Settlement and adhesion of algal cells to hexa(ethylene glycol)-containing self-assembled monolayers with systematically changed wetting properties

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Protein resistance of self-assembled monolayers (SAMs) of hexa(ethylene glycols) (EG_6) has previously been shown to be dependent on the alkoxyl end-group termination of the SAM, which determines wettability [S. Herrwerth, W. Eck, S. Reinhardt, and M. Grunze, J. Am. Chem. Soc. **125**, 9359 (2003)]. In the present study, the same series of hexa(ethylene glycols) was used to examine the correlation between protein resistance and the settlement and adhesion of eukaryotic algal cells, *viz*., zoospores of the macroalga *Ulva* and cells of the diatom *Navicula*, which adhere to the substratum through the secretion of protein-containing glues. Results showed that the initial settlement of *Ulva* zoospores was highest on the hydrophilic EG_6OH but that cells were only weakly adhered. The number of *Ulva* zoospores and *Navicula* cells firmly adhered to the SAMs systematically increased with decreasing wettability, as shown for the protein fibrinogen. The data are discussed in terms of hydration forces and surface charges in the SAMs. © *2007 American Vacuum Society.* [DOI: 10.1116/1.2806729]

I. INTRODUCTION

The common green seaweed *Ulva* (syn. *Enteromorpha*) which is found on seashores all over the world is well-known for fouling submerged structures, especially ships' hulls.[1] Diatoms (unicellular algae) such as *Navicula* spp. also contribute to the biofouling problem through the formation of biofilms, often referred to as "slimes." Biofouling is a consequence of the initial adhesion of organisms (spores in the case of *Ulva*, single cells in the case of *Navicula*) to surfaces, through the secretion of polymeric adhesives. Both of these organisms have been used as model organisms to study fundamental aspects of adhesion of these "soft-fouling" systems.[2,3] The economic cost of fouling and stricter global regulations on the use of biocidal antifouling paints have led to an increasing demand for environmentally benign solutions to fouling control over the last few years. Research has focused on surfaces that minimize settlement and/or adhesion of fouling organisms, but in order to inform the design of effective coatings, it is essential to understand which properties of surfaces directly influence settlement and adhesion of the colonizing stages of organisms.

Ulva reproduces by the production of vast numbers of microscopic, "naked" (i.e., without a cell wall) zoospores, $5-7$ μm in length, which swim through the water using four flagella. In order to complete their life cycle, the zoospores need to locate a surface, settle on it, and then firmly adhere to it. On locating a suitable surface, the zoospore undergoes "settlement" and permanent attachment, involving loss of motility and secretion of adhesive, which anchors the spore to the substratum.[2] The *Ulva* spore adhesive is a polydisperse, self-aggregating hydrophilic glycoprotein, resembling the group of hydroxyproline-rich extracellular matrices of both plants and animals.[2] Swimming zoospores respond to a number of physical surface cues including wettability and topography.[4–6] Once settled, adhesion of the attached spores is also influenced strongly by wettability and surface friction.[7,8]

Diatoms are unicellular algae characterized by their elaborately ornamented silica cell walls composed of overlapping halves or "valves." Diatoms are not motile in the water column, and those that colonize surfaces rely on water movement and currents to transport them to substrata to which they adhere. Raphid diatoms adhere to surfaces through the secretion of sticky extracellular polymeric substances (EPS) comprising a range of complex proteoglycans (recently reviewed by Chiovitti *et al.*, 2006) through one or two slits in the silica cell wall called raphes.[9] Contact with the substratum initiates a set of processes that facilitate primary adhesion and reorientation of the cell. Secretion of EPS also provides a mechanism for "gliding" motility via an adhesion complex that links the extracellular adhesive strands through the plasma membrane to an actin-myosin system inside the cell.[10,11] Like *Ulva* zoospores, adhesion of raphid diatoms is also strongly influenced by surface wettability and friction.[7,8,12]

Self-assembled monolayers (SAMs) are a highly versatile tool to tailor surface properties including wettability and many other properties for cell and protein-adhesion studies.[13] The interfacial interaction between biologically relevant molecules and SAMs has been investigated from a fundamental standpoint for many years,[14–18] and has led to the

