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Novel antifouling coatings by zwitterionic silica grafting on glass substrates

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Keywords

Zwitterionic coatings, hybrid silica, anti-fouling, sol-gel, biocompatibility

Abstract

Zwitterionic silica coatings for surface functionalization are greatly prominent because of their simple and fast preparation, high availability and effective antifouling properties. In this work, two zwitterionic sulfobetaine silane coatings, *i.e.*, *mono*-SBSi and *tris*-SBSi, were deposited on glass surfaces and tested for antifouling of biological material and biofilm by using human cancer cell and seawater, respectively. The used zwitterionic precursors *mono*-SBSi and *tris*-SBSi differ by the number of hydrolysable silane groups: whereas *mono*-SBSi contains one trimethoxysilane group, *tris*-SBSi contains three of these functions. First, X-ray photoelectron spectroscopy indicates the successful grafting of zwitterionic coatings on a glass surface. Characterization using atomic force microscopy shows the different morphology and roughness of the two coatings. The glass surface became more hydrophilic after the grafting of zwitterionic coatings compared with bare glass substrate. The antifouling properties of two coatings were evaluated *via* human cancer cell adsorption. Interestingly, the *tris*-SBSi coating displays a significantly lower level of cell adsorption compared to both *mono*-SBSi coating and the not modified control surface. The same trend was observed for biofilm formation in seawater. Finally, the toxicity of *mono*-SBSi and *tris*-SBSi coatings was evaluated on zebrafish embryos, indicating good biocompatibility of both coatings. Our results indicate interesting antifouling properties of zwitterionic coatings. The chemical constitution of the used precursor has an impact on the anti-fouling properties formed coating: the *tris*-SBSi based zwitterionic silica coatings displays improved anti-fouling properties compared to the *mono*-SBSi based coating. Besides, the use of trisilylated precursors should result in the formation of more resistant and robust coatings due to the higher number of grafting functions. For all these reasons, we anticipate that *tris*-SBSi coatings will open new perspectives for anti-fouling applications for biological environments and implants.

1. Introduction

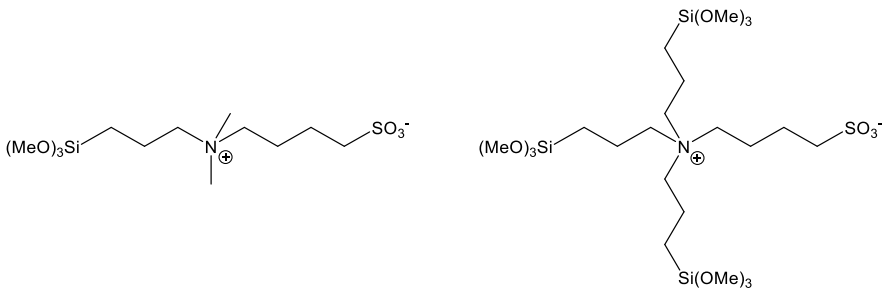
Biofouling is an important issue in numerous fields such as biosensing, membrane sciences, marine industries, biomedical implants, and other medical devices. Biofouling designates the irreversible adhesion of biofilm on surfaces.[1-3] As examples for its high economic impact, biofilm formation on medical devices may cause the rejection of the implanted biomaterial and adverse health effects.[4] Biofilm on marine vessels may increase the hydrodynamic drag forces and reduce the sailing speed, resulting in increased fuel consumption.[5] Therefore, it is strongly required to endow a material's surface with antifouling properties.[6-8] Following the worldwide prohibition of tributyltin based coatings in 2003, efficient strategies that limit bio-adhesion and fouling are still a challenge.

