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Physicochemical Properties of (Ethylene Glycol)-Containing Self-Assembled Monolayers Relevant for Protein and Algal Cell Resistance

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The influence of the number of repeating units in self-assembled monolayers (SAMs) of ethylene glycol and of their end-group termination on the settlement and adhesion of two types of algal cells, viz., zoospores of the macroalga *Ulva* and cells of the diatom *Navicula*, was studied. The findings are related to the resistance of these surfaces against fibrinogen adsorption. Results showed that settlement and adhesion of algal cells to oligo(ethylene glycol) (OEG; 2−6 EG units) and poly(ethylene glycol) (PEG; MW = 2000, 5000) SAMs was low, while resistance was less effective for mono(ethylene glycol) (EG_1OH)-terminated surfaces. These findings concur with former protein adsorption studies.[1,2] In situ microscopy showed that PEG surfaces inhibited the settlement of zoospores, i.e., zoospores did not attach to the surfaces and remained motile. In contrast, on $EG_{2-6}OH$ surfaces, although zoospores settled, i.e., they secreted adhesive and lost motility, adhesion between secreted adhesive and the surface was extremely weak, and the settled spores were unable to bond to the surfaces. The influence of surface properties such as hydration, conformational degrees of freedom, and interfacial characteristics of the SAMs is discussed to understand the underlying repulsive mechanisms occurring in (ethylene glycol)-based coatings.

Introduction

Biofouling, the undesired growth of marine organisms on submerged surfaces, is a global problem with both economic and environmental penalties.[3,4] Since the use of biocidal antifouling paints is increasingly being restricted, science and industry are seeking environmentally benign coatings to control fouling.[5] Basic research can contribute a fundamental understanding by determining the surface properties that inhibit settlement of the colonizing stages of fouling organisms or that reduce the adhesion strength of the attached organisms so that they are more easily removed by shear forces. One group of model surfaces that can provide insight into the mechanism of interface interactions are self-assembled monolayers (SAMs), which allow the tailoring of surface properties in a reproducible and well-defined manner.[6,7] Such surfaces have been used for protein and cell adhesion studies for many years, and their surface properties have been characterized in detail by various techniques. More recently, studies with zoospores of the green seaweed *Ulva,* a major fouling organism, provided information about the surface properties which influence the settlement processes and the strength of adhesion.[2,8,9] "Settlement" here is defined as the process whereby motile spores attach irreversibly to a surface, rapidly (within minutes), secrete a glycoprotein adhesive, and lose their flagella.[10] "Adhesion strength" refers to the ease with which settled spores are removed from a surface when exposed to a calibrated hydrodynamic shear stress.

The microscopic, motile zoospores of *Ulva* respond to a number of physical surface cues including wettability,[2,11,12] topography,[13] lubricity,[14] and charge.[15] A systematic change of wettability using varying compositions of mixed OH/CH_3-terminated alkanethiols revealed that the resulting SAMs on gold inhibited settlement of zoospores on the hydrophilic surfaces. Only low numbers of spores settled on these surfaces, which therefore could be said to have antifouling properties.[8,11,16] However, the adhesion *strength* of zoospores was much higher on the less attractive, hydrophilic, OH-terminated surfaces compared with the more attractive, hydrophobic, CH_3-terminated surface.[16] Wettability is a surface property that also has been correlated with the protein resistant properties of surfaces.[1,17] SAMs containing oligo- (EG_Y) and

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poly(ethylene glycol) (PEG) have been used in the biomedical field for a number of years.[18] A wettability series of SAMs based on hexa(ethylene glycol)-containing alkanethiol SAMs on gold with different end-group terminations (EG_6OX; X = H, CH_3, C_2H_5, C_3H_7) was used previously to investigate the effect of varying surface wetting properties on the attachment of two types of algae, viz., zoospores of *Ulva* and cells of the diatom *Navicula perminuta*.[2] For both types of cells, it was found that the number of spores of *Ulva* or cells of *Navicula* that attached to the SAMs increased systematically with the increase in contact angle.[2] This trend paralleled the results of an earlier study on fibrinogen adsorption for the same set of EG_6OX SAMs.[1] Careful observation of the surfaces exposed to spores of *Ulva* indicated that the most hydrophilic EG_6OH surface, which also had the highest protein resistance, was extremely *attractive* to spore settlement with rafts of settled spores formed on the surface. However, the strength of adhesion of these settled spores was so low that even the minimal shear forces created by a small disturbance of the slides in the assay dishes was sufficient to remove the attached spores from the surface, i.e., the adhesive secreted by spores was unable to bond to the surface.