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development of many medical and biotechnological applications.[19–21] SAMs have also been used to investigate the response of marine organisms to a number of physicochemical attributes of surfaces. A systematic change of wettability using varying compositions of mixed OH/CH_3 alkanethiol SAMs on gold indicated that *Ulva* zoospores avoided settling on hydrophilic surfaces.[4,7,22] However, although more cells settled on the hydrophobic CH_3-rich SAMs, they were less strongly adhered than to the hydrophilic surfaces.[7] Interestingly, the adhesion of diatom cells appears to be different from that of *Ulva* zoospores; diatom cells adhered more strongly to the hydrophobic methyl SAM and less strongly to the hydrophilic OH SAM.[7] An issue using mixed SAMs to produce surfaces of differing wettability can be formation of "patches," since partial demixing might occur.[23] It is therefore preferable to change the surface energy by varying the chemical nature of the individual alkanethiols to produce homogeneous SAMs rather than by mixing different chemistries.

Another important merit of varying the chemical nature of the individual alkanethiols is not only the alternation between hydrophilicity and hydrophobicity but also the protein resistance of the hydrophilic moieties. As mentioned above, previous investigations indicated hydrophilicity inhibited the settlement of *Ulva* zoospores, although those that attached to the OH SAM were strongly adhered,[7] but these surfaces are not resistant to protein adsorption. However, both low settlement density and low adhesion strength of zoospores was found for mPEG-$DOPA_3$ surfaces,[24] which also display protein resistance.[25] Thus, it appears that hydrophilicity alone may not be sufficient to reduce adhesion strength, protein resistance being an additional requirement.[26]

The purpose of the present study was to systematically examine the correlation between resistance to protein adsorption, and the adhesion of zoospores of *Ulva* and cells of *Navicula*, on a range of hexa(ethylene glycol)-containing undecanethiols with different end-group terminations, which had previously been shown to exhibit a high dependence between the surface wettability and adsorption of fibrinogen.[27] The use of fibrinogen as protein allows comparison of our results with previous studies on protein resistance of oligo(ethylene glycol) (OEG).[14,15] The two algal cell types were chosen because of their opposing preferences in terms of wettability for strong adhesion.

II. MATERIALS AND METHODS

A. Preparation and characterization of the EG_6OX SAMs (X=H, CH_3, C_2H_5, C_3H_7)

All chemicals, such as ethanol, *N*,*N*-dimethylformamide (DMF), fibrinogen, phosphate-buffered saline (PBS) were purchased from Sigma-Aldrich (Munich, Germany). Deionized water was purified with a MilliQ plus system (Millipore, Schwalbach, Germany). PBS buffer was used at *p*H 7.4 and a concentration of 0.01 M. Float glass slides were obtained from Schott (Mainz, Germany). α-undecyl-mercapto-ω-alkoxyl-hexa(ethylene glycol)s with different alkoxyl terminations were synthesized in a three-step reaction according to a protocol by Prime *et al.*[28,29]

Thin films of polycrystalline gold were prepared by thermal evaporation of 9 nm of titanium as an adhesion promoter and subsequent deposition of 30 nm of gold (99.99% purity) onto Schott float glass slides. Evaporation was performed at a pressure of 2×10^{-7} Torr and a deposition rate of 0.5 nm/s. The gold-coated glass slides used for the experiments were of extra-smooth quality. The rms roughness value was about 1 nm, which lies in the area of a gold-coated silicon wafer that was used for previous experiments.[27] The gold-coated slides were prepared in Heidelberg and stored under argon until SAMs were prepared in Birmingham immediately prior to assay. For the SAM formation, four solutions of 30 mg EG_6OX in 60 ml DMF were prepared, respectively. Prior to immersion, the samples were placed in a UV light-emitting reactor (150 W mercury-vapor lamp, Heraeus Noblelight, Germany, model TQ150) for a minimum of 2 h in order to remove organic adsorbates from the surface, 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 DMF and then with ethanol, and finally dried in a flow of nitrogen. The samples were stored under pure nitrogen until used for the experiments.