In this context, the surface functionalization with antifouling polymer coatings is a widely used method to inhibit fouling and reduce biofilm formation. In addition to the traditional grafting of uncharged hydrophilic polymers such as poly (ethylene glycol) (PEG) or poly (hydroxyethyl methacrylate) (PHEMA), zwitterionic polymers have recently emerged as an alternative and promising antifouling material.[9-11] Zwitterionic polymers have an equal number of homogeneously distributed anionic and cationic groups, which can form a tightly bound hydration layer through electrostatic interactions and ionic solvation between the water molecules and zwitterionic coating, resulting in significantly reduced protein and/or biomolecule adsorption. Zwitterionic polymer coatings generated from typical zwitterionic monomers such as sulfobetaine methacrylate (SBMA) and carboxybetaine methacrylate (CBMA) were grafted onto the surfaces by a variety of methods.[12, 13] Jiang et al. reported a typical method of grafting zwitterionic polymers brushes *via* atom transfer radical polymerization (ATRP).[14-16] They have successfully demonstrated the excellent antifouling properties and long-term stability of the zwitterionic polymers modified surfaces. In addition, the mussel-inspired approaches were widely used.[17, 18] Zwitterionic polymers were effectively immobilized onto the surfaces by either a "grafting to" or "grafting from" method with the help of mussel-inspired dopamine chemistry.[19, 20] These zwitterionic polymer coatings have shown good antifouling performance and resistance to protein, bacterial and cell adhesion.

Surface chemical functionalization can be achieved either using polymer deposition directly on the substrate's surface or by the grafting of low-molecular-weight molecules bearing a chemical functional group that can react with the material surfaces to form an organolayer by formation of covalent bonds.[21] Here, the formation of self-assembled monolayers (SAMs) of zwitterionic molecules attracted considerable attention.[22, 23] Ordered structures and high packing density of

zwitterionic functional organolayer coatings results in good antifouling properties as indicated by low protein and bacterial adsorption. Huang et al. reported a facile approach for the development of zwitterionic mixed SAMs on gold surface comprising carboxybetaine-thiols (CB-thiols) and sulfobetaine-thiols (SB-thiols).[24] Compared to the CB-thiol SAM, the SB-thiol SAM was packed more densely and exhibited pH-insusceptible antifouling properties as shown by protein and bacterial adsorption tests. The antifouling property of the mixed SAM was successfully implemented for sensitive molecular detection. Gu et al. demonstrated a zwitterionic cysteine SAMs coating on gold surface, which provided significant capabilities of reducing the adsorption of bovine serum albumin, fibrinogen and human blood.[25]

Besides thiol-gold reaction, zwitterionic silica molecules were developed for the surface modification of silica surfaces, metal oxides, and oxidized silicone. It was reported that zwitterionic sulfobetaine silane coatings display excellent performance for fouling repellence, antifogging properties, oil-water separation, and colloidal stability.[26-29] Molino et al. presented hydrophilic low-fouling surface coatings by zwitterionic sulfobetaine functionalized SiO_2 nanoparticles with excellent fouling-resistant properties against protein, bacterial and fungal spore adhesion.[30, 31] Tsao et al. reported smart surfaces with switchable wettability for both aqueous and non-aqueous drops based on zwitterionic sulfobetaine silane.[32] The previously reported zwitterionic silica coatings form a zwitterionic layer on the surface that reduces the adhesion of biomolecules and the formation of biofilms.[33, 34]



Scheme 1 Structures of the zwitterionic precursors *mono*-SBSi (*left*) and *tris*-SBSi (*right*)

In this work, we report a facile and efficient strategy to enhance the antifouling properties of glass surfaces *via* the generation of coating with anti-adherent zwitterionic layers. The coatings were generated using surface sol-gel reactions on glass substrates using two zwitterionic sulfobetaine silane precursors: *mono*-SBSi and *tris*-SBSi (scheme 1). The two zwitterionic sulfobetaine compounds differ by the number of hydrolysable alkoxysilyl groups: whereas *mono*-SBSi contains one trimethoxysilyl group, *tris*-SBSi contains three of these grafting units

and therefore offers nine hydrolysable alkoxysilane anchoring groups. Both compounds were used for the formation of zwitterionic surface coatings on glass substrates *via* surface sol-gel reactions. The resulting systems were characterized and the anti-adhesion properties were investigated against the adhesion of human breast cancer cells and the formation of biofilm and microorganisms from seawater. Finally, we also monitored the biocompatibility of these modified surfaces against zebrafish embryos.

