Potential roles of fluorine-containing sol-gel coatings against adhesion to control microbial biofilm

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Abstract. Treatment of materials by sol-gel technique can be an excellent tool to convey new properties to surfaces, therefore the hybrid organic-inorganic materials show the properties of both phases, contributing for example to the obtainment of an anti-fouling coating. In this research, the explored procedure includes the co-condensation of silane coupling agents with epoxide and amine tail-groups, (*3*-Glycidyloxypropyl)-trimethoxysilane (GPTMS) and (*3*-Aminopropyl)-triethoxysilane (APTES), respectively, in combination with two perfluorosilane precursors, namely glycidyl-2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-hexadecafluorononylether and trimethoxy-(*3,3,3*-trifluoropropyl)-silane, either individually or together. This synthetic approach allows collecting stable hydrophobic, non-toxic, anti-fouling coatings that were investigated to study their morphology and chemical structure by different physical-chemical techniques. The anti-fouling properties were evaluated through test on treated glass slides in different microbial suspension in seawater-based medium per 24 h at room temperature. During tests, each suspension was maintained in continuous agitation to simulate the natural movement of seawater, and the attachment of cells on bare degreased glass slides is compared with that occurring on the treated slides. Results show that the fluorinated coatings have good antimicrobial activities and low adhesive properties, no biocidal effects towards the studied microorganisms were observed.

1. Introduction

The undesired colonization of submerged surfaces by marine micro- and macro-organisms such as bacteria, diatoms, algae, barnacles, and seaweeds is called biofouling [1]. It has detrimental effects among which on aquaculture systems and oceanographic sensors. Moreover, this natural phenomenon on shipping and leisure vessels causes severe problems for marine industries due to corrosion and hydrodynamic drag, which leads to elevated fuel consumption and higher maintenance costs [2-4]. In order to reduce both economic and environmental penalties, the primary strategy for inhibit marine fouling is to use biocide-containing paints [5-8]. At the same time, environmental business and legislation are driving science and technology towards non-biocidal solutions based only on physical-chemical and materials properties of coatings [9-14]. From this point of view, advances in

nanotechnology and recent knowledge of marine chemistry and biology are giving a significant contribution to the development of a new generation of bioinspired surface designs by the nature [15-17]. An approach to the development of novel coatings is to create a 'deterrent' surface that inhibits the initial stage of microorganism settlements. Currently, deepen research has been realized on newer anti-fouling (AF) technologies using fluoropolymers [18-19]. Fluoropolymers can be used to form anti adhesion surfaces with low critical surface energy, thanks to the presence of the exposed CF_2 and CF_3 moieties at the interface, which reduce the attachment of fouling (i.e. FR or fouling release coatings) depending also on the fluorine atoms limited mobility resulting around the backbone bonds [20-21]. Moreover, amphiphilic coatings, which incorporate some of the benefits of both hydrophobic and hydrophilic functionalities, have been developed in order to create an engineered surface with local variations in surface chemistry, topography and mechanical properties. Sol-gel technique for the treatment of materials can be an attractive tool to impart new and interesting properties to their surfaces, particularly if organic components are incorporated into the formulation.

2. Results and Discussion

2.1. *Sol-gel synthesis and characterization*

The newly synthesized sol–gel coating was made from silicon trialkoxide [R'Si(OR)$_n$] (**Figure 1**) undergoing simultaneously hydrolysis–condensation reactions in several steps. Associated condensation reactions are directly related to the removal of water and corresponding alcohol from the polymer matrix resulting in the formation of more rigid silica networks [7-8]. Four separate solutions, namely G/A, G/A_F3, G/A_F16, G/A_F3_F16, all containing GPTMS and APTES in 2/1 molar ratio, and F3 and/or F16 at a total 0.5 wt% were prepared.