Sessile drop (Millipore) water contact angles were measured by goniometry under ambient conditions. Droplets were dispensed from a micropipette. 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 x-ray photoelectron spectroscopy (XPS) using a Leybold-Heraeus MAX 200 x-ray photoelectron spectrometer with an aluminum anode as x-ray source ($K\alpha$=1486.4 eV). To determine the thickness of the adsorbed fibrinogen layer, spectral ellipsometry measurements were performed with a Horiba Jobin Yvon UVISEL spectral ellipsometer operating in a wavelength range between 280 and 800 nm. The organic film was modeled as a single Cauchy layer.

The protein affinity of all samples was tested by a fibrinogen assay.[30] For each slide coated with EG_6 SAM, 10 mg of fibrinogen (from human plasma; 58% protein, ~15% sodium citrate, ~25% sodium chloride/98% clottable protein, Sigma) were dissolved in 10 ml of PBS buffer solution (0.01 M, *p*H 7.4) by sonicating for 20 min. The solution was filtered using a syringe filter (Rotilabo®, pore size 0.45 μm). The test samples were immersed in 5 ml Millipore water (MW) for 5 min, and then 10 ml of the fibrinogen solution were added. After 15 min, the solution was diluted with 400 ml of MW, rinsed with MW, and dried in a stream of nitrogen. The amount of adsorbed fibrinogen was measured from ellipsometric constants that were obtained from two points on the sample by the spectral ellipsometer described above.

B. Settlement and adhesion assays for *Ulva* zoospores

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.[1] Ten ml of freshly released spores in Tropic Marin® artificial seawater (ASW) (1.5×10^6 spores per ml) were added to individual compartments of a sterile Quadriperm dish, each containing a test surface. Six replicates of each test sample were immersed simultaneously. The slides were incubated in darkness for 45 min and then washed gently in ASW to remove unsettled, i.e., motile, spores. Three replicates were used to determine the number of settled (initially attached) spores. Spores were fixed in 2.5% glutaraldehyde in ASW, washed in deionized water, and dried. Spore counts were taken using a Kontron 3000 image analysis system attached to a Zeiss epifluorescence microscope. Spores were visualized by autofluorescence of chlorophyll and counts were recorded for 30 fields of view on each slide as described by Bowen *et al.*[8]

To determine the adhesion strength of attached spores, the remaining three replicates were exposed to a shear stress in a calibrated water channel using methods described previously.[31] The apparatus was run at maximum velocity creating a wall shear stress of 53 Pa. The number of spores remaining after flow was compared to the unexposed samples.

C. Visualization of secreted spore adhesive by indirect immunofluorescence

Spores were stained with a monoclonal antibody (mAb) Ent 6, an antibody specific to the *Ulva* adhesive, following procedures described in detail by Stanley *et al.* in which settled spores fixed in glutaraldehyde are incubated with primary antibody (Ent 6) followed by a fluorescent second antibody, FITC-RAMIG (fluorescein isothiocyanate conjugated rabbit antimouse immunoglobulin).[32] Control staining was carried out in the absence of the primary antibody (i.e., using FITC-RAMIG alone) or with a primary antibody that does not recognize algal antigens (UBIM22, a mAb raised against rat bone cells). Staining was visualized in a Zeiss Axioplan epifluorescence microscope with standard FITC filters.

D. Diatom adhesion assay

Navicula perminuta cultures were grown according to the protocols detailed by Pettitt *et al.* in Guillard's F/2 medium made up using natural seawater.[33,34] Log phase cells were resuspended using a pipette to a chlorophyll *a* content of 0.3 mg ml^{-1}. Chlorophyll was extracted in DMSO and quantified using the equations of Jeffrey and Humphrey.[35,36]

The *Navicula* settlement assay followed the general principles as for *Ulva*. Details are given in Pettitt *et al.*[33] The slides were incubated with a suspension of diatom cells for 2 h, on the bench. Cells were counted using image analysis similar to that described for *Ulva*. For cell detachment studies, slides were exposed to a wall shear stress of 8.2 Pa in a water channel apparatus. Percentage removal was calculated as described for *Ulva*.

