**Designing biomimetic antifouling surfaces**


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Marine biofouling is the accumulation of biological material on underwater surfaces, which has plagued both commercial and naval fleets. Biomimetic approaches may well provide new insights into designing and developing alternative, non-toxic, surface-active antifouling (AF) technologies. In the marine environment, all submerged surfaces are affected by the attachment of fouling organisms, such as bacteria, diatoms, algae and invertebrates, causing increased hydrodynamic drag, resulting in increased fuel consumption, and decreased speed and operational range. There are also additional expenses of dry-docking, together with increased fuel costs and corrosion, which are all important economic factors that demand the prevention of biofouling. Past solutions to AF have generally used toxic paints or coatings that have had a detrimental effect on marine life worldwide. The prohibited use of these antifoulants has led to the search for biologically inspired AF strategies. This review will explore the natural and biomimetic AF surface strategies for marine systems.

**Keywords:** biomimetics; antifouling coatings; natural products; surface physical features; hydrodynamics

1. Introduction

Biomimetics is defined as the study of the structure and function of biological systems and processes as models or inspiration for the sustainable design and engineering of materials and machines. One of the interesting developments in the area of green tribology over the past 10 years is the recognition that nature has developed many highly optimized tribological surfaces that are: (i) typically multifunctional, (ii) reactive to their environment, and (iii) use a combination of physical and biological design strategies. Often these biological surfaces are multilayered with specific hierarchical structures, i.e. mother of pearl (nacre) (Barthelat 2007). Natural surfaces are self-healing and self-cleaning and possess

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the ability to produce cell-to-cell communication signals to prevent colonization. In this context, tribology includes the interfacial science of flowing fluid over a solid surface and the friction induced by this flow. Drag (resistance) reduction of ships’ hulls would, for example, increase fuel efficiency, vessel availability and invasive species translocation and reduce life-cycle costs. There are three main resistance forces that have to be overcome when a vessel moves through the sea: the form (hull shape), wave making and skin friction. Figure 1a illustrates the resistive component contributions with respect to a range of Froude numbers, Fr (a dimensionless number relating inertia and gravitational forces to quantify the resistance of an object moving through water, allowing comparison of objects of different sizes). At low to moderate speeds (Fr < 0.25) the frictional resistance is the largest component of the total drag. Frictional resistance is affected by the roughness of the underwater hull, which is controlled by either subsequent surface deterioration or biofouling (figure 1b).

This paper reviews the state-of-the-art research and understanding of natural and biomimetic antifouling (AF) surfaces with a focus on marine systems. The review will look at approaches to translate the functionality of natural AF systems into engineering systems and designs, building upon recent reviews of new non-toxic AF strategies (Clare & Aldred 2009; Maréchal & Hellio 2009). One strategy is inspired by the low-drag performance of sharkskin surfaces made to mimic their grooved scales (placoid scales, which resemble tiny spines that protrude from the surface). These scales are almost parallel to the longitudinal body axis of the shark and their presence has been shown to reduce drag by 5–10%. Likewise, pilot whale skin has been shown to resist micro-organisms because of the presence of microscopic pores and nano-ridges surrounded by a secreted enzymatic gel that denatures proteins and carbohydrates (Baum et al. 2002). Surface topography is also used in biological surface design, where surface roughness at the micro- or nanoscale hierarchical level can change the properties of a system and introduce multiple equilibria, instability and dissipation. In particular, a small change of surface roughness can lead to large changes in the capillary force. The capillary effects are crucial for small-scale applications and the functionality of engineered
devices, such as micro-/nano-electromechanical systems (Nosonovsky & Bhushan 2009). Alternatively, the shell of the blue mussel *Mytilus edulis* can remain free of fouling organisms as long as it possesses an intact periostracum, which is a multiple AF defence that comprises a ripple-like microtopography and the production of chemical AF compounds (Bers *et al.* 2006). However, once the periostracum deteriorates, fouling settlement then often occurs (figure 1c).

Biological inspiration for AF surfaces has directed researchers into looking at a wide range of natural systems such as sharks and whales, geckos (Hui *et al.* 2006), garden peas (Bauer & Ulrike Mathesius 2004), lotus leaves, and marine brown algae (Bazes *et al.* 2009). However, one of the main challenges facing surface engineers who wish to mimic such surfaces is the disparate surface areas between natural systems and man-made structures or shipping, for example. This raises the issue of scale-up and the volume required of any natural AF product that may be incorporated into a coating or bulk material.

(a) Implications for shipping

With an estimated 300 million tonnes of bunker fuel oil consumed annually by the world’s fleet, there is an ever increasing focus on shipping’s environmental footprint. The International Maritime Organization (2009) estimates that, without corrective action and the introduction of new technologies, air emissions due to increased bunker fuel consumption by the world’s shipping fleet could increase by between 38 and 72 per cent by 2020. It is estimated that AF coatings provide the shipping industry with annual fuel savings of $60 billion and reduced emissions of 384 million and 3.6 million tonnes, respectively, for carbon dioxide and sulphur dioxide per annum.

2. Mechanisms of action

(a) Surface physical features

We believe that a holistic approach is a fundamental requirement for any future AF development, implying a much better understanding of the ecology drivers and adaptations for natural AF strategies, with a greater emphasis on the synergistic effect of combined mechanisms. From a biomimetic standpoint, the intertidal zone is an extreme environment where wave-swept rocky coasts place substantial hydrodynamic forces (drag, lift and acceleration) on plants and animals. Within this habitat, breaking waves routinely reach water velocities of between 10 and 15 ms$^{-1}$ (O’Donnell & Denny 2008), and storm waves can be greater than 25 ms$^{-1}$; in addition, there are often accompanying accelerations in excess of 400 ms$^{-2}$ (Denny *et al.* 1985). The strength of tidal currents and waves has been shown to influence morphological differences and biomechanical properties. Although these areas might be expected to place strong constraints on fouling, the primary productivity and thus the species richness is high owing to the increased nutrient supply (Leigh *et al.* 1987). The strong hydrodynamic forces within the intertidal zone impact organism morphology in a similar manner to that found on ships’ hulls, i.e. 10–15 ms$^{-1}$ is equivalent to about 19–29 knots. Likewise, when a ship is moving, a significant mass of water, of the order of a quarter or even a third of the vessel’s total mass, is accelerated to a speed close to that of the ship.
At lower speeds (i.e. cruising), viscous drag dominates, with a minimal influence from wave making. As a result, surface merchant and naval shipping generally operates within this speed range (approx. 15 knots; figure 2).