2. Experimental section

2.1 Materials

N-ethyl diisopropylamine, (3-aminopropyl)trimethoxysilane, (3-chloropropyl)trimethoxysilane and (N,N-dimethylaminopropyl)trimethoxysilane were purchased from ABCR GmbH. 1,4-butane sultone was obtained from Sigma-Aldrich. Anhydrous ethanol and acetone were purchased from VWR PROLABO.

2.2 Synthesis of tris(3-(trimethoxysilyl)propyl)amine (TrisN)

The synthesis of tris(3-(trimethoxysilyl)propyl)amine (TrisN) was realized by mixing (3-aminopropyl)trimethoxysilane (84.5 mL, 0.36 mol, 1 equiv.) with (3-chloropropyl)trimethoxysilane (232 mL, 1.28 mol, 3.5 equiv.) in the presence of N-ethyldiisopropylamine (248 mL, 1.46 mol, 4 equiv.). Argon was circulated in the system and the transparent homogenous solution was stirred magnetically (600 rpm, RT) during five minutes prior heating the solution to 160 °C during 30 h. Then, the reaction mixture was cooled to room temperature and then to 0°C by the use of an ice bath. Solid white crystals of N-ethyldiisopropyl ammonium chloride precipitated that were eliminated by successive recrystallizations and filtrations. The resulting solution was purified by medium vacuum distillation ($< 1 \times 10^{-3}$ mbar) starting from room temperature until 200 °C where no more unreacted departing reagents were recovered as distillates. Then the obtained liquid was distilled at 250 °C as the final liquid was the desired compound. TrisN was obtained in a yield of 61 % with the density of 1.0904 g/cm$_3$.^{1}H NMR: ($CDCl_3$, 400MHz) δ (ppm): 3.34 (s, 27H); 2.18 (m, 6H); 1.30 (m, 6H); 0.37 (m, 6H).

2.3 Synthesis of 3-(mono(3-(trimethoxysilyl)propyl)ammonio)butane-1-sulfonate (*mono*-SBSi)[35]

24 mmol of (N,N-dimethylaminopropyl)trimethoxysilane and 25 mmol of 1,4-butane sultone were dissolved in 25 mL of anhydrous acetone and stirred for 12 h under nitrogen protection at

40 °C. A white powder was produced. The solution was filtered with a G3 glass gooch filter. The white *mono*-SBSi was washed with acetone and finally dried under reduced pressure. Yield: 69 %. ^{1}H NMR (400 MHz, D_2O): δ 0.69 (t, 2H), 1.75 (m, 2H), 1.85 (m, 2H), 2.0 (m, 2H), 3.11 (t, 2H), 3.19 (s, 6H), 3.3 (m, 2H), 3.39 (s, 9H), 3.4 (m, 2H).

2.4 Synthesis of 3-(Tris(3-(trimethoxysilyl)propyl)ammonio)butane-1-sulfonate (*tris*-SBSi)[36]

4 mmol of tris(3-(triethoxysilyl)propyl)amine (TrisN) and 6mmol of 1,4-butane sultone were mixed and stirred for 72h under argon at 140°C. After cooling to room temperature, the reaction mixture was washed with diethyl ether in order to remove excess of sultone. After drying under vacuum, the title compound was obtained as highly viscous oil. Yield: 90%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.69 (m, 6H), 1.71 (m, 6H), 1.78 (m, 2H), 1.86 (m, 2H), 2.81 (m, 2H), 3.11 (m, 6H), 3.33 (m, 2H), 3.58 (m, 27H).