TABLE I. Sessile drop contact angles of the different EG_6OX samples (error: ±4°). The middle column refers to the samples used in the *Ulva* assays, the right column to the samples used in the *Navicula perminuta* assays.

SAM	Contact angle (*Ulva* assay)	Contact angle (*N. perminuta* assay)
EG_6OH	35°	32°
EG_6OMe	60°	63°
EG_6OEt	70°	81°
EG_6OPr	87°	95°

III. RESULTS

A. Surface characterization

For each experiment, seven replicates with four different EG_6 terminations were prepared by the protocols described above. Six replicates were used for the bioassay and one slide was used for surface characterization to verify successful SAM formation. Sessile water drop contact angles obtained for the different surfaces are listed in Table I. In line with previous studies, the hydroxyl-terminated SAMs with oligo ether end groups on gold had the lowest contact angles of water (32–35°, Table I) due to the terminating hydroxyl-group.[27] The steady increase of contact angle is due to the increased length of the hydrophobic, outermost alkyl chains going from methoxyl-, to ethoxyl-, to propoxyl-end groups. X-ray photoelectron spectroscopy was used to verify the chemical composition of the organic films as well as to determine the thickness of the SAMs. The latter was done by quantifying the attenuation of the gold substrate signal due to the adsorption of the oligo(ethylene glycol) alkanethiols following the description in previous investigations.[37,38] The obtained film thicknesses are shown in Table II and all values agree with previous measurements, e.g., 2.5 nm for EG_6OH. This verifies that a sufficient amount of OEG-thiol has been adsorbed to form a densely packed monolayer on the surface. As the C1*s* ether carbon can be distinguished from the C1*s* alkyl carbon signal by their different chemical shift in the XPS measurements, their intensities were quantified and compared to the stoichiometric ratios. While C_{ether}/C_{alkyl} ratios close to the stoichiometric ratios indicate an arbitrary orientation of the molecules on the surface, higher ratios are caused by the attenuation of the photoelectrons from the alkyl spacer close to the surface by the ethylene oxide groups

TABLE II. Layer thicknesses of the different EG_6OX samples, determined by XPS.

SAM	Thickness (Å)
EG_6OH	28±3
EG_6OMe	25±3
EG_6OEt	22±3
EG_6OPr	22±3

TABLE III. Experimental and stoichiometric ratios of ether carbon to alkyl carbon XPS intensities.

SAM	C1*s* ether/C1*s* alkyl	Stoichiometric ratio
EG_6OH	1.64/1	1.3/1
EG_6OMe	1.85/1	1.4/1
EG_6OEt	2.1/1	1.3/1
EG_6OPr	1.65/1	1.2/1

above. The results for the OEG films are shown in Table III and reveal, in agreement with previous measurements by Harder *et al.,* C_{ether}/C_{alkyl} peak ratios higher than the stoichiometric ones, thus indicating a densely packed and ordered SAM.[37] Concluding, all observed spectroscopic data and wetting properties are consistent with previous findings for high-quality self-assembled monolayers consisting of hexa(ethylene glycol)-terminated undecanethiols.[27] The affinity of the SAMs toward fibrinogen adsorption was tested for each set of samples by measuring the amount of adsorbed protein with spectral ellipsometry. The results are shown in Fig. 1 and reveal, in agreement with previous reports, a steady increase of adsorbed protein with rising contact angle.[27]

