ADVANCED MATERIALS INTERFACES

Supporting Information

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Self-Stratifying Porous Silicones with Enhanced Liquid Infusion and Protective Skin Layer for Biofouling Prevention

Alex Vena, Stefan Kolle, Shane Stafslien, Joanna Aizenberg, and Philseok Kim**

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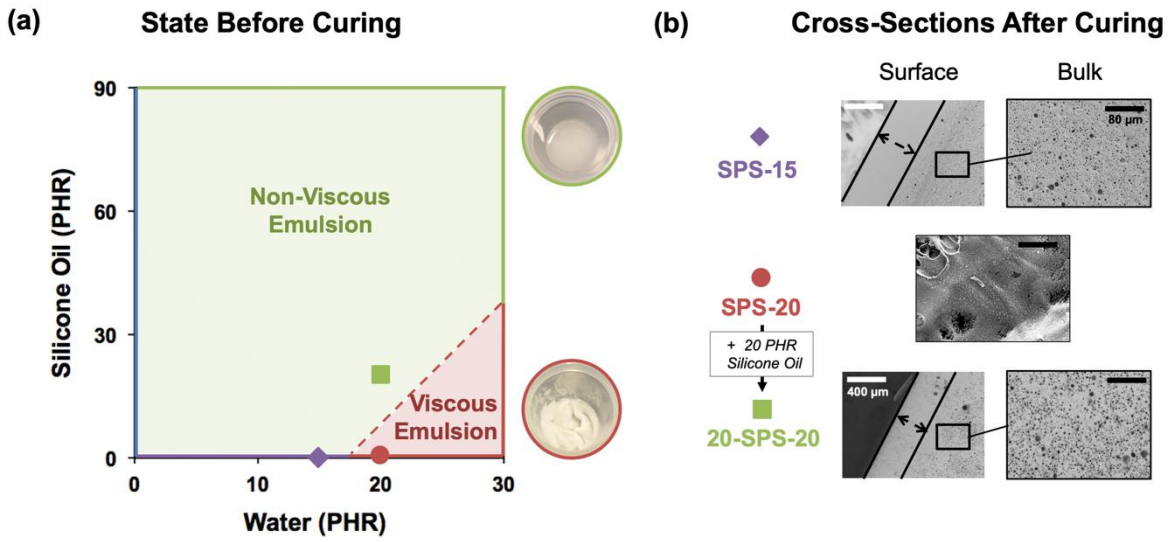

Figure S1. Overview of formulation design space investigated before and after SPS curing. (a) State diagram of SPS emulsions prepared with different amounts of water and silicone oil. A high viscosity paste is produced at high water concentration and low oil concentration. (b) SEM images of cured SPS vertical cross-sections near surface (left) and in bulk (right). While SPS-20 has a distinct pore morphology compared to SPS-15, the addition of silicone oil to the emulsion (20-SPS-20) created a similar pore morphology to SPS-15 at higher water concentration.

