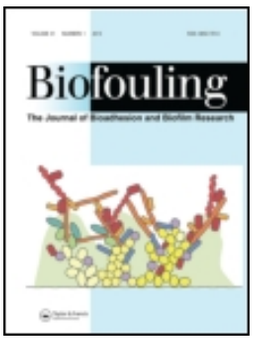

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Polysulfone and polyacrylate-based zwitterionic coatings for the prevention and easy removal of marine biofouling

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A series of polysulfone and polyacrylate-based zwitterionic coatings were prepared on epoxy-primed aluminum substrata and characterized for their antifouling (AF) and fouling-release (FR) properties towards marine bacteria, microalgae and barnacles. The zwitterionic polymer coatings provided minimal resistance against bacterial biofilm retention and microalgal cell attachment, but facilitated good removal of attached microbial biomass by exposure to water-jet apparatus generated hydrodynamic shearing forces. Increasing the ion content of the coatings improved the AF properties, but required a stronger adhesive bond to the epoxy-primed aluminum substratum to prevent coating swelling and dissolution. Grafted poly(sulfobetaine) (gpSBMA), the most promising zwitterionic coating identified from microfouling evaluations, enabled the removal of four out of five barnacles reattached to its surface without incurring damage to their baseplates. This significant result indicated that gpSBMA relied predominately on its surface chemistry for its FR properties since it was very thin (~1–2 μm) relative to commercial coating standards (>200 μm).

Keywords: zwitterionic polymer; coating; antifouling; fouling release; biofouling; poly(sulfobetaine methacrylate)

Introduction

Marine and hydrokinetic (MHK) technology is currently being developed to produce energy from wave, current and tidal resources. Based on the resource, several MHK designs are being explored, but key technological challenges common to all are designs that can survive the marine environment, are reliable, and are easy to operate and maintain (Beaudoin et al. 2010). Similar to other marine industries, MHK technology must contend with biofouling and corrosion. Biofouling prevention (antifouling, AF) and the removal of foulants (fouling-release, FR) are long-standing issues that lie at the heart of several contemporary research initiatives such as coatings for ships' hulls and materials for biomedical implants (Qian et al. 2007; Krishnan et al. 2008). Fouling by marine organisms such as bacteria, algae, tubeworms and barnacles is of particular concern to naval and commercial vessels because of the substantial increase it causes to hydrodynamic drag on the ship (Schultz et al. 2011). For MHK technology, similar occurrences of fouling may be detrimental to performance and could significantly increase operational and maintenance costs. In addition, to meet environmental concerns, deployments of MHK technology around the world have also driven the need for effective nontoxic AF and FR coatings or materials (Bedard 2008; Grippo & Hlohowskyj 2012).

Zwitterionic polymers constitute one class of materials studied for its AF and FR properties (Hayward & Chapman 1984; Ishihara et al. 1990; Jiang & Cao 2010). These coatings have fixed pairs of both positive and negative ions that have electrostatic interactions with nearby water molecules. Unlike most other ionic or polar surfaces, zwitterionic polymers surfaces do not perturb the native hydrogen bonded network of water molecules (Kitano et al. 2005, 2007; Leng et al. 2014). This is a critical attribute since long-range ordering of water molecules has been shown to be responsible for exclusion of colloidal and molecular solutes from the surface vicinity (Zheng et al. 2006).

Several different zwitterionic polymer structures and surface attachment schemes have been investigated for various AF applications. For instance, phosphorylcholine-containing polymers demonstrated resistance to both protein and cell adhesion (Lewis 2000; Hirota et al. 2005; Patel et al. 2005). Poly(sulfobetaine methacrylate) (poly-SBMA) has shown excellent AF performance and has been grafted onto gold and glass using both siloxane (Zhang, Chen, et al. 2006; Cheng et al. 2007) and catechol (Li et al. 2008) chemistries for adhesion. Similarly, poly (2-carboxy-N,N-dimethyl-N-[2′-(methacryloyloxy)ethyl] ethanaminium) has also been prepared as a grafted zwitterionic polymer with siloxane (Cheng et al. 2008) and catechol (Gao et al. 2010) attachment methods.

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Several poly(norbornene)-based zwitterionic polymers have also been synthesized and shown to be more resistant to protein adsorption than methacrylate-based zwitterionic coatings (Colak et al. 2009; Colak & Tew 2012). Another approach, the preparation of charge-balanced hydrogels by the polymerization of comonomers with opposite charges, has led to materials with good resistance to protein adsorption (Chen & Jiang 2008).

Zwitterionic coatings have also been shown to possess good anti-adhesive properties towards some microorganisms. Resistance to nonspecific protein adsorption is highly dependent on surface hydration. Zwitterionic coatings exhibit electrostatically induced hydration which holds water molecules strongly on the surface. Because of this strong hydration layer, some biomolecules cannot displace the strongly bound water molecules and adsorb to the underlying, zwitterionic coating surface. In particular, two recent studies showed that microalgal cells were easily detached from surfaces coated with polySBMA when exposed to hydrodynamic shearing (Finlay et al. 2013; Bodkhe et al. 2015). PolySBMA has also been shown to completely inhibit the settlement of barnacle cyprids (Aldred et al. 2010). How the zwitterionic chemistry achieved its resistance to macrofouling was not determined, but it was hypothesized to be similar, in principle, to that proposed for single proteins or cells. Since the placement and distribution of ions within a polymer structure can greatly influence its water uptake properties, a better understanding of how zwitterionic polymer structure affects AF and FR properties is needed.

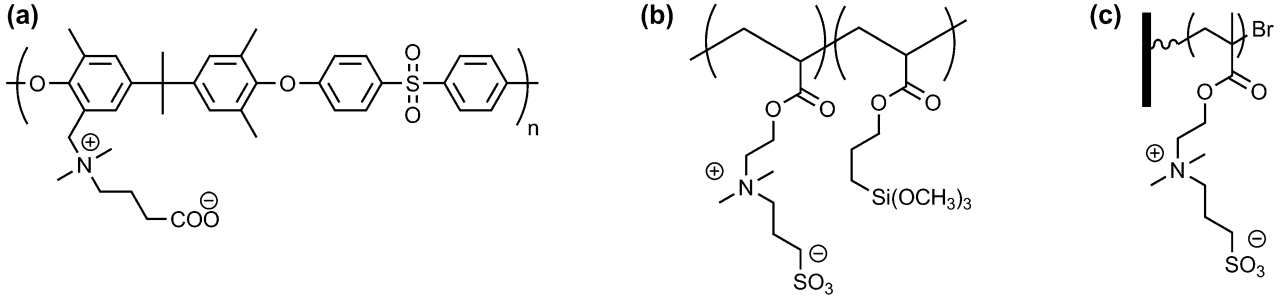
In this study, three distinct zwitterionic polymer structures (Figure 1) were prepared and evaluated for their AF and FR properties against cells of a marine bacterium (*Cellulophaga lytica*), a diatom (microalga; *Navicula incerta*), and adult barnacles (*Amphibalanus amphitrite*) using high throughput techniques. Each coating was applied to primed aluminum substrata. The first coating system discussed is based on a poly(arylene ether sulfone) that was applied to a surface with the subsequent addition of zwitterions randomly along the polymer chain. This system is labeled as zwitterionic tetramethylpoly(sulfone) or (ZTMPS) and is shown in Figure 1a. The second coating comprised of a polyacrylate with both zwitterionic and trimethoxysilane functional groups designed to impart AF and adhesive properties, respectively. Figure 1b illustrates the poly(sulfobetaine methacrylate-co-trimethoxysilyl propyl methacrylate) or (pSBMA-co-TMSMA) coating evaluated.

