Sol-gel based Hybrid Materials as Antifouling and Fouling-Release Coatings for Marine Applications

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Abstract

Hybrid materials (HMs) offer unique properties as they combine inorganic and organic components into a single material. Here we developed HM coatings for marine antifouling applications using sol-gel chemistry and naturally occurring polysaccharides. The coatings were characterized by spectroscopic ellipsometry, contact angle goniometry, AFM, ATR-FTIR, and their stability was tested in saline media. Marine antifouling and fouling-release properties were tested in laboratory assays against the settlement of larvae of the barnacle *Balanus improvisus* and against the settlement and removal of the diatom *Navicula incerta*. Furthermore, laboratory data were confirmed in short-term dynamic field assays in Florida, USA. All hybrid coatings revealed a superior performance in the assays compared to a hydrophobic reference. Within the hybrids, those with the highest degree of hydrophilicity and negative net charge across the surface performed best. Alginate and heparin showed good performance making these hybrid materials promising building blocks for fouling-resistant coatings.

Introduction

In recent years, hybrid materials (HMs) have received great attention in various industries, in particular for medical applications.^{1,2} HMs combine advantages of both inorganic and organic polymers and represent a unique class of materials.³ In times of climate change, species extinction, and global plastic pollution, there is a growing demand for novel, sustainable and biodegradable materials. Marine antifouling biocides like tributyltin oxide (TBTO) were used extensively for many years in biocidal ablative and self-polishing coatings due to their high efficiency in preventing the settlement of fouling organisms. The main drawback of biocides is the potential harm to non-target organisms and the accumulation of the biocides and degradation products in nature.^{4,5} To protect the oceans and marine ecosystems, metal-free and nontoxic materials are desired.⁶ Biomaterials should be environmentally friendly, biodegradable, based on renewable compounds, and biocompatible. All of these requirements are fulfilled by HMs, which are defined as a polymer fused with other material classes.^{7,8} A notable factor making these different to other composite materials is that the monomer structures are chemically connected at the molecular scale and therefore the materials achieve high mechanical strength through durable networks.^{9,10}

Based on the bond between the inorganic and the organic species, HMs can be divided into two classes.¹¹ Class I hybrids have weak interactions between the two segments, such as Van der Waals, hydrogen bonding or weak electrostatic interactions. Class II hybrids possess strong chemical interactions that connect the components by covalent bonds. HMs based on sol-gel chemistry belong to class II hybrids and are commonly studied because of their convenient and simple synthesis.^{8,10} Not only in HMs but in general, sol-gel based coatings have demonstrated highly effective antifouling and fouling release properties in recent years.¹²

The Detty group was one of the first to realize the potential of HMs for marine antifouling applications.^{13–15} Sol-gel chemistries were investigated using varying ratios of silanes with different alkyl-chain length, or residual organic functional groups, showing that hard sol-gels with low surface energy are advantageous. While classical polydimethylsiloxane (PDMS) still outperformed most of the sol gel chemistries, a mixture of C₁₈/C₈/TEOS (1:49:50) showed the most promising results and was successfully engineered into a commercial coating.^{16,17} The sol-gel process allows organic polymers such as sugar derivatives, proteins and peptides, as well as synthetic polymers like porphyrin-structures, to be combined with inorganic compounds to obtain HMs.^{18–20} Synthesis parameters like temperature, pH, concentration, and solvent can additionally be used to tailor the properties of the HM. A major challenge for the use of biopolymers in a sol-gel system is compatibility in terms of solubility, pH, hydrophilicity, and charge.

Among the different bioinspired building blocks for hybrid materials, polysaccharides are an interesting class of biocompatible and renewable biopolymers, which are biologically active, degradable, and environmentally friendly. Polysaccharides are frequently used in cosmetics and medical applications, but also show very interesting properties as low-fouling materials.²¹ Grafted to surfaces as self-assembled monolayers (SAMs), they were established as antifouling coatings to suppress nonspecific adsorption of proteins, hematopoietic cells, and various marine hard- and soft-fouling organisms.^{22–28} Promising anionic candidates were hyaluronic acid, alginate, and chondroitin sulfate. In particular, for anionic polysaccharides, a blocking of the carboxylates with fluorinated capping agents led to enhanced marine antifouling properties due to the amphiphilic character and the reduced affinity to complex bivalent ions.^{26–28} Further interesting polysaccharides for low fouling applications are chitosan and heparin, which reduced attachment of marine organisms and performed well in medical applications.^{29–32} In nature, heparin acts as an

anticoagulant preventing hemostasis and is broadly used to store blood samples. The stabilizing effect seems to be connected to the high density of negative charge created by the carboxylic acid and sulfate groups, which make heparin interesting for antifouling applications.³³ Chitosan is antigenic, antibacterial, and has a broad relevance for medical applications.^{34,35} In contrast to all other mentioned polysaccharides, chitosan is positively charged due to its high amount of primary amine groups.⁹

The most convenient way to synthesize hybrid materials based on polysaccharides is by the use of sol-gel chemistry. Both compounds react by forming strong covalently Si-O bonds (av. 440 kcal/mol).⁹ Shchipunov *et al.* studied polysaccharide-based hybrid materials extensively for immobilization with enzymes. For that purpose a hydrophilic tetrakis(2-hydroxyethyl) orthosilicate (THEOS) was developed.^{36,37} Besides carrageenan, many different organic polymers were tested like alginate, xanthan, or PVA and PEO but not all of them formed hybrid hydrogels.^{18,38} In addition to THEOS, the more hydrophobic tetraethyl orthosilicate (TEOS) is also capable of forming hybrids with polysaccharides.^{9,39,40} Many studies investigated chitosan as the building block, which is soluble in acidic conditions and well suited for the construction of HMs by sol-gel chemistry. Most of the other above-mentioned polysaccharides are negatively charged and tend to precipitate at lower pH.

Here we used a simple and efficient synthesis route that allows cationic and also anionic polysaccharides to be incorporated into a sol-gel system to form hybrid materials. While acidic catalysts are used rather commonly, basic catalysts are less popular but well suited to prevent precipitation of anionic polysaccharides. Five different HMs were synthesized and characterized by state-of-the-art surface analysis. The polysaccharides used (chitosan, hyaluronic acid, alginate, chondroitin sulfate and heparin ([Figure 1](#)) have different net charges and carry different functional

98 groups. For the first time, the marine antifouling properties of hybrid materials based on
99 polysaccharides were tested in laboratory barnacle and diatom settlement assays, as well as in a
100 dynamic short-term field exposure.

Experimental Section

Preparation of hybrid materials

Figure 1. Schematic synthesis route to prepare the hybrid materials. Polysaccharides were dissolved in water. The sol-gel process took place after adding TEOS and HCl/NaOH as a catalyst. The hydrolysis required between 2-6 hours.

Table 1. List of polysaccharides with their origin and molecular weight used to synthesize the HMs.

Name	Abbreviation	Origin	Molecular Weight	Functional Groups
Chitosan	CH	not specified	50-190 kDa	OH / NH ₂
Hyaluronic acid	HA	sodium salt from <i>Streptococcus equi</i>	1.5-1.8 mDa	OH / COOH / NHCOCH ₃
Alginate	ALG	sodium salt from brown algae	100–200 kDa	OH / COOH
Chondroitin-6-sulfate	CS	sodium salt from shark cartilage	not specified	OH/ COOH / SO ₄ H / NHCOCH ₃

Heparin	HEP	sodium salt from porcine intestinal mucosa	≥ 180 USP units/mg	OH / COOH / N ₂ SO ₃ H / SO ₄ H / NH ₂
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Polysaccharides (PSs, [Table 1](#), all purchased from Sigma Aldrich St. Louis, USA, and used as received) were dissolved in Milli-Q water (MQ, deionized water purified by a Siemens Water Technologies system, conductivity: 0.056 μ S/cm) by stirring overnight. To dissolve chitosan, acidic conditions were required. All other PSs were dissolved in distilled water and the sol-gel process took place under alkaline conditions. In the case of chitosan, HCl (VWR Chemicals, Radnor, USA, 12 M) was added to increase the solubility. After stirring, NaOH (12 M in MQ, Fisher Chemicals, Hampton, USA) was added to the other four PS solutions as a catalyst. Tetraethyl orthosilicate (TEOS, 98%, Sigma Aldrich, St. Louis, USA, used as received) was added. TEOS was initially insoluble in the PS solution, the mixture was vigorously stirred and the hydrolysis of TEOS was completed when a clear solution consisting of a single phase was obtained ([Figure 1](#)). The hydrolysis required between two and six hours depending on the polysaccharide. Hybrid solutions with a PS/silicone mass-ratio of 0.2 were used ([Table 2](#)).

Table 2. Synthesis protocol for a 3 ml HM precursor solution. Due to different viscosity, the water content had to be adjusted while maintaining the PS to silane mass ratios at 0.2.