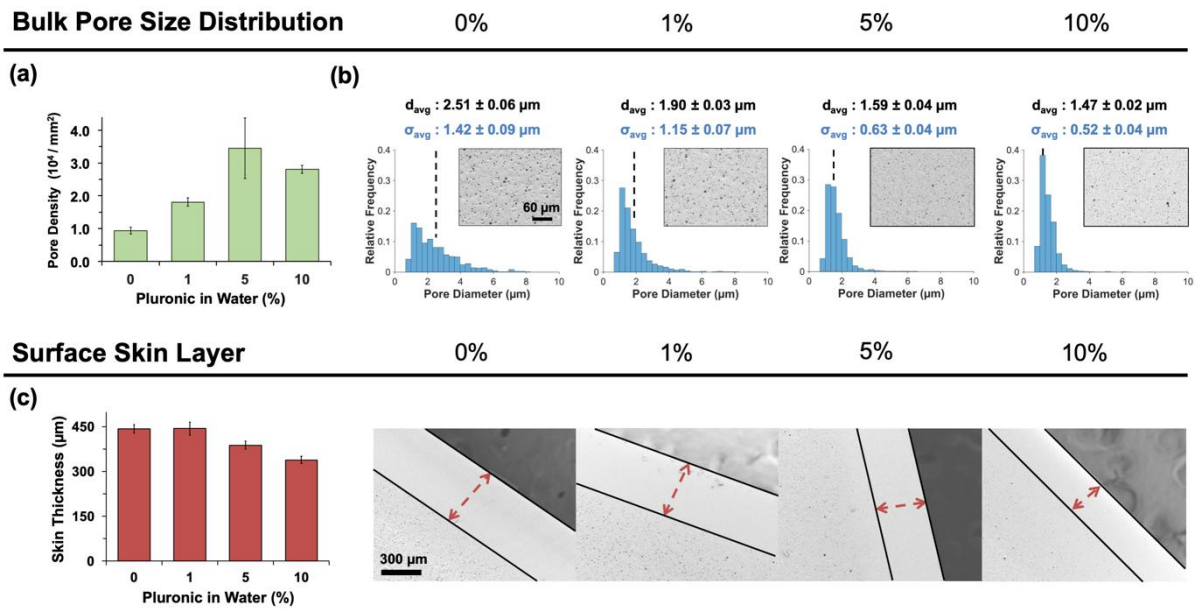

Figure S2. Pore size distribution and skin layer thickness of SPS resins prepared with surfactant concentrations from 0 to 10 wt.% in water [n = 5]. (a) Bulk pore density in SPS-10 increased with surfactant concentration. (b) SEM images and analyzed pore size distribution of bulk porosity in SPS-10. The mean pore diameter (d_{avg}) and pore size distribution (σ_{avg}) decreased at higher surfactant concentration. (c) Skin thickness of SPS-10 decreased as surfactant concentration in water was increased. SEM images of SPS-10 vertical cross-sections confirmed the presence of a surface skin layer at different surfactant concentrations.