The final coating, Figure 1c, is a grafted poly(sulfobetaine) or (gpSBMA) prepared by initially attaching a polymerization initiator to the surface using siloxane chemistry. The synthesis and characterization is described below.

Materials and methods

Chemical reagents and materials

Chloroform, anhydrous methanol, anhydrous isopropanol, 4-(dimethylamino)butyric acid hydrochloride (DMABA), sulfobetaine methacrylate (SBMA), 3-(trimethoxysilyl)propyl methacrylate (TMSMA), azobisisobutyronitrile (AIBN), copper(I) bromide, ascorbic acid, 2,2′-bipyridine (bpy), sodium carbonate and sea salts were purchased and were used as received from Sigma-Aldrich, St Louis, MO, USA. Marine broth, peptone, yeast extract and crystal violet dye were purchased from VWR International, Radnor, PA, USA. Reducer No. 15 was purchased from Sherwin-Williams, Cleveland, OH, USA. Intergard®264, Intersleek®700 (IS 700) and Intersleek® 900 (IS 900) coating systems were purchased from International Paint, Houston, TX, USA. Silastic-T2 (T2) was purchased from Ellsworth Adhesives, Germantown, WI, USA. Guillard's F/2 medium was purchased from Bigelow Laboratory for Ocean Sciences, Boothbay, ME, USA. Brine shrimp cysts were received from Florida Aqua Farms, Inc., Dade City, FL,



ZTMPS **pSBMA-coTMSMA** **gpSBMA**

Figure 1. Structures of the zwitterionic coatings synthesized for this study.

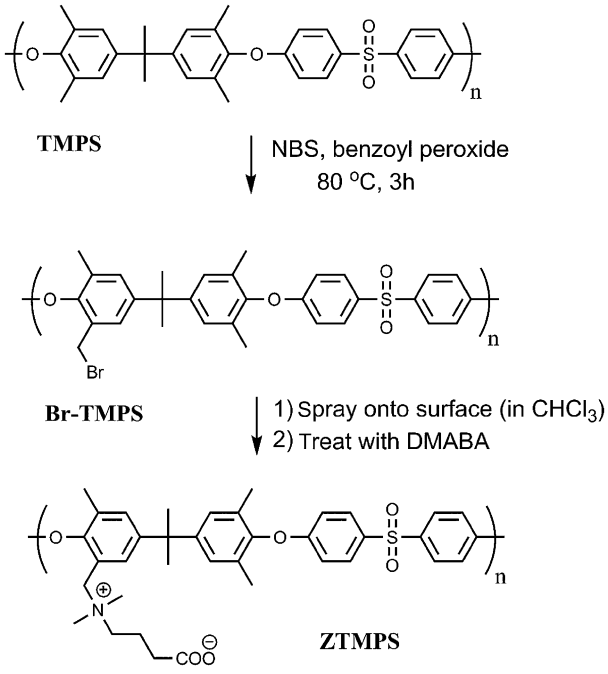


Figure 2. Synthesis and application of ZTMPS coatings.

USA. Artificial seawater (ASW) was prepared by dissolving 38.5 g of sea salts into 1 l of deionized H_2O. The substrata used were aluminum panels with 60 aluminum disks attached and aluminum Q-panels® (76×152 mm, 0.6 mm thickness, alloy 3,003 H14, obtained from Q-lab). Both types of substrata were primed by air-assisted spray with Intergard® 264 epoxy at a thickness of 70–80 µm.

Synthesis and application of coatings

Zwitterionic tetramethylpoly(sulfone) (ZTMPS)

Tetramethylpoly(sulfone) homopolymer (TMPS) and brominated tetramethylpoly(sulfone) (Br-TMPS) were prepared as previously reported (Yan & Hickner 2010; Yan et al. 2013). Br-TMPS samples with varying levels of bromination were dissolved in chloroform (5 wt%) and sprayed onto substrata using an airbrush with nitrogen at 15 psi. The mass of Br-TMPS used was calculated to give an average coverage of 0.8 mg of polymer per cm^2. After drying in a fume hood for 2 h, coated panels were immersed in an aqueous solution of DMABA (0.15 M) and Na_2CO_3 (0.25 M) for 24 h at room temperature. The panels were then soaked in deionized water for 24 h and dried at room temperature under a constant air flow. For biofouling studies with bacteria and microalgae, 12 replicate disks were removed from the coated disk-panels and adhered to the bottom of 24-well plates using double-sided adhesive tape.

Poly(sulfobetaine methacrylate-co-trimethoxysilyl propyl methacrylate) (pSBMA-co-TMSMA)

SBMA (23.7 g, 85.0 mmol), TMSMA (3.73 g, 15.0 mmol), and AIBN (0.14 g, 0.82 mmol) were combined in a flask with dry methanol (100 ml) and stirred under argon. After 22 h, the solution was poured into a pan containing substratum panels and was allowed to sit, covered, for 2.5 h at room temperature. The panels were then removed from the dish and placed in a vacuum oven at 100°C for 2 h.

The panels were then soaked in deionized water for 24 h and dried at room temperature under a constant air flow. For biofouling studies with bacteria and microalgae, 12 replicate disks were removed from the coated disk-panels and adhered to the bottom of 24-well plates using double-sided adhesive tape.