Abbreviation	TEOS / ml	H ₂ O / ml	PS / mg	Catalyst / μ l	Hydrolysatation / h
CH	0.4	2.6	10	8 HCl	2
HA	0.3	2.7	7.5	12 NaOH	4
ALG	0.4	2.6	10	16 NaOH	4
CS	0.6	2.4	15	28 NaOH	6
HEP	0.6	2.4	15	28 NaOH	6

After sonication and filtration (0.45 μm) of the solutions, thin films were applied by spin-coating (WS-650MZ-23NPP/Lite, Laurell Technologies Corporation). For the bioassays, Nexterion B® Glass Slides (cleanroom quality, Schott, Mainz, Germany) were used as substrates. Pieces of silicon wafers ($\approx 2\text{ cm} \times 2\text{ cm}$, Siegert Wafer, Aachen, Germany) were used as reference samples for spectroscopic ellipsometry measurements. Prior to spin-coating, the substrates were placed in an oxygen plasma (*GaLa miniFlecto-PC-MFC*; 0.4 mbar, 80 W, 22 kHz) for three minutes to clean and hydroxylate the surfaces. While the silane to PS ratio was kept constant, the water content was adjusted for the different PSs to obtain a similar viscosity and thus comparable coating thicknesses in the spin-coating process. After spinning, the prepared surfaces were cured for 3 days at 65°C in a drying oven. Before determination of the thickness and contact angle, the surfaces were thoroughly rinsed with MQ and dried in a N₂-flow.

Preparation of octadecyltrichlorosilane (OTS) monolayer coatings

Glass slides coated with a thin monolayer of octadecyltrichlorosilane (OTS) were used as hydrophobic, negative references. In order to coat the glass slides (Nexterion B®, cleanroom quality, Schott, Mainz, Germany) and the silicon substrates (Siegert Wafer, Aachen, Germany) with the silane, the substrates were first cleaned in an oxygen plasma before being transferred into a desiccator and thoroughly purged with nitrogen. The OTS deposition was performed by a wet-chemical procedure: a 0.5 M solution of octadecyltrichlorosilane (OTS; $\geq 90\%$, Sigma-Aldrich, St. Louis, USA) was prepared by adding the silane to a mixture of 75 vol% cyclohexane ($\geq 99.98\%$) and 25 vol% chloroform ($\geq 99.8\%$; both Fisher Chemicals, Hampton, USA) at $\sim 5^\circ\text{C}$. The OTS mixture was added to the desiccator and sonicated (*Bandelin Sonorex Super* RK 106, 480 W) for 30 min while keeping the temperature below 10 °C. After silanization, the samples were removed from the desiccator, sonicated in cyclohexane and toluene for 3 min, and dried in a nitrogen flow.

Spectroscopic ellipsometry

The film thickness was determined by spectroscopic ellipsometry. Measurements were carried out using a M-2000 V (J.A Woolam, Lincoln, USA), which measures in the spectral range between 370 and 1000 nm. All measurements were carried out at three different incidence angles (65°, 70° and 75°). The thickness of the spin-coated coatings was modeled by a nontransparent layer (absorbing film) model using a β -spline to describe the wavelength-dependent refractive index and absorption coefficient by using the software package CompleteEASE Version 4.98 for data analysis. Data obtained from at least three replicates, with a minimum of three measurements from each sample, were averaged to determine the thickness. The error bars represent the standard deviation.

Contact angle goniometry

The static water contact angles (WCAs) were measured using a custom-built goniometer. The water droplets ($\approx 50 \mu\text{L}$) were deposited on the surfaces of the coated samples and their shapes were recorded via a charged-coupled device camera. The implemented contour tracing algorithm automatically distinguished between drop and mirror image. The shape analysis was accomplished using Young's equation. The reported values were obtained from three independent measurements on each of the three replicate samples. Error bars represent the standard deviation.

Attenuated total reflection - Fourier-transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR spectra were acquired using a nitrogen purged Vertex 70 from Bruker (Billerica, USA) with Platinum Diamond ATR accessory (50 scans, 4 cm^{-1} resolution, wavenumber range $4000\text{-}650 \text{ cm}^{-1}$). FTIR spectra were recorded by a RT-DLaTGS detector.

Stability assay in aqueous media

The stability of the three prepared coatings in aqueous solutions was tested by incubating the samples for defined durations up to 1 week in MQ water or artificial seawater (ASW; Tropic

Marin). After incubation, the samples were rinsed thoroughly with MQ water, dried in a nitrogen flow, and analyzed by spectroscopic ellipsometry. Relative changes in the coating thicknesses were averaged and reported.

Atomic force microscopy (AFM)

The AFM images were measured with a NanoWizard 3 (JPK, Germany). For tapping mode, a MULTI-75GD-G cantilever (Budget Sensors, Bulgaria) was used. Typical settings for a 10 μm x 10 μm scan area were 1V target amplitude, 0.5 V setpoint, 350 Hz igain, and 11.2 $\mu\text{m/s}$ tip velocity. The data analysis was done with Gwyddion (Version 2.47). The roughness values presented were obtained from a 10 μm x 10 μm scan with a resolution of 512 x 512 pixels corresponding to 262.144 data points.

Laboratory diatom attachment and removal assay

The settlement and removal of cells of *Navicula incerta* were studied following published protocols.⁴¹ After three days of culturing, the cells were in the log growth phase. Cells were washed three times in fresh ASW (Tropic Marin) before they were harvested and diluted to yield a suspension with a chlorophyll a content of approximately 0.25 $\mu\text{g/ml}$. All coatings were pre-immersed for 30 min in 0.22 μm filtered ASW prior to the assays. Cells were allowed to settle on three replicates of each coating in individual quadriPERM dishes containing 10 ml of suspension, at $\sim 20^\circ\text{C}$, on the laboratory bench. After 2 h, the slides were placed on an orbital shaker for 5 min (60 rpm) followed by a submerged wash in ASW to remove cells which had not attached. Samples were fixed in 2.5 vol% glutaraldehyde solution, air-dried, and the densities of cells attached to the surfaces of each slide were counted either manually, or with the aid of image analysis software using fluorescence microscopy images captured on a Zeiss Axioscope 2 plus microscope. Counts were made for 30 fields of view (each 0.15 mm^2) on each slide. The removal of diatoms was tested with an additional set of three coated slides of each coating chemistry settled with cells of *N.*

incerta, as described above. Slides with the attached cells were then exposed to a turbulent flow in a water channel exerting a wall shear stress of 26 Pa.⁴² Samples were fixed and the number of cells remaining attached was counted, as described above. A one-way ANOVA analysis with a post hoc Tukey test was carried out to test the statistical significance ($\alpha = 0.05$) of the observed differences.

Barnacle settlement assay

Barnacle larvae were obtained from stocks of *Balanus improvisus* that are kept in semi-continuous culture at Newcastle University. These stocks were originally sourced from the Sven Lovén Centre for Marine Sciences, Tjärnö, Sweden. Adult *B. improvisus* were kept in a 19°C recirculating aquarium in brackish conditions (25 ppt ASW) and fed daily with *Artemia* sp. and *ad libitum* with the chlorophyte *Tetraselmis suecica*. To collect larvae, the adult barnacles were removed from water overnight. On re-immersion, nauplius larvae released into the water column were collected by attraction to a light source. Nauplii ($\approx 10,000$) were transferred into 10-L buckets containing aerated ASW at a salinity of 25 ppt. Nauplii of *B. improvisus* were initially fed a 50:50 mixture of *T. suecica* and *Thalassiosira pseudonana* with the proportion of *T. pseudonana* reducing to zero by the third day of culture. The nauplii took between 4-5 days to metamorphose into cyprids, at which point they were collected by filtration and stored in the dark at 6°C for 3 days. Three-day-old cyprids of *B. improvisus* were used in settlement experiments. Due to the very hydrophilic nature of some of the coatings, an assay geometry was developed, inspired by and simplified from that of Petrone *et al.*⁴³ Briefly, a ‘sandwich’ of two coated microscope slides was arranged, with the slides separated by ≈ 1.5 mm using plastic spacers. Into this space, 10 cyprids were introduced in 100 μ L of ASW. The water droplet never contacted the spacers and the test samples were thus the only material onto which cyprids could settle. Sealed and placed in a humidified incubator for 48 h, settlement of cyprids onto positive reference surfaces was found to

be very high using this technique. After 48 h, the assay was disassembled, and the number of barnacles attached to each pair of slides was counted. Settlement was expressed as a proportion of the 10 larvae originally added per slide pair and averaged over the six replicate pairs of slides for each chemistry. A one-way ANOVA analysis with a post hoc Tukey test was carried out to test the statistical significance ($\alpha = 0.05$) of the observed differences. This assay was conducted twice, with sample sets prepared specifically for each evaluation.⁴⁴