Many marine organisms within these zones often remain remarkably free from epibionts (sessile organisms attached to the surface of a living organism), although they are subject to the same fouling pressures as the inorganic non-living substrates, i.e. the submerged rocks and man-made underwater structures. Such organisms, with surface topography and favourable hydrodynamics, show exemplar non-toxic AF strategies. However, these organisms are likely to be the most troublesome foulers, owing to their attachment strength and substrate selection criteria. Thus, a greater understanding is required of the complex multi-dimensional chemical, physical and mechanical mechanisms (Scardino et al. 2009a,b). A further possible mode of AF is through the production of natural chemicals that could act by modifying the surface of organisms, decreasing the incidence of epibiosis. The thalli of many marine algae and the surfaces of invertebrates are covered in mucilage or slime, which could render the attachment of fouling spores difficult or effect the removal of epibionts by continuous or periodic surface renewal (Wahl 1989). The role of surface sloughing in the removal of bacterial fouling in Chondrus crispus has been observed (Sieburth & Tootle 1981), and the colonial ascidian Polysyncraton lacazei has been found to shed a thin surface cuticle with its attached epibionts at irregular intervals (Wahl & Banaigs 1991).

Reynolds number is a measure of the ratio of inertial forces to viscous forces and quantifies the relative importance of these forces for given flow conditions. Figure 3 illustrates that the influence of hydrodynamic flow on the biofouling process is fundamental and cannot be ignored (Patel et al. 1985; Dusenbery 2009). The movement of different organisms involves Reynolds numbers spanning a huge
range of order of magnitude. Micro-organisms at Reynolds numbers around $10^{-5}$ are in an environment where viscous forces significantly dominate over inertial forces. These viscous hydrodynamic interactions almost completely influence the settlement processes, allowing the micro-organism to identify an appropriate substratum surface. This contrasts with engineered underwater structures, where Reynolds numbers are usually between $10^3$ and $10^9$. A challenge to biomimicry is to adapt natural systems that are effective at relatively low Reynolds numbers over small surfaces to man-made systems that operate under parameters orders of magnitude greater.

(b) Topography

Hoipkemeier-Wilson et al. (2004) have demonstrated the influence of surface topography on biological settlement. Many organisms have evolved surface topographies that deter settlement, for example, pilot whale skin (Baum et al. 2002), mussel/bivalve surfaces (Scardino & de Nys 2004), crabs and eggshell casings (Bers & Wahl 2004). The roughness scale of a coating significantly influences its wettability (Nosonovsky & Bhusan 2009). Microtopography replicas are rarely as effective as the organism in situ, inferring that other contributors to AF behaviour are active in the organism’s lifetime. However, the marked effect of surface topography has stimulated new efforts to develop bioinspired AF surfaces. Notably, the AMBIO project (Advanced Nanostructured Surfaces for the Control of Biofouling) has developed a range of nanostructured surfaces, including sol–gel coatings with nanoparticle inclusions, and nanoscaled self-assembled surfaces (Callow & Callow 2009). Nevertheless, surface design
should be approached with caution as a means for cell adhesion resistance, given
the mixed community nature of settling organisms, whose planktonic spores range
in size by orders of magnitude.

Customized surface nano-architecture has yielded a commercial product,
Sharklet, inspired by the overlapping, ridged platelet structures of sharkskin
(Carman et al. 2006). The topography of this structure, which features a
gradient of microscale change in three dimensions, offers a more hydrophobic
surface compared with isolated topography gradients. Among other applications,
Sharklet is undergoing development as an AF coating. The ability of a spore to
settle is compromised when topographical features are separated by size scales
smaller than that of the spore, and when it cannot support its entire mass on
a single feature (Carman et al. 2006; Schumacher et al. 2007b). The resultant
‘bridging’ behaviour and hydrophobicity of the surface result in weakened
adhesion and tension in the unsupported cellular membranes. Settlement of Ulva
intestinalis zoospores was reduced by 86 per cent compared with similar, non-
patterned topographies. Schumacher et al. (2007a) integrated a Sharklet surface
topography, known to repel Ulva spores, within a ridge system repellent to
barnacle cyprids. However, Ulva settled prolifically at the base of ridges, avoiding
the Sharklet topography. Efimenko et al. (2009) tested a series of more complex,
wrinkled hierarchical structures. These remained clean for over a year in trials,
although diatom settling occurred between ridges on the smallest size scale.