However, the previous study was concerned solely with SAMs based on EG_6 with different terminations to provide a range of wettabilities. In order to further expand our understanding of how spores interact with protein-resistant surface coatings based on ethylene glycol (EG), we have explored the effect of EG chain length (with OH termination), and expanded our study to include PEG (OH- and OMe-terminated). This enabled us to test how other film properties, such as packing density, chain length, or amount of bound water in the oligo(ethylene glycol) (OEG) film affect adhesion of algal cells. The EG_6OH and EG_6OMe SAMs investigated previously were compared to a set of polymeric homologues of PEG (PEG thiol) with higher molecular weight. Although protein resistance of the latter coatings is retained, EG_6 and PEG differ subtly with respect to their surface properties. For example, hydration, surface charge, elasticity, conformational degrees of freedom, surface density, and interfacial characteristics are significantly different.[18−22] In addition to long chain length PEGs, a set of short chain length OH-terminated EG SAMs (EG_YOH, Y = 1−5) was prepared in order to determine the minimal threshold of EG units required to maintain resistance to settlement and/or adhesion of spores of *Ulva*. Lowering the number of EG units causes a decrease in hydration of the SAMs, while the wettability is kept constant. If the EG units in the SAM are reduced to one, the hydration energy is no longer strong enough to prevent displacement of hydration water molecules with the result that adhesion becomes irreversible.[19,23−25]

In addition to studying interactions with spores of *Ulva*, in the present study we have performed a parallel study with cells of the diatom (unicellular alga) *Navicula perminuta*. Diatoms are not motile in the water column, and in laboratory assays reach a surface by gravity, i.e., they cannot explore the surface for adhesion sites. They adhere to surfaces through the secretion of sticky extracellular polymeric substances (EPSs) comprising a range of complex proteoglycans. Adhesion of cells to a surface requires bonding of the EPS with the surface.[26]

Materials and Methods

Preparation and Characterization of the EG_6OX, EG_YOH, PEG2000-OX, PEG5000-OX SAMs (X = H, CH_3; Y = 1−6). Ethanol, *N*,*N*-dimethylformamide (DMF), fibrinogen, and phosphate-buffered saline (PBS) were purchased from Sigma-Aldrich (Munich, Germany). Deionized water was purified with a Milli-Q plus system (Millipore, Schwalbach, Germany). PBS buffer was used at pH 7.4 and a concentration of 0.01 mol/L. Float glass slides were obtained from Schott AG (Mainz, Germany). Thin films of polycrystalline gold were prepared by thermal evaporation deposition of 30 nm gold (99.99% purity) onto Schott float glass slides primed with a 9 nm titanium adhesion layer. Evaporation was performed at a pressure of 2×10^{-7} mbar and a deposition rate of 0.5 nm/s, deriving a root-mean-square (rms) roughness of about 1 nm. The gold-coated slides were prepared in Heidelberg and stored under argon until SAMs were prepared in Birmingham immediately prior to assay. The molecular structure of EG_6OX and EG_YOH is HS-$(CH_2)_{11}(OCH_2CH_2)_YOX$ (X = H, CH_3; Y = 1−6). The PEG molecules comprise HS-$(CH_2)_2(OCH_2CH_2)_YOX$ (X = H, CH_3; $Y \approx 44$, 112) and are a distribution of different chain lengths centered around 44 and 112 EG units, respectively. EG_6OMe was synthesized in a three-step reaction according to a protocol by Prime et al.[27,28] EG_6OH was bought from Obiter Research, LLC (Champaign IL). All PEGs were bought from Rapp Polymere GmbH (Tuebingen, Germany), and $EG_{1-5}OH$ was obtained from ProChimia Surfaces Sp. z o.o. (Sopot, Poland).

