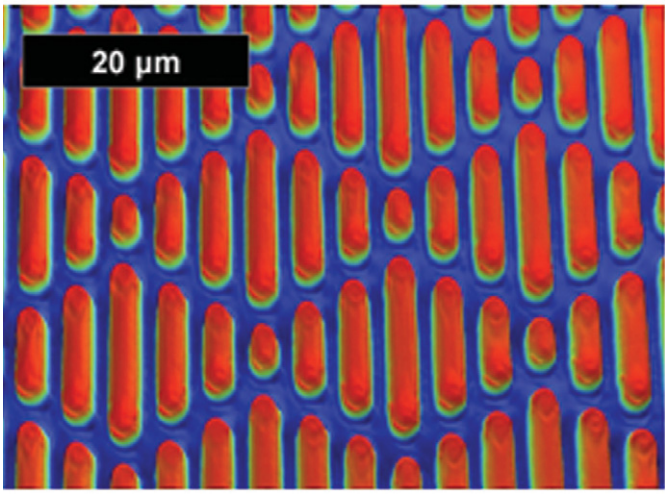

Fig. 6 White light optical profilometry image of Sharklet AF ™.

(5 or 1.5 μm) and spacings (5 or 20 μm) using photolithographic techniques. These particular patterns were found to systematically enhance settlement of the spores of *Ulva* when created in poly(dimethyl siloxane) elastomer (PDMSe)[79]. The addition of silicone oils to the PDMSe reduced overall *Ulva* settlement, but did not decrease settlement on microtopographies compared to smooth control surfaces[80,81]. Carman *et al.* demonstrated in 2006 that a bio-inspired surface, Sharklet AF™ (Fig. 6), reduced *Ulva* settlement by 86% compared to smooth when feature width and spacing were 2 μm[74]. These dimensions are smaller than the average diameter of the spore body of *Ulva* (~5 μm). These experiments implied that the width and spacing of topographical features necessary to deter biofouling must be tailored to the size of the organism.

Contact guidance was observed for endothelial cells cultured on ridges, pillars, and Sharklet AF™ topographies[74,80]. Additionally, Feinberg[82] demonstrated that a pattern of 3 μm diameter circles of the ECM protein fibronectin on PDMSe could be used to direct formation of focal adhesions and grow an endothelial cell monolayer with density and morphology similar to that of the native artery[83]. Hatcher and Seegert[84] showed that scaffolds of various porosities made from polyvinylpyrrolidone modified bioactive glass fibers could increase proliferation of rat mesenchymal stem cells preceding differentiation. Chung and others demonstrated that the Sharklet AF™ topography inhibited biofilm formation of *Staphylococcus aureus* over a period of 21 days[85].

The change in wettability of a surface due to microtopographical roughness is also likely to be a contributing factor to antifouling properties. The topic of wetting and dewetting on rough surfaces has been thoroughly reviewed by Quéré and colleagues[86-88]. The application of surface roughness to alter wettability for antifouling coatings especially superhydrophobic coatings has also been reviewed extensively[10,13,14]. Long and others reported recently that seven different engineered microtopographies exhibited contact angle anisotropy between contact angles measured parallel and perpendicular to the features[89]. This work demonstrates the importance of anisotropy in the design and study of antifouling surfaces.

An engineered roughness index was developed that demonstrated a negative correlation between the settlement behavior of the zoospore of *Ulva* with wettability of engineered microtopographies (Fig. 7). The original ERI empirically ratios the product of Wenzel's roughness factor[90] (r) and the degrees of freedom of the pattern (*df*) to the depressed surface area fraction (1- Φ_S)[91]. Bico, Quéré, and others[86-88] described the surface solid fraction (*1*- Φ_S) as the ratio of the depressed surface area between features and the projected planar surface area. The surface solid fraction is equivalent to 1-f_1, the solid-liquid interface term of the Cassie-Baxter equation for wetting[92].