2.5 Preparation of zwitterionic organosilicon coatings

The glass substrates were placed sequentially in a sonication bath of acetone, ethanol and water for 10 min, followed by drying in a stream of nitrogen. The glasses were cleaned by a standard plasma system (PicoPCCE, Diener electronics) to expose O_2 plasma with a power of 100 W for 5 min to remove trace amounts of contaminants and to obtain a hydroxyl-terminated surface. The clean glasses were immersed in 5 mM *mono*-SBSi or *tris*-SBSi anhydrous ethanol solution. The coating solutions were kept for 12 h at room temperature. The modified glasses were removed and cleaned in a sonication bath of ethanol, followed by drying in a stream of nitrogen. The glass substrates were baked in an oven at 80 °C for 1 h.

2.6 Surface characterizations

Chemical elemental spectra of coatings were carried out with X-ray photoelectron spectroscopy (XPS) system (Thermofischer Scientific ESCALAB 250 Xi) using focused monochromatized Al Kα radiation (1486.6 eV). The analyzed samples surface area was500µm diameter disk. The takeoff angle was set at 90°. The charge is compensated by a low energy electron beam (-2 eV). The energetic resolution was 0.2 eV. The spectra were analyzed using CasaXPS software. The surface morphology and roughness of coatings was explored by using an atomic force microscope (AFM). AFM images were acquired with a Bruker Nanoman (Bruker SAS, Palaiseau, France) driven by Nanoscope 3A Quadrex electronics in tapping mode under ambient conditions. Images were processed using Gwyddion software. Contact angle measurements for *mono*-SBSi and *tris*-SBSi coatings were estimated by using a contact angle goniometer (Digidrop Goniometer, GBX, France). At least five about 0.5 µL deionized water

droplets were used. Contact angle values were averaged from five measurements at random positions.

2.7 Analysis of human cells adhesion on zwitterionic silica coatings

Human breast cancer cells (MCF-7) were purchased from ATCC and maintained in culture 75 cm^2 tissue-culture flasks (T75) at 37 °C in a humidified atmosphere with 5 % CO_2. They grew in DMEM F12 supplemented with antibiotics (100 UmL^{-1} penicillin, 100 $\mu g\ mL^{-1}$ streptomycin) and 10% fetal calf serum. Before the experiment, all glasses coated or not with zwitterionic silica were incubated in EtOH for 10 min and then washed trice with Phosphate Buffered Saline (PBS). Then, glasses were placed at the bottom of the wells of 6-well plates and culture medium for cell growth was added (2 mL/well). Cells were then seeded at a density of 10^6 cells/cm^2. For 3 days, cell growth was analyzed by the amount of confluence on each glass. Pictures were taken for 3 days using a microscope (Axiovert 40 CFL, Zeiss) and all images were performed with a 10X magnification. Pictures analysis with ImageJ software allowed us to compute quantitative data.

2.8 Analysis of micro-organisms of sea water adhesion on zwitterionic silica coatings

Glasses coated or not with zwitterionic silica were placed at the bottom of the wells of 6-well plates and 5 mL of sea water (from Palavas-les-Flots, France) were added in each well. Plates were maintained at room temperature in a humidified atmosphere. For 8 days, the coating level was observed and analyzed for each glass. Pictures were taken at 8 days using a microscope (Axiovert 40 CFL, Zeiss) and all images were performed with a 10X magnification. Pictures analysis with ImageJ software allowed us to compute quantitative data.