For the SAM formation, 1 mM solutions of EG_6OX and EG_YOH in ethanol, and 50 μM solutions of PEG in DMF were prepared, respectively. Prior to immersion, the samples were placed in a UV light-emitting photochemical reactor (Southern New England Ultra Violet Company, Branford CT) for 2 h for surface cleaning, and then immersed into the thiol solution for 36 h in the dark at room temperature. After removal from the thiol solution, the SAMs were first rinsed with the same solvent that was used for the assembly procedure, then with ethanol, and finally dried in a flow of nitrogen. The samples were stored under nitrogen.

To verify a successful SAM formation, the samples were analyzed with three different methods; contact angle goniometry, spectral ellipsometry, and X-ray photoelectron spectroscopy (XPS). Sessile drop (Millipore) water contact angles were measured by goniometry under ambient conditions. Droplets were dispensed from a micropipet. The reported values are the average of three measurements taken for different samples with the tip not being in contact with the droplet. Film purity and thickness were analyzed by XPS using a Leybold-Heraeus MAX 200 X-ray photoelectron spectrometer with a magnesium anode as the X-ray source, Kα = 1253.6 eV. The calculation of the film thickness with XPS was done by quantifying the attenuation of the gold substrate signal due to the adsorption of the SAMs following the description in previous investigations.[19,29] SAM thickness measurements were also performed with a Horiba Jobin Yvon UVISEL spectral ellipsometer operating in a wavelength range

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between 250 and 800 nm. The organic film was modeled as a single Cauchy layer at a constant refractive index of 1.5.

Protein Adsorption Assay. The protein resistance of all samples was tested by a fibrinogen assay as described in detail by Schilp et al.[2] Each sample was immersed in 10 mL of a PBS-buffered solution of fibrinogen (1 mg/mL) for 15 min. After rinsing and drying, the amount of adsorbed fibrinogen was measured by spectral ellipsometry from two points on the sample.

Settlement and Adhesion Assays for Zoospores of *Ulva*. Fertile plants of *Ulva linza* were collected from the beach at Llantwit Major, South Wales, U.K. (51° 40′N, 3° 48′W). Zoospores were released and prepared for settlement and adhesion experiments as described previously.[30] The settlement assay and method of counting spores is summarized in the work of Schilp et al.[2] Briefly, six replicates of each surface were placed in Quadriperm dishes, and 10 mL of a spore suspension was added (1.5×10^6 spores/mL). After 45 min in darkness, the slides were washed gently to remove unsettled, i.e., motile spores. Careful visual observations of the slides were made at this time to determine whether rafts of settled spores were disturbed.[2] Three replicates were used to determine the number of settled (attached) spores. To determine the adhesion strength of the attached spores, the remaining three replicates were exposed for 5 min to a shear stress in a calibrated water channel at a wall shear stress of 53 Pa (equivalent shear rate 4.9×10^4 s^{-1}) using methods described previously.[31] The data are expressed as percentage removal, calculated from the number of spores remaining after flow compared to the unexposed samples. Nexterion glass slides (Schott, Germany) were included as standards in the assays.

Diatom (*Navicula perminuta*) Adhesion Assay. *Navicula perminuta* cultures were grown in Guillard's F/2 medium made up using natural seawater.[32,33] The assay for settlement of *Navicula* followed the general principles as for *Ulva*. Details are given in the work of Pettitt et al.[33] For cell detachment studies, slides were exposed to a wall shear stress of 8.2 Pa (equivalent shear rate 7.5×10^3 s^{-1}) in a water channel apparatus. Percentage removal was calculated as described for *Ulva*.

Results

Adhesion of Zoospores of *Ulva* and Cells of *Navicula* on Long Chain Length PEG and EG_6-SAMs with Hydroxyl and Methyl End-Group Termination. Part of the EG_6OX series with systematically changed wettability described previously[2] was extended with a set of commercially available polymeric homologues of PEG (PEG thiol) with different molecular weights and hydroxyl and methoxyl end-group terminations. Varying the chain length of the EG units retains the protein resistance of these coatings, but, in comparison to EG_6OH, properties such as hydration, conformational degrees of freedom, and interfacial characteristics are changed, which may potentially influence the behavior of spores in terms of either settlement and/or strength of adhesion.[18−21]