(c) Textures

Biofouling resistance and high levels of fouling removal have been studied for
a wide range of marine organisms, and are often associated with the growth of
surface topographies to prevent fouling and facilitate its removal when fouled.
Attachment point theory predicts lower settlement on microtextures with a
size smaller than the width of the settler; and higher settlement where the
texture is wider than the settler is thought to be the mechanism when the
micro-organism exhibits choice during settlement (Scardino et al. 2008). In a
recent review, characterizing the key biomimetic surface features related to
resistance and fouling removal from the shells of 36 species of marine molluscs,
five parameters were positively correlated with increased fouling resistance, in
the following order of importance: (i) low fractal dimension, (ii,iii) high skewness
of both roughness and waviness profiles, (iv) higher values of isotropy and
(v) lower values of mean surface roughness (Scardino et al. 2009a,b). The only
surface parameter that strongly correlated with percentage fouling removal was
waviness, with the highest waviness profiles having the weakest fouling adherence
(Scardino et al. 2009a,b). Another review highlights the importance of the
structure and mechanical properties of mollusc shells from the perspective of
synthetic biomimetic materials (Barthelat et al. 2009). Foul-release coatings
(FRCs), i.e. coatings based on silicone elastomers with low surface free energies,
are generally only successful for fast-moving vessels, as the decrease in adhesion
strength facilitates removal at speeds greater than 20 knots (Brady 2001).
However, diatoms have higher attachment strength on hydrophobic surfaces
(Holland et al. 2004), remaining on FRCs in excess of 30 knots (15.4 m s\(^{-1}\);
Candries et al. 2001), with reports of persistent colonization over 50 knots
(25.7 m s\(^{-1}\); Towsin & Anderson 2009). Alternatively, superhydrophobic surfaces

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(a combination of micrometre-scale and nanometre-scale roughness, along with a low surface free energy, resulting in high water contact angles greater than 150°) have been studied for their AF performance (Zhang et al. 2005; Scardino et al. 2009a,b) and are known to exhibit reduced viscous drag due to slip associated with a layer of air trapped at the liquid–solid interface. However, loss of superhydrophobicity is due to the so-called Cassie–Wenzel transition, where it has been shown that both Cassie (air trapped) and Wenzel (surface roughness) regimes can coexist on the same surface. Moreover, the Cassie state can switch to the Wenzel state under external stimuli such as pressure or vibration (Bormashenko et al. 2007).

(d) Mechanical

The impact of fouling on ships’ hulls has been extensively reviewed, including the effects of biofilms (Schultz & Swain 1999), filamentous algae (Schultz 2000) and calcareous fouling (Leer-Andersen & Larsson 2003; Schultz 2004). These studies indicate that fouling leads to a significant increase in drag resistance, although the overall magnitude of the drag increase depends on the overall fouling type and coverage (Schultz 2007). The primary role of the AF coating of a ship’s underwater hull is to minimize the increase in drag due to limiting the influence of biofouling accumulation (Schultz 2007). An effective coating is critical to the sea-going performance of ships, as inadequate coatings can result in increased drag, leading to either increased propulsive power to maintain a given speed or to reduced speed at a given input power compared with a hydraulic smooth and unfouled hull. The skin friction on some ship hull types can account for as much as 90 per cent of the total drag even when the hull is free of fouling (Schultz 2007). For mid-sized merchant and naval vessels, such as frigates and destroyers that are typically 150 m in length, at cruising speed (7.7 m s$^{-1}$ or 15 knots) or near maximum (15.4 m s$^{-1}$ or 30 knots), an 8–18% penalty in propulsive power has been attributed to a mature slime (bacteria and diatom film) and up to 80 per cent for heavy calcareous deposits. This is in good agreement with a recent paper on full-scale ship resistance and powering predictions, based on laboratory drag measurements and boundary layer similarity law analysis (Schultz 2007).

(e) Wall shear stress

When water flows over an immersed solid-body surface (i.e. a ship’s hull), a velocity gradient known as the boundary layer develops in the water between the surface and the free-stream ambient flow. The water flowing in the boundary layer not only delivers water-borne larvae and particles to the substratum but also dislodges them from it. The greater the distances from the leading edge of a body (e.g. a ship, dock or fouling plate), the thicker the boundary layer. For a ship, wall roughness due to fouling and coating defects results in increased turbulence and fluid mixing within the boundary layer (a near-wall region characterized by its fluid velocity profile), leading to increased turbulent and wall shear stress that is directly linked to increased power requirements (Munk et al. 2009). Similarly, the boundary layers in marine habitats over macroscopic surfaces are turbulent, although there is a very thin (viscous) sublayer of water next to any smooth surfaces in which the viscosity (resistance to being sheared) of the water damps the turbulence. Bumps on rough surfaces can disrupt the viscous sublayer.
The velocity gradient in a turbulent boundary layer is steepest at the solid surface. As eddies swirl around in a turbulent boundary layer, water and the materials it carries (e.g. larvae, particles, dissolved chemical cues) are transported to and from the surface. Hydrodynamic forces can also induce indirect biological effects on larval settlement. Barnacle larvae (cyprids) are reported to attach much more tightly to surfaces covered with an established biofilm that had developed when the surfaces were exposed to high shear stresses than for biofilms grown in low shear flow (Neal et al. 1996). The impact that biofouling has on drag is well known (Callow et al. 2002; Holm et al. 2004); a biofilm slime of even a few tens of micrometres can double the friction factor (figure 4) in a smooth pipe (Stoodley et al. 1998). However, what is less known is how biofilms can adapt to hydrodynamic conditions. The fact that bacterial biofilms can streamline their forms under high flow to reduce drag suggests an adaptive process (Stoodley et al. 1998; Taherzadeh et al. 2009). It has also been reported that biofilms grown under higher flow have higher storage (elastic) and loss (viscous) moduli, and require the application of a greater shear stress to remove them from surfaces, than biofilms grown at lower flow conditions (Stoodley et al. 2002). The conclusion is that biofilms grown at high flows adhere more firmly and are mechanically stronger than biofilms grown at low flows. It is not known whether this occurs from selection (i.e. only the stronger individuals can attach and grow in high flows) or whether bacteria can sense and respond to the hydrodynamic forces. Preliminary data suggest that bacteria in biofilms express specific genes when exposed to elevated fluid shear (P. Stoodley 2010, unpublished data). These genes might also play a role in sensing and adapting to mechanical stresses. The possibility that biofilms can adapt to shear stress presents a greater challenge consideration in AF strategies, but also presents an opportunity for exploitation as a target for manipulation.