2.9 Toxicological study on zebrafish embryos

Wild-type AB zebrafish embryos were purchased from Zebrafish International Resource Center. Embryos were raised to adulthood in circulating aquarium system in controlled room at 28 °C, 80 % humidity, 14 h light/10 h dark cycle, at lab's facilities of Molecular mechanisms in neurodegenerative dementia, Inserm U1198, Montpellier University, Montpellier. The night before the start of the experiment, male and female zebrafish were placed in a breeding tank and were spent the night together. The following morning, zebrafish were mated and the fertilized embryos were collected and maintained at 28 °C. At 5 hours post fertilization (hpf), embryos were examined under the microscope, and only embryos that developed normally were selected for the study. Glasses coated with zwitterionic silica (*mono*-SBSi, *tris*-SBSi) were placed in 6-well plate. Glasses with no coating was considered as a control. One well of the 6-

well plates with no glass inside was considered as another negative control. Thirty embryos were placed in each well in 6 mL of water, the development of embryos including chorionated, hatching, mortality and malformation were recorded at different time intervals (24, 48, 52, 57 and 72 hpf) using Carl Zeiss stereomicroscope Stemi 508.[37, 38] Experiments with zebrafish embryos until 96 hpf are considered as *in vitro* studies according to the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

3. Results and discussions

First, glass substrate was treated with the two zwitterionic compounds *mono*-SBSi and *tris*-SBSi. Prior to the grafting reaction, the glass substrates were cleaned and activated *via* plasma treatment. The surface sol-gel reaction was then performed in an ethanolic solution containing either *mono*-SBSi or *tris*-SBSi at room temperature. The functionalized glass substrate was washed with ethanol and finally dried at 80 °C.

To examine the formation of two zwitterionic silane coatings, the surface of the pristine and functionalized glass surfaces were analyzed via X-ray photoelectron spectroscopy (XPS). Survey spectra of bare surface, *mono*-SBSi and *tris*-SBSi coated surfaces are shown in **Figure 1A**. Both *mono*-SBSi and *tris*-SBSi coatings surfaces show distinct N 1s (401.0 eV) and S 2p (167.0 eV). The existence of N 1s and S 2p confirms the success of the grafting. The N/S ratios (nearly 1) are in good agreement with the chemical structures of the two molecules.[39] XPS signatures originating from N and S atoms within both coatings were measurable in the high-resolution spectra. N 1s and S 2p core levels are shown in **Figure 1B**. N peak components with a binding energy at 402.5 eV appear in the XPS N 1s core-level spectra. The peak components are associated with the quaternary ammonium cations $-N^{+}(CH_2)_3$. In addition, a spin-orbit split doublet is observed in the S 2p core-level spectra. The S 2p doublets at the binding energy of 167.0 and 168.2 eV are attributed to sulfonate species in the zwitterionic moiety $-SO_3^{-}$.[27, 40] Neither N nor S is detected found in the sample of bare glass surface. As a result, the XPS spectra clearly demonstrate the formation of the sulfobetaine organosilicon layers on glass surfaces.