Grafted poly(sulfobetaine) (gpSBMA)

The atom transfer radical polymerization (ATRP) initiator, BrTMOS, was prepared as previously reported (Zhang, Chao, et al. 2006). Substrata were immersed in a solution of BrTMOS (19.7 g, 60.0 mmol) in dry isopropanol (120 ml) at room temperature for 4 h. The panels were then removed from the dish and placed in a vacuum oven at 100°C for 4 h, cooled to room temperature, soaked in ethanol for 24 h, and dried at room temperature under a constant air flow. SBMA (24.0 g, 120 mmol), CuBr (464 mg, 3.23 mmol), bpy (1.14 g, 7.30 mmol), and ascorbic acid (5.67 g, 32.3 mmol) were charged to a flask that was evacuated and refilled with argon twice. A syringe was used to transfer 120 ml of a degassed water/methanol mixture (4:1, v/v) into the flask and the resulting solution was stirred under argon for 5 min. The solution was transferred by cannula into a sealed container with the substrata. The substrata were immersed in the solution for 24 h. Finally, the substrata were rinsed with water, soaked in methanol for 24 h and dried at room temperature under a constant airflow. For biofouling studies with the bacterium and microalga, 12 replicate disks were removed from the coated disk-panels and adhered to the bottom of 24-well plates using double-sided adhesive tape.

Standard commercial coatings

For comparison measurements of AF and FR properties, commercial coating solutions of Intersleek 700 (IS 700), Intersleek 900 (IS 900) and T2 Silastic (Dow Corning) were deposited into wells of 24-well plates modified with Intergard 264 primed aluminum disks in each well (Majumdar et al. 2011; Stafslien et al. 2011). The IS 700 and IS 900 FR coatings were prepared according to the manufacturers specifications while T2 was thinned in

Sherwin-Williams Reducer No. 15 (35 wt%). 0.25 ml of each coating solution were deposited into three columns of a modified 24-well plates (12 replicate samples). The coatings were allowed to sit for 24 h at room temperature and then for an additional 24 h at 50°C to ensure they had fully cured (Stafslien et al. 2011).

Coating characterization

X-ray photoelectron spectroscopy (XPS) was performed using a Kratos Axis Ultra DLD instrument with a monochromatic Al Kα (1486.7 eV) source purchased from Kratos Analytical Ltd (Manchester, UK), a wholly owned subsidiary of Shimadzu Corporation (Kyoto, Japan). Survey spectra for elemental compositions were collected with an analyzer pass energy of 80 eV and a step size of 1 eV. The analyzer was used in hybrid mode with an elliptical analysis area of 300 µm by 700 µm. Charge neutralization was required for these samples. Data processing was performed with CasaXPS Version 2.3.15 (Casa Software Ltd., www.casaxps.com). Quantifications were performed using the built-in relative sensitivity factors.

Water uptake measurements were performed on free-standing films. Films of ZTMPS were prepared by casting 5% solutions of Br-TMPS in chloroform in glass dishes and allowing the solvent to evaporate at room temperature. The dry films were then immersed in an aqueous solution of DMABA (0.15 M) and Na_2CO_3 (0.25 M) for 24 h at room temperature. Samples of pSBMA-co-TMSMA were prepared by casting the as-polymerized solution on Teflon-lined dishes. All films were soaked in deionized water for at least 24 h prior to measuring water uptake. The wet mass of each film was determined by wiping the excess water from the surface and weighing. The films were then dried under vacuum in the presence of P_2O_5 at room temperature for 24 h and then re-weighed to determine the dry mass. Water uptake values were calculated as the difference in the hydrated and dry masses of a film divided by the mass of the dry film.

The contact angle (CA) was measured using a VCA Optima XE (AST Products) with a drop size of 2 µl. The CA was determined by averaging two angles of two separate drops.

Coating preconditioning and leachate toxicity assessments

Prior to conducting the biological laboratory assays for assessing AF and FR performance, all coatings were subjected to water immersion preconditioning in a flowing tank system for seven days. Preconditioning facilitates the leaching of any potential toxic impurities that may have been present in the cured coating films (ie residual monomer, catalyst, solvent). Toxicity assessments of coating leachates were performed after the water immersion preconditioning protocol to ensure that any residual toxins were adequately removed. The specific methods used to assess the coating leachate toxicity towards the suite of fouling organisms employed in this study are detailed in Bodkhe et al. (2015).

Microalgal cell attachment and adhesion

Microalgal cell attachment and adhesion evaluations on coatings prepared in 24-well plates have been previously described in detail (Casse et al. 2007; Stafslien et al. 2015). Briefly, three-day-old cultures of the diatom *Navicula incerta* were rinsed three times with ASW and re-suspended in Guillard's F/2 medium to achieve a final cell density of 10^5 cells ml^{-1}. One ml of the suspension was added to each well of the coated plates and incubated statically at 18°C for 2 h in an illuminated growth chamber (photon flux density 46 µm m^{-2} s^{-1}). The plates were then transferred to the deck of an automated water-jet apparatus and the coatings were subjected to water-jet treatments at 20 psi for 10 s. The coatings were immediately extracted after water-jet treatments with 1.0 ml of dimethyl sulfoxide for 15 min. The resulting eluates were transferred to 96-well plates and measured for fluorescence of chlorophyll (Ex: 360 nm; Em: 670 nm). Percentage removal calculations were determined by comparing the total biomass on the coating surfaces before and after water-jet treatments as follows:

$$\%\ \text{Removal} = (1 - (\text{TBM}_J/\ \text{TBM}_{NJ})) \times 100$$

where TBM_J is the mean fluorescence value of three replicate jetted samples and TBM_{NJ} is the mean fluorescence value of three replicate non-jetted samples.

Bacterial biofilm retention and adhesion

Bacterial biofilm retention and adhesion characterization on coatings prepared in 24-well plates has been previously reported in detail (Stafslien et al. 2006, 2015; Stafslien, Daniels et al. 2007, Stafslien, Bahr et al. 2007). Overnight cultures of the marine bacterium *Cellulophaga lytica* in marine broth were then harvested by centrifugation (10,000×g for 10 min) and rinsed three times with sterile ASW. The bacterial cells were then re-suspended in ASW supplemented with 0.5 g l^{-1} of peptone and 0.1 g l^{-1} of yeast extract to achieve a final cell density of 10^7 to 10^8 cells ml^{-1}. One ml of bacterial suspension was added to each well of the coating plates and incubated at 28°C for 24 h under static conditions. The plates were then transferred to the deck of an automated water-jet apparatus and the coatings were subjected to water-jet treatments at 10 psi for 10 s. Following water-jet treatments, the coatings were stained with a crystal violet (CV) solution (0.3% w/v) for 15 min, rinsed three times with ASW and air dried for

1 h at ambient laboratory conditions. The CV was extracted from the biofilms on the coating surfaces by adding 0.5 ml of 33% acetic acid for 15 min and the resulting eluates transferred to a 96-well plate and measured for absorbance at 600 nm using a multi-well plate spectrophotometer. Percentage removal calculations were determined by comparing the total biomass on the coating surfaces before and after water-jet treatments as described for the evaluation of microalgal adhesion using mean CV absorbance values.