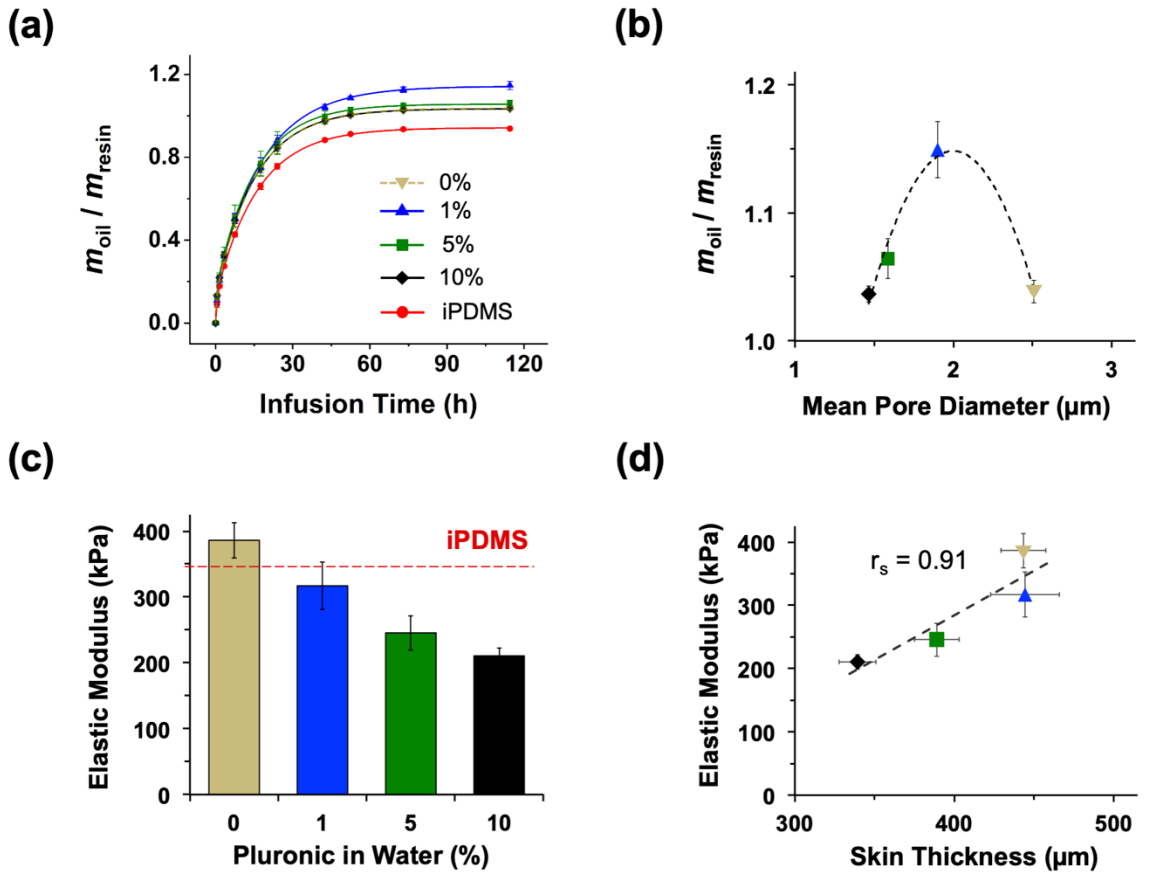

Figure S3. Equilibrium oil capacity and elastic modulus of iSPS-10 prepared with surfactant concentrations from 0 to 10 wt.% in water [n = 4]. (a) Infusion kinetics of SPS-10 immersed in silicone oil for 120 h. At equilibrium, all iSPS-10 variants had more silicone oil per initial mass of dry resin compared to iPDMS. (b) Equilibrium oil loading of iSPS-10 vs. mean pore diameter. Silicone oil infusion was maximized for iSPS-10 when the mean pore diameter is 1.9 μm, which was produced using a 1% Pluronic solution. (c) Elastic modulus of iSPS-10 with varying surfactant concentration. Elastic modulus decreased as surfactant concentration in water was increased. (d) Elastic modulus of iSPS-10 positively correlated to skin thickness with Pearson coefficient (r_s) of 0.91.