A biological attachment model based on a modified ERI was recently proposed by Long *et al*[93]. In this model the ERI was changed by replacing the degrees of freedom (*df*) of the pattern with the number of distinct features in the pattern (n). The number of attached organisms per area was normalized to the number of organisms attached to a smooth control. The data were transformed by taking the natural logarithm (Eq. 1).

$$\ln\left(\frac{A}{A_{SM}}\right) = m * \frac{r * n}{1 - \varphi_s} - b \qquad (1)$$

This transformation unified the data from numerous experiments onto a single plot. The attachment density of spores of *Ulva* for all of the experiments showed a high statistical correlation (R^2=0.88) to the attachment model. The attachment model also correctly predicted a further reduction of *Ulva* attachment on a newly designed topography with a higher ERI value[93]. This relationship can be used

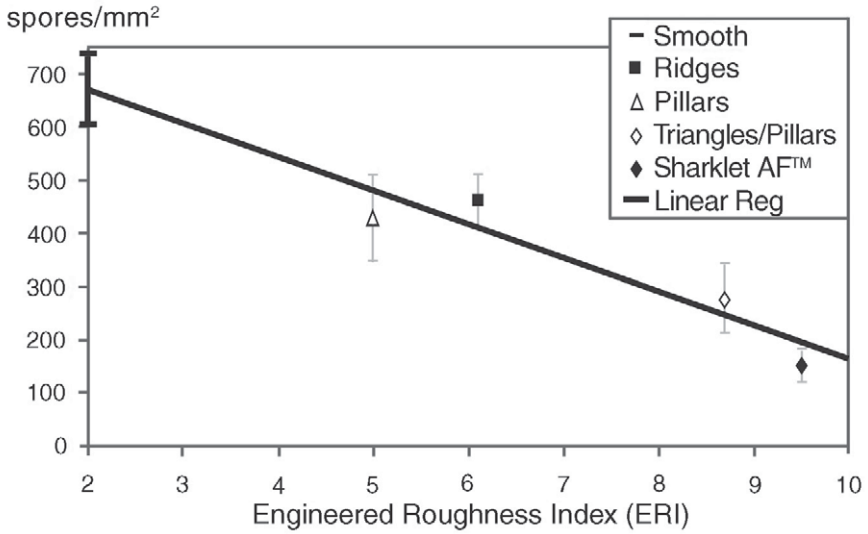

Fig. 7 Correlation of Ulva spore settlement density and Engineered Roughness Index (ERI). The calculated ERI for the tested PDMSe surfaces is plotted against the experimental mean spore density (spores/mm2)+SE (n=3). Reprinted from[77] with permission from the publisher Taylor & Francis Group (http://www.informaworld.com).

to create new engineered microtopographies that further reduce *Ulva* attachment.

The elastic modulus of a substratum is another physical factor that has been shown to influence bioadhesion. The adhesion strength of a disc to an elastomeric substrate was proposed by Kendall[94] as:

$$P = \frac{F_c}{\pi a^2} = \left(\frac{8\pi\gamma E a^3}{(1-\nu^2)}\right)^{1/2} \quad (2)$$

In which P = critical stress for removing the disc, F_c = critical force, a = radius of the disc, γ = interfacial energy between the disc and substreate, E = elastic modulus of the substrate, and ν is Poisson's ratio. A similar analysis by Brady demonstrated a correlation between elastic modulus and surface energy of a material[94,95]. Vascular graft research has shown that intimal hyperplasia can be caused by compliance mismatch between the graft and the vessel wall and poor re-endothelialization of the luminal surface[96]. It has also been reported that substratum elasticity directs stem cell differentiation into specific lineages[97]. Likewise, in the area of marine biofouling it has been proposed that the release behavior of pseudobarnacles and spores from various coatings is inversely proportional to the pull-off stress and scales with elastic modulus ($E^{1/2}$)[98,99].

The importance of hydrodynamics to the fouling process cannot be overlooked. Work by Crisp in 1955 showed that there is a critical velocity gradient at the surface for barnacle cyprids to attach[100]. A critical observation by Purcell[101] states that our physical intuition of swimming does not apply to microorganisms. Bacteria and cells swim in an environment of very low Reynolds number (*E. coli*, Re~10^{-5}). As a result, these organisms live in a world where viscous forces dominate over inertial forces. It has been demonstrated both empirically and experimentally that *E. coli* is attracted to the walls of a container purely by hydrodynamic interactions[102,103]. This hydrodynamic attraction is similar to other phenomena described by Vogel[104]. For instance, if two spheres fall next to each other in a fluid, they are attracted to each other by viscous forces. These hydrodynamic interactions may initiate the settlement process by allowing the organism to "find" the surface.