Sessile drop contact angles were in good agreement with literature values,[1,34] as well as the film thicknesses, which were obtained by XPS and spectral ellipsometry.[2,34,35] The contact angle for EG_6OMe (68°) was significantly higher than that for EG_6OH (33°) because of the terminal methyl group.[1,2] In agreement with literature results, the wetting properties of the PEG surfaces did not depend on the chain length or termination and were similar to those of EG_6OH.[34]

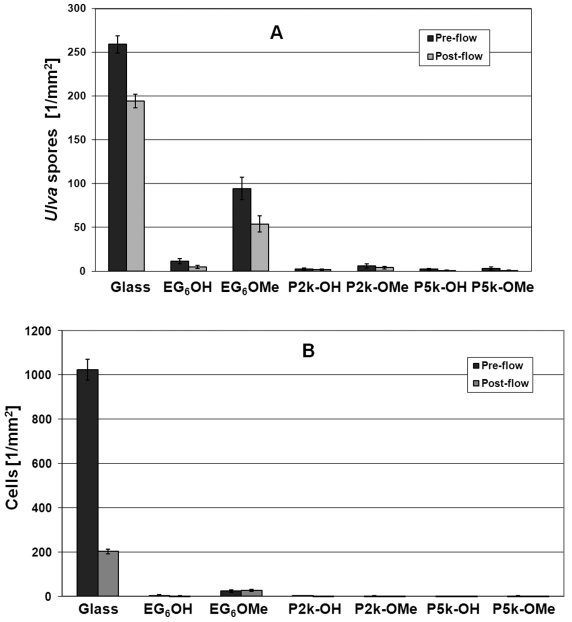

Figure 1. Settlement density of zoospores of *Ulva linza* (A) and cells of *Navicula perminuta* (B) on long-chain PEG-OH/OMe and short-chain EG6OH/OMe before (preflow) and after (postflow) exposure to a wall shear stress of 53 Pa (*Ulva*) or 8.2 Pa (*Navicula*). The points represent the mean number of cells attached. Bars are means of 90 counts, 30 from each of three replicates; bars show 2× the standard error of the mean (SEM).

The results of the settlement and adhesion bioassays for spores of *Ulva* are shown in Figure 1A. Regarding end-group termination, spore settlement on all three hydroxyl-terminated surfaces (EG_6OH, PEG2000-OH, and PEG5000-OH) was similar, all surfaces having a low number of attached spores. In the case of the methoxyl-terminated SAMs, only the polymeric homologues PEG2000-OMe and PEG5000-OMe had low numbers of attached spores, whereas a significant amount of spores settled on the oligomeric EG_6OMe. The extremely low settlement on all PEG surfaces (between 0 and 2 spores per field of view) precluded any statistically reliable quantification of the adhesion strength of the settled spores since such measures rely on comparisons of spore density before and after shear. Comparison of EG_6OH and EG_6OMe confirm the same trend as reported before,[2] which showed that more spores settled on the methyl-terminated EG_6OMe compared to the hydroxyl-terminated surface (Figure 1A). Similar results were obtained for cells of *Navicula perminuta* (Figure 1B), as no cells remained attached to any of the surfaces after gentle washing except for EG_6OMe to which a few cells adhered. The data for *Navicula* for the EG_6 surfaces concurred with previous data.[2] Interestingly, increasing the number of EG units from EG_6OMe to PEG-OMe (MW 2000 and 5000) caused the surface to become fully resistant to cell attachment, and both the hydroxyl- and the methoxyl-terminated PEG coatings prevented any diatom cells from attaching. This result is in line with the observation that PEG-OH and PEG-OMe had similar contact angles.[34] An explanation for the similar properties of the differently terminated PEGs with respect to algae and protein adhesion is that the high degree of conformational freedom allows the end-groups of the PEG chains to be buried in the film, which is not possible for the crystalline and densely packed EG_6 SAMs.

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The Influence of OEG Chain Length on Settlement and Adhesion of Zoospores of *Ulva*. The results obtained for the EG_6 and PEG surfaces raised the question of whether a minimum number of EG units was required for resistance to attachment of algal cells. Irreversible adsorption of protein is inhibited if strong hydration bonds are present in the SAM.[24,25] Furthermore, Klose et al. found that the effective water binding energy per mole of EG-terminated alkane linearly rises by 7 kJ/mol per added EG unit.[23] In order to investigate the influence of hydration on *Ulva* adhesion experimentally, shorter chain length SAMs of EG_YOH with Y = 1−5 subunits were prepared.