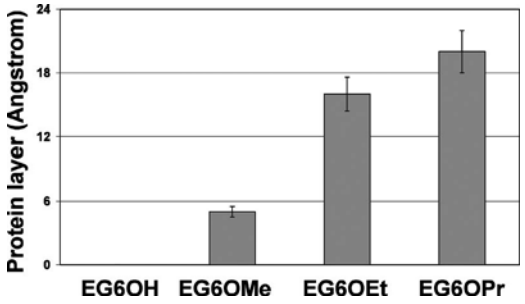


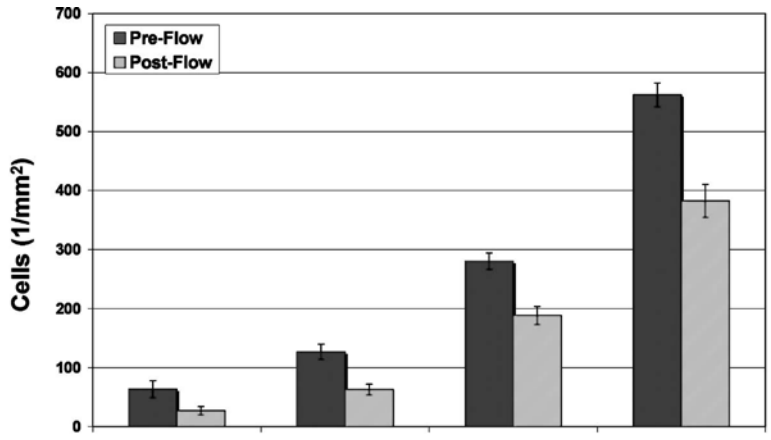
FIG. 1. Thickness of protein film adsorbed on a given hexa(ethylene glycol) SAM on gold (error: ±15%).

B. Settlement and adhesion strength of zoospores of *Ulva*

The settlement assay quantifies the density of spores attached to the surface after gentle washing to remove residual, unattached, i.e., motile, spores. The number of attached spores increased with an increase in contact angle from the hydrophilic EG_6OH and EG_6OMe up to the hydrophobic EG_6OEt and EG_6OPr (Fig. 2). The number of cells remaining attached to the surface after exposure to flow indicates how strongly the spores are adhered to the surface (Fig. 2). The proportion of spores removed decreases with increasing hydrophobicity from EG_6OH (57% removed) > EG_6OMe (50% removed) > EG_6OEt (33% removed) > EG_6OPr (32% removed).

C. Observations on settled spores

Observation of EG_6OH surfaces at the end of the spore settlement period suggested that *Ulva* spores had settled onto this SAM in very high numbers, since the green spore suspension was depleted of color and a green layer was visible on the slide surfaces. Slight movement of the dishes during handling and subsequent washing resulted in large rafts (ca. 0.7 cm^2) of spores peeling from the green surface layer, rising through the water, and coming to rest at the air-water interface. This suggests first that the density of settled spores was sufficient for spore adhesive to form a semicontinuous film encompassing multiple spores, adhering them together laterally. Second, the low number of spores settled on EG_6OH, as recorded in Fig. 2, is not due to inhibition of settlement *per se*, but rather to the extremely weak adhesion strength of spores that had gone through the processes associated with normal settlement (secretion of adhesive and loss of motility) and were subsequently removed during handling. Detachment of rafts of cells was also observed for the EG_6OMe, but not for the EG_6OEt or EG_6OPr samples.



Sample	EG6OH	EG6OMe	EG6OEt	EG6OPr
Contact angle	35°	60°	70°	87°

FIG. 2. Density of *Ulva* spores (mm^2) on EG_6OX SAMs with different end-group chemistries, after 45 min settlement, washing, and fixation. Preflow counts show the number of cells present before exposure to 53 Pa wall shear stress. Postflow counts show the number of cells remaining after exposure to shear stress. $N=90$, 30 from each of 3 replicates, error bars = ±2 × standard error.

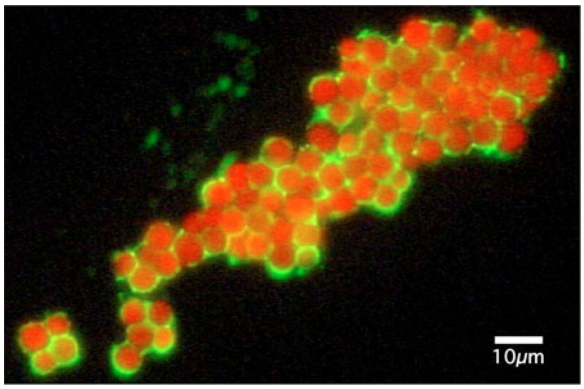


FIG. 3. A raft of *Ulva* spores removed from the surface of a dish containing an EG_6OH-terminated alkanethiol SAM. Spores were stained with a mAb, Ent 6. Scale bar=10 μm.