Dynamic short-term field test

The field test was carried out at the biofouling test site at Port Canaveral (28°24'29.4"N 80°37'37.7"W, Florida, USA) of the Florida Institute of Technology (FIT). A dynamic immersion setup described previously was used, which allows immersion into the ocean under well-defined hydrodynamic conditions.⁴⁵ The rotating disk setup was mounted on a floating raft to keep the immersion depth throughout the measurements constant at 0.6 m. A constant rotation speed of 12 rpm was applied to achieve a wall shear stress of 0.18 Pa and all surfaces were immersed for seven days. To retrieve the samples, the disk was unscrewed and lifted out of the water. The samples were placed in a glass compartment containing seawater with 0.5 % glutaraldehyde (Thermo Fisher Scientific, Waltham, United States of America). After 15 min fixation, the majority of the solution was removed without dewetting the slides, followed by three washing steps with distilled water (3 min each). Finally, the samples were air-dried. Samples were subsequently analyzed by fluorescence microscopy (Eclipse Ti, Nikon Instruments, Japan) using a 10x magnification objective and a BV-2A filter set (both Nikon Instruments) to image autofluorescence of the attached marine organisms. Each hybrid material and the reference coatings were tested with four replicates deposited on glass slides (Nexterion B, 75.6 mm × 25.0 mm) and the assay was repeated with two independent sets to confirm reproducibility of the observed trends. Sixty fields of view, each 1.0 mm x 1.0 mm, were recorded automatically on every sample using bright field and

fluorescence imaging (fluorescence light: ExFO XCite-121, Exceilias Technologies Corp, Germany; filter: BV-2A, EX: 400-440; DM: 455; BA: 470, Nikon Instruments, Japan) to obtain sufficient power for the statistical analysis. The locations of the fields of view relative to the edges of the microscopy slides were the same on every sample. Bright field and fluorescence images were overlaid, and the surface coverage was calculated using NIS-Elements (Version AR 4.30.02 64-bit). Since most of marine fouling organisms exhibit autofluorescence molecules like chlorophyll the surface coverage was used to determine the antifouling efficiency of the coatings. Automatic detection of single organisms was hampered due to the presence of sand, dirt, bacteria, and diatom agglomerates. A one-way ANOVA analysis with a post hoc Tukey test was carried out to test the statistical significance ($\alpha = 0.05$) of the observed differences.

Silt adsorption assay

Natural sand sediment collected at Port Canaveral (FL, USA) was autoclaved and dispersed in sterile filtered seawater (CaribSea Pure, Fort Pierce, FL, USA) using a concentration of 37.5 g/L. Besides hydrophilic glass and hydrophobic OTS monolayers, the zwitterionic polymer poly-3-[N-(2-methacryloyloxyethyl)-N,N-dimethylammonio] propanesulfonate (PSPE) was used as a highly hydrated polymer, which is known to have excellent antifouling properties under laboratory conditions but fails due to its high susceptibility for silt accumulation.⁴⁶ All pristine coatings were pre-immersed for 20 min in CaribSea Pure and UV-Vis spectra (Agilent Cary 60, Santa Clara, CA, USA) were recorded. To test the silt accumulation, the coatings were immersed in a sediment suspension on a linear shaker at 60 rpm (Laboshake Heavy-load Shaker, Gerhardt Analytical Systems, Germany) for 20 min. To remove loosely attached sand, all samples were immersed twice for 2 min in Milli-Q water and again investigated by UV-Vis spectroscopy. The change in absorbance of the sediment immersed coating compared to the pristine one was determined at

266 440 nm as the fraction of the absorbance after immersion in the sediment suspension (A_{silt}) and the
267 one of the pristine pre-incubated samples (A_{pristine}).⁴⁶

Results

Hybrid material coating preparation and characterization

Figure 2. AFM images used to determine the morphology, distribution, and roughness of the coatings. Image size: 10 μm x 10 μm .

Table 3. Summary of the properties of the hybrid material coatings. Thickness was determined by spectroscopic ellipsometry, roughness by AFM, and wettability by water contact angle goniometry. All values were determined using three measurements on each of three independent samples. Errors represent the standard deviation.

Abbreviation	Thickness / nm	Roughness / nm	Contact angle / °
OTS	3 $\pm$ 0	0.01	110 $\pm$ 3
CH	117 $\pm$ 1	0.20	45 $\pm$ 1
HA	129 $\pm$ 2	9.50	<10°
ALG	134 $\pm$ 2	22.12	<10°
CS	156 $\pm$ 1	13.73	<10°

HEP	140±3	7.63	<10°
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The viscosity was adjusted for each polymer individually by changing the water content while maintaining the silane / PS ratio to obtain coatings with a thickness between 120 nm and 150 nm. In particular, hyaluronic acid is known for its enormous uptake of water, which leads to a high viscosity, demanding higher dilutions.

Spectroscopic ellipsometry revealed that all hybrid coatings had a homogeneous interface with similar height differences across the surface (standard deviation between 1-3 nm, [Table 3](#)). Hydrophobic silane monolayers consisting of OTS were prepared as negative references because they are known to induce high settlement in fouling assays.^{28,44,47} CH-based HM showed a very low roughness (root mean square, RMS) of only 0.2 nm with structures only on the nanoscale. HA and CS revealed a very fine, porous morphology with randomly distributed spots and a roughness of ≈ 10 nm ([Figure 2](#)). A more distinct surface morphology was found on the ALG coatings, which had a hill-and-valley structure resulting in a roughness of 22 nm. HEP showed a very porous structure and features reminiscent of micro-donuts but with fairly low RMS values of 7.6 nm. All hybrid coatings had very low contact angles, indicating the hydrophilic properties of the interface. Only CH revealed higher contact angles of $45^\circ \pm 1^\circ$. The hydrophobic reference, OTS, had a contact angle of $110^\circ \pm 3^\circ$ ([Table 3](#)).

Figure 3. ATR-FTIR spectra of the HMs. The sol-gel related vibrations dominated the spectra due to their high ratio in the material. A dedicated area (zoom-in) was shown to determine differences between HMs, in the graph spectra were normalized to the largest vibration mode of the $\nu(\text{Si-O-Si})$ stretch vibration between 1100 - 1073 cm^{-1} .

ATR-FTIR was applied for chemical characterization and to verify the formation of HMs (Figure 3). The overall spectra of the HMs showed no major differences, and all were dominated by the $\nu(\text{Si-O-Si})$ stretch vibration between 1100 - 1073 cm^{-1} . The vibrations of $\nu(\text{Si-O-C})$ and $\nu(\text{C-O-C})$, which arise with incorporation of the polysaccharides, have vibrational modes in the same energy region and cannot be clearly separated. Other vibrations are usually located around 1150 - 1070 cm^{-1} .⁴⁸ Here they appear as a shoulder of the silicone peak. The peaks between 950-1200 can be attributed to the polysaccharides^{49,50} used as building blocks for the hybrid materials. The peaks differ slightly in terms of intensity, but also small shifts were observed for the different PSs. All HMs showed small OH/NH-stretch vibration regions between 3500 - 3000 cm^{-1} .⁵⁰ To see

differences between the HMs, the inset in [Figure 3](#) shows a magnification of the area between 1900 - 1350 cm^{-1} in which most vibrations of the functional groups of the PSs are located. Despite a peak that can be assigned to an overlapping vibration of amine I and amide II around 1650-1550 cm^{-1} , chitosan had the most individual spectrum due to its high number of primary amines $\nu(\text{C-NH})$, showing a broad peak at 1527 cm^{-1} and OH-deformation vibrations at 1389 cm^{-1} .⁵⁰ In contrast, all other hybrids, but in particular alginate, bore a significant peak between 1414-1426 cm^{-1} due to the symmetric stretch vibration of the carboxylate anion $\nu_s(\text{R-COO}^-)$.⁴⁸

Table 4. Stability test in MQ and ASW over 7 days. Positive values indicate larger thicknesses due to water uptake. Negative values suggest coating degradation. Values presented are the average of three independent samples measured three times each by spectroscopic ellipsometry. Errors are the standard error of the mean.

Sample	CH	HA	ALG	CS	HEP
Degradation in MQ / %	-0.4 ± 4.5	0.7 ± 3.5	-4.2 ± 1.8	-3.0 ± 0.2	-10.8 ± 1.3
Degradation in ASW / %	5.1 ± 1.1	8.3 ± 5.1	-26.8 ± 4.8	-13.3 ± 11.3	-20.9 ± 3.8

The stability test in distilled water showed a high solidity of the hybrids in aqueous media. Except for HEP, which showed a decline from 123 nm to 110 nm (10.8 %), all coatings remained stable with a decrease in thickness between 0 - 4 % ([Table 4](#)). While CH and HA tended to take up water and swelled weakly (5 – 8 %), ALG, CS, and HEP tended to degrade slightly. The thickness of CS decreased the least while ALG had the highest decrease at 27 %. Still, the majority of the coatings remained on the substrate after one week in saline water. Based on these results, the coatings seemed to be appropriate for laboratory and short-term field accumulation assays.