The wetting properties of EG_YOH were similar to EG_6OH and are shown in Table 1.[2] Thicknesses of EG_YOH SAMs indicate a crystalline, upright chain structure, which is also reflected by a steady increase of film thickness with rising number of EG units. As Table 1 also shows, the protein adsorption strongly depends on the numbers of EG repeat units. EG_1OH adsorbed a fibrinogen layer of almost 19 Å thickness, which concurs with earlier reports.[1] EG_2OH is in the transition range, as the formed protein layers are significantly lower than for EG_1OH. From EG_3OH-EG_6OH and for long chain PEGs, no protein adsorption was detected. The observed protein deposition of EG_2OH-EG_6OH SAMs is in line with previous reports.[1] An explanation is the degree of hydration of EGs with one EG unit. Because of the existence of only one hydrogen bridge donor oxygen atom in EG_1OH SAMs, it is rather low and causes water molecules in the film to be displaced more easily by the approaching proteins.[24,25]

Figure 2 shows the results of the settlement assay with zoospores of *Ulva*. In line with PEG and EG_6OH, shorter EGs were resistant down to EG_2OH. This again concurs with the protein resistance of the SAMs shown in Table 1 and earlier reports.[1] Although EG_1OH did not inhibit settlement of spores, the strength of attachment of the spores was weak since exposure to a wall shear stress of 53 Pa resulted in 92.8% of the attached spores being removed (compare preflow and postflow bar in Figure 2).

In Situ Microscopy of Settled Zoospores of *Ulva*. In view of the settlement behavior of spores of *Ulva* on EG_6OH, i.e., large numbers of spores settled, but the secreted adhesive was unable to firmly bond to the SAMs,[2] the surfaces coated by PEG and EG_YOH were inspected in greater detail at the end of the settlement assay. In the case of all EG_YOH SAMs, the green spore suspension was depleted of color, and a green layer of settled spores was visible on the test surfaces. As shown previously for EG_6OH,[2] the spores were easily dislodged from all surfaces by gentle rinsing except for EG_1OH. The situation for PEG was different; visual inspection of the assay dishes indicated that the spores were swimming in the seawater solution (the liquid was green) and there was no evidence of a green layer on the test surfaces. For visualization, a series of microscopy images was taken after the settlement period in situ, prior to washing the samples, in the focal plane of the surface.

Figure 3 shows a comparison of the distribution of settled spores on EG_1, EG_2, EG_3, EG_6, and PEG2000-OH coated surfaces. These images can be compared with the quantitative data shown in Figure 2. Three types of surface can be recognized. The first refers to EG_1OH, the second to $EG_{2-6}OH$, and the third to PEG2000-OH (representing all the other investigated PEGs). The visual observation of the assay dishes at the end of the assay concurs with these microscopy images. The spore solution was depleted as a result of the high number of spores settled on the EG_YOH surfaces; the spore solution covering the PEG SAMs looked unchanged, i.e., was still green, since almost no spores had settled on these surfaces. Interestingly, the spores settled on EG_1OH were mostly individuals, whereas on $EG_{2-6}OH$ the formation of rafts (large two-dimensional groups of spores in contact with the surface) occurred. The observation of raft formation on EG_6OH concurs with our previous observations.[2] Interestingly, initial settlement as observed by in situ microscopy shows approximately the same number of spores on all EG_YOH surfaces (Figure 3). However, the adhesion strength was significantly different between EG_1OH and $EG_{2-6}OH$, which became obvious after gentle washing and taking the samples through the air−water interface (preflow in Figure 2). While on $EG_{2-6}OH$, a slight motion of the slide or its transfer through the air−water interface was enough to remove nearly all settled spores, EG_1OH needed to be exposed to turbulent shear in the water channel apparatus to remove the settled spores (postflow in Figure 2).