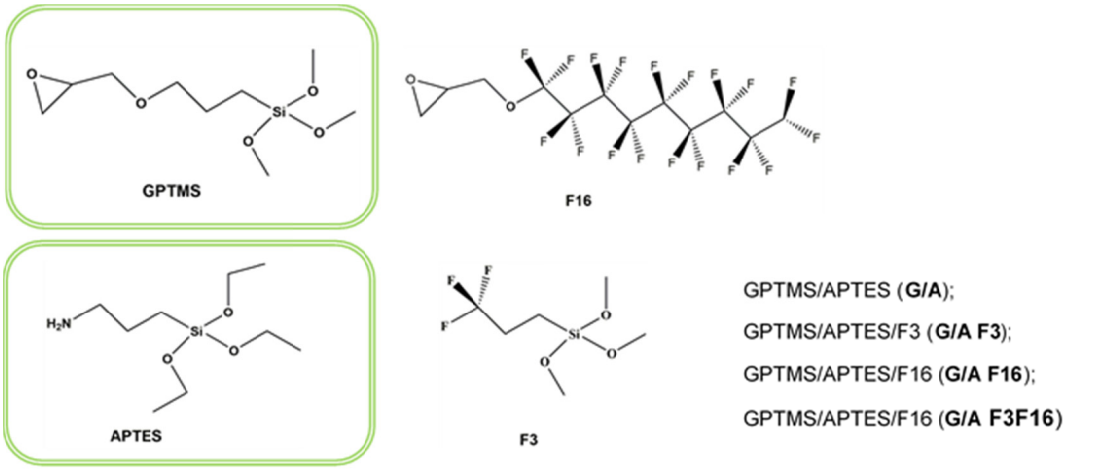

Figure 1. Chemical structures of the employed functional alkoxysilane reagents, together with the adopted acronyms, employed for the tested anti-fouling coatings.

The four G/A, G/A_F3, G/A_F16 and G/A_F3_F16 sols were fully characterized by FT-IR spectroscopy and Nuclear magnetic resonance (NMR). Coated glass was analyzed through FT-IR spectroscopy. ^{1}H and ^{13}C NMR provide a useful tool to detect the epoxy consumption and the hydrolysis reaction. The first step of the reactions, namely the opening of the oxirane ring of GPTMS, has been followed by ^{1}H and ^{13}C NMR spectra on freshly prepared samples from the bulk reaction mixtures that have been taken and dissolved in $CDCl_3$ [22-25]. The formation of the R'N(H)CH_2-fragment is associated to the progressive lowering of the signals due to the epoxy rings. The spectra also reveal a concomitant but partial hydrolysis of the methoxy and ethoxy groups of GPTMS and APTES which occurs at a lower rate [24]. Before application of coatings, the glass slides were cleaned

with a concentrate sulfuric acid/ potassium permanganate solution, then washed several times with ultrapure water and dried in an oven at 80°C for 24 hours.

2.2 Application on glass slides

In order to test the efficacy of the products and the lack of toxicity, plate tests have been set up by testing treated and non-controlled slides immersed in microbial suspensions. In particular, the toxicity was evaluated by comparing the number of cells/mL in the suspension both before and after the test, while the antifouling capacity comparing the number of microbial cells adhered on the control slides compared to those treated with the various products. Preparation of the slides was carried out starting from a thorough cleaning of the latter: the glass slides were cleaned with a concentrate sulfuric acid/potassium permanganate solution, then washed with ultrapure water and stored at 80°C for 24 hours. Dip coatings were performed with a NIMA Instruments dip coater at a 20 mm/min speed for a total of 20 immersion/withdrawal cycles for each. After each coating the glass slides have been thermally cured at 80°C for 10 minutes, then at 160 °C for 3 minutes. To explore the nature of the coatings and to confirm their successful deposition, ATR FT-IR spectra of silane xerogels applied and annealed on glass slides were registered and investigated. The nature of the hybrid structure of silica precursors (**G, A, F3, F16)** is strongly influenced by the epoxy ring opening that can succeed different reaction pathways: (a) hydrolysis with diol formation; (b) alcoholysis with ethyl ether terminal groups; (c) consecutive polymerization steps to give a hybrid poly(ethylene)oxide 3D-network; (d) reaction with primary amino group, thus transformed in secondary one. Actually, during the final thermal curing step, each silanol group can react with each other to form stable siloxane bonds (Si-O-Si). At the same time, glycidoxy groups can react both with themselves and with hydroxyl groups of hydrolyzed precursors. Therefore, the presence of Si-O-C bonds can increase the flexibility of the structure, favoring a great homogeneity between the organic and inorganic components of the 3D network [26-28]. These kind of coatings have the advantages of being environmentally friendly, non-toxic and can be obtained by mild temperature and pressure conditions.

2.3 Evaluation of anti-fouling properties

Results obtained by testing different sets of glasses in contact to microbial strains were shown in table 1 and Figures 2 and 3. As can be deduced from the data collected, all the FR formulations tested resulted in a decrease of the adhered cells compared to the control (only slide), in particular the G/A_F3 formulation showed the best fouling-release capacity against the Gram negative strain (Table 1). The coating containing a single G/A matrix reduced the number of adhered cells on slide to 76.5% compared to the control. The G/A_F3 and G/A_F16 formulations significantly reduced cell adhesion to 29.5% and 53%, respectively. The formulation G/A_F3_F16 albeits to a lesser extent than the other formulated, showing fouling release activity, with a decrease of adherent cells equal to 71% compared to the control, but almost similar to the behaviour of the G/A matrix. Thus, the G/A_F3 formulation showed the best fouling-release ability against the Gram negative strain.