Adult barnacle reattachment assay

The accelerated testing method used to determine adult barnacle adhesion strength to coated surfaces can be found in Rittschof et al. (2008) and Stafslien et al. (2012, 2015). Five adult barnacles (*Amphibalanus amphitrite*) of a testable size (>5 mm basal diameter) were dislodged from glass panels coated with Silastic T2 and then placed on all coating surfaces. Immobilization templates were applied to each panel (Stafslien et al. 2012) and then transferred to an artificial salt water aquarium tank system. The reattached barnacles were fed daily with freshly hatched brine shrimp nauplii (*Artemia* sp.). After reattachment for 14 days in the aquarium system, the coatings were removed and the barnacles were dislodged with a hand-held force gage in shear to measure the peak force at release. Once the force gage measurements were completed, the areas of the barnacle base plates were measured using a Sigma Scan Pro software package (SigmaScan Pro 5.0, Systat Software Inc., Richmond, CA, USA) and the adhesion strengths were calculated by dividing the force required to remove the barnacles by the basal area. Barnacle adhesion for each coating was reported as the mean value of the total number of barnacles that had a measurable detachment force. Barnacles that had no measurable force for detachment were counted as 'not attached' and not included in adhesion calculations.

Statistical analysis

Statistical analysis was performed with JMP 7.0.2 statistical software, SAS Institute Inc., Minneapolis, MN, USA. One-way ANOVAs were used to determine the differences in AF/FR performance among the experimental coatings for each marine organism evaluated. The *p*-values were reported and a Tukey–Kramer HSD *post hoc* test was used to compare individual coatings within each data-set (α=0.05).

Results and discussion

ZTMPS coatings

Figure 2 shows the synthetic scheme for bromination of the poly(arylene ether sulfone) TMPS and its subsequent addition of zwitterionic groups to make ZTMPS. The fraction of methyl groups that are converted into bromomethyl groups during the synthesis of Br-TMPS is controlled by the amount of brominating agent used. For simplicity, the structure of Br-TMPS in Figure 2 is drawn such that exactly one methyl group on each repeat unit was brominated. For this study, Br-TMPS samples with 31, 41, and 62% methyl to bromomethyl conversion levels were prepared to study how the density of zwitterions on a surface affects its biofouling prevention/release properties. For perspective, a Br-TMPS sample with a 100% conversion level would have four bromomethyl groups per repeat unit.

Br-TMPS was hand-sprayed onto substrata as evenly as possible to provide an average coverage of 0.8 mg cm^{-2}, or a thickness of about 7–8 μm. When Br-TMPS coatings were exposed to the aqueous DMABA solutions, the bromomethyl groups underwent nucleophilic attack by the tertiary amines to form quaternary ammonium cations with pendant carboxylate anions (Figure 2). As the ionic groups were formed, the polymer became more hydrophilic and allowed more DMABA to penetrate such that the entire thickness of the coating was converted from Br-TMPS to ZTMPS. XPS analysis of the ZTMPS coatings revealed no measurable amount of bromine, confirming the complete conversion of bromomethyl groups to ammonium groups. The mass fractions of bromine in the three Br-TMPS coatings were 16.6, 21.0, and 27.2%, in close agreement with the theoretical values of 17.5, 20.9, and 28.5%. This high conversion to ammonium groups is in agreement with previous studies of freestanding films of Br-TMPS in which treatment with an aqueous solution of a tertiary amine resulted in nearly complete conversion of bromomethyl groups to quaternary amines (Yan & Hickner 2010).

The Br-TMPS coating with the highest level of bromination swelled visibly when exposed to aqueous DMABA due to its increased hydrophilicity. After a few hours, it completely delaminated from the substratum illustrating an inherent limitation of zwitterionic coatings that are not covalently bound to the surface.

Table 1 gives the characterization of the polymer chemistry of the coatings used in this study. The ion exchange capacity, or IEC, indicates the number of ion pairs per gram of each polymer studied. IEC is calculated from the bromination level (measured by ^{1}H NMR) and the polymer structure assuming a complete conversion of bromomethyl groups to zwitterions. IEC is expressed as milliequivalents of ion pairs per gram of polymer and thus is directly proportional to the zwitterion density in the coatings. Because the different polymers in this study have large variations in the sizes of their repeat units, IEC is a useful way to compare zwitterion density among different coatings.

Table 1. Characterization of the zwitterionic coatings.

Coating	IEC[a] (meq g^{-1})	Contact angle (°)	% Atomic compositionmeasured[b] (theoretical) N	S	Si
Primed Al control	0	82	2.0 (N/A)	0.2 (N/A)	1.5 (N/A)
ZTMPS	2.40	50	2.2 (2.6)	4.5 (4.9)	0.1 (0)
ZTMPS	3.05	46	2.3 (3.2)	4.1 (4.5)	0.1 (0)
ZTMPS	4.29	—[c]	—[c]	—[c]	—[c]
pSBMA-co-TMSMA	3.09	33	3.6 (4.4)	10.1 (10.2)	2.5 (1.6)
gpSBMA	3.58	22	4.1 (5.0)	10.8 (11.5)	0.8 (0[d])
T2	0	110	—[e]	—[e]	—[e]
IS 700	0	108	—[e]	—[e]	—[e]
IS 900	0	104	—[e]	—[e]	—[e]

[a]IEC = ion exchange capacity. [b]Determined by XPS. [c]Coating delaminated during conversion to ionomer. [d]Assuming the base siloxane layer is completely covered by pSBMA. [e]Not measured.

The bulk water contents of freestanding films of ZTMPS with IEC values of 2.40 and 3.05 were determined to be 72 and 81 wt%, respectively. A determination of the water content at the surface of the films was not possible. However, given the rigidity of the polysulfone backbone and the entangled nature of the sprayed-on polymer chains, it is reasonable to assume that the concentration of water does not vary much through the thickness of the ZTMPS films and coatings.

Contact angle measurements were used to further investigate the hydrophobic nature of the polymer and XPS was used to identify compositional changes. Predictably, the water contact angle for the ZTMPS coatings decreased as the IEC increased from 2.40 to 3.05, due to the hydrophilicity of the zwitterions. The XPS analysis showed small changes in the nitrogen and sulfur contents in agreement with the theoretical values for the two different IECs. The very small difference in nitrogen content between the control and either of the ZTMPS coatings was determined to be due to a nitrogen impurity on the control since the nitrogen content of the Br-TMPS coating (prior to conversion to ZTMPS) was very low (0.2%).