Table 1. Results of Surface Analysis (XPS and Spectral Ellipsometry) of the SAMs[a]

SAM	Contact angle	SAM thickness [Å] (XPS)	SAM thickness [Å] (Ellipsometry)	Thickness of adsorbed fibrinogen [Å] (Ellipsometry)
EG_1OH	(28 ± 4)°	13 ± 3	17 ± 2	19 ± 2
EG_2OH	(33 ± 4)°	15 ± 3	18 ± 2	5 ± 2
EG_3OH	(31 ± 4)°	16 ± 3	18 ± 2	0 ± 2
EG_4OH	(33 ± 4)°	18 ± 3	22 ± 2	0 ± 2
EG_5OH	(34 ± 4)°	21 ± 3	23 ± 2	0 ± 2

[a] Results of the fibrinogen deposition assay (last column) are calculated as protein thickness on the SAM as determined by spectral ellipsometry.

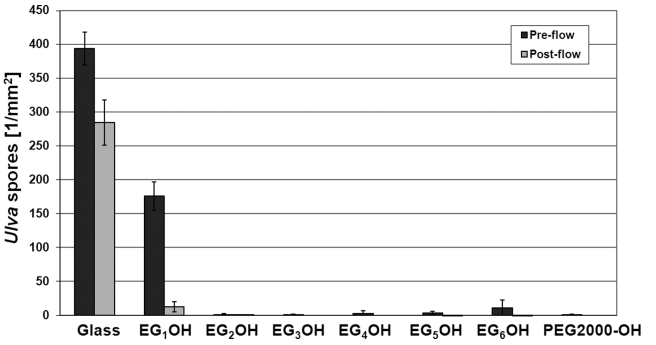

Figure 2. Density of attached zoospores of *Ulva* on short chain EG_YOH with different chain lengths and PEG2000-OH before (preflow) and after (postflow) exposure to a wall shear stress of 53 Pa. Each point is the mean of 90 counts, 30 from each of three replicates; error bars show 2× SEM.

Discussion

Although adhesion of spores of *Ulva* on the EG_6OX SAM series varied with changing end-group termination,[2] the change from hydroxyl to methyl termination was of no consequence in terms of either settlement or adhesion to all high molecular weight PEGs. As described above, the similar contact angles and the resistance toward protein and algal cell adhesion in the polymeric films can be ascribed to the lower order of the films. As one consequence, flexible hydrophobic end-groups are buried in the PEG film. Steric repulsion along with hydration of the films is most likely responsible for the high resistance toward proteins and the adhesion of spores.[36,37] The swelling in water and the degree of hydration in the films should increase with the number of EG

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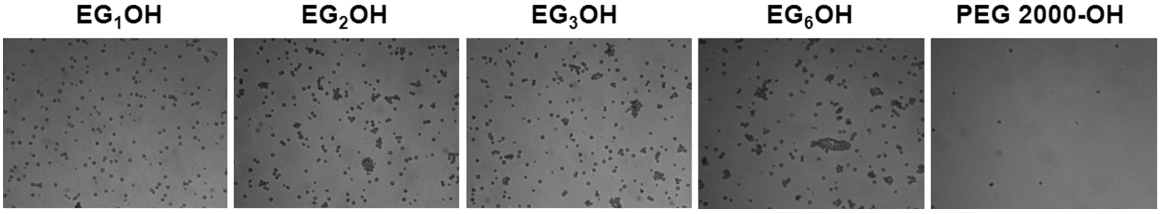

Figure 3. Images (450 × 338 μm) showing in situ distribution of settled spores on various short chain EG_YOH and PEG2000-OH. The majority of spores on EG_1 are present as single spores; many spores on EG_2, EG_3, and EG_6 are present in groups, with almost no spores present on PEG2000-OH.

units (EG_6 < PEG2000 < PEG5000).[25] In line with these results are recent hydrogel experiments that reveal a high resistance of these highly hydrated gels.[38,39]