D. Observations on rafts of detached spores using an immunofluorescent probe to the spore adhesive

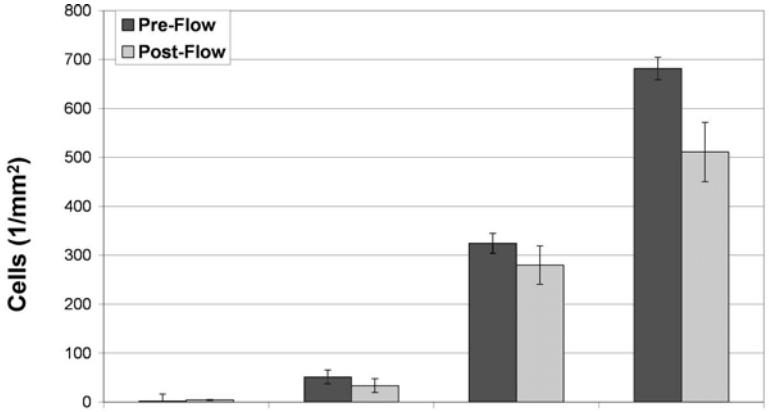
In order to confirm that the zoospores had settled "normally," i.e., had gone through the process of adhesive secretion, a floating raft of spores recovered from the air-water interface of the assay dish was stained with a monoclonal antibody (mAb probe) specific for spore adhesive. Figure 3 shows a fluorescent micrograph of a detached raft of *Ulva* spores recovered from the surface of a dish containing a slide coated with hydroxyl-terminated SAM. The red color channel represents autofluorescence of chlorophyll in the spore body; the green fluorescence shows the secreted adhesive stained by the mAb Ent 6. Controls stained in the absence of the primary antibody, or the rat bone cell mAb UBIM22 (which does not recognize algal cell antigens) were both negative.

E. *Navicula* adhesion assay

Diatom cells, unlike *Ulva* spores, are not motile in the water column; hence, cells reach a surface by falling through the water by gravity. Thus, at the end of the incubation period, in principle the same number of cells will be present on every surface. Therefore, any differences in the number of cells attached after the slides have been manipulated following the settlement period, including the gentle washing step, represents differences in the ability of cells to adhere initially to the substratum. Figure 4 shows the number of cells of *N. perminuta* before and after exposure to a shear stress in a flow channel. The number of cells attached initially to the surface apparently increased with contact angle with almost no cells attaching to the EG_6OH surface and few attaching to the EG_6OMe surface (Fig. 4). As for *Ulva* zoospores, slight vibration of the dish revealed that almost none of the cells that had come into contact with the SAM had been able to adhere to the EG_6OH and only a small number had adhered to the EG_6OMe surface. Exposure of the more firmly attached cells on the more hydrophobic surfaces to 8.2 Pa shear stress in the flow channel resulted in only a small proportion (<25%) of the cells being removed (Fig. 4).

IV. DISCUSSION

If we consider first the results obtained for *Ulva* spores, it is important to note that two facets of the overall process of adhesion are being assessed in these experiments. First, in order to colonize a surface, swimming spores have to recognize that surface as being suitable; a consequence of that is selectivity in settlement behavior in relation to surface properties such as wettability.[4,5,22] Second, having settled on a surface and made the transition to a permanently attached, sessile stage of their life history, the strength of that attachment is also determined by interfacial properties. Surface wettability affects both of these processes; previous experiments with mixed OH/COOH and CH_3-terminated alkanethiols demonstrated that spores tend to prefer to settle on a



Sample	EG6OH	EG6OMe	EG6OEt	EG6OPr
Contact angle	32°	63°	81°	95°

FIG. 4. Density of *N. perminuta* cells on EG_6OX SAMS with different end-group chemistries. Preflow counts show the number of cells present before exposure to 8.2 Pa wall shear stress. Postflow counts show the number of cells remaining after exposure to shear stress. N=90, error bars are ±2 × standard error.