(f) Natural chemical defences (antifouling)

Sessile marine organisms often lack the possibility of exploiting dynamic or mechanical strategies to deter the settlement of fouling upon them. However, the surfaces of many of these organisms remain remarkably free from macrofouling.
A good example is the fronds or thalli of macroalgae (seaweeds), which are usually uncolonized. This has led to the hypothesis that chemical substances produced by seaweeds could play a part in deterring the settlement of fouling organisms. Secondary metabolites produced by several species of marine macro- and microalgae have been shown to exhibit antibacterial, anti-algal, antimacrofouling and antifungal properties, which could be effective in the prevention of biofouling (Bhadury & Wright 2004). A wide variety of such compounds have been reported, and nearly 20,000 new compounds have been isolated from marine organisms since the early 1970s (Fusetani 2008). Many of these have unusual chemical structures not seen in terrestrial plants and animals, and the evolution of these in the marine environment has been discussed (Cimino & Ghiselin 2001). While the specific mode of action of the majority of these compounds is unclear, the AF mechanisms of natural products include low pH, deterrents, anaesthetics, attachment and/or metamorphosis inhibitors or toxic chemicals (Ralston & Swain 2009).

Secondary metabolites can be arranged in classes depending on the biosynthetic pathway used to form them. More than half of reported marine natural products are derived from the terpenoid pathway (Harper et al. 2001). The role of terpenoid metabolites in mediating interactions among organisms has been reviewed (Gershenzon & Dudareva 2007). Sesquiterpenes from the red seaweed Laurencia caduciramulosa inhibited fouling by mussels (Cassano et al. 2008), and linear geranylgeraniol-derived diterpenes have been identified in the brown alga Bifurcaria bifurcata (Culioli et al. 2000) and shown to have AF properties (Hellio et al. 2001). A number of marine organic secondary metabolites contain bromine in their structure, an element that is ubiquitous in the oceans, but rare on land. Brominated furanones derived from the Australian red seaweed Delisea pulchra have been extensively investigated for their AF and antibacterial potential (de Nys et al. 1995).

(g) Biological signals

Givskov et al. (1996) first hypothesized that bacterial growth on surfaces might be controlled using chemicals that interfered with the natural cell signalling mechanisms employed by bacteria for biofilm attachment and development by showing that the marine seaweed D. pulchra produced halogenated furanones that blocked swarming (the movement of cells over a surface) of Serratia liquefaciens, a medical pathogen. It was hypothesized that D. pulchra used this strategy to prevent bacterial fouling, and the exploitation of this natural defence was suggested as an AF strategy for marine surfaces and structures, thus establishing an early bioinspired concept to reduce biofouling. The link between the regulation of bacterial biofilm growth through secreted diffusible cell signals was further strengthened by Davies et al. (1998), who showed that all Pseudomonas aeruginosa cells within a biofilm population secreted signals (in this case, N-acyl homoserine lactones (AHLs)) at a low basal rate, but once the signal concentration reached a threshold ‘quorum sensing’ (QS) level, there was a phenotypic switch in which many biofilm genes were up-expressed, leading to accelerating biofilm formation. This led to the notion that biofilms could be controlled by manipulating these ‘communication’ systems through the exogenous addition of extracted natural signals or synthesized signal blockers (Manefield et al. 1999) to arrest, or even reverse, biofilm formation. However, while initial results in the laboratory were promising and marine trials showed comparable
results to conventional biocides, long-term efficacy has remained elusive, largely owing to the problem of uncontrolled leaching (de Nys et al. 2006). Ralston & Swain (2009) point out that the key to using biomimicry more effectively for the control of marine fouling lies not in taking any one factor (such as a secreted chemical or an aspect of surface topography) in isolation, but rather in identifying and reproducing combined strategies deployed by an organism in a synergistic manner, in the context of the environment in which the organism lives. Living organisms have the advantage over abiotic surfaces in that they can continually produce and replenish antimicrobial/antibiofilm agents, in a controlled manner. In an attempt to replicate the ability to avoid depletion from passive leaching, Yee et al. (2007) used a ‘living paint’ that incorporated live marine bacteria in carageenan beads, which was effective in reducing fouling for up to seven weeks in the field (Yee et al. 2007). It remains to be seen whether the control of two living systems—the coating and the foulers—will present more of a problem than controlling the foulers alone.

Other issues concerning natural diffusible signals need to be considered with respect to biofilm control. Based on the initial findings with the AHLs that a threshold concentration had to be achieved to initiate QS, it was assumed that high concentrations were always desirable. However, Rickard et al. (2006) have shown with a different signal, auto-inducer 2 (AI2), that there was an inverse relationship between concentration and the extent of biofilm formation, and that the greatest biofilm formation was at concentrations in the micromolar range. Fluid flow can also modulate QS effects on biofilm formation. Purevdorj et al. (2002) showed that the correlation between flow rate and biofilm structure was just as important as the production of AHL signals, and Kirisits et al. (2007) reported that cell signals only built up to threshold concentration under stagnant conditions due to the ‘wash-out’ effect, even under laminar flow. This is of particular relevance to wetted ship surfaces, which experience a wide range of hydrodynamic flows, not only across different locations, but also as a function of the ship’s speed. While initial strategies with cell signals focused on blocking biofilm formation, more recently signals have been discovered that initiate dispersal of biofilms. Nitrous oxide is one such signal that at high concentrations is toxic, but at low (nanomolar) concentrations acts as a bacterial dispersal signal (Barraud et al. 2006). Thus, from a design perspective, control of the release of an AF agent is an important consideration to ensure that appropriate concentrations are maintained at the surface to achieve the desired effect. Another potential dispersal signal for AF consideration is the fatty acid signal, cis-2-decenoic acid, which is reported to have very broad bacterial activity and whose dispersal effect is effective over a wide range of concentrations (0.01–10 000 μm; Davies & Marques 2009). Such a signal makes a good candidate for AF as it is non-toxic, is broad acting and does not require precise release control for efficacy. While the search for a ‘universal’ biofilm prevention or dispersal signal is attractive, the existence of such a signal might be elusive. First, it seems unlikely from an evolutionary perspective that a diverse multitude of species would so easily give up control of their biofilm development to potential competitor species. Second, while it is reasonable to expect that an animal or plant might have developed such signals targeted against foulers in a given environment, such a signal might not necessarily be expected to be effective against organisms in other environments.
3. Biomimetic approaches already tried

The strategies evolved by marine organisms to resist epibiosis rely on four main mechanisms that can be broadly classified as employing chemical, physical, mechanical or behavioural effects (Ralston & Swain 2009). The latter two are outside the scope of coatings technology, and current biomimetic approaches aim to mimic the physical and/or chemical AF effects displayed by living organisms.