Shorter chain length OEGs ($EG_{2-6}OH$) were also able to prevent irreversible adsorption of the spore adhesive secreted at settlement, whereas EG_1OH was a nonresistant surface, which is in agreement with previous reports of protein adsorption.[1] Hydrophilicity cannot account for failure of EG_1OH since all surfaces showed similar contact angles, with EG_1OH (28°) being more hydrophilic than the $EG_{2-6}OH$ SAMs (on average 33°). The strength of hydration bonds in the film appears to be the main reason for resistance of the EG_{2-6} surfaces. The premise for this is the availability of at least two proton acceptor oxygen atoms in the OEG chain, allowing strong hydration bonds in a double hydrogen bridge bonding configuration,[24,25] which is the case for all $EG_{Y>1}OH$. This is supported by the fact that physisorbed proteins are present on OEG surfaces, which was recently confirmed by neutron reflectivity studies.[40] If chain length finally drops to EG_1OH, the degree of hydration is no longer sufficient to resist the adhesion of proteins or *Ulva* spores. In conclusion, the remarkable strength of the bifurcated hydration bond of water to the OEG chain can be considered as the key surface property responsible for the dependence of spore adhesion of *Ulva* on the EG-containing coatings.

Although $EG_{2-6}OH$ and PEG coatings all show a low number of spores (Figures 1 and 2), there are profound differences in the manner by which the low observed density of settled spores was achieved. Spores initially settled in high numbers on an EG_6OH SAM, as shown by in situ microscopy (Figure 3), but their adhesion strength was so weak that the slightest movement of the assay dish caused them to detach from the surface and float to the air−water interface as rafts of spores held together by spore−spore adhesion.[2] By contrast, on the PEG surfaces, spores were *inhibited* from settling, i.e., they did not secrete their adhesive and continued swimming above the surface of the SAM. Both behaviors are illustrated by the images shown in Figure 3. On polymeric PEG, only a few spores can be seen, but many spores had settled on all of the other surfaces. A reason for this observation may lead back to the underlying resistance mechanisms of both systems. PEG not only resists attachment (adhesion) of *Navicula*, but it also *inhibits* settlement of spores of *Ulva,* presumably due to steric repulsion of the loosely packed chains forming a diffuse interphase.[19] OEG SAMs ($EG_{Y>1}OH$) cannot prevent settlement since they cannot offer such a deterrent surface because of their short, well-defined, and densely packed EG chains forming a sharp and well-defined interface. This interface property seems to render the surfaces attractive in the first place and leads to a significant amount of initially settled spores, as shown by in situ microscopy.

Another observation made in Figure 3 is relevant when discussing the attractive but nonadhesive properties of the EG surfaces: spores settled individually on EG_1OH, but mostly clustered on $EG_{2-6}OH$. In a previous publication[2] we reported the formation of loosely adherent "rafts" of spores on EG_6OH, and this was attributed to the inability of settled spores to irreversibly bind to this surface, so that when the slides were disturbed by manipulation, or by passage through an air−water interface, the weakly attached spores formed rafts through spore−spore adhesion, i.e., the "rafts" were secondary, postsettlement consequences of inherently weak adhesion. While this interpretation sustains for the occurrence of extensive "rafts", the observations made in the present study by in situ microscopy, in which samples are not disturbed by manipulation, suggest that there are other routes by which the formation of small groups of settled spores on $EG_{2-6}OH$ can occur. The density-dependent, cooperative settlement of dispersal stages of marine organisms (i.e., larvae, spores) in groups is known as "gregarious settlement", and it is thought to be advantageous in nature because it enhances local population densities, thus facilitating sexual interactions and niche exploitation, e.g., by providing a measure of protection against turbulent forces. Gregariousness in some marine species is known to be based on chemical signaling. Spores of *Ulva* exhibit gregarious settlement at high spore densities, especially on hydrophobic surfaces.[11,30] The extensive occurrence of groups of spores on $EG_{2-6}OH$ in the present study was therefore unexpected. The reason for this observation is not clear since the physicochemical basis of gregarious settlement in *Ulva* is not understood. However, a possible explanation is that we are observing a form of "pseudogregariousness" on $EG_{2-6}OH$ whereby small groups are formed during settlement as a consequence of the impact of one spore against another as they actively explore a surface.[30] Since the settled spores are only weakly attached, such impacts may be sufficient to "nudge" adjacent spores more closely so that their coronas of discharged adhesive make contact, thus promoting spore−spore adhesion. On EG_1OH surfaces, spores are more strongly attached, and such an effect is not observed, even though spores settled in appropriate densities. To explain this, we may speculate that the strength of adhesive−surface bonding is stronger for EG_1OH units, as the binding energy of water is reduced as compared to its longer homologues. As the spore adhesive is a hydrophilic and hygroscopic glycoprotein,[10] a shorter EG chain length makes it easier for the glue to displace the water from the interface, thus promoting strong adhesion. The longer chain length EG homologues are less suited for firm adhesion, as here more water per EG unit is stored with a higher binding energy,[24,25] thus making it more difficult to exclude water from the bonding interface, allowing the attached spores to "slide" across the surface and to form groups if they contact each other.