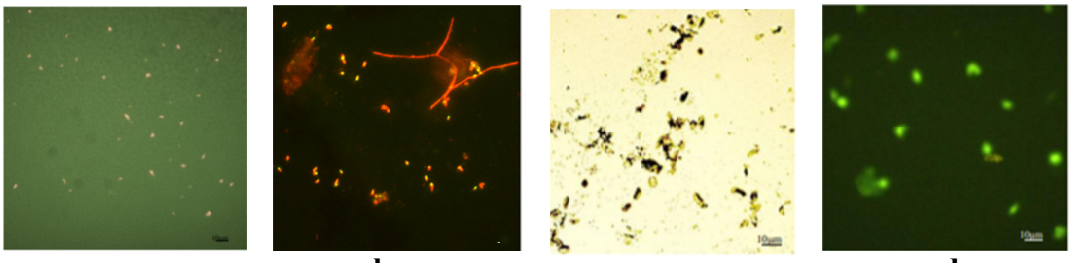

Figure 2. Images of the glass slide treated with **G/A_F3** showing diffuse cells of the Gram- strain *S. maltophilia BC 658* observed under EM 40x (**a**). Glass slide treated with **G/A_F16** observed under EM with the Gram+ strain *B. aquimaris BC 660.* Note the formation of spores and chain of cells 40x (**b**). Images of the treated slides

with **G/A_F3** in contact with the cells of *Navicula sp.* obtained with the LM **(c)** and EM microscopes **(d)** in which is evident cells sufference. Magnification 20X.

Table 1. Percentage of the adhered cells onto the untreated and treated glass slides after incubation with the strains *S. maltophilia (BC 658)* and *B. aquimaris (BC 660)*, and *Navicula sp.*

	S. maltophilia (BC 658)	*B. aquimaris* (BC 660)	*Navicula* sp.
Samples	% of adhering cells*		
CTRL	100	100	100
G/A	76.5	4.3	149
G/A F3	29.5	0	10.5
G/A F16	53	2.7	25.5
G/A F3F16	71	0	181

*As determined by direct counts under LM and/or EM microscopy. Values above 100% mean a higher number of cells adhering to the treated slides than to the glass slide controls.

Regarding the toxicity, none of the products seems to show a detrimental effect on the growth of bacteria and diatoms as clearly shown in Figure 3.

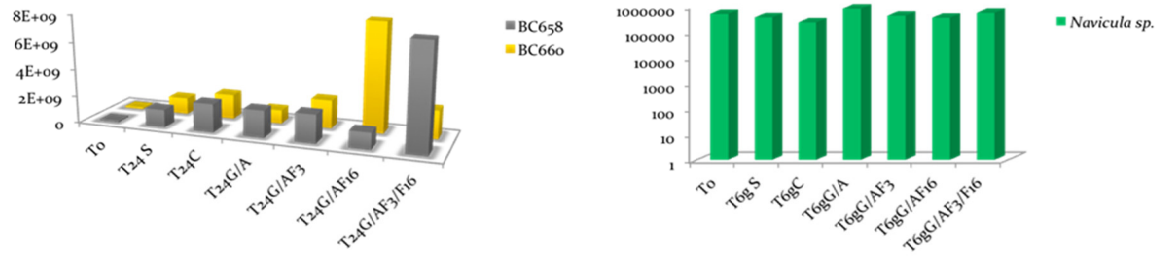

Figure 3. Number of viable cells of the strains *S. maltophilia (BC 658)* and *B. aquimaris (BC 660)* (**left**) and *Navicula sp.* (**right**), before and after incubation with the treated and untreated glass slides.