One approach to create new antifouling surfaces may be to utilize the concept of fluid slip. Fluid slip is the boundary condition in which the fluid has a finite velocity at an interface[105]. This is in contrast to the "no slip" boundary condition which is commonly assumed in fluid mechanics. The no slip boundary condition is relevant to a fluids moving over air, which occurs in the case of superhydrophobic materials in the "non-wetted" or Cassie-Baxter state[106-108]. It may be possible to prevent hydrodynamic attraction of swimming organisms through the use of fluid slip.

Fluid hydrodynamics also contributes to the antifouling character of biological tissues. In the case of vascular implants, thrombus formation is a common problem. Thrombogenesis follows a typical biofouling cascade in which proteins that are present in blood adsorb to the surface, followed by platelets and red blood cells. Therefore, a healthy endothelium requires a constant supply of both thrombogenic and anti-coagulant factors. These factors are maintained by fluid flow through the blood vessel[109]. Fluid shear affects platelet and red blood cell physiology and subsequent thrombus formation[110]. The disruption of native fluid flow in a vessel – either by injury or placement of an implant – influences the balance of these thrombogenic factors. Therefore, fluid flow plays an integral role in the fouling process.

Conclusion

Biofouling is a dynamic process which spans numerous length scales and involves a complex variety of molecules and organisms. Antifouling strategies, therefore, must include both chemical and physical concepts. Nature provides examples of antifouling and fouling-release surfaces that emphasize the importance of these factors. Physical cues, such as surface roughness and fluid hydrodynamics, can act singularly or in concert with surface chemistry to enhance or inhibit the attachment of organisms to a surface. Chemical cues, especially surface energy, influence not only the ability of an organism to initially attach to a surface, but also the degree of fouling-release from the surface once adhesion has been established. At this point, no single technology has been demonstrated universally effective at either antifouling or fouling-release. The environmental impacts of biofouling demonstrate the need to continue the development of strategies that are truly non-toxic and broadly effective. Confronting the complexity of biofouling requires the cooperative effort of industry and academia in all disciplines of science and engineering. mt

Acknowledgements

Authors thank the Office of Naval Research for financial support – Contract #N00014-02-1-0325.

REFERENCES

1 Yebra, D. M., *et al., Prog Org Coat* (2004) **50** (2), 75.
2 Grozea, C. M., and Walker, G. C., *Soft Matter* (2009) **5** (21), 4088.
3 Genzer, J., and Efimenko, K., *Biofouling* (2006) **22** (5), 339.
4 Ralston, E., and Swain, G., *Bioinspir Biomim* (2009) **4** (1), 15007.
5 Callow, M. E., and Callow, J. A., *Biologist* (2002) **49** (1), 10.
6 Wahl, M., *Mar Ecol-Prog Ser* (1989) **58**, 175.
7 Chambers, L., *et al., Surf Coat Tech* (2006) **201**, 3642.
8 Ingle, M., presented at the ONR Program Review, Charleston, SC, 2008 (unpublished).
9 Callow, M. E., and Callow, J. A., *Biologist* (2002) **49** (1), 1-5.
10 Howell, D., and Behrends, B., *Biofouling* (2006) **22** (6), 401.