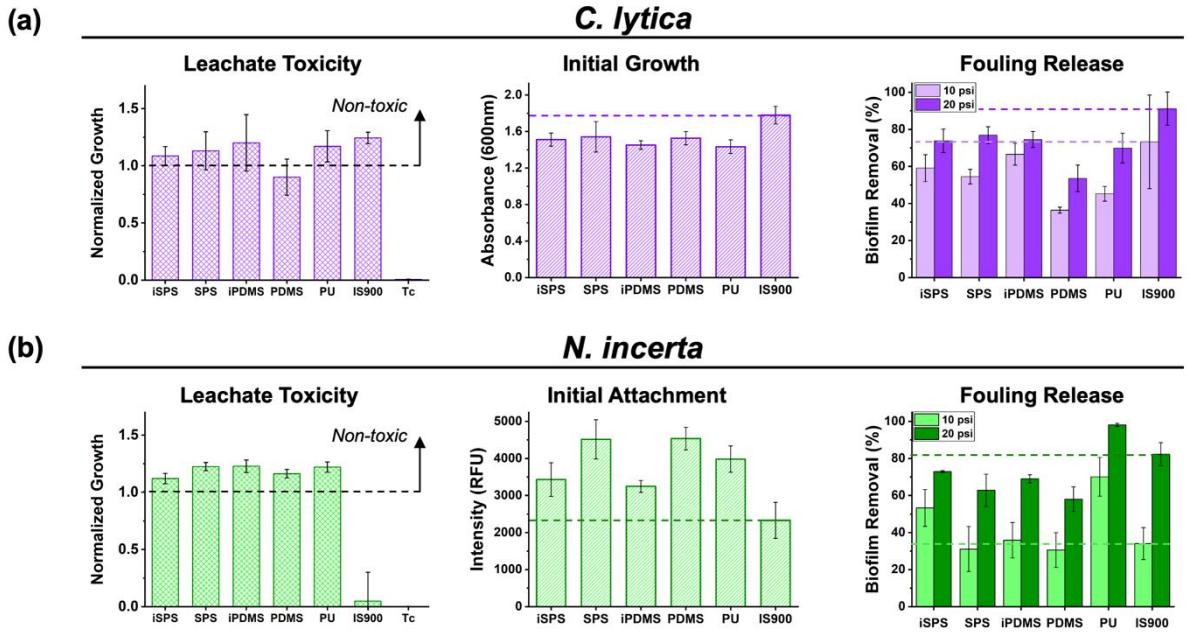

Figure S4. Leachate toxicity, initial cell attachment/biofilm formation and fouling release properties of iSPS-15 using model marine fouling organisms; bacterium *C. lytica* and microalgae *N. incerta* [n = 3]. For comparison, the performance of commercial standard Intersleek 900 (IS900) is denoted by a horizontal dashed line. (a) iSPS coatings were not toxic to *C. lytica* and had comparable attachment and removal efficiency to iPDMS and IS900. (b) iSPS coatings were not toxic to *N. incerta* and had comparable initial attachment to iPDMS, but slightly higher attachment than IS900. iSPS had higher *N. incerta* removal efficiency than iPDMS and IS900 under low pressure cleaning (10 psi). Error bars represent one standard deviation of the mean.

Table S1. Summary of skin thickness for different SPS compositions and curing conditions [error bars = 1σ, *n*=5]. Slower curing kinetics (regulated by lower cure temperature or less curing agent), low surfactant concentrations and low water concentrations independently produced a thicker skin layer for SPS.

Water Content [PHR]	Pluronic in Water [wt.%]	Base to Curing Agent	Cure Temperature [˚C]	Skin Thickness [μm]
0	0	10:1	70	2500 ± 50
5	1	10:1	70	615 ± 25
10	1	10:1	70	445 ± 22
15	1	10:1	70	320 ± 13
10	**0**	10:1	70	445 ± 14
10	**1**	10:1	70	445 ± 22
10	**5**	10:1	70	390 ± 14
10	**10**	10:1	70	340 ± 12
10	1	**10:1**	**40**	495 ± 15
10	1	**10:1**	**70**	405 ± 7
10	1	**10:3**	**70**	310 ± 6
10	1	**10:1**	**85**	325 ± 20
10	1	**10:1**	**110**	255 ± 18

Theoretical Oil Capacity for iSPS

The oil capacity of a liquid-infused resin is defined as the equilibrium mass of oil divided by the mass of the resin:

$$u_{infused\ resin} = \frac{m_{oil}}{m_{resin}} \tag{S1}$$

For iPDMS, the oil capacity can be rewritten as:

$$u_{iPDMS} = \frac{m_{oil}}{m_{PDMS}} = \frac{m_{iPDMS} - m_{PDMS}}{m_{PDMS}} \tag{S2}$$

The density difference between iPDMS and PDMS is negligible as silicone oil has comparable density to PDMS resin and proportionally increases the volume of PDMS during infusion. This was confirmed via experiment [n = 4] by measuring the volume and mass of PDMS before (0.96 ± 0.01) and after oil infusion (1.00 ± 0.01). As such, oil capacity of iPDMS can be expressed in terms of volume. Given a representative sample of uninfused as-polymerized PDMS with volume V that is infused with oil to a volume v in the swollen state, the oil capacity of iPDMS can therefore be written as:

$$u_{iPDMS} = \frac{v - V}{V} \tag{S3}$$

For a representative sample of SPS with the same PDMS volume V and a porosity p, the volume in the uninfused state is $V(1 + p)$. When SPS and PDMS are prepared with identical crosslinking stoichiometry and cured under the same environmental conditions, the silicone component of SPS swells by the same amount as PDMS (v). Assuming the swelling is isotropic, SPS porosity will stay the same, resulting in a pore volume(vp) that increases the same amount as the surrounding silicone matrix. Therefore, the total infused SPS volume is $v(1 + p)$. The oil capacity of iSPS is thus:

$$u_{iSPS} = \frac{v(1 + p) - V}{V} \tag{S4}$$

Combining Equation S3 and S4, the oil capacity of iSPS can be expressed in terms of iPDMS oil capacity, leading to the final result:

$$u_{iSPS} = u_{iPDMS}(1 + p) + p \tag{S5}$$

The theoretical oil capacity of iSPS for a given porosity p can be calculated using Equation S5 and the measured oil capacity of iPDMS (0.94). A comparison of the theoretical and measured oil capacity for iSPS variants is provided in **Table S2.**

Table S2. Comparison of the theoretical and measured oil capacity for iSPS variants.

	iSPS-5	**iSPS-10**	**iSPS-15**
Porosity (*p*)	0.05	0.10	0.15
Oil Capacity (*Observed*)	1.06 ± 0.02	1.15 ± 0.02	1.20 ± 0.01
Oil Capacity (*Theoretical*)	1.04	1.13	1.23
Absolute Percent Difference (%)	1.92	1.77	2.44

Overall, the measured oil capacity of iSPS is in good agreement with the theoretical oil capacity (Equation S5) for SPS porosities between 5 to 15%.

Statistical Analysis Tables for Biofouling Assays

Table S3. Statistical analysis tables for (a) *C. lytica* biofilm retraction assay, (b) *G. demissa* mussel adhesion assay and (c) *A. amphitrite* barnacle adhesion assay. PU was excluded from the barnacle adhesion assay analysis as 4 out of 5 barnacles broke during testing (i.e. the substrate has little fouling release potential). [*NS = Not Significant, * = p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p < 0.0001*]

a) *Cellulophaga lytica* Retraction [%]			
Treatment	**N**	**Mean**	**SD**
PDMS	3	100	0
SPS	3	100	0
PU	3	99.76	0.33
iPDMS	3	20.16	5.01
iSPS	3	25.06	9.01
IS900	3	31.07	6.53
ANOVA Summary			
F	476.3		
P value	<0.0001		
P value summary	****		
R square	0.995		
Brown-Forsythe Test			
F (DFn, DFd)	1.412 (5, 12)		
P value	0.2882		
P value summary	NS		
Tukey's Multiple Comparisons Test	**Mean Diff.**	**Summary**	**Adjusted P Value**
PDMS vs. SPS	0	NS	>0.9999
PDMS vs. PU	0.052	NS	0.8591
PDMS vs. iPDMS	1.368	****	<0.0001
PDMS vs. iSPS	1.317	****	<0.0001
PDMS vs. IS900	1.254	****	<0.0001
SPS vs. PU	0.052	NS	0.8591
SPS vs. iPDMS	1.368	****	<0.0001
SPS vs. iSPS	1.317	****	<0.0001
SPS vs. IS900	1.254	****	<0.0001
PU vs. iPDMS	1.315	****	<0.0001
PU vs. iSPS	1.265	****	<0.0001
PU vs. IS900	1.202	****	<0.0001
iPDMS vs. iSPS	-0.051	NS	0.8706
iPDMS vs. IS900	-0.113	NS	0.2105
iSPS vs. IS900	-0.062	NS	0.7504

b) Adhesion Strength of *Geukensia demissa* [N]			
Treatment	**N**	**Mean**	**SD**
PDMS	6	5.025	1.720
SPS	6	6.432	2.587
PU	5	5.845	2.043
iPDMS	6	0	0
iSPS	6	0	0
IS900	6	0	0
ANOVA Summary			
F	220.9		
P value	<0.0001		
P value summary	****		
R square	0.9736		
Brown-Forsythe Test			
F (DFn, DFd)	2.738 (3, 18)		
P value	0.0737		
P value summary	NS		
Tukey's Multiple Comparisons Test	**Mean Diff.**	**Summary**	**Adjusted P Value**
PDMS vs. SPS	-0.092	NS	0.5892
PDMS vs. PU	-0.059	NS	0.8841
PDMS vs. iSPS/iPDMS/IS900	1.482	****	<0.0001
SPS vs. PU	0.033	NS	0.9757
SPS vs. iSPS/iPDMS/IS900	1.573	****	<0.0001
PU vs. iSPS/iPDMS/IS900	1.540	****	<0.0001