hydrophobic surface, but once settled the adhesion strength is lower than on the hydrophilic surface.[4,7,22] We have speculated this may be consequent on secreted adhesive spreading more on a hydrophilic surface.[39] It should also be emphasized that low settlement on these alkanethiol SAMs was a consequence of swimming spores avoiding settling on the hydrophilic surfaces.[4]

If we consider the current data in terms of settlement, at first sight it seems that the results presented in this article are entirely consistent with these previous reports, since it appears that few spores settled on the more wettable EG_6OH surface. However, careful observation established that in fact the hydrophilic EG_6OH surface, and to a lesser extent the EG_6OMe surface, was extremely attractive to spores— so attractive that the green spore suspension was visibly depleted of color at the end of the settlement period because so many spores had settled. However, the strength of adhesion of the settled spores was so low that even the minimal shear forces created by moderate disturbance of the slides were sufficient to remove the attached spores *en masse*. The use of the mAb immunofluorescent probe clearly demonstrated that these weakly attached spores had gone through the normal settlement processes involving released adhesive (Fig. 3). In contrast, although many spores settled on the more hydrophobic ethoxyl- and propoxyl-terminated EG_6, their attachment strengths were relatively high. In both respects, therefore, the results presented here more closely resemble those for another (ethylene glycol)-based substrate, mPEG-$DOPA_3$, which also showed low settlement and high release properties, and for which it was noted that weakly attached cells were removed in rafts.[24] The apparent difference in the strength of spore adhesion on alkanethiol and hexa(ethylene glycol) SAMs can be explained in terms of the well-known protein resistance of the latter compared with other hydrophilic surfaces.

Numerous different approaches have tried to explain the protein resistance of oligo(ethylene glycol)-terminated SAMs. Harder *et al.* reported that EG_6OH is able to resist adsorption of, e.g., fibrinogen due to the helical or amorphous structure of the ethylene glycol chain which projects into the water phase.[37,40,41] This structure of the EG chain then enables strong hydrogen bond interactions between the EG chain and water which prevent the approaching protein from excluding the water from the EG chain/water interface and thus attachment to the surface. Reasons for weaker hydrogen bonds and final loss of protein resistance are the alkyl end groups that protect the water from deeper penetration into the EG chain.[27]

Kreuzer *et al.* investigated the influence of surface charges on hydration of water at the EG chain.[42] Although SAMs are nonionic surfaces, density functional calculations resulted in SAMs based on ethylene glycol exhibit surface charges. These are created by adsorption of hydroxide ions (from autoionization of water or in alkaline media) to the terminal hydroxyl groups. Thus, the repellent effect of these SAMs against proteins is ascribed to electrostatic repulsion between both the negatively charged surface and proteins,

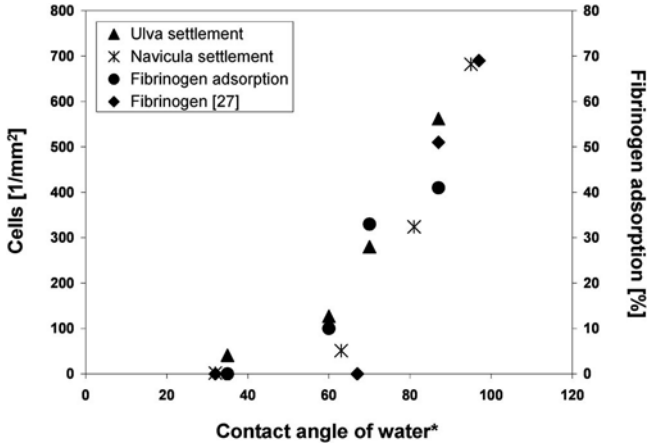


FIG. 5. Combined plot showing the number of cells of *Ulva/Navicula* attached and fibrinogen adsorbed vs surface energy. *Advancing contact angles for values taken from Ref. 27. Sessile drop contact angles for all other values.

respectively (fibrinogen and the algal adhesive glycoprotein).[43] Furthermore, the presence of surface charge and electrostatic repulsion was confirmed by atomic force microscopy and streaming potential measurements.[44–47] In the case of the SAM series used for this study, the effect declines with the addition of each methylene group, but is still intact for the methoxyl termination. Beyond this threshold, though, adhesion strength of algal cells increases rapidly, which can be ascribed to displacement of the surface charges and removal of water at the interface in consequence.