324 *Settlement and removal of Navicula incerta*

325 **Figure 4.** Initial attachment and removal assay for the diatom *Navicula incerta* . **A:** Mean diatom
326 density from three replicate coating samples for each chemistry. A repeat assay is shown in the
327 Supporting Information (Figure S1). The blue bars show the density of settled diatoms on the
328 references and hybrid surfaces after 2 h. The light grey parts of the bars indicate the density of
329 diatoms remaining after exposure to a turbulent flow exerting a wall shear stress of 26 Pa. Each
330 data point is the mean from 90 counts on three replicate slides. Error bars show the standard error.
331 **B:** Percentage removal of diatoms from HM coatings by the turbulent flow with a wall shear stress
332 of 26 Pa. Error bars show the standard error of the mean derived from arcsine transformed data.
333 For both, a one-way ANOVA with post-hoc Tukey test (significance level of $\alpha = 0.05$) was applied
334 to determine if the observed differences were statistically significant, which were then marked
335 with different letters.

336 The HMs coatings were tested against the attachment of diatoms and their removal. In the assay,
337 the coatings were exposed to suspensions containing the same concentration of diatoms for 2 hours
338 (Figure 4A). A one-way analysis of variance with post hoc Tukey test ($p < 0.05$) indicated that
339 settlement on the OTS references was significantly lower (212 ± 17 diatoms/mm²) than on the
340 other samples. The lower settlement was unexpected as OTS is known as a strongly fouling

surface.^{28,47} On all hybrid coatings, initial settlement was similar to, or slightly higher than that on glass (283 ± 17 diatoms/mm²).

In order to measure how strongly the diatoms adhered to each of the coatings, their attachment was challenged by the application of a turbulent water flow in a calibrated channel exerting a wall shear stress of 26 Pa.⁴² A one-way analysis of variance with post hoc Tukey test ($p < 0.05$) showed statistical differences between removal from the coatings (Figure 4B). OTS as a negative reference coating, showed only 2 % removal, indicating that the diatoms attached strongly to its surface. Removal from all the hybrid coatings, except from the CH, was greater than from the glass reference surface (42%). Attachment of the diatoms was weakest on the CS and HEP coatings with removal of up to 77%. This was followed by the ALG (65%) and the HA (53%) coatings.

352 *Settlement of Balanus improvisus cyprids*

353 **Figure 5.** Settlement data of 3-day-old cypris larvae after 48 hours on five different hybrid coatings
354 and on hydrophobic OTS references. The assay was done with six replicates of each coating. A
355 repeat of the assay (Figure S3) is shown in the Supporting Information showing a similar trend
356 within the HMs. Statistical differences are marked by different letters ($\alpha = 0.05$). Error bars
357 represent the standard error.

358 Settlement of three-day-old cypris larvae was high on the hydrophobic OTS references
359 ($88 \pm 5\%$), indicating a high settlement competence of the cypris larvae (Figure 5).⁴⁴ Settlement
360 on CH ($60 \pm 15\%$) and HA ($47 \pm 21\%$) was high compared the other HMs ($28 \pm 3\%$). The lowest
361 settlement was found on HEP. Only $23 \pm 8\%$ larvae settled on these coatings. A one-way ANOVA
362 with post hoc Tukey test (significance level of $\alpha = 0.05$) indicated that OTS was statistically
363 significantly different to ALG, CS, and HEP but not to HA and CH.

364

366 **Figure 6.** Biomass accumulation after dynamic field exposure and sand uptake in a silt exposure
367 assay in the laboratory. **A:** Dynamic rotating-disk field assay (spring 2019) at the Port Canaveral
368 test site of the Florida Institute of Technology. Four samples of each chemistry were immersed.
369 Sixty fields of views (1 mm²) were recorded by fluorescence microscopy and the fluorescence
370 coverage on the surfaces was determined. Values presented are the average of 240 fields of view
371 in total. A second individual assay (autumn 2018) with similar trends is shown in the Supporting
372 Information. A one-way ANOVA with post hoc Tukey test (significance level of $\alpha = 0.05$) was
373 applied to observe statistically significant differences between the coatings depicted in separate
374 letters. The error bars represent the standard error of the mean. **B:** Silt accumulation on the coatings
375 after a 20-min sediment immersion. The absorbance of the coatings at a wavelength of 440 nm
376 was measured before and after immersion into a suspension of sediment collected from the Florida
377 test site. Absorbance increase was calculated as the fraction of the absorbance of the coatings after
378 immersion and that of the pristine coatings. Reported values are the average of three independent
379 measurements. Error bars are the standard error of the mean.

380 After dynamic immersion on a rotating disk for 7 days in the harbor of Port Canaveral, samples
381 were fixed and analyzed by fluorescence microscopy. On all surfaces, an accumulation of
382 microorganisms and silt was detected. The coverage by attached microorganisms like diatoms was
383 determined by analyzing chlorophyll autofluorescence. The hydrophobic OTS reference had the
384 highest coverage of 25 ± 1 % (Figure 6A). CH and CS performed similarly with 12 - 14 %
385 coverage, while HA, ALG, and HEP had comparable coverages of 5 – 7 %. A one-way ANOVA
386 with post hoc Tukey test (significance level of $\alpha = 0.05$) revealed that OTS was significantly
387 different from all hybrid coatings. CH/CS had significantly higher coverages compared to
388 HA/ALG/HEP. The silt accumulation (Figure 6B) was calculated as the change in absorbance of
389 the coatings in UV-Vis spectroscopy due to the immersion in a silt suspension. The references
390 glass and OTS revealed very low sediment uptake (Absorbance increases between 1.1 – 1.4).
391 Comparably low silt attachment was seen on CS and HEP HMs (1.3 – 1.8). The group of CH, HA,
392 and ALG performed slightly worse with increases between 2.2 – 2.8. PSPE is shown for
393 comparison representing the class of well hydrated hydrogel coatings. Such coatings are known
394 for their excellent antifouling properties under laboratory conditions but fail in natural conditions
395 due to silt accumulation.⁴⁶ In contrast to low silt accumulation of the hybrid materials in this study,
396 the UV-Vis absorbance of the previously tested photo-crosslinked PSPE coatings increased up to
397 13.2-fold after immersion into a silt suspension.

Discussion

We synthesized five different HM coatings based on naturally occurring, environmentally friendly, and renewable polysaccharides. The thickness of the coatings was adjusted to similar heights to enable direct comparison of their antifouling and fouling-release properties. While the coating thickness was similar, wettability, morphology, roughness, and IR spectra differed. In IR, the presence of both polysaccharides and of the silane building blocks was evident. All hybrids had very hydrophilic properties except for CH, which is not unexpected as in previous studies grafted CS, ALG, and HA showed similarly low WCAs of $<13^\circ$,^{26,27} while CH, a polysaccharide rich in amino groups, was less hydrophilic and showed a WCA of $45 \pm 1^\circ$. As indication for the presence of hydrophilic groups at the interface, the data can be compared to literature values for SAMs as model surfaces with controlled surface termination. Monolayers assembled from hydroxyl-terminated undecanthiol (HUDT) have WCAs between $24\text{--}33^\circ$ while the amino-terminated undecanthiol (AUDT) revealed values between $57\text{--}60^\circ$.^{51,52} Carboxyl-groups, which are not present in CH, show intermediate WCAs of 29° .⁵³ To conclude, the absence of carboxy- and the presence of amino-groups, seems to be the main factor for the high CA of CH. Thus, the use of sol-gel chemistry allowed the synthesis of stable hybrid material coatings in which the original properties of the polysaccharides are preserved.

To test the antifouling and fouling-release properties of the polysaccharide-based hybrid materials, the coatings were challenged by settlement and removal assays against different marine species and in a dynamic short-term field test. In tests against the marine diatom *N. incerta*, all HMs showed higher initial settlement than the hydrophobic reference, which was unexpected.^{28,47} After the hybrid surfaces were exposed to a turbulent water flow, the most hydrophobic hybrid, CH, had the lowest performance of all hybrid coatings with a slightly higher removal ($43 \pm 3\%$) than found on the hydrophilic glass references ($42 \pm 1\%$). All other hybrids were highly

422 hydrophilic ($\text{WCA} < 10^\circ$) and showed higher removal between $52 \pm 4 \%$ for HA and $76 \pm 2 \%$ for
423 HEP/CS. Thus, the fouling-release performance (removal) decreased in the order
424 $\text{HEP} \approx \text{CS} > \text{ALG} > \text{HA} > \text{CH}$. The easy detachment of the diatoms from the hydrophilic HMs is
425 in line with literature reporting that diatoms attach weaker on hydrophilic surfaces.^{47,54} Static
426 settlement assays with barnacle larvae revealed that HEP attracted the lowest settlement ($23 \pm 8\%$),
427 which was 3.8-fold lower than on the hydrophobic OTS references ($88 \pm 5\%$). All other
428 hydrophilic hybrids ranged between 28-47%, while CH ($60 \pm 15\%$) performed only slightly better
429 than OTS, which is nevertheless a reduction of 32%. Settlement on the different coatings increased
430 in the order: $\text{HEP} < \text{ALG} \approx \text{CS} < \text{HA} < \text{CH}$. Field tests on a rotating disk at Port Canaveral showed
431 that ALG-based HM showed the lowest coverage ($5 \pm 1\%$), followed by HA/HEP with 6-7 % and
432 CH/CS with 12-15 %. The biofouling coverage, measured by the autofluorescence of chlorophyll
433 in biomaterials increased on the HMs in the order $\text{ALG} \approx \text{HA} \approx \text{HEP} < \text{CS} \approx \text{CH}$.