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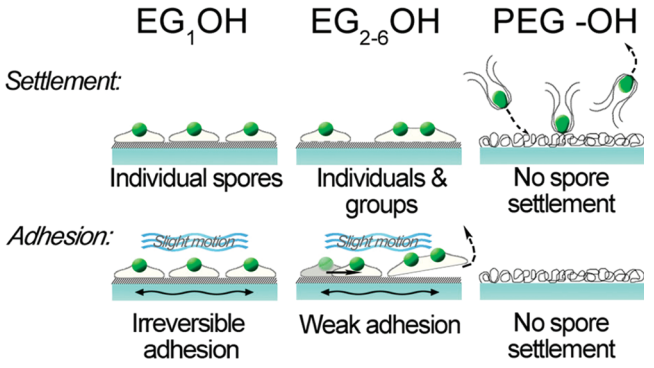

Figure 4. The different responses of spores of *Ulva* to OEG and PEG surfaces. (Left) *Nonresistant surfaces*: spores settle onto EG_1OH and continue to adhere during mild disturbance. (Middle) *Adhesive-resistant surfaces*: spores settle onto $EG_{2-6}OH$, but the adhesive is unable to bond with the surface; settled spores slide along and float off these surfaces under mild disturbance. (Right) *Inhibitory surfaces*: the number of spores settled on PEG surfaces is minimal.

We can now distinguish the different spore−surface interactions observed on the EG surfaces composed of different chain length and ascribe them to different categories. An *inhibitory surface* inhibits spore settlement, observed for the long-chain PEG coatings (see Figure 3, picture on the far right). The inhibitory effect of PEG coatings has also been observed by digital in-line holography, which revealed intense swarming of spores above the surfaces without any surface exploration, e.g., spores spinning on the surface or settlement events.[41,42] For these longer chain length molecules, the high degree of hydration and steric repulsion probably contributes to the surface being inhospitable.

EG_2OH to EG_6OH coated surfaces do not inhibit spore settlement and are more correctly referred to as *adhesive-resistant surfaces*, since spores are able to settle on them, but the secreted glycoprotein adhesive is unable to form a strong bond with the surface. The EG_1OH surface is neither an inhibitory nor a resistant surface; spores settle on this *nonresistant surface*, and they require the application of a wall shear stress to dislodge them. The three different settlement categories described above are illustrated schematically in Figure 4.

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Conclusion

OH/OMe-terminated PEG SAMs with high molecular weight and a set of short chain length OEG SAMs (EG_YOH, Y = 1−6; EG_6OMe) were prepared on gold to investigate the correlation between EG chain length-dependent surface properties and the settlement and adhesion strength of algal cells. Attachment of zoospores of *Ulva* and fibrinogen adsorption follow the same general trend and show a dependence on the degree of hydration which, in the case of PEG, is independent of its end-group termination. If the number of EG units per molecule is greater than one, the hydration is sufficiently strong to prevent irreversible binding of the secreted glycoprotein glue. However, subtle changes in surface properties have a great influence on the settlement behavior of spores. While PEG thiols are inhibitory toward spore settlement, $EG_{2-6}OH$ SAMs allow settlement, but adhesion between spore and surface is minimal as spores detach through a slight hydrodynamic force. Reasons appear to be the transition from a sharp and well-defined interface (EG_YOH) to a diffuse polymeric interphase (PEG), accompanied by enhancement of the resistance mechanism by the number of hydrated EG units[24,25] *plus* steric repulsion of the diffuse polymeric network, which EG_YOH SAMs with a better defined interface lack. The reason why spores commit to settlement on the $EG_{2-6}OH$ SAMs is not known and is counter to a strategy to optimize survival, since firm adhesion to the substrate is a necessity for survival, growth, and reproduction.

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