11 Yebra, D. M., *et al., Prog Org Coat* (2004) **50**, 75.
12 Sonak, S., *et al., J Environ Manage* (2009) **90**, (1), S96.
13 Genzer, J., and Efimenko, K., *Biofouling* (2006) **22** (5), 339.
14 Marmur, A., *Biofouling* (2006) **22** (2), 107.
15 Goupil, D. W., DePalma, V.A., & Baier, R.E., presented at the Marine Industries: Problems & Opportunities, Washington, D.C., 1973 (unpublished).
16 Klevens, R. M., *et al., Public Health Rep* (2002) **122**, 160.
17 Scott, R. D. I., *The Direct Medical Costs of Healthcare-Associated Infections in U.S.* Hospitals and the Benefits of Prevention, (2009).
18 Libby, P., and Theroux, P., *Circulation* (2005) **111**, 3481.
19 Heart Disease & Stroke Statistics, (2009).
20 Abarzua, S., and Jakubowski, S., *Mar Ecol-Prog Ser* (1995) **123**, 301.
21 Ostuni, E., *et al., Langmuir* (2001) **17** (18), 5605.
22 Vroman, L., *et al., Adv Chem Ser* (1982) **199**, 265.
23 Williams, E. C., *et al., J Biol Chem* (1982) **257** (24), 14973.
24 Bergkvist, M., *et al., J Biomed Mater Res* (2003) **64A**, 349.
25 Stewart, P. S., and Franklin, M. J., Nat Rev Microbiol (2008) **6**, 199.
26 Costerton, J. W. *et al., Ann Rev Microbiol* (1987) **41**, 435.
27 Zobell, C. E., and Allen, E. C., *J Bacteriol* (1935) **29** (3), 239.
28 Joint, I., *et al., Science* (2002) **298**, 1207.
29 Patel, P., *et al., Environ Microbiol* (2003) **5** (5), 338.
30 Marshall, K., *et al., Microbial Ecology* (2006) **52**, 302.
31 Hadfield, M. G., and Paul, V. J., N*atural Chemical Cues for Settlement and Metamorphosis of Marine-Invertebrate Larvae in Marine Chemical Ecology*, (eds.) James B. McClintock & Bill J. Baker (CRC Press, Boca Raton, FL, 2001), pp 431-461.
32 Zardus, J. D., *et al., Biol Bull* (2008) **214**, 91.
33 Dobretsov, S., *Inhibition and Induction of Marine Biofouling by Biofilms in Marine and Industrial Biofouling,* (eds.) Flemming, H. -C., Sriyutha Murthy, P., Venkatesan, R., and Cooksey, K. E., (Springer-Verlag, Berlin Heidelberg, 2008), Vol. 4.
34 Qian, P. -Y., *et al., Mar Biotechnol* (2007) **9** (4), 399.
35 Bone, Q. and Moore, R. H., *Biology of Fishes*. (Taylor & Francis Group, New York, NY, 1995).
36 Lang, A. W., *et al., Bioinsp Biomim* (2008) **3**, 046005.
37 Raschi, W., & Tabit, C., *Aust J Mar Freshwater Res* (1992) **43**, 123.
38 Gucinski, H., *et al., presented at the 6th International Congress on Marine Corrosion and Fouling,* Athens Greece, 1984 (unpublished).
39 Scardino, A., *et al., Biofouling* (2002) **19**, 221.
40 Bers, V. A. and Wahl, M., *Biofouling* (2004) **20** (1), 43.
41 Biology of the Arterial Wall. (Kluwer Academic Publishers, Dordrecht/Boston/London, 1999).
42 Xue, L. and Greisler, H. P., *Blood Vessels in Principles of Tissue Engineering*, (eds.) Lanza, R. P., Langer, R., and Vacanti, J. P., (Academic Press, New York, 2000).
43 Baier, R. E., *J Mater Sci-Mater M* (2006) **17**, 1057.
44 Ostuni, E., *et al., Langmuir* (2001) **17**, 5605.
45 Chang, Y., *et al., Langmuir* (2008) **24**, 5453.
46 Ekblad, T. *et al., Biomacromolecules* (2008) **9**, 2775.
47 Balamurugan, S., *et al., J. Am. Chem. Soc.* (2005) **127**, 14548.
48 Schilp, S., *et al., Biointerphases* (2007) **2** (4), 143.
49 Jeon, S. I., *et al., J Colloid Interf Sci* (1991) **142** (1), 149.
50 Herrwerth, S., *et al., J Am Chem Soc* (2003) **125**, 9359.
51 Callow, M. E., *et al., Appl Environ Microb* (2000) **66** (8), 3249.
52 Finlay, J. A., *et al., Biofouling* (2002) **18** (4), 251.
53 Bowen, J., *et al., J R Soc Interface* (2007) **4** (14), 473.
54 Ista, L. K., *et al., FEMS Microbiology Letters* (1996) **142** (1), 59.
55 Ista, L. K. *et al., Appl Environ Microb* (2004) **70** (7), 4151.