Figure 3 displays the results of the AF and FR assessments for the ZTMPS (IEC = 2.40 and 3.05) coatings as well as T2, IS 700, and IS 900 standards. Biofilm retention (Figure 3a) and removal (Figure 3b) of the marine bacterium *C. lytica* and cell attachment (Figure 3c) and removal (Figure 3d) of the marine microalgae *N. incerta* were measured to determine the respective AF and FR properties towards microfouling. As shown in Figure 3a and c, there was no statistically significant difference in the amount of fouling that occurred on the two ZTMPS coatings (*C. lytica* ANOVA $p = 0.0094$; *N. incerta* ANOVA $p < 0.0001$) despite the difference in their zwitterion contents. This may indicate that there is a threshold ion concentration that is required to achieve the proper network of hydrogen bonded water molecules necessary for good AF properties. If the ion concentrations of the two ZTMPS coatings are below that putative threshold, AF properties are not observed. For bacterial biofilm retention, the two ZTMPS coatings were statistically equivalent to all three control coatings (*C. lytica* ANOVA $p = 0.0094$). In addition, the amount of microalgal cell attachment was significantly lower on the ZTMPS coatings than on T2 and IS 700 and significantly higher than on the IS 900 (*N. incerta* ANOVA $p < 0.0001$).

The *C. lytica* biofilm removal results are shown in Figure 3b. The two ZTMPS coatings performed similarly, although only the removal result for the ZTMPS with an IEC of 3.05 was statistically distinguishable from the IS 700 result. Both ZTMPS coatings showed significantly less removal when compared to the IS 900 control (ANOVA $p < 0.0001$), which enabled 97% of the biofilm to be removed by exposure to hydrodynamic forces generated by the impinging water-jet apparatus. In the microalgal cell removal test (Figure 3d), the ZTMPS coatings performed extremely well by allowing > 90% removal of the attached cells. Overall the ZTMPS coatings were shown to be significantly better than all three controls (ANOVA $p < 0.0001$) which had ~45% removal under the same water-jetting conditions. These good FR results observed with respect to *N. incerta* are in agreement with a recent report in which the same diatom was shown to release more easily from zwitterionic surfaces with higher surface energies (Finlay et al. 2013). The diatom biofilms were unstable in both cases because the adhesive molecules secreted from the cells were unable to bond with the highly hydrated surfaces.

The AF and FR properties of the two ZTMPS coatings were nearly identical. The similar performances of the two ZTMPS coatings are likely due to the fact that the ion concentrations are fairly close. The number of zwitterion pairs per repeat unit on the 2.40 and 3.05 IEC coatings are 1.3 and 1.6, respectively. This difference of 0.3 ion pairs per repeat unit is only enough to have a small effect on the water uptake, contact angle, and

microorganism adhesion properties. Also, because of the rigidity of the polysulfone backbone, these coatings are unable to self-organize into a phase-separated order with higher concentrations of ions at the water interface. These results were encouraging and led to the development of other zwitterionic coatings with higher IEC values.

pSBMA-co-TMSMA

In order to avoid the delamination issue that prevented testing of ZTMPS with an IEC > 3.05, the pSBMA-co-TMSMA coating was designed to covalently bind to the substratum. SBMA and TMSMA were first copolymerized in an 85:15 mol% ratio and then the Intergard® 264 primed substrata were immersed in the reaction solution without isolating the polymer (Figure 4). Intergard® 264 is an epoxy-based primer, so it had many residual hydroxyl groups that could engage in condensation reactions with the trimethoxysilyl groups on pSBMA-co-TMSMA. The resulting bonds help the hydrophilic copolymer adhere tightly to the surface in the presence of water. As indicated in Table 1, the IEC of SBMA-co-TMSMA (3.09) is very similar to that of the ZTMPS coating with the higher IEC (3.05). The polyacrylate backbone of SBMA-co-TMSMA is much more flexible than that of ZTMPS and it was intended that this backbone mobility would allow the aggregation of zwitterions to the outer (water side) surface of the coating. The bulk water uptake value for pSBMA-co-TMSMA and the ZTMPS with a similar IEC were similar (86% *vs* 81%, respectively). However, the contact angles of these two coatings are significantly different (33° for pSBMA-co-TMSMA versus 46° for ZTMPS). This indicates that a clustering of ions at the surface of the pSBMA-co-TMSMA may have taken place since the average density of ions throughout the two film types is nearly identical. The significant increases (relative to the primed control) in sulfur and silicon content (10.1 and 2.5%, respectively) served to verify that the coating was attached to the surface and could not dissolve in the water.

Figure 5 shows the AF and FR test results for the pSBMA-co-TMSMA coating as well as T2, IS 700, and IS 900 standards and an Intergard® 264 primer control. Due to severe CV dye binding to the pSBMA-co-TMSMA coatings, an alternative quantification technique based on ATP bioluminescence was utilized to assess bacterial biofilm retention and adhesion (Sule et al. 2009). The pSBMA-co-TMSMA coating, unlike ZTMPS, showed a significantly lower amount of bacterial biofilm retention than either of the control coatings (ANOVA $p < 0.0001$) (Figure 5a). The T2 and the primer coating performed as well as the zwitterionic coating. As for bacterial biofilm removal (Figure 5b), the results were similar to those for ZTMPS, as the zwitterionic coating (and the primer only coating in Figure 5b) was indistinguishable from the T2 and IS700 standards, while the IS900 showed significantly more removal (ANOVA $p = 0.0002$).

In the microalgal cell attachment study (Figure 5c), pSBMA-co-TMSMA enabled significantly less cell attachment than T2 or IS700, but significantly more cell attachment than IS900 (ANOVA $p < 0.0001$). The water jetting results displayed in Figure 5d show that the pSBMA-co-TMSMA coating was superior to all of the standards. Interestingly however, the primer control showed statistically equivalent cell removal when compared to the zwitterionic coating (ANOVA $p < 0.0001$). Following these tests, separate samples of pSBMA-co-TMSMA were prepared for the reattached barnacle adhesion test. The strength of attachment of reattached adult barnacles was measured in shear. Five barnacles were tested on each coating with a shear force applied until each barnacle was either removed cleanly or broken during the effort. None of the barnacles were removed from the pSBMA-co-TMSMA or primed surfaces without breaking. In comparison, all five barnacles on each of the IS700 and IS900 surfaces were removed cleanly and at relatively low force values (< 0.5 kg).

gpSBMA

To improve the FR properties of the zwitterionic coatings, a third composition with a higher IEC was prepared. Samples of grafted poly(sulfobetaine) or (gpSBMA), shown in Figure 6, were produced on primed aluminum substrata using synthetic schemes similar to those previously described (Zhang, Chao, et al. 2006; Zhang, Chen, et al. 2006). Due to the oxygen sensitivity of the CuBr catalyst in ATRP, ascorbic acid was used as a reducing agent to ensure Cu^{+1} was available to catalyze the reaction (Min et al. 2007). As with pSBMA-co-TMSMA, gpSBMA was covalently attached to the epoxy primer by siloxane linkages, although each gpSBMA polymer chain was only attached at one end to give a brush-type polymer coating.

gpSBMA has the highest IEC (3.58) listed in Table 1 since it has a polyacrylate backbone and a zwitterion pair on every repeat unit. This should have made it the most hydrophilic coating tested and its contact angle of 22° was in agreement with that expectation. A bulk water content measurement was not possible for this coating because its structure requires a substrate for attachment and initiation. Nevertheless, it can be speculated that the flexibility and brush structure of gpSBMA should allow for a water content that is higher than any of the other zwitterionic coatings in this study. The XPS analysis of gpSBMA showed significant increases in sulfur and silicon content (in agreement with theoretical values) after

Table 2. Zwitterionic and standard coatings normalized to IS 900 for each FR biological laboratory screening assay. Values indicate ratio to IS 900 ± one SD.