In summary, for *Ulva* spores a reasonable explanation of our data is that, like the adsorption of proteins such as fibrinogen, an intact water shell prevents effective interaction of the proteoglycan adhesives used by *Ulva* to the OH-terminated surface. However, this does not explain why the EG_6OH surfaces are so attractive to the initial settlement of *Ulva* spores; at present we have no mechanistic explanation or testable hypothesis other than to speculate that the basis of this effect presumably lies in the interactions between EG_6OH and those proteins in the plasma membrane of the spore that must play a role in surface recognition.

The results with the diatom *Navicula* generally concur with those for *Ulva*. Diatoms typically show weak adhesion to hydrophilic surfaces.[7,12,48] Although, with the possible exception of mPEG-$DOPA_3$, no other surfaces evaluated previously appear to have such marked anti-adhesive properties as the EG_6OH.[24] Diatom mucilages are complex, multicomponent polysaccharides and proteoglycans,[3] although these data indicate that initial attachment is most likely moderated by glycoproteins.[49] When diatoms such as *Navicula* settle on a surface, they typically land on their girdle side, which facilitates weak attachment via cell surface mucilages. For attachment that facilitates gliding, the cells have to flip over, thereby bringing the raphe in contact with the surface. This process requires traction to be established between the extracellular adhesive strands that traverse the plasma membrane to an actin-myosin-based intracellular adhesion

complex.[9,11,49] The inability of the adhesive glycoprotein strands to adhere sufficiently to the EG_6OH surface is probably the reason for the low numbers of cells on this surface.

The cell attachment and protein adsorption data obtained from the present investigation and the protein adsorption data from Herrwerth *et al.* are all presented in an overview graph (Fig. 5).[27] Here, the contact angle of water is plotted against cell/spore attachment and percent fibrinogen adsorption, respectively. For comparison, 100% adsorption stands for a 4.92 nm thick monolayer of fibrinogen adsorbed on a hexadecane thiol SAM, which is used as reference value. Although the values of the two *y*-axes are not directly comparable, they indicate a common trend for attachment of *Ulva* zoospores, cells of *Navicula*, and adsorption of fibrinogen. All show increasing attachment/adsorption with increasing contact angle on densely packed SAM surfaces that consist of hexa(ethylene glycol)-containing alkanethiols with systematically changing end-group termination. The data suggest there is a strong interrelation between surface energy, fibrinogen adsorption, and adhesion of both types of marine cells that adhere through protein-based glues.

V. CONCLUSION

Four types of self-assembled monolayers based on hexa(ethylene glycol)-containing alkanethiols with systematically changing end-group termination were prepared on gold to examine the correlation between surface energy and the adhesion of two types of algal cells that adhere by different mechanisms. Adsorption of the protein fibrinogen, and attachment of both *Ulva* zoospores and cells of *Navicula* follow the same general trend and show a dependence of the wettability of the surfaces. EG_6OH, and to a lesser extent EG_6OMe, initially have high densities of settled *Ulva* spores but the low numbers that adhere to the surface reflects the fact that the secreted glycoprotein adhesive is unable to bond to the surface. The most likely reason is because water cannot be displaced from the interface, which is essential to bring the bioadhesive molecules in direct contact with the SAM surface. Possible reasons for this behavior are discussed in terms of ongoing research on mechanisms of protein resistance to surfaces composed of ethylene glycol. The most likely point relevant to our observations is the hydration of the EG chains and adsorption of hydroxide ions to the end group of the SAM. This effect declines with increasing alkyl chain length and appears to moderate protein adsorption as well as the adhesion of higher organisms such as zoospores of *Ulva* and cells of *Navicula*.

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