56 Rasmussen, K., *et al., Biofouling* (2002) **18** (3), 177.
57 Rasmussen, K., and Ostgaard, K., *Water Research* (2003) **37**, 519.
58 Cowling, M. J., *et al., Sci Total Environ* (2000) **258**, 129.
59 Ju, H., *et al., J Membrane Sci* (2009) **330**, 180.
60 Grozea, C. M., and Walker, G. C., *Soft Matter* (2009) **5**, 4088.
61 Krishnan, S., *et al., J Mater Chem* (2008) **18**, 3405.
62 Dobretsov, S., *et al., Mar Biotechnol* (2007) **9** (3), 388.
63 Olsen, S. M., *et al., Biofouling* (2007) **23** (5), 369.
64 McMaster, D. M. *et al., Biofouling* (2009) **25** (1), 21.
65 Kristensen, J. B. *et al., Biotechnol Adv* (2008) **26** (5), 471.
66 Pawlik, J. R., *An Annual Review* (1992) **30**, 273.
67 Clare, A. S., *Biofouling* (1996) **9** (3), 211.
68 Rittschof, D., *Biofouling* (2000) **15** (1-3), 119.
69 Fusetani, N., *Nat Prod Rep* (2004) **21**, 94.
70 Dalsin, J. L., and Messersmith, P. B., *Materials Today* (2005) **8** (9), 38.
71 Harrison, R., *J Exp Zool* (1914) **17** (4), 521.
72 Weiss, P., *J Exp Zool* (1945)**100** (3), 353.
73 Curtis, A. S. G., and Wilkinson, C. D., *J Biomater Sci Polymer Edn* (1998) **9** (12), 1313.
74 Carman, M. L. *et al., Biofouling* (2006) **22** (1), 11.
75 van Kooten, T. G., and von Recum, A. F., *Tissue Eng* (1999) **5** (3), 223.
76 Berntsson, K., *et al., J Exp Mar Biol Ecol* (2000) **251**, 59.
77 Schumacher, J. F. *et al., Biofouling* (2007) **23** (5), 307.
78 Scheuerman, T. R., *et al., J Colloid Interf Sci* (1998) **208**, 23.
79 Callow, M. E., *et al., Biofouling* (2002) **18** (3), 237.
80 Feinberg, A. W., *et al.,* 2003 (unpublished).
81 Hoipemeier-Wilson, L., *et al., Biofouling* (2004) **20** (1), 53.
82 Feinberg, A. W., *Dissertation*, University of Florida, 2004.
83 Feinberg, A. W., *Acta Biomater* (2009) **5**, 2013.
84 Hatcher, B. M., *et al., J Biomed Mater Res A* (2002) **66A** (4), 840.
85 Chung, K. K., *et al., Biointerphases* (2007) **2** (2), 89.
86 Bico, J., *et al., Europhys Lett* (1999) **47** (2), 220.
87 Bico, J., *et al., Colloid Surface A* (2002) **206** (1-3), 41.
88 Quere, D., *Annu Rev Mater Res* (2008) **38**, 71.
89 Long, C. J., *et al., Langmuir* (2009) **25** (22), 12982.
90 Wenzel, R. N., *Ind Eng Chem* (1936) **28** (8), 988.
91 Schumacher, J. F. *et al., Biofouling* (2007) **23** (1), 55.
92 Cassie, A. B. D., and Baxter, S., *T Faraday Soc* (1944) **40**, 546.
93 Long, C. J. *et al., Biofouling* (2010) **26** (4), 411.
94 Kendall, K., *J Phys D: Appl Phys* (1971) **4** (8), 1186.
95 Brady, R. F., *Prog Org Coat* (1999) **35**, 31.
96 Tai, N. R., *et al., Brit J Surg* (2000) **87**, 1516.
97 Engler, A. J., *et al., Cell* (2006)**126** (4), 677.
98 Brady, R. F., *Prog Org Coat* (2001) **43**, 188.
99 Chaudhury, M. K., *et al., Biofouling* (2005) **2** (1), 41.
100 Crisp, D. J., *J Exp Biol* (1955) **32** (3), 569.
101 Purcell, E. M., *Am J Phys* (1977) **45** (1), 3.
102 Berke, A. P., *et al., Phys. Rev. Lett.* (2008) **101** (3), 4.
103 Lauga, E. and Powers, T. R., *Rep Prog Phys* (2009) **9**, 096601.
104 Vogel, S., *Life in Moving Fluids: The Physical Biology of Flow.* (Princeton University Press, Princeton, NJ, 1994).
105 Granick, S., *et al., Nat Mater* (2003) **2** (4), 221.
106 Ybert, C., *et al., Phys Fluids* (2007) **19** (12), 10.
107 Voronov, R. S., *et al., Ind Eng Chem Res* (2008) **47** (8), 2455.
108 Lee, C. and Kim, C. -J., *Langmuir* (2009) **25** (21), 12812.
109 Edmunds, L. H., Jr., *Tex Heart Inst J* (1996) **23** (1), 24.
110 Spijker, H. T., *et al., Biomaterials* (2003) **24** (26), 4717.
111 Pickford, A. R., *et al., EMBO J* (2001) **20** (7), 1519