Coating	*C. lytica* biofilm removal	*N. incerta* cell removal
ZTMPS TIEC = 2.40	0.42 ± 0.09	2.01 ± 0.04
ZTMPS TIEC = 3.05	0.46 ± 0.06	2.05 ± 0.04
pSBMA-co-TMSMA	0.27 ± 0.23	1.22 ± 0.03
gpSBMA	0.69 ± 0.05	2.09 ± 0.02
Primed Al	0.15 ± 0.01	1.56 ± 0.52
T2	0.61 ± 0.10	0.60 ± 0.21
IS 700	0.36 ± 0.26	0.46 ± 0.20

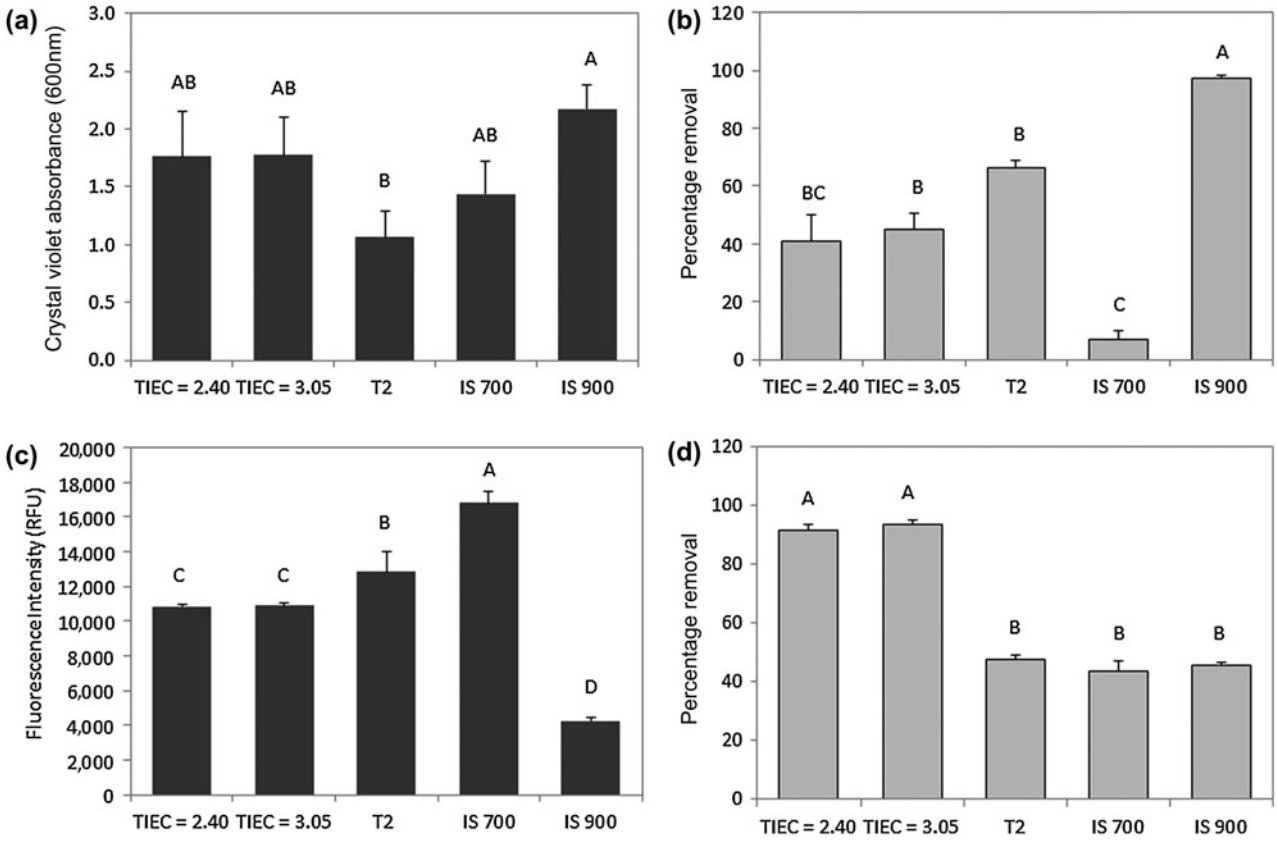


Figure 3. Evaluation of *C. lytica* biofilm (a) retention and (b) removal; and *N. incerta* cell (c) attachment and (d) removal on ZTMPS and control coatings. Data points that share a letter are not statistically different from one another (ANOVA, $\alpha = 0.05$).

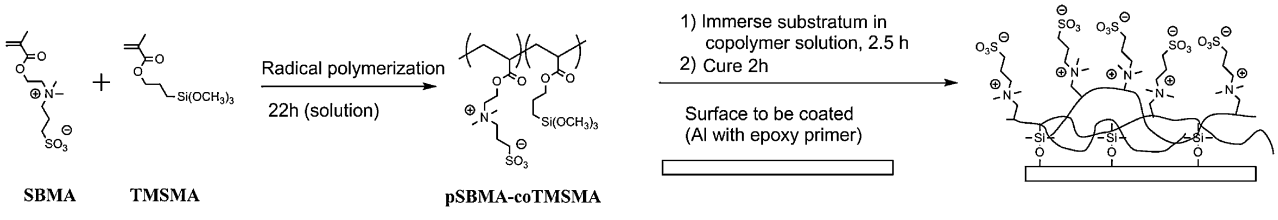


Figure 4. Synthesis and application of the pSBMA-co-TMSMA coating.

thorough washing with water and served to verify that the coating was attached to the surface.