434 Thus, in all biological tests the HM coatings were able to reduce the settlement or enhance the
435 removal of the tested marine fouling organisms compared to the hydrophobic references. Some of
436 the coatings based on the different polysaccharides performed very well, while CH showed, in all
437 cases, the worst performance. While for the diatoms HEP and CS performed best in reducing
438 attachment strength of *N. incerta*, HEP showed lowest settlement of *B. improvisus*, despite no
439 statistically significant differences being found within the HMs. In the dynamic field tests,
440 HA/ALG/HEP showed the best performances with lower settlement than CS/CH. As mentioned
441 before, all HMs revealed extraordinary hydrophilicity and wettability except CH, which performed
442 worst in all biological assays. The importance of hydrophilicity is well documented in the
443 literature. Many studies showed that contrary to contemporary theories, high surface energy
444 coatings often outperform low-energy ones. This was shown several times by SAMs terminated

with methyl- (CH₃) or hydroxyl- (OH) groups or mixtures of them against model fouling organisms and protein adsorption.⁵⁵⁻⁵⁷

Besides the substantial number of hydroxyl groups, which are in part responsible for the hydrophilicity of the coatings, the PSs used here carry protonatable groups, which are charged in sea water. The distribution of the sulfate groups (SO₃⁻, pK_a: 0.5 – 1.5), carboxyl groups (COO⁻, pK_a: 3 - 5), amine groups (NH₃⁺, pK_a: 7 – 10) and N-acetyl amine groups (NH₂⁺COCH₃, pK_a ≈ 11)^{58,59} per four monosaccharide building block, which is the smallest repetition unit, are summarized in [Table S1](#) and can be seen in the molecular structures in [Figure 1](#). At the pH of ≈8 of sea water, the degree of protonation/deprotonation of the different groups leads to a net charge of the biomacromolecules. In order to make a relative comparison between the different polysaccharides, the average net charge per monosaccharide building block was calculated based on the pK_a values of the individual protonatable groups. Estimated by the average net charge of the PSs, they can be sorted by decreasing negative charge density (at pH 8) in the order of HEP (-1.4) > ALG (-1.0) ≈ CS (-1.0) > HA (-0.5) > CH (+0.3). It is obvious that the positive charge on CH is connected with the high colonization of the obtained hybrid materials in both laboratory and field assays. This high attachment on CH correlates well with literature results, as positively charged amino groups were frequently reported to promote high colonization by marine fouling organisms. *Navicula incerta*, for example, is known to settle and adhere strongly on quaternary ammonium-rich SAMs.²⁷ Barnacle larvae also prefer to settle and metamorphose on positively charged surfaces,⁶⁰ albeit also reports of low^{57,61} or similar settlement of both, *Balanus amphitrite* and *B. improvisus* can be found in literature.⁶² Adhesive deposits from exploring barnacle cyprids of *B. amphitrite* as determined by iSPR were slightly larger on negatively charged carboxyl groups compared to positively charged amino groups, but in both cases much higher than on uncharged,

hydrophilic ethylene glycols.⁶³ For algal zoospores a strong interaction with positively charged quaternary ammonium groups⁶⁴ and arginine-rich peptides was found.^{65,66} The coverage of fluorescent biomaterial in the dynamic field exposure experiments was enhanced on CH. This is in line with previous field tests in which coatings dominated by quaternary amino groups showed higher coverages by fouling organisms.²⁷

While wettability and the density of charged groups seem to contribute substantially to the antifouling properties, we did not see a clear correlation with the initial roughness of the coatings. In the dry state, CH had a smooth surface (RMS: 0.2 nm) comparable to an organic monolayer coating, while all other hybrids had rougher surfaces (RMS: 10-20 nm). Considering the thickness of the coating ≈ 140 nm, the roughness was between 7 - 14% of the total coating height, which can be considered as a very smooth surface. In all cases, the size of the tested fouling organisms was much larger than the structures on the surface of the coatings. While the diatoms used in this study have lengths of ≈ 14 μm , barnacle larvae are larger (≈ 600 μm).⁶⁷ It was shown previously that both barnacle larvae and diatoms respond to feature sizes (roughness, surface patterning) in the range of the size of the organism and that this affects the probability of them settling. While smoother surfaces lead to longer periods of surface exploration and higher settlement, attachment was found to be reduced with a decreasing number of attachment points.⁶⁸⁻⁷²

In previous work, we noted that zwitterionic hydrogel coatings tested in the field were prone to accumulating silt, which masked the original properties of the coatings.⁴⁶ It was also found that enhancing the crosslink density in zwitterionic hydrogel coatings reduced the susceptibility to silt uptake and enhanced the antifouling performance.⁷³ Furthermore, amphiphilic polymer coatings showed even stronger resistance against silt uptake and antifouling performance in the field was superior to that of the purely hydrophilic homopolymers.⁷⁴ Both results showed that hydrogels

with a low degree of crosslinking tended to form interphases with a density gradient between the polymer and the water phase, which allowed silt and inorganic material to become easily incorporated into the highly diffuse, terminal interphase of the hydrogels. The HMs showed a very low tendency for silt accumulation in the sediment exposure assays with values comparable to hydrophobic OTS and hydrophilic glass references. While the above-mentioned photocrosslinked, zwitterionic hydrogels tend to swell strongly upon exposure to water due to their low degree of crosslinking, the hybrid materials store less water ($< 5\%$), which is most likely due to the strong crosslinking (only 20 w% of the hybrid coatings consist of PS) by TEOS. Similar effects were reported previously, showing high water uptake for HA and ALG powders by up to 250-fold mass weight. Interestingly, increased crosslinking also led to lower water uptake (100-fold).⁷⁵ In comparison with the polysaccharide-based materials, which are able to store large amounts of water, the conversion into a HM seems to limit silt uptake. This is due to the higher degree of crosslinking, which in comparison reduces water uptake, whilst preserving hydration and hydrophilicity to a degree that is sufficient to enable the coatings to remain effective against marine biofouling. This theory is in good agreement with the results of the Detty research group showing that it is advantageous in marine biofouling to use hard sol-gel-based coatings without a diffuse, terminal interphase.^{16,17}

While settlement and release properties could in the future be further improved by fine-tuning the composition of the hybrid materials, the sol-gel process itself is an easy and practical way to create polysaccharide-based coatings. We showed that even small changes in the functional groups of the polysaccharides can influence their physicochemical properties and their antifouling and fouling-release properties against marine model organisms and in short term field exposures.

ALG and HEP gave particularly promising results and highlight the demand for further investigations and modification to generate fouling-release coatings.

Conclusion

Using a sol-gel process, we were able to produce five different hybrid materials based on polysaccharides as building blocks. The coatings showed good antifouling and fouling-release performance and polysaccharides rich in anionic groups showed superior performance compared to those with amine groups. In developing HM coatings charge, hydration, and wettability were relevant physicochemical properties for consideration, while morphological factors, such as surface roughness, were less important. Despite their hydrophilic properties, silt uptake and fouling accumulation in the field was successfully suppressed. The approach opens the opportunity to screen further HMs based on renewable and biodegradable compounds for environmentally benign coatings.

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References

(1) Chin, S. M.; Synatschke, C. V.; Liu, S.; Nap, R. J.; Sather, N. A.; Wang, Q.; Álvarez, Z.; Edelbrock, A. N.; Fyrner, T.; Palmer, L. C.; Szleifer, I.; La Olvera de Cruz, M.; Stupp, S. I. Covalent-Supramolecular Hybrid

536 Polymers as Muscle-Inspired Anisotropic Actuators. *Nature communications* **2018**, 9 (1), 1–11. DOI:
537 10.1038/s41467-018-04800-w.

538 (2) Wang, B.; Wu, Z.; Zhang, D.; Wang, R.-M.; Song, P.; Xiong, Y.; He, Y. Antibacterial Silicylacrylate
539 Copolymer Emulsion for Antifouling Coatings. *Progress in Organic Coatings* **2018**, 118, 122–128. DOI:
540 10.1016/j.porgcoat.2018.01.025.

541 (3) Singh, V.; Srivastava, P.; Singh, A.; Singh, D.; Malviya, T. Polysaccharide-Silica Hybrids: Design and
542 Applications. *Polymer Reviews* **2016**, 56 (1), 113–136. DOI: 10.1080/15583724.2015.1090449.

543 (4) Olivares, R. U.; Tabeta, S.; Sombrito, E. Z. Tributyltin in Marine Sediments and Philippine Green
544 Mussels (*Perna viridis*) in Manila Bay. *J Mar Sci Technol* **2013**, 18 (2), 213–219. DOI: 10.1007/s00773-
545 012-0201-2.