c) Adhesion Strength of *Amphibalanus Amphitrite* [MPa]			
Treatment	**N**	**Mean**	**SD**
PDMS	5	0.0836	0.0452
SPS	3	0.0887	0.0300
iPDMS	4	0.0263	0.0189
iSPS	3	0.0220	0.0030
IS900	3	0.0320	0.0139
ANOVA Summary			
F	5.866		
P value	0.0064		
P value summary	**		
R square	0.6435		
Brown-Forsythe Test			
F (DFn, DFd)	0.7417 (4, 13)		
P value	0.5803		
P value summary	NS		
Tukey's Multiple Comparisons Test	**Mean Diff.**	**Summary**	**Adjusted P Value**
PDMS vs. SPS	-0.0171	NS	0.9943
PDMS vs. iPDMS	0.1347	*	0.0335
PDMS vs. iSPS	0.1401	*	0.0437
PDMS vs. IS900	0.1081	NS	0.1531
SPS vs. iPDMS	0.1518	*	0.0356
SPS vs. iSPS	0.1573	*	0.0428
SPS vs. IS900	0.1253	NS	0.1324
iPDMS vs. iSPS	0.0055	NS	>0.9999
iPDMS vs. IS900	-0.0266	NS	0.9751
iSPS vs. IS900	-0.0320	NS	0.9619

Experimental Methods

Toxicity Assessments of Coating Leachates

Toxicity assessments of coating leachates were performed after 7 days of water immersion preconditioning in an aquarium tank system supplied with a continuous flow of tap water at a rate of 7.57 liters h^{-1} to ensure that all potentially toxic impurities had been removed from the cured coating films prior to conducting surface property assessments of anti-biofouling performance. The specifics of the methods used to assess the toxicity of coating leachates towards the suite of fouling organisms employed in this study have been reported in detail in a recent publication by the authors.[1]

Bacterial Biofilm Retraction and Removal

The analysis of *Cellulophaga lytica* biofilm retraction was performed on the coatings deposited in 24-well plates as previously described.[2,3] Briefly, the coatings were inoculated with 1.0 ml of a 10^7 cells ml^{-1} suspension of *C. lytica* in biofilm growth medium (BGM; 0.5 g peptone + 0.1 g yeast extract per liter of artificial sea water) and the plates were incubated statically for 24 h at 28°C. Subsequently, the BGM and planktonic bacterial growth were discarded, and the wells were rinsed with artificial sea water (ASW; 38.5 g per liter of Sigma sea salts) three times to remove unattached or weakly adhered biofilm. The plates with retained bacterial biofilm were dried for 1 h under ambient conditions and subsequently stained with crystal violet (0.3% w/v in deionized water) for 15 min. The plates were rinsed three times with ASW to remove excess crystal violet, tapped firmly on an absorbent pad and allowed to dry at ambient laboratory conditions. The amount of biofilm retraction on each coating surface (i.e., percent surface coverage) was quantified using a customized, automated image-based software program as described in detail in a previous publication by the authors.[4]