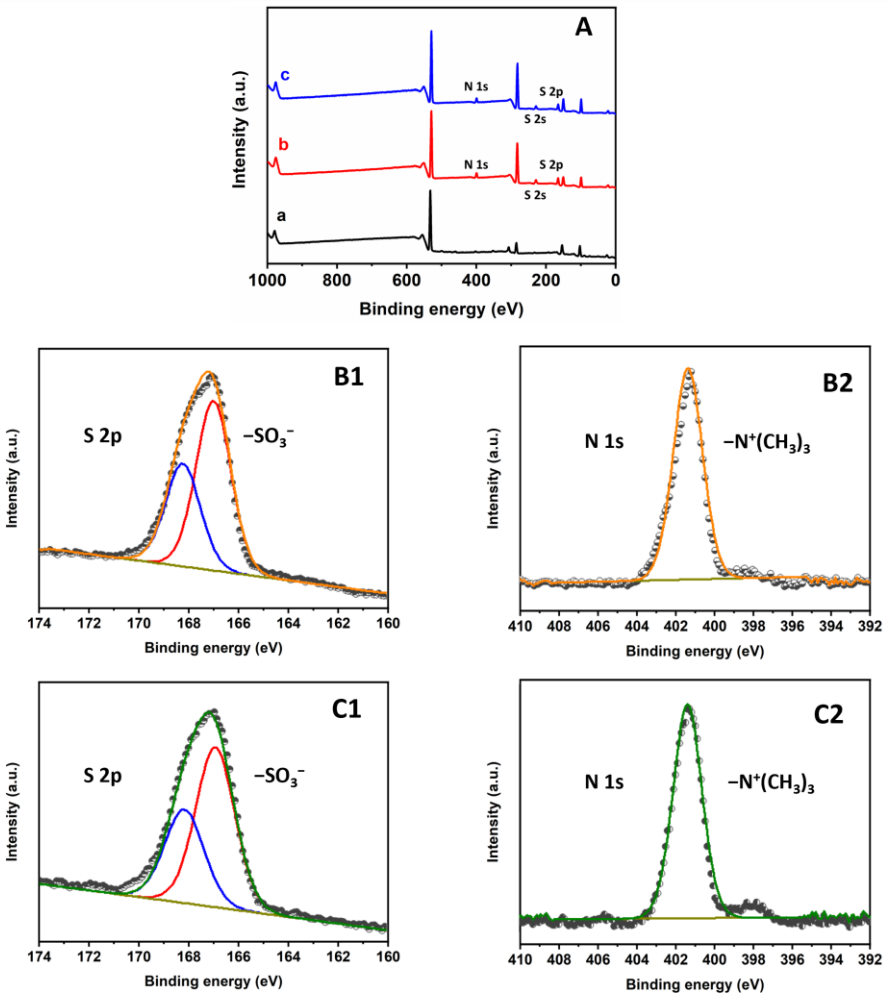


Figure 1. XPS survey scans of bare glass (a), mono-SBSi (b) and *tris*-SBSi (c) coated glasses surface (A); high-resolution XPS spectra for S 2p (B1) and N 1s (B1) of mono-SBSi coated glass surfaces and S 2p (C1) and N 1s (C1) of *tris*-SBSi coated glass surfaces.

The surface morphology and roughness values of coatings were measured by atomic force microscope (AFM). The AFM images of unmodified, *mono*-SBSi and *tris*-SBSi coated glasses are shown in **Figure 2**. The surface features of bare and *mono*-SBSi coated surfaces are generally flat and smooth with the comparable roughness values of 0.51 and 0.43 nm, respectively (**Figure 2a, b**). Our results show a smooth coating by the deposition of *mono*-SBSi, which is in good agreement with the smooth zwitterionic silane coatings reported before.[41, 42] The surface feature of *tris*-SBSi coating is shown in **Figure 2c**. Compared with the *mono*-SBSi coating, the *tris*-SBSi coating exhibits a significantly rougher surface morphology with a roughness value of 6.53 nm. It seems that the morphologies of the coating are associated with the different numbers of hydrolysable groups of two zwitterionic sulfobetaine silane precursors. Whereas the *mono*-SBSi precursor results in the formation of a monolayer coating, the coating with the *tris*-SBSi-precursor presents a much rougher and porous spatial structure

on the surface, which may be the result of the surface hydrolysis-polycondensation reaction of the multifunctional *tris*-SBSi molecules, resulting in the formation of a thicker zwitterionic silica hybrid layer.

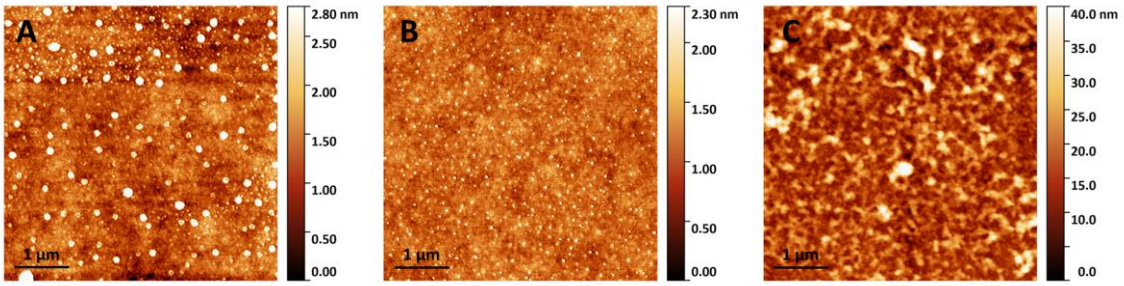


Figure 2. AFM images for a bare glass (a), mono-SBSi (b) and *tris*-SBSi (c) coatings surfaces.