(a) Coatings

Most work on biomimetic coatings has taken place in the biomedical field with the aim to increase the compatibility of artificial substrates with biological tissue, for example, by coating titanium dental and orthopaedic implants with hydroxyapatite (Zhang et al. 2009). However, in some biomedical devices such as stents, an AF, antibiofilm, surface is required. Biomimetic AF membranes for biomedical use have been produced by creating phospholipid groups on the surface of synthetic polymer membranes (Huang et al. 2006). Ultra-low-fouling peptide surfaces derived from natural amino acids have also been created (Chen et al. 2009). However, few examples of bioinspired coatings for marine AF use have been described. One mechanism that combines a biochemical effect with synthetic polymer film-formers is the use of enzymes in coatings. A recent example (Olsen et al. 2010) is an AF coating that uses enzymes to generate hydrogen peroxide bursts, mimicking one defence mechanism exploited by marine algae (Nylund & Pavia 2003). However, the need to incorporate an expendable starch substrate within the coating led to a maximum effective lifetime of only 67 days in cold ocean exposures (and less in warm waters). Bioinspired AF coatings might not just come from looking at the external environment. Loose et al. (2006) report borrowing aspects from the human innate immune system by exploring the incorporation of small antimicrobial peptides into surfaces. In a similar vein, aspects of mucosal immunity, which is important in preventing bacteria sticking to human epithelial cells, might be exploited for large engineered systems.

(b) Surface energy

Operators of high-activity vessels such as ferries, which travel at high speed and have rapid turnarounds, often employ FRCs. FRCs are ultra-smooth, hydrophobic poly(dimethylsiloxane) or fluoropolymer surfaces with low friction and surface energy. Baier (1973) studied the relationship of bioadhesion with surface energy, demonstrating that the pessimal surface energy for bioadhesion is around 23 mN m$^{-2}$ (figure 5; Brady 2001; Zhao et al. 2005). This hydrophobicity reduces the ability of any fouling organism larger than a bacterium to adhere to the vessel, and shear stress at the surface can easily dislodge any weakly bonded foulers when the boat is in motion. Hydrophilic coatings are also of interest in AF design. Polyethylene glycol (PEG) functionalized polymers tend to be hydrophilic with a high degree of hydration. Both linearly aligned and hyperbranched PEG side chains have been effective in reducing diatom adhesion and weakening protein adsorption. Effective new coatings have recently been developed to combine the low surface energy effect of hydrophobicity and the resistance to protein adsorption characteristic of hydrophilicity, with adjacent, heterogeneous regions.
Figure 5. The Baier curve illustrating relative bacterial adhesion versus surface free energy.

generated on the nanoscale (1–10 nm). Many of these coatings have been adapted in novel, biomimetic ways; for instance, Statz et al. (2006) describe a mimic of mussel adhesive proteins conjugated with hydrophilic PEG segments.

(c) Superhydrophobic surfaces

Superhydrophobic (i.e. extremely non-wettable) surfaces have also been considered for AF use (Genzer & Efimenko 2006). The preferred definition of a superhydrophobic surface (Koch et al. 2009) is one that only has an apparent contact angle of 150° or more but also a low hysteresis of contact angle, and many such surfaces are found in nature. Two hundred plant leaves were examined (Neinhuis & Barthlott 1997) and the majority were found to have superhydrophobic surfaces. The leaves of the lotus plant are known to be superhydrophobic and self-cleaning because of their unique surface structure (Cheng 2006). Some structured superhydrophobic plant surfaces entrap a thin layer of air that enables them, as well as replicas of their surfaces, to stay dry under water for several days (Koch et al. 2009). Synthetic nano-engineered superhydrophobic surfaces that retain a layer of air have been investigated for their potential marine fouling resistance properties (Scardino et al. 2009b). Such a layer of air is unlikely to persist for extended periods on a moving ship’s hull, so would be difficult to exploit for AF purposes. Genzer & Efimenko’s (2006) analysis of whether various types of superhydrophobic surface can deter marine fouling concluded that the situation is complex, because, in addition to correlations between substrate wettability and adhesion for some organisms, there are indications that contact angle hysteresis is decisive (Nosonovsky & Bhushan 2007). However, in biomimetic design, it is important not to take a natural AF strategy out of context. With the ‘lotus leaf effect’, the leaf is not submerged and the self-cleaning relies on the combination of the superhydrophobicity of the surface with the rolling action of water droplets, which pick up debris.
Review. Biomimetic antifouling surfaces

Figure 6. Biofilm growing on the upper surface of a submerged lotus leaf in (a) plan view and (b,c) cross section. The biofilm was stained so that the individual bacteria (bright white rods, green in colour version) were clearly seen in biofilm clusters. Diffuse grey (red in colour version) staining between the rods is indicative of biofilm slime. Some of the biofilm clusters formed ‘droplet’-shaped structures with ‘contact angles’ ranging between 45° and 123°. Bacteria also colonized the stomata on the leaf, forming distinctive circular patterns (white arrow in (a)). The leaf was freshly harvested and was provided by Dr J. C. Post, Allegheny-Singer Research Institute, Pittsburgh, PA. Scale bar, 20 µm.

owing to the surface tension of the water drop. Bos et al. (2000) showed that hydrophobicity had little correlation with initial attachment of bacteria but was positively correlated with flow-induced detachment; the same group reported that the effective removal of bacteria from surfaces by the passage of an air–water interface (i.e. an air bubble) was highly dependent on the physical nature of the surface and the species of bacteria (Gómez-Suárez et al. 2001). Figure 6 shows a P. aeruginosa biofilm growing on a lotus leaf submerged in nutrient-rich water, demonstrating that biofilms can grow on hydrophobic surfaces.
4742
M. Salta et al.