3. Conclusions

The aim of the present work was to investigate the most significant factors to obtain efficient and stable hybrid sol-gel GPTMS-based FR coatings [22-23]. The FR effects of the silica containing coatings depend on the possibility to realize chemical interactions between the hydroxyl group and/or epoxy group of the precursor with functional groups belonging to the polymers [24-25]. Room temperature reaction of the GPTMS with the bifunctional APTES leads to the formation of a hybrid silica-epoxy polymers. In whose network the amine acts as a network former while the epoxy compounds mainly acts as a network modifier leading to a different development of the polymeric backbone. The structural differences reflect the thermal properties and hydrophobic properties of material particularly influenced by the addition of perfluorinated species. The nature of the surface of the materials obtained from GPTMS/APTES. 2:1 following the addition of the long-chained F16 and F3, changes to more hydrophobic state, whereas GPTMS/APTES 5:1 exhibits an opposite trend. The different hydrorepellent activity is explained with a different assembly and alignment of the fluorinated chains at the polymer-air interphase. These formulations have been properly designed for fouling release purposes. These sol-gels (G/A, G/A_F3, G/A_F16, G/A_F3_F16) were applied on slides using a dip coater and anti-fouling and toxicity tests were performed by using pure microbial cultures. Cell adhesion experiments using microorganisms isolated from marine environment such as *Bacillus aquimaris*, *Stenotrophomonas maltophilia,* and *Navicula* indicate the considerable anti-fouling capacity against both Gram-positive and Gram-negative bacteria as well as against diatoms

[29]. These properties were tested on treated glass slides in different microbial suspension in seawater-based medium for 24 h at room temperature. The fluorinated coatings have good antimicrobial activities and low adhesive properties towards the studied bacteria, furthermore the eventual biocide effect due to the product release in the liquid medium evaluated by counting the microbial cells before and after the period of incubation showed no biocidal effects [30].

4. Experimental Details

(3-aminopropyl)-triethoxysilane (APTES, labelled **A**), (3-glycidyloxypropyl)-methyldiethoxysilane (GPTMS, **G**), 3,3,3-trifluoropropyl-trimethoxysilane (**F3**), glycidyl-2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-hexadeca fluorononyl ether (**F16**) (**Figure 1**), absolute Ethanol (HPLC grade) were purchased from Sigma Aldrich and used without any other purification. In a typical procedure of G/A_F3, 2.013 g of GPTMS were mixed with 0.943g of APTES in 37.48 g of ethanol under stirring. Then 0.2 g of F3 was added to the clear ethanol solution and left at room temperature under stirring for 24 h. The other functional alcoxysilane sol gels, G/A, G/A_F16, G/A_F3_F16, were synthetized in the same way.
FT-IR spectra were acquired by means of a Thermo Avatar 370, equipped with Attenuated Total Reflection (ATR) accessory. A diamond crystal was used as internal reflectance element on the ATR accessory. Spectra were recorded, at rt, in the range from 4000 to 650 cm^{-1}, with 32 scans and a resolution of 4 cm^{-1}. ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were performed in methanol-d_4 at 298.2 (±0.1) K with a Varian 500 spectrometer, equipped with a 5 mm OneNMR (TM) probe operating at 500.1 and 125.7 MHz.

4.1. Evaluation of anti-fouling properties

Different sets of glasses were placed in separate sterile glass Petri dishes (∅ 120 mm) and sterilized overnight under UV. Twenty ml of each microbial suspension were then added and Petri dishes, previously coated with G/A, G/A_F3, G/A_F16 and G/A_F3_F16 sols, were placed in a horizontal shaker and incubated for 24h at room temperature set at 25°C for bacteria and for 6 days in the light at 30°C for the diatoms. The evaluation of the anti-fouling properties was carried out with a Gram negative strain *Stenotrophomonas maltophilia* BC658, a Gram positive spore forming strain *Bacillus aquimaris* BC 660, a diatom strain *Navicula* sp, in sea-based culture media; all isolated from marine habitat. Antifouling properties of the coatings was determined comparing cells adhesion to the untreated and treated glasses observed under Light microscopy, (LM) and Epifluorescence microscopy (EM) a. The toxicity of the coatings was evaluated by the measurement of the density and/or by counting the cells before and after the incubations in the liquid medium.
After incubation time, glasses were rinsed with sterile PBS and prepared for three different treatments: one set was air dried by placing the glass directly to the Bunsen flame for 30 seconds, observation was carried out in LM or under epifluorescence microscope (EM) after staining with Acridine Orange (AO).
The second set was prepared for epifluorescent microscopy and fixed in a solution of a fixation buffer (4% paraformaldehyde in PBS, pH 7.2) with PBS (3:1 molar ratio) and incubated for 90 min at 4 °C. Bacterial cells were dyed with Acridine Orange (AO)(0.1mg/ml) diluted in sterile distilled water 1:2 (v/v) for 3-4 min, [11] and the last set of glasses was prepared for SEM microscopy by fixing in glutaraldehyde 2,5% in Phosphate buffer (PB) 0.1M pH 7.4 per 6h at 4°C, and then air dried.

Acknowledgments

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