Then, the wettability and hydrophilicity of *mono*-SBSi and *tris*-SBSi coatings was determined via water contact angle (CA) measurement, as shown in **Figure 3**. The results showed a CA of about 33° for the bare glass substrate. A very low CA of less than 5° indicated increased wettability of the *mono*-SBSi coated glass surface. The surface became more hydrophilic because of the introduction of highly hydrophilic zwitterionic moieties, in agreement with previous works with related zwitterionic materials.[43] On the other side, the *tris*-SBSi coated glass showed a CA of 19°. This value indicates an increased hydrophilicity compared with the bare glass surface, but a lower hydrophilicity compared to the *mono*-SBSi coating. This result may be attributed to the surface polymerization of the *tris*-SBSi precursor and the formation of a silica hybrid layer as already stated above.

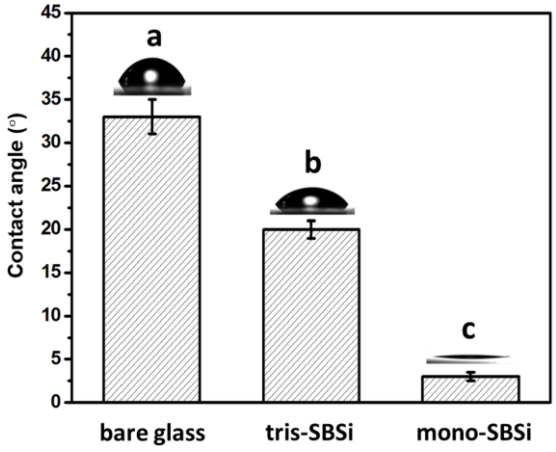


Figure 3 Contact angles of bare glass (a), *mono*-SBSi (b) and *tris*-SBSi (c) coated glass surfaces.

Our results indicate the successful grafting of the mono- and trisilylated precursors *mono*-SBSi and *tris*-SBSi on the surface of glass substrates. The chemical constitution of the zwitterionic precursor has a large impact on the morphologies of the formed coatings. Whereas the use of the monosilylated *mono*-SBSi leads to the formation of a monolayer that homogeneously covers the surface of the glass substrate, the use of the trisilylated *tris*-SBSi yields a thicker layer of an amorphous zwitterionic silica hybrid material. This difference also has consequences on the interfacial properties of the surface of the materials, *i.e.*, their hydrophilicity. The more organized monolayer of *mono*-SBSi graft results in a high hydrophilicity whereas the formation of a thicker and disordered sol-gel coating from *tris*-SBSi monomers gives surfaces with relatively lower hydrophilicity. Compared with bare glass surfaces, the grafting of zwitterionic precursors results in increased hydrophilicity, independent of the used precursor.