(d) Natural products as antifoulants

The use of natural products as AF agents is attractive from an environmental point of view, since such compounds exist naturally in the environment. As sessile marine organisms lack the ability to actively evade predation and epibiosis, they have instead evolved a variety of chemical defences against such threats. Chemicals with AF activity have been isolated from marine invertebrates, as well as from many macroalgae (seaweeds) by extraction of the whole plant. While these extracts may show strong activity in laboratory bioassays, the relevance of these tests to AF performance in vivo is far from clear. Compounds isolated from macroalgae by extraction of the crushed whole plant may not be present at the surface of the intact living thallus (or frond) in effective quantities, especially if they are highly water-soluble polar metabolites. Steinberg et al. (1998) reported that non-polar metabolites accumulated at the surface of macroalgal fronds had significant AF activity, which was apparently due to chemical effects per se rather than to any physico-chemical modification of the surface. A few attempts have been made to incorporate isolated natural products into coating systems for AF use. Zosteric acid from eelgrass was incorporated into silicone coatings (Barrios et al. 2005). Direct mixing of zosteric acid and silicone led to rapid leaching of the additive, but the rate was greatly reduced by using a co-solvent in the formulation. Oroidin, a marine natural product isolated from sponges of the Agelasidae family, was incorporated into a generic marine paint without biocides and found to exhibit antibiofilm activity over three weeks (Melander et al. 2009). However, studies that demonstrate long-term AF coating performance from natural products are lacking. A further hurdle to the introduction of such products is the need to comply with legislation, such as the European Biocidal Products Directive (BPD 98/8/EC), which entered into force on 14 May 2000. However, the introduction of BPD 98/8/EC, which is well intended in response to tributyl tin, may now be too restrictive and may possibly hinder biomimetic AF coating innovation, as the regulatory authorities now require testing of a new active substance before marketing authorization, leading to associated prohibitive costs.

4. Bioassay testing

A biological assay, often referred to as a bioassay, is a means of measuring the response of living organisms to a chemical substance of a specific concentration and time. Bioassays are the principal laboratory experiments conducted to test new antifoulant efficacy against marine organisms. Alternatively, direct evaluation of coatings in the natural environment is an expensive procedure requiring large quantities of test products and prolonged immersion times. The choice of species to assay can greatly affect the comparative success of an experimental AF coating, as the seasonal and biogeographical variance of shipping routes means that an AF compound that is effective in temperate waters may be ineffectve in tropical waters and vice versa. Thus, it is important to test the AF efficacy against a broad spectrum of organisms and marine environments. For bioassay tests, pioneering species often used are: Pseudoalteromonas sp., Vibrio sp., Marinobacter sp. and Cobetia sp. for the bacteria; Navicula sp., Amphora sp., Nitzschia sp. and Cylindrotheca closteriunm for diatoms; Ulva sp. as a macroalgae; and Balanus sp. as a test barnacle. The

Phil. Trans. R. Soc. A (2010)
optimal target species commonly used in bioassays would be those that occur in both temperate and tropical locations and should include representative species from the microfouling community (i.e. mainly bacteria and diatoms) and the main macrofouling groups of algae, annelids, ascidians, barnacles, hydroids, mussels and bryozoans (Salta et al. 2009).

Generally, screening assays for novel AF compounds are separated into two categories; toxicity and AF assays (Dhams & Hellio 2009). Toxicity is examined using the LC50, where the lethal concentration to kill 50 per cent of the organisms is used as an indicator of the compound’s acute toxicity. Another routinely used assay is the EC50, the effective concentration at which 50 per cent of the organisms are affected when administrated in a dose–response manner. These tests are necessary to obtain toxicological information on natural products for AF efficacy; however, for a natural product to be selected for its AF activity, the EC50 used needs to be lower than LC50 (Dhams & Hellio 2009). Increasingly, there is evidence that active compounds affect organisms at non-toxic concentrations (Maximilien et al. 1998), hence the necessity for more insightful AF testing. In future, AF assays should also focus on the mechanisms used by the organisms (biological, chemical, physical and behavioural) to facilitate their initial attachment/adhesion/colonization onto a surface (Briand 2009; Dhams & Hellio 2009).