546 (5) Rodríguez Grimón, R. O.; Arroyo Osorio, M. F.; Freitas, D. M. de; Castro, Í. B. Tributyltin Impacts in
547 Galapagos Islands and Ecuadorian Shore: Marine Protected Areas under Threat. *Marine Policy* **2016**, 69,
548 24–31. DOI: 10.1016/j.marpol.2016.03.017.

549 (6) Barletta, M.; Aversa, C.; Pizzi, E.; Puopolo, M.; Vesco, S. Design, Development and First Validation of
550 “Biocide-Free” Anti-Fouling Coatings. *Progress in Organic Coatings* **2018**, 123, 35–46. DOI:
551 10.1016/j.porgcoat.2018.06.007.

552 (7) Owens, G. J.; Singh, R. K.; Foroutan, F.; Alqaysi, M.; Han, C.-M.; Mahapatra, C.; Kim, H.-W.; Knowles,
553 J. C. Sol–Gel Based Materials for Biomedical Applications. *Progress in Materials Science* **2016**, 77, 1–79.
554 DOI: 10.1016/j.pmatsci.2015.12.001.

555 (8) Schottner, G. Hybrid Sol–Gel-Derived Polymers: Applications of Multifunctional Materials. *Chem.*
556 *Mater.* **2001**, 13 (10), 3422–3435. DOI: 10.1021/cm011060m.

557 (9) Spirk, S.; Findenig, G.; Doliska, A.; Reichel, V. E.; Swanson, N. L.; Kargl, R.; Ribitsch, V.; Stana-
558 Kleinschek, K. Chitosan-Silane Sol-Gel Hybrid Thin Films with Controllable Layer Thickness and
559 Morphology. *Carbohydrate polymers* **2013**, 93 (1), 285–290. DOI: 10.1016/j.carbpol.2012.04.030.

560 (10) Montheil, T.; Echalié, C.; Martinez, J.; Subra, G.; Mehdi, A. Inorganic Polymerization: An Attractive
561 Route to Biocompatible Hybrid Hydrogels. *J. Mater. Chem. B* **2018**, 6 (21), 3434–3448. DOI:
562 10.1039/C8TB00456K.

563 (11) Wojcik, A. B.; Klein, L. C. Transparent Organic/Inorganic Hybrid Gels: A Classification Scheme. *Appl.*
564 *Organometal. Chem.* **1997**, 11 (2), 129–135. DOI: 10.1002/(SICI)1099-0739(199702)11:2<129:AID-
565 AOC559>3.0.CO;2-O.

566 (12) Hu, P.; Xie, Q.; Ma, C.; Zhang, G. Silicone-Based Fouling-Release Coatings for Marine Antifouling.
567 *Langmuir* **2020**, 36 (9), 2170–2183. DOI: 10.1021/acs.langmuir.9b03926.

568 (13) Tang, Y.; Finlay, J. A.; Kowalke, G. L.; Meyer, A. E.; Bright, F. V.; Callow, M. E.; Callow, J. A.; Wendt,
569 D. E.; Detty, M. R. Hybrid Xerogel Films as Novel Coatings for Antifouling and Fouling Release. *Biofouling*
570 **2005**, 21 (1), 59–71. DOI: 10.1080/08927010500070935.

571 (14) Detty, M. R.; Ciriminna, R.; Bright, F. V.; Pagliaro, M. Environmentally Benign Sol-Gel Antifouling
572 and Foul-Releasing Coatings. *Accounts of chemical research* **2014**, 47 (2), 678–687. DOI:
573 10.1021/ar400240n.

574 (15) Ciriminna, R.; Bright, F. V.; Pagliaro, M. Ecofriendly Antifouling Marine Coatings. *ACS Sustainable*
575 *Chem. Eng.* **2015**, 3 (4), 559–565. DOI: 10.1021/sc500845n.

576 (16) Sokolova, A.; Cilz, N.; Daniels, J.; Stafslie, S. J.; Brewer, L. H.; Wendt, D. E.; Bright, F. V.; Detty, M.
577 R. A Comparison of the Antifouling/Foul-Release Characteristics of Non-Biocidal Xerogel and Commercial
578 Coatings toward Micro- and Macrofouling Organisms. *Biofouling* **2012**, 28 (5), 511–523. DOI:
579 10.1080/08927014.2012.690197.

(17) Detty, M. R.; Ciriminna, R.; Bright, F. V.; Pagliaro, M. Xerogel Coatings Produced by the Sol-Gel Process as Anti-Fouling, Fouling-Release Surfaces: From Lab Bench to Commercial Reality. *ChemNanoMat* **2015**, *1* (3), 148–154. DOI: 10.1002/cnma.201500056.

(18) Shchipunov, Y. A.; Karpenko, T. Y. Hybrid Polysaccharide–Silica Nanocomposites Prepared by the Sol–Gel Technique. *Langmuir* **2004**, *20* (10), 3882–3887. DOI: 10.1021/la0356912.

(19) Regina, V. R.; Sørhoel, H.; Lokanathan, A. R.; Bischoff, C.; Kingshott, P.; Revsbech, N. P.; Meyer, R. L. Entrapment of Subtilisin in Ceramic Sol-Gel Coating for Antifouling Applications. *ACS applied materials & interfaces* **2012**, *4* (11), 5915–5921. DOI: 10.1021/am301554m.

(20) El Abiad, C.; Radi, S.; Faustino, M. A. F.; Neves, M. G. P. M. S.; Moura, N. M. M. Supramolecular Hybrid Material Based on Engineering Porphyrin Hosts for an Efficient Elimination of Lead(II) from Aquatic Medium. *Molecules* **2019**, *24* (4). DOI: 10.3390/molecules24040669.

(21) Lee, H.-Y.; Kim, H.-E.; Jeong, S.-H. One-Pot Synthesis of Silane-Modified Hyaluronic Acid Hydrogels for Effective Antibacterial Drug Delivery via Sol-Gel Stabilization. *Colloids and surfaces. B, Biointerfaces* **2019**, *174*, 308–315. DOI: 10.1016/j.colsurfb.2018.11.034.

(22) Kerchove, A. J. de; Elimelech, M. Calcium and Magnesium Cations Enhance the Adhesion of Motile and Nonmotile *Pseudomonas Aeruginosa* on Alginate Films. *Langmuir* **2008**, *24* (7), 3392–3399. DOI: 10.1021/la7036229.

(23) Cao, X.; Pettit, M. E.; Conlan, S. L.; Wagner, W.; Ho, A. D.; Clare, A. S.; Callow, J. A.; Callow, M. E.; Grunze, M.; Rosenhahn, A. Resistance of Polysaccharide Coatings to Proteins, Hematopoietic Cells, and Marine Organisms. *Biomacromolecules* **2009**, *10* (4), 907–915. DOI: 10.1021/bm8014208.

(24) Ederth, T.; Ekblad, T.; Pettitt, M. E.; Conlan, S. L.; Du, C.-X.; Callow, M. E.; Callow, J. A.; Mutton, R.; Clare, A. S.; D'Souza, F.; Donnelly, G.; Bruin, A.; Willemsen, P. R.; Su, X. J.; Wang, S.; Zhao, Q.; Hederos, M.; Konradsson, P.; Liedberg, B. Resistance of Galactoside-Terminated Alkanethiol Self-Assembled Monolayers to Marine Fouling Organisms. *ACS applied materials & interfaces* **2011**, *3* (10), 3890–3901. DOI: 10.1021/am200726a.

(25) Fyrner, T.; Lee, H.-H.; Mangone, A.; Ekblad, T.; Pettitt, M. E.; Callow, M. E.; Callow, J. A.; Conlan, S. L.; Mutton, R.; Clare, A. S.; Konradsson, P.; Liedberg, B.; Ederth, T. Saccharide-Functionalized Alkanethiols for Fouling-Resistant Self-Assembled Monolayers: Synthesis, Monolayer Properties, and Antifouling Behavior. *Langmuir* **2011**, *27* (24), 15034–15047. DOI: 10.1021/la202774e.

(26) Bauer, S.; Arpa-Sancet, M. P.; Finlay, J. A.; Callow, M. E.; Callow, J. A.; Rosenhahn, A. Adhesion of Marine Fouling Organisms on Hydrophilic and Amphiphilic Polysaccharides. *Langmuir* **2013**, *29* (12), 4039–4047. DOI: 10.1021/la3038022.

(27) Bauer, S.; Alles, M.; Arpa-Sancet, M. P.; Ralston, E.; Swain, G. W.; Aldred, N.; Clare, A. S.; Finlay, J. A.; Callow, M. E.; Callow, J. A.; Rosenhahn, A. Resistance of Amphiphilic Polysaccharides against Marine Fouling Organisms. *Biomacromolecules* **2016**, *17* (3), 897–904. DOI: 10.1021/acs.biomac.5b01590.

(28) Jakobi, V.; Schwarze, J.; Finlay, J. A.; Nolte, K. A.; Spöllmann, S.; Becker, H.-W.; Clare, A. S.; Rosenhahn, A. Amphiphilic Alginates for Marine Antifouling Applications. *Biomacromolecules* **2018**, *19* (2), 402–408. DOI: 10.1021/acs.biomac.7b01498.