Bacterial biofilm removal was carried out by exposing retained biofilms to a pressurized stream of ASW using an automated water-jet apparatus.[5] The coatings were inoculated with 1.0 ml of a 10^5 cells ml^{-1} suspension of *C. lytica* in BGM and incubated statically for 24 h at 28 ºC. Subsequently, the plates were then transferred to the deck of an automated water-jet apparatus. Three replicate wells for each coating composition were excluded from water-jet treatments and were used to measure the initial amount of biofilm retained on each coating surface. Three additional replicate wells were treated with the water-jet apparatus at a 10 and 20 psi nozzle pressure for 5 s. After water-jet treatments, the plates were stained with 0.5 mL of crystal violet, rinsed three times with ASW water and allowed to dry at ambient laboratory conditions for 1 h. Digital images were captured of each plate and 0.5 mL of 33% acetic acid was then added to each well for 15 min to solubilize the crystal violet dye retained in the adherent biofilm on the coating surfaces. 0.15 mL of the eluate from each well was 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 the water-jet treatments as follows:

$$\%\text{Removal} = (1-(TBM_J / TBM_{NJ})) \times 100,$$

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

Microalgae Cell Attachment and Removal

Microalgae cell attachment and removal on coatings prepared in 24-well plates has been described previously in detail.[6] Briefly, the coatings were inoculated with 1.0 ml of

Navicula incerta suspension in F/2 (0.03 OD_{660} or ~10^5 cells ml^{-1}) and incubated statically for 2 h at room temperature to facilitate cell attachment. The plates were subsequently transferred to the deck of an automated water-jet apparatus and three replicates of each coating were water jetted at 10 and 20 psi nozzle pressure for 10 s. Three replicates wells of each coating were left untreated and served as initial cell attachment measurements. 0.5 ml of DMSO was added to each well and the plates were incubated statically in the dark for 20 min to extract chlorophyll *a* from the attached microalgae biomass. The resulting eluates were transferred (0.15 ml) to a 96-well plate and fluorescence measurements were acquired using a multi-well plate spectrophotometer (excitation wavelength: 360 nm, emission wavelength: 670 nm). Percentage removal calculations were determined by comparing the total biomass on the coating surfaces before and after the water-jet treatments as follows:

$$\%\text{Removal} = (1-(TBM_J / TBM_{NJ})) \times 100,$$

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.

Adult Barnacle Reattachment and Adhesion

The method of assessing the reattachment and adhesion of adult barnacles has been previously described in detail.[7,8] Adult barnacles (*Amphibalanus amphitrite* = (*Balanus amphitrite*)) of a testable size (>5mm basal diameter) were dislodged from glass panels coated with a silicone elastomer (Silastic-T2 on glass) and placed on the surface of the coatings produced for the study on 10 cm x 20 cm panels. Immobilization templates were applied to each panel and then transferred to an artificial saltwater aquarium tank system. The reattached barnacles were fed daily with freshly hatched brine shrimp nauplii (*Artemia* sp.). After 14 days of reattachment in the aquarium system, the coatings were removed and the barnacles were dislodged with a hand-held force gauge in shear to measure the peak force at release (lbs). Subsequently, the area of the barnacle base plates was measured using Sigma Scan Pro 5.0 and the adhesion strength was calculated by dividing the force required to remove the barnacles by the basal area and reported as megapascals (MPa). 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 excluded from average adhesion calculations. Barnacle attachment was reported as the percent of barnacles that successfully reattached to the coating surface (n = 5 attempted) as exhibited by a measurable force gauge value.

Adult Mussel Attachment and Adhesion

The assessment of marine mussel attachment and adhesion to the coating surfaces was evaluated using a method reported previously.[9] Prior to the attachment study, a 4 cm long acetal plastic rod was adhered to each mussel (*Geukensia demissa*) perpendicular to the ventral edge using an acrylic adhesive. Subsequently, six mussels were immobilized onto each coating surface using a custom-designed template fabricated from PVC sheets. The coatings containing immobilized mussels were then placed in an ASW aquarium system and fed daily with live marine phytoplankton. The coatings were subsequently removed from the ASW aquarium tank system after 3 days of immersion and the total number of mussels exhibiting attachment of byssus threads was recorded for each coating. The plastic rod of each attached mussel was then affixed to individual 5 Newton load cells of a custom-built tensile force gauge that enabled all attached mussels to be pulled off simultaneously (1 mm s^{-1} pull rate). The total force (Newtons) required to completely detach all byssus threads for each mussel was recorded and the mean adhesion value of the total number of attached mussels for each coating was calculated.

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