Here, we present preliminary work conducted using two natural products (B. bifurcata and C. crispus crude extracts) tested against marine bacteria using a unique combination of screening AF bioassays. For the first time, the biofilm formation on a paint system containing natural products was studied using a multi-detection microplate reader, which allows real-time monitoring of bacterial growth kinetics, in addition to in situ quantifiable measurements of the biofilm proliferation using a viability staining technique (Molecular Probes Live/Dead kit). In this study, natural-product-containing coatings were applied to polycarbonate coupons, placed in a 24-well plate and then inoculated with the marine biofilm-forming bacterium Pseudoalteromonas sp. NCIMB 2021 for 24 h (figure 7a,b). Figure 7a shows the fluorescence intensity emitted by the bacteria when excited at wavelengths of 520 nm (for live bacteria) and 620 nm (for dead bacteria). The un-inoculated control intensities (without bacteria) were subtracted from the test samples. These data suggest that biofilm formation occurred on the control (coating with paint), while the natural product coatings gave no evidence of a biofilm (negative values are due to measurement noise). Figure 7b shows the optical densities and establishes that planktonic bacteria were present in all well fluids. In addition, we used the imaging capability of the microplate reader for the novel application of visualizing biofilm on the coupons, allowing identification of heterogeneous biofilm structures. Each well, containing the natural product coating, was scanned to produce an image of the biofilm formation. This technique provided the opportunity to observe live and dead bacteria in biofilms directly on the coated surfaces (figure 7c,d). There was good agreement between the fluorescent intensity readings and the images. We have also used confocal laser scanning microscopy (CLSM) to obtain high-resolution three-dimensional imaging of biofilms. Figure 8 shows a biofilm-colonized polycarbonate surface compared with a clean surface. CLSM is useful for biofouling studies since it allows three-dimensional imaging of fully hydrated living biofilms on various spatial scales, from those relevant to the single cell up
Figure 7. Polycarbonate coupons coated with natural products (CC, *C. crispus*; BB, *B. bifurcata*) and paint (TiO2 and poly(methyl methacrylate)) as a control tested against the marine bacterium *Pseudoalteromonas* sp. NCIMB 2021. (a) Biofilm attachment on the surface of the coupons measured by fluorescence intensity (using the Live/Dead kit): pale bar, live; dark bar, dead. (b) Optical density measurements at 620 nm show that planktonic bacterial growth was not inhibited. (c, d) Imaging of the bacterial biofilm formed on the coupon surface as a function of fluorescence intensity at (c) $\lambda = 520$ nm (live bacteria) and (d) $\lambda = 620$ nm (dead bacteria). In (c, d), columns 1 and 2 show coupons that were inoculated and column 3 the un-inoculated negative control.

Figure 8. Confocal microscopy images of (a) a clean polycarbonate coupon and (b) a polycarbonate coupon with bacteria that are stained with nucleic acid Live/Dead dyes attached to the surface. The arrow in (a) and lower arrow in (b) indicate the autofluorescing signal from the material. The upper arrow in (b) is pointing at bacteria. Scale bars, (a) 15 µm and (b) 25 µm.

to those for large biofilm clusters. In addition, specific stains can be used to view the biofilm slime and to physically quantify biofilm thickness, volume, surface area coverage, etc.
We are developing these novel techniques to be used in combination with traditional methods in order to gain greater insight into the processes of marine biofouling and to allow us to assess the efficacy of our various AF strategies. Since many of the organisms that we are looking at for bioinspired AF strategies live in flowing systems, and indeed we wish to prevent fouling under stagnation and flow, it is imperative to test under controlled hydrodynamic conditions. We are currently adapting flow cells to accommodate various bulk materials, coatings and natural materials that can be tested over a wide range of Reynolds numbers. We believe that looking at the surface interactions directly has a greater relevance for AF, which is a surface-related phenomenon, than using conventional techniques, such as the EC$_{50}$, in which the AF agent is predominantly applied in solution. Although we will initially concentrate on marine AF, these techniques will have much broader utility in medical and industrial applications.

(a) Biofilm modelling

A variety of biofilm growth models and simulations have been reported in the literature, although not specifically for marine AF technology. Biofilm structure (Picioreanu et al. 2000), biofilm pattern formation (Gonpot et al. 2000), impact of antimicrobials (Chambless et al. 2006), biofilm detachment mechanisms (Chambless & Stewart 2007) and effect of hydrodynamic conditions on biofilm growth (Eberl et al. 2000) have been studied and modelled for flat surfaces using two- and three-dimensional models. Kreft et al. (1998, 2001) have developed a simulator, using a bottom-up individual-based modelling approach for biofilm growth, that aims to understand the complex organization of the interactions of individual cells with their environment. The algorithm simulated the growth and behaviour of individual bacteria as autonomous agents, as well as the substrate and product diffusion and reaction. Picioreanu et al. (2000) studied biofilm heterogeneity with a two-dimensional model including hydrodynamic processes, biomass growth, diffusive and convective transport, and substrate transport. Their model is based on the Navier–Stokes equations, substrate mass balances and kinetic laws for biomass growth. Gonpot et al. (2000) described a two-dimensional diffusion model using a Monte Carlo simulation to model the growth of _Escherichia coli_ biofilms on different substrates by taking into account the diffusion rate of the nutrient solution that covers the biofilm. Modelling has design and predictive utility with respect to biomimetic strategies. Some of these might be borrowed from other disciplines; for example, the elution of antibiotics from bulk materials such as poly(methyl methacrylate) (Seeley et al. 2004) might be extrapolated to predict the lifetime of a surface that incorporates the delivery of a signal. Usually, such models are run under static conditions and assume an infinite boundary layer (i.e. laminar), or assume no boundary layer limitation (i.e. fully turbulent), although they could be tailored using computational fluid dynamics to assess the potential influence of a range of flows on elution kinetics. Also, cell signalling reaction–diffusion models have been used to predict the build-up of signalling concentrations in biofilms (Chopp et al. 2003; Horswill et al. 2007). However, in these cases, the signal is assumed to be produced endogenously and homogeneously in the biofilm. Nutrients and waste products are also generally assumed to diffuse in to and out of the biofilm from the overlying bulk fluid. Mathematical and multi-scale modelling and/or simulations...
are powerful tools for designing and developing bio-AF systems, despite the complexities of the system and the difficulties of taking into account the several and various parameters, such as the sea water conditions, the physical/chemical behaviour of the micro-organisms, the attachment/detachment process of biofilms and the operational conditions for the AF models. Molecular-level simulations can also be useful in order to understand the physical/chemical interactions and reaction mechanisms between the small organisms, organic molecules and substrates. For biomimicry purposes using secreted signals, it will be useful to measure the concentration at the surface of the organism and then develop predictive mathematical models that can be used to design surfaces that release similar concentrations over relevant time scales. It will also be useful to couple growth models, where nutrients are delivered by the bulk liquid, with elution models, where signals or other agents are delivered from the substratum, to predict how the concentration profiles, and therefore the signalling response, may vary within the biofilm, and how the presence of a biofilm may influence the elution kinetics.