(29) Kim, S.; Ko, S.; Kang, S. M. Adhesive Heparin Coating for Marine Antifouling Applications. *Macromol. Res.* **2016**, *24* (7), 645–649. DOI: 10.1007/s13233-016-4091-8.

(30) Buzzacchera, I.; Vorobii, M.; Kostina, N. Y.; los Santos Pereira, A. de; Riedel, T.; Bruns, M.; Ogieglo, W.; Möller, M.; Wilson, C. J.; Rodriguez-Emmenegger, C. Polymer Brush-Functionalized Chitosan Hydrogels as Antifouling Implant Coatings. *Biomacromolecules* **2017**, *18* (6), 1983–1992. DOI: 10.1021/acs.biomac.7b00516.

- (31) Xu, G.; Liu, P.; Pranantyo, D.; Neoh, K.-G.; Kang, E.-T. Dextran- and Chitosan-Based Antifouling, Antimicrobial Adhesion, and Self-Polishing Multilayer Coatings from pH-Responsive Linkages-Enabled Layer-by-Layer Assembly. *ACS Sustainable Chem. Eng.* **2018**, *6* (3), 3916–3926. DOI: 10.1021/acssuschemeng.7b04286.
- (32) Yu, W.; Koc, J.; Finlay, J. A.; Clarke, J. L.; Clare, A. S.; Rosenhahn, A. Layer-by-layer Constructed Hyaluronic Acid/Chitosan Multilayers as Antifouling and Fouling-Release Coatings. *Biointerphases* **2019**, *14* (5), 51002. DOI: 10.1116/1.5110887.
- (33) Yuan, W.; Ji, J.; Fu, J.; Shen, J. A Facile Method to Construct Hybrid Multilayered Films as a Strong and Multifunctional Antibacterial Coating. *Journal of biomedical materials research. Part B, Applied biomaterials* **2008**, *85* (2), 556–563. DOI: 10.1002/jbm.b.30979.
- (34) Elsabee, M. Z.; Abdou, E. S. Chitosan Based Edible Films and Coatings: A Review. *Materials science & engineering. C, Materials for biological applications* **2013**, *33* (4), 1819–1841. DOI: 10.1016/j.msec.2013.01.010.
- (35) Yılmaz Atay, H.; Çelik, E. Investigations of Antibacterial Activity of Chitosan in the Polymeric Composite Coatings. *Progress in Organic Coatings* **2017**, *102*, 194–200. DOI: 10.1016/j.porgcoat.2016.10.013.
- (36) Shchipunov, Y. A. Sol–Gel-Derived Biomaterials of Silica and Carrageenans. *Journal of colloid and interface science* **2003**, *268* (1), 68–76. DOI: 10.1016/S0021-9797(03)00457-0.
- (37) Shchipunov, Y. A.; Karpenko, T.'y. Y.; Bakunina, I. Y.; Burtseva, Y. V.; Zvyagintseva, T.'y. N. A New Precursor for the Immobilization of Enzymes Inside Sol–Gel-Derived Hybrid Silica Nanocomposites containing polysaccharides. *Journal of Biochemical and Biophysical Methods* **2004**, *58* (1), 25–38. DOI: 10.1016/S0165-022X(03)00108-8.
- (38) Shchipunov, Y. A.; Kojima, A.; Imae, T. Polysaccharides as a Template for Silicate gGenerated by Sol-Gel Processes. *Journal of colloid and interface science* **2005**, *285* (2), 574–580. DOI: 10.1016/j.jcis.2004.11.026.
- (39) Ayers, M. R.; Hunt, A. J. Synthesis and Properties of Chitosan–Silica Hybrid Aerogels. *Journal of Non-Crystalline Solids* **2001**, *285* (1-3), 123–127. DOI: 10.1016/S0022-3093(01)00442-2.
- (40) Wang, G.-H.; Zhang, L.-M. A Biofriendly Silica Gel for *in situ* Protein Entrapment: Biopolymer-Assisted Formation and its Kinetic Mechanism. *The journal of physical chemistry. B* **2009**, *113* (9), 2688–2694. DOI: 10.1021/jp810736v.
- (41) Finlay, J. A.; Bennett, S. M.; Brewer, L. H.; Sokolova, A.; Clay, G.; Gunari, N.; Meyer, A. E.; Walker, G. C.; Wendt, D. E.; Callow, M. E.; Callow, J. A.; Detty, M. R. Barnacle Settlement and the Adhesion of Protein and Diatom Microfouling to Xerogel Films with Varying Surface Energy and Water Wettability. *Biofouling* **2010**, *26* (6), 657–666. DOI: 10.1080/08927014.2010.506242.
- (42) Schultz, M. P.; Finlay, J. A.; Callow, M. E.; Callow, J. A. A Turbulent Channel Flow Apparatus for the Determination of the Adhesion Strength of Microfouling Organisms. *Biofouling* **2000**, *15* (4), 243–251. DOI: 10.1080/08927010009386315.
- (43) Petrone, L.; Lee, S. S. C.; Teo, S. L. M.; Birch, W. R. A Novel Geometry for a Laboratory-Based Larval Settlement Assay. *Biofouling* **2013**, *29* (2), 213–221. DOI: 10.1080/08927014.2012.762643.
- (44) Wanka, R.; Aldred, N.; Finlay, J. A.; Amuthalingam, A.; Clarke, J. L.; Clare, A. S.; Rosenhahn, A. Antifouling Properties of Dendritic Polyglycerols against Marine Macrofouling Organisms. *Langmuir* **2019**, *35* (50), 16568–16575. DOI: 10.1021/acs.langmuir.9b02720.

- (45) Nolte, K. A.; Koc, J.; Barros, J. M.; Hunsucker, K.; Schultz, M. P.; Swain, G. W.; Rosenhahn, A. Dynamic Field Testing of Coating Chemistry Candidates by a Rotating Disk System. *Biofouling* **2018**, *34* (4), 398–409. DOI: 10.1080/08927014.2018.1459578.
- (46) Koc, J.; Simovich, T.; Schönmann, E.; Chilkoti, A.; Gardner, H.; Swain, G. W.; Hunsucker, K.; Laschewsky, A.; Rosenhahn, A. Sediment Challenge to Promising Ultra-Low Fouling Hydrophilic Surfaces in the Marine Environment. *Biofouling* **2019**, *35* (4), 454–462. DOI: 10.1080/08927014.2019.1611790.
- (47) Wanka, R.; Finlay, J. A.; Nolte, K. A.; Koc, J.; Jakobi, V.; Anderson, C.; Clare, A. S.; Gardner, H.; Hunsucker, K. Z.; Swain, G. W.; Rosenhahn, A. Fouling-Release Properties of Dendritic Polyglycerols against Marine Diatoms. *ACS applied materials & interfaces* **2018**, *10* (41), 34965–34973. DOI: 10.1021/acsami.8b12017.
- (48) van Hoogmoed, C. G.; Busscher, H. J.; Vos, P. de. Fourier Transform Infrared Spectroscopy Studies of Alginate-PLL Capsules with varying compositions. *Journal of biomedical materials research. Part A* **2003**, *67* (1), 172–178. DOI: 10.1002/jbm.a.10086.
- (49) Maréchal, Y.; Chanzy, H. The Hydrogen Bond Network in I β Cellulose as Observed by Infrared Spectrometry. *Journal of Molecular Structure* **2000**, *523* (1-3), 183–196. DOI: 10.1016/S0022-2860(99)00389-0.
- (50) Alkrad, J. A.; Mrestani, Y.; Stroehl, D.; Wartewig, S.; Neubert, R. Characterization of Enzymatically Digested Hyaluronic aAcid using NMR, Raman, IR, and UV–Vis Spectroscopies. *Journal of Pharmaceutical and Biomedical Analysis* **2003**, *31* (3), 545–550. DOI: 10.1016/S0731-7085(02)00682-9.
- (51) Thome, I.; Pettitt, M. E.; Callow, M. E.; Callow, J. A.; Grunze, M.; Rosenhahn, A. Conditioning of Surfaces by Macromolecules and its Implication for the <settlement of Zoospores of the Green Alga *Ulva linza*. *Biofouling* **2012**, *28* (5), 501–510. DOI: 10.1080/08927014.2012.689288.
- (52) Thome, I.; Bauer, S.; Vater, S.; Zargiel, K.; Finlay, J. A.; Arpa-Sancet, M. P.; Alles, M.; Callow, J. A.; Callow, M. E.; Swain, G. W.; Grunze, M.; Rosenhahn, A. Conditioning of Self-Assembled Monolayers at Two Static Immersion Test Sites along the East Coast of Florida and its Effect on Early Fouling Development. *Biofouling* **2014**, *30* (8), 1011–1021. DOI: 10.1080/08927014.2014.957195.
- (53) Koc, J.; Scharadt, L.; Nolte, K.; Beyer, C.; Eckhard, T.; Schwiderowski, P.; Clarke, J. L.; Finlay, J. A.; Clare, A. S.; Muhler, M.; Laschewsky, A.; Rosenhahn, A. Effect of Dipole Orientation in Mixed, Charge-Equilibrating Self-assembled Monolayers on Protein Adsorption and Marine Biofouling. *ACS Appl. Mater. Interfaces* **2020** (accepted). DOI: 10.1021/acsami.0c11580.
- (54) Krishnan, S.; Wang, N.; Ober, C. K.; Finlay, J. A.; Callow, M. E.; Callow, J. A.; Hexemer, A.; Sohn, K. E.; Kramer, E. J.; Fischer, D. A. Comparison of the Fouling Release Properties of Hydrophobic Fluorinated and Hydrophilic PEGylated Block Copolymer Surfaces: Attachment Strength of the Diatom *Navicula* and the Green Alga *Ulva*. *Biomacromolecules* **2006**, *7* (5), 1449–1462. DOI: 10.1021/bm0509826.
- (55) Callow, M. E.; Callow, J. A.; Ista, L. K.; Coleman, S. E.; Nolasco, A. C.; López, G. P. Use of Self-Assembled Monolayers of Different Wettabilities to Study Surface Selection and Primary Adhesion Processes of Green Algal (*Enteromorpha*) Zoospores. *Applied and environmental microbiology* **2000**, *66* (8), 3249–3254. DOI: 10.1128/AEM.66.8.3249-3254.2000.
- (56) Rosenhahn, A.; Ederth, T.; Pettitt, M. E. Advanced Nanostructures for the Control of Biofouling: The FP6 EU Integrated Project AMBIO. *Biointerphases* **2008**, *3* (1), 1–5. DOI: 10.1116/1.2844718.
- (57) Petrone, L.; Di Fino, A.; Aldred, N.; Sukkaew, P.; Ederth, T.; Clare, A. S.; Liedberg, B. Effects of Surface Charge and Gibbs Surface Energy on the Settlement Behaviour of Barnacle Cyprids (*Balanus Amphitrite*). *Biofouling* **2011**, *27* (9), 1043–1055. DOI: 10.1080/08927014.2011.625474.