In the bacterial biofilm and microalgal cell attachment assays (Figure 7a and c), gpSBMA did not distinguish itself from the primed aluminum control or the T2 standard, and statistically outperformed only the IS700 standard (*C. lytica* and *N. incerta* ANOVA $p < 0.0001$). The IS 900 was clearly better than all other samples at mitigating the retention of bacterial biofilm in this trial (Figure 7a). The gpSBMA coating performed very well in both of the removal assays (Figure 7b and 7d), enabling 70% removal of the bacterial biofilm and 99% removal of the attached microalgal cells. The gpSBMA was the only coating to achieve > 50% removal for both microorganisms. In particular, the primed aluminum showed relatively poor FR properties with respect to bacterial biofilm (15% removal) and IS 900 only permitted 48% microalgal cell removal. It is important to note that the cell attachment and biofilm retention on the IS 900 control for this trial was not consistent with the

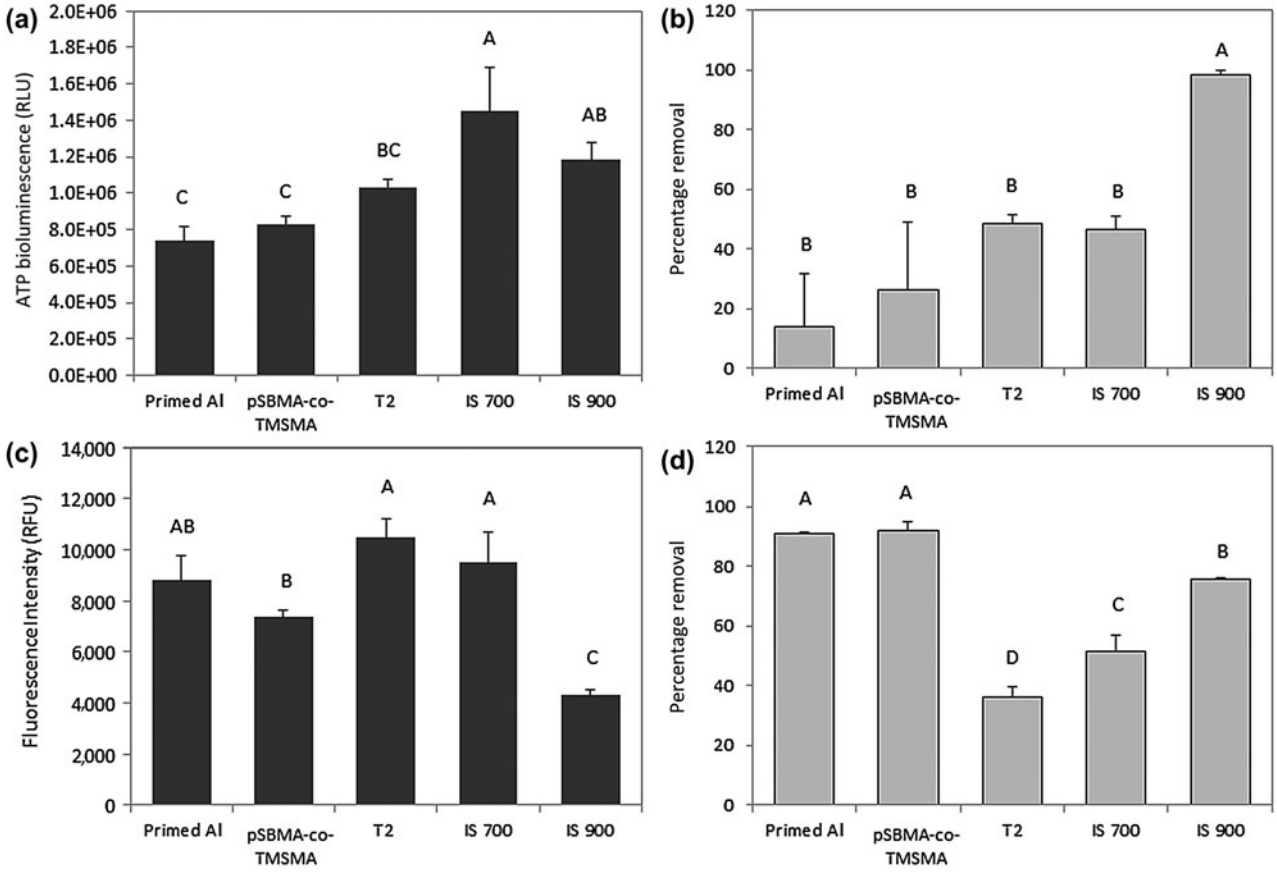


Figure 5. Evaluation of *C. lytica* biofilm (a) retention and (b) removal and *N. incerta* cell (c) attachment and (d) removal on pSBMA-co-TMSMA and control coatings. Data points that share a letter are not statistically different from one another (ANOVA, $\alpha = 0.05$).

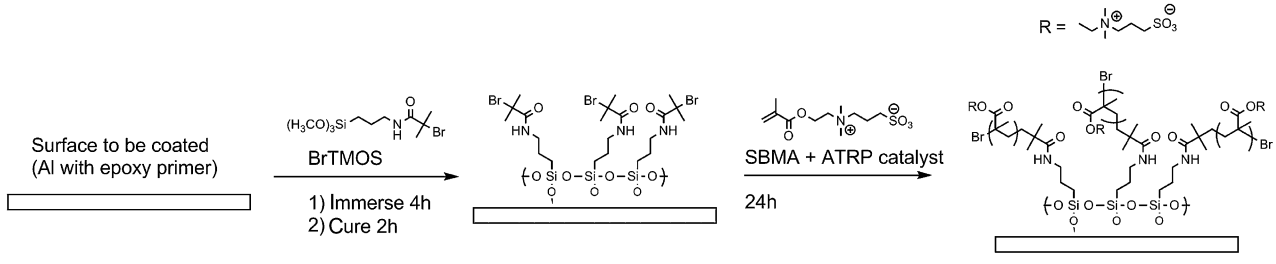


Figure 6. Synthesis and application of the gpSBMA coating.