(b) Biofilm monitoring

In addition to underwater hull structures, a vessel’s sea water handling pipelines and heat exchangers can also suffer from the detrimental effects of biofouling, as a result affecting system availability and performance (Zhao et al. 2005). Since these systems generally have exposed metallic surfaces (usually copper-based and titanium alloys), biofouling can lead to biocorrosion, resulting in increased safety and financial concerns (Busalmen et al. 2004; Flemming et al. 2009). Biofouling not only significantly reduces heat transfer, but also can cause a considerable pressure drop, resulting in higher pumping requirements. Although there are various methods of biofouling control within sea-water-handling systems, these routine control strategies are not always successful owing to insufficient knowledge of the biofouling processes (Janknecht & Melo 2003). This is because of the variations in the rate of biofouling in different systems under different environmental conditions. Hence, there is a need for effective biofilm monitoring that will enable optimization of the biocide dosing strategies within marine piping systems (Flemming et al. 2009). Various approaches to biofilm analysis, including microscopy, microbiological, molecular biological, biochemical and physical methods, have recently been extensively reviewed (Denkhaus et al. 2007). Among the various techniques, electrochemical sensors (Tribollet 2003) have shown promise in the detection of very small changes in the interfacial metal–solution properties, for instance the interfacial capacitance. Studies have demonstrated that this parameter is sensitive to the bacterial attachment and colonization of sensor surfaces from the initial attachment until the formation of mature biofilms (Munoz-Berbel et al. 2007; Dheilly et al. 2008). However, greater effort is still required to characterize different electrochemical signals more fully and to miniaturize the sensors and/or sensor arrays. Commercialized electrochemical sensors, which rely on electrical polarization methods, are used for on-line monitoring of biofilm and biocorrosion, and the effectiveness of biocide dosing strategies within marine piping systems (Flemming et al. 2009). The most commonly used electrochemical sensors currently are BIoGEORGE and BioX (Mollica & Cristiani 2003).
AF coatings must meet all the normal requirements for heavy-duty paints, such as good adhesion, film hardness, abrasion resistance and corrosion resistance. Multiple coating properties can be simultaneously optimized using statistical design of experiments (DOE). DOE enables the building of empirical models in areas where a theoretical understanding is not available. One commonly used experimental design is the full factorial experiment in which the values of the input variables, or factors, are set at two (or more) different levels and data gathered from observations made at every possible combination of all factors. Because of the large number of experiments generated when several factors are involved, partial subsets of the experimental matrix can be taken. These are referred to as fractional factorial designs, and are carefully selected to maximize the predictive ability of models constructed from the data. An annotated bibliography of illustrative application papers using fractional factorial designs has been published (Prvan & Street 2002). DOE using factorial designs has been used in the development of bioinspired, mineralized polysaccharide coatings (Peng et al. 2009).

When the levels of each factor are not independent, as is the case for each component contained in a mixture, factorial experiment designs cannot be used. In a mixture, the constraint exists that the sum of the fractional amount of all components is always unity, and increasing the proportion of one component of the mixture necessarily decreases the amount of the others. Mixture designs have been used to study the influence of solvent composition on the formation of surface microtopography in a thermoset siloxane–urethane system (Majumdar & Webster 2006). However, the use of mixture design experiments has not been widely reported in the development of AF coatings. While DOE is invaluable for optimizing a coating’s physical properties, which can be accurately and reproducibly measured, difficulty is encountered when attempting to analyse AF performance. Marine fouling is a complex biological phenomenon consisting of the cumulative growth of communities of various organisms, and as such it is resistant to quantitative analysis in all but the coarsest fashion. The most controllable way to test AF performance of biocides and surfaces is in the laboratory, by bioassay against single organisms. An automated 96-well plate reader has been used to study the microbial stability of latex paint formulations in a mixture design in conjunction with bioassays (Rhoades et al. 2005). Ultimately, the performance of all AF coatings needs to be validated by long-term exposure to their intended environment. Visual estimation of the degree of coverage by various fouling organisms can be carried out by following appropriate standards, e.g. ASTM 6990-03. Image analysis has also been used to estimate the coverage of organisms but is most appropriate where single species are investigated in a controlled environment (Bellas et al. 2006). Because the growth of fouling organisms is highly affected by local conditions, attachment and growth vary greatly between different locations and seasons of the year. Thus, inert control substrates are always immersed at the same time as the test panels and AF performance is rated by comparison with these controls.

Owing to the length of time necessary for exposure testing under service conditions, various accelerated test methods for AF paints have been devised. Many factors must be taken into account, including the effect of sea water
parameters on performance. Rotor testing, in which paint samples are attached to a cylinder that is rotated at high speed in sea water, is commonly used for testing AF paints. The total run time of such tests is at least two months, but typically three to four months (Kiil et al. 2001). Trentin et al. (2001) have compared the results of rotor testing with extended exposure testing for conventional and self-polishing paints containing cuprous oxide as biocide and found that laboratory tests proved to be significantly shorter, with a reduction in time from several years for field tests to just a few months and days for laboratory tests.

5. Summary

This exciting new branch of AF research is truly multi-disciplinary, drawing from expertise in microbiology, engineering and materials science, in order to establish modern biomimetic AF technologies. The design of novel AF coatings has broadened to encompass natural products research, surface chemistry modulation and biomimetic surface development. However, as others have remarked, it is probable that any future broad-spectrum fouling-resistant surface will draw on several, if not all, of these diverse research areas to facilitate its efficacy. Ideally, an effective biomimetic AF coating will have the following properties: at least 5 years biofouling life-cycle control, durable and resistant to damage, repairable, low maintenance, easy to apply, hydraulically smooth, compatible with existing anticorrosion coatings, cost effective, non-toxic to non-target species and effective in port and at sea (Ralson & Swain 2009).

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