- (58) Wang, H. M.; Loganathan, D.; Linhardt, R. J. Determination of the pKa of Glucuronic Acid and the Carboxy Groups of Heparin by ^{13}C -Nuclear-Magnetic-Resonance Spectroscopy. *The Biochemical journal* **1991**, 278 (Pt 3), 689–695. DOI: 10.1042/bj2780689.
- (59) Guarino, V.; Caputo, T.; Altobelli, R.; Ambrosio, L. Degradation Properties and Metabolic Activity of Alginate and Chitosan Polyelectrolytes for Drug Delivery and Tissue Engineering Applications. *AIMS Materials Science* **2015**, 2 (4), 497–502. DOI: 10.3934/matensci.2015.4.497.
- (60) Maleschlijski, S.; Bauer, S.; Di Fino, A.; Sendra, G. H.; Clare, A. S.; Rosenhahn, A. Barnacle Cyprid Motility and Distribution in the Water Column as an Indicator of the Settlement-Inhibiting Potential of Nontoxic Antifouling Chemistries. *Biofouling* **2014**, 30 (9), 1055–1065. DOI: 10.1080/08927014.2014.966097.
- (61) Di Fino, A.; Petrone, L.; Aldred, N.; Ederth, T.; Liedberg, B.; Clare, A. S. Correlation between Surface Chemistry and Settlement Behaviour in Barnacle Cyprids (*Balanus Improvisus*). *Biofouling* **2014**, 30 (2), 143–152. DOI: 10.1080/08927014.2013.852541.
- (62) Gatley-Montross, C. M.; Finlay, J. A.; Aldred, N.; Cassady, H.; Destino, J. F.; Orihuela, B.; Hickner, M. A.; Clare, A. S.; Rittschof, D.; Holm, E. R.; Detty, M. R. Multivariate Analysis of Attachment of Biofouling Organisms in Response to Material Surface Characteristics. *Biointerphases* **2017**, 12 (5), 51003. DOI: 10.1116/1.5008988.
- (63) Aldred, N.; Ekblad, T.; Andersson, O.; Liedberg, B.; Clare, A. S. Real-Time Quantification of Microscale Bioadhesion Events *in situ* using Imaging Surface Plasmon Resonance (iSPR). *ACS applied materials & interfaces* **2011**, 3 (6), 2085–2091. DOI: 10.1021/am2003075.
- (64) Bauer, S.; Finlay, J. A.; Thomé, I.; Nolte, K.; Franco, S. C.; Ralston, E.; Swain, G. E.; Clare, A. S.; Rosenhahn, A. Attachment of Algal Cells to Zwitterionic Self-Assembled Monolayers Comprised of Different Anionic Compounds. *Langmuir* **2016**, 32 (22), 5663–5671. DOI: 10.1021/acs.langmuir.6b00839.
- (65) Ederth, T.; Nygren, P.; Pettitt, M. E.; Ostblom, M.; Du, C.-X.; Broo, K.; Callow, M. E.; Callow, J.; Liedberg, B. Anomalous Settlement Behavior of *Ulva Linza* Zoospores on Cationic Oligopeptide Surfaces. *Biofouling* **2008**, 24 (4), 303–312. DOI: 10.1080/08927010802192650.
- (66) Vater, S. M.; Finlay, J.; Callow, M. E.; Callow, J. A.; Ederth, T.; Liedberg, B.; Grunze, M.; Rosenhahn, A. Holographic Microscopy provides New Insights into the Settlement of Zoospores of the Green Alga *Ulva Linza* on Cationic Oligopeptide Surfaces. *Biofouling* **2015**, 31 (2), 229–239. DOI: 10.1080/08927014.2015.1022534.
- (67) Callow, J. A.; Callow, M. E. Trends in the Development of Environmentally Friendly Fouling-Resistant Marine Coatings. *Nature communications* **2011**, 2, 244. DOI: 10.1038/ncomms1251.
- (68) Aldred, N.; Scardino, A.; Cavaco, A.; Nys, R. de; Clare, A. S. Attachment Strength is a Key Factor in the Selection of Surfaces by Barnacle Cyprids (*Balanus Amphitrite*) during Settlement. *Biofouling* **2010**, 26 (3), 287–299. DOI: 10.1080/08927010903511626.
- (69) Berntsson, K. M.; Jonsson, P. R.; Lejhall, M.; Gatenholm, P. Analysis of Behavioural Rejection of Micro-Textured Surfaces and Implications for Recruitment by the Barnacle *Balanus Improvisus*. *Journal of Experimental Marine Biology and Ecology* **2000**, 251 (1), 59–83. DOI: 10.1016/S0022-0981(00)00210-0.
- (70) Scardino, A. J.; Guenther, J.; Nys, R. de. Attachment Point Theory revisited: The Fouling Response to a Microtextured Matrix. *Biofouling* **2008**, 24 (1), 45–53. DOI: 10.1080/08927010701784391.
- (71) Scardino, A. J.; Harvey, E.; Nys, R. de. Testing Attachment Point Theory: Diatom Attachment on Microtextured Polyimide Biomimics. *Biofouling* **2006**, 22 (1-2), 55–60. DOI: 10.1080/08927010500506094.

(72) Xiao, L.; Finlay, J. A.; Röhrig, M.; Mieszkina, S.; Worgull, M.; Hölscher, H.; Callow, J. A.; Callow, M. E.; Grunze, M.; Rosenhahn, A. Topographic Cues Guide the Attachment of Diatom Cells and Algal Zoospores. *Biofouling* **2018**, *34* (1), 86–97. DOI: 10.1080/08927014.2017.1408801.

(73) Koc, J.; Schönemann, E.; Wanka, R.; Aldred, N.; Clare, A. S.; Gardner, H.; Swain, G. W.; Hunsucker, K.; Laschewsky, A.; Rosenhahn, A. Effects of Crosslink Density in Zwitterionic Hydrogel Coatings on their Antifouling Performance and Susceptibility to Silt Uptake. *Biofouling* **2020**, *36* (6), 1–14. DOI: 10.1080/08927014.2020.1796983.

(74) Koschitzki, F.; Wanka, R.; Sobota, L.; Koc, J.; Gardner, H.; Hunsucker, K. Z.; Swain, G. W.; Rosenhahn, A. Amphiphilic Dicyclopentenyl/Carboxybetaine-Containing Copolymers for Marine Fouling-Release Applications. *ACS applied materials & interfaces* **2020**, *12* (30), 34148–34160. DOI: 10.1021/acsami.0c07599.

(75) Barbucci, R.; Consumi, M.; Magnani, A. Dependence of Water Uptake and Morphology of Hyaluronan- and Alginate-Based Hydrogels on pH and Degree of Crosslinking. *Macromol. Chem. Phys.* **2002**, *203* (10-11), 1292–1300. DOI: 10.1002/1521-3935(200207)203:10/11<1292:AID-MACP1292>3.0.CO;2-I.

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