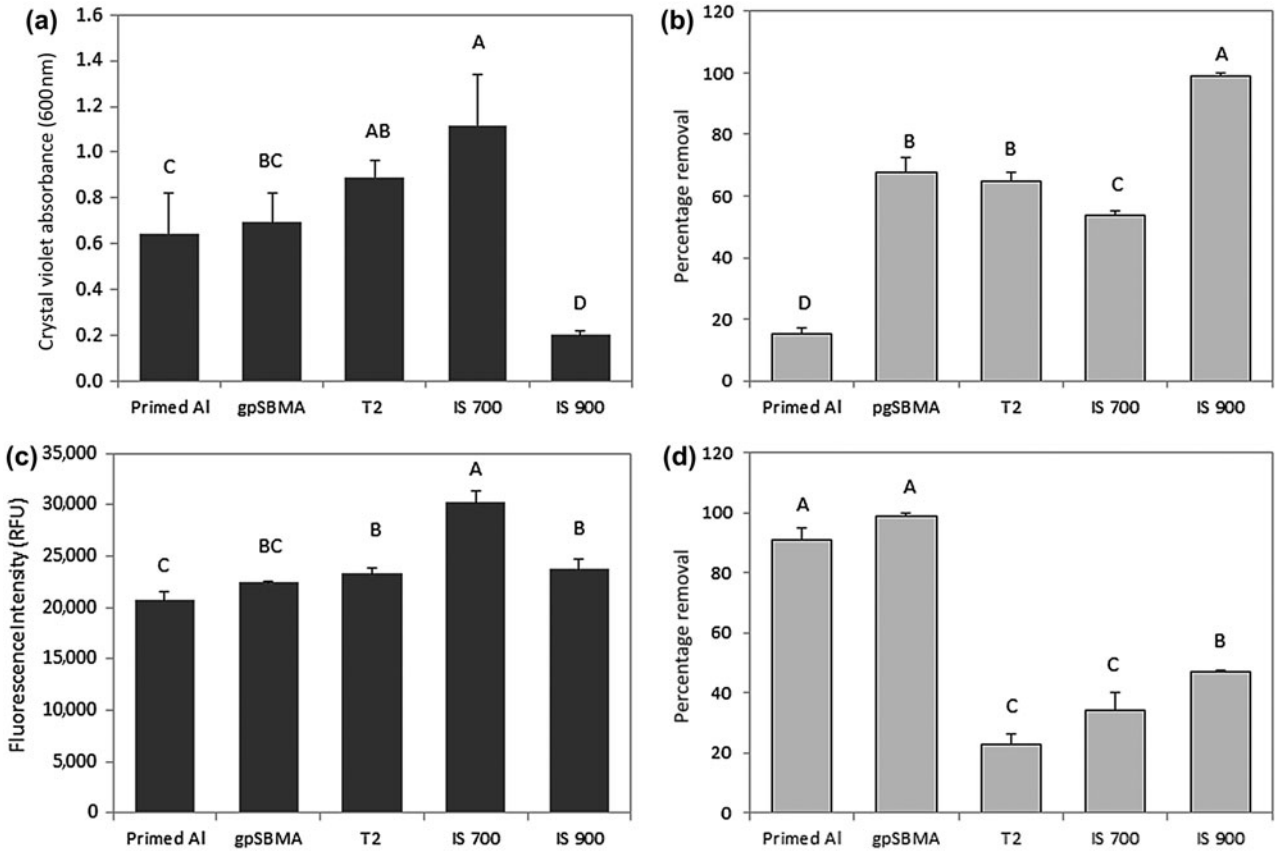


Figure 7. Evaluation of *C. lytica* biofilm (a) retention and (b) removal and *N. incerta* cell (c) attachment and (d) removal on gpSBMA and control coatings. Data points that share a letter are not statistically different from one another (ANOVA, $\alpha = 0.05$).

results observed for the first two experiments (Figures 3a and 3c, and 5a and c). However, the trend in bacterial biofilm and microalgal cell removal was very consistent for the IS 900 in all three trials; which may be expected as the IS 900 is a FR coating rather than an AF marine paint.

For a better comparison of FR performance among the zwitterionic coatings, Table 2 summarizes the microfouling removal data (from Figures 3, 5 and 7) with the results normalized to the IS 900 standard. In both the *C. lytica* and *N. incerta* removal studies, the gpSBMA zwitterionic coating was the best performing coating relative to IS 900. None of the zwitterionic or control coatings, however, outperformed IS 900 in bacterial biofilm removal assay, while all four zwitterionic compositions showed higher algal cell removal than IS 900.

Given the positive outcome of the water-jet removal assays, a barnacle adhesion assay was performed on the gpSBMA coating. Figure 8 shows the average adhesion value for adult barnacles reattached to the coating surfaces. Of the five barnacles that were subjected to force gage removal on gpSBMA, one animal broke and the other four were cleanly removed from this surface and exhibited an adhesion value that was statistically equivalent to the T2 control (ANOVA $p < 0.0001$). This result

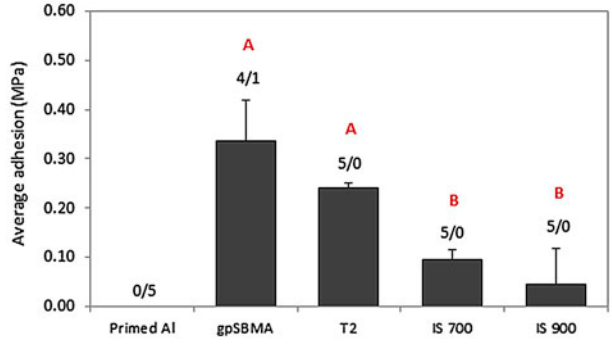


Figure 8. Average adhesion values for reattached adult barnacles that were dislodged from the coating surfaces without incurring visible damage or breakage to baseplates. The ratios above each bar indicate the total number of barnacles removed without baseplate damage *vs* the number of barnacles that exhibited baseplate damage during force gage removal. Data points that share a letter are not statistically different from one another (ANOVA, $\alpha = 0.05$).

was clearly better than the primed substratum which did not enable the removal of any barnacles without breaking or damaging their baseplates. The commercial standards (eg IS 900, IS 700, and T2) facilitated the removal of all five barnacles with lower shear forces (0.05–0.25 MPa) than was required for the gpSBMA coating (0.34 MPa).

The FR performance of gpSBMA against both microorganisms and barnacles is noteworthy as this coating had a thickness of only 1–2 μm and thus relied purely on surface chemistry to prevent adhesion. In Figures 5 and 7, the performances of the zwitterionic coatings are indistinguishable from the samples with only primer in almost all cases, but the barnacle adhesion results clearly show a great difference in the FR properties of a surface dominated by hydrogen-bonding –OH groups (epoxy primer) and one dominated by anion/cation pairs (gpSBMA). The T2, IS700, and IS900 standards are all paints with thicknesses > 200 μm. In the case of these thick coatings, elasticity plays an important role in determining AF and FR properties (in addition to surface chemistry). The development of thin AF and FR coatings would be particularly useful for applications that require some degree of optical transparency such as sensors or solar panels.

Conclusions

The three zwitterionic coatings prepared for this study demonstrated varying levels of AF and FR activity. The AF properties improved as the density of zwitterions on the surface was increased. The high hydrophilicity of these coatings required that they were strongly adhered to the substratum surface in order to prevent swelling and delamination. The zwitterionic systems examined were shown to be more effective as FR coatings rather than as AF coatings, with the best coating (gpSBMA) enabling the removal of four out of five reattached barnacles. The most effective structure was a brush-like polymer with a flexible backbone extending away from the surface. This type of structure has been well documented as a low-fouling coating in studies for primarily biomedical applications. It is expected that the brush structure of gpSBMA allows for greater surface hydration than the other polymers in this study and this results in a greater resistance to cell adhesion. The extension of zwitterionic coatings into the field of AF and FR in marine environments is promising but will probably require additional synthetic and application strategies.

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