After having ascertained the successful grafting of the zwitterionic compounds, we analyzed the ability of adhesion of human cells in culture and also of micro-organisms from sea water, in order to determine the antifouling potential of these materials. In the first trial, we investigated the adhesion of MCF-7 human breast cancer cells. To the best of our knowledge, our study is the first one that investigates the adhesion of cancer cells on zwitterionic sulfobetaine silane coatings. MCF-7 cells were seeded in 6-well plates in which glasses, coated or not, were previously added at the bottom of the well to force cells to grow on it. **Figure 4a** shows the representative areas of the different conditions during 3 days of culture. It can be seen that the *mono*-SBSi surface coating significantly reduces cell adhesion. Very interestingly, no cell adhesion could be detected on the surface grafted *tris*-SBSi coating (green arrow). This demonstrates that the *tris*-SBSi coating totally inhibits the cell adhesion on the glass substrates. The cell fouling levels were quantitatively analyzed with the ImageJ software, and the results are shown in **Figure 4b.** The control glass shows increasing coverage by MCF-7 cells with increasing number of incubation days (red bars). In contrast, both zwitterionic functionalized glass substrates show significantly lower MCF-7 cell adhesion, whereupon the *tris*-SBSi coated surface shows considerably higher cell repellence than the one coated with the *mono*-SBSi precursor. In the case of the surface treated with *tris*-SBSi, less than 1 % of the coated surface is covered by MCF-7 cells after three days. This result can mainly be attributed to the roughness and morphology of the *tris*-SBSi coating that affects the surface functionality and results in strongly reduced cell adhesion.

The *mono*-SBSi and *tris*-SBSi resulted in reduced MCF-7 cell adhesion. According to previous reports, zwitterionic coatings can effectively repel the adhesion of proteins, bacteria, fungal

spores and algae.[25, 26, 30] This is mainly due to the fact that zwitterionic functionalization can lead to electrostatically induced hydration that enhances water binding and provides a hydrophilic surface coating.[44] This is the first time that a zwitterionic self-assembled monolayer coating has been found to be resistant to the adhesion of human cells. The positive and negative charges on the surface of *mono*-SBSi monolayer coating significantly improve the surface hydrophilicity due to its strong hydration ability through electrostatic interactions.[45] Notably, the *tris*-SBSi coating exhibited higher resistance to MCF-7 cell adhesion, although the tris-SBSi coating was less hydrophilic than the *mono*-SBSi coating, possibly due to the exposure of unhydrolyzed ethoxysilane.[46] The roughness of coatings is known to affect the adhesion strength of microorganisms, and certain coatings that mimic microscale and nanoscale topographic features have been shown to limit interactions and reduce bonding strength.[31, 47] The active *tris*-SBSi undergoes a hydrolysis-condensation polymerization reaction on the surface, forming a rougher polymer coating, which may lead to better antifouling properties.[48-50]

Various functionalization methods can generate zwitterionic coatings with diverse thicknesses. Zwitterionic SAMs, for instance, can have thicknesses as minimal as 1 nm.[23, 25] ATRP can yield thicker coatings, reaching tens of nanometers.[44, 51-53] Zwitterionic coatings constructed through layer-by-layer assembly can achieve thicknesses exceeding 100 nm.[54, 55] Initiated chemical vapor deposition can produce zwitterionic antifouling coatings with thicknesses extending into hundreds of nanometers.[56, 57] The antifouling performance of zwitterionic coatings is closely related to the coating thickness.[58] While zwitterionic SAMs demonstrate robust resistance to protein adsorption, their sustained prevention of attachment to mammalian cells encounters challenges. This may stem from inadequate film stability and insufficient hydration layer thickness to resist adsorption in complex media. Conversely, surface-grafted zwitterionic polymers, characterized by higher packing density and steric repulsion, exhibit exceptional anti-adsorption properties.[14, 59] In this study, the *tris*-SBSi coating exhibited superior resistance to cell adhesion compared to the *mono*-SBSi coating, which may also be attributed to the coating thickness. The *mono*-SBSi is expected to form a covalent monolayer through the hydrolysis and condensation of ethoxysilane group on the substrate, as already described in the literature.[31, 60] On the other hand, *tris*-SBSi incorporates three trimethoxysilyl graft units, providing nine hydrolyzable alkoxysilane anchoring groups. Consequently, the high chemical activity of *tris*-SBSi induces sol-gel polymerization on the substrate surface, resulting in a thicker polymer surface compared to the *mono*-SBSi coating. Furthermore, the deposition of organosilane

coatings generally correlates with deposition time, temperature and water content, [61, 62] but also with the concentration of the precursor. Profilometry measurements indicated a relatively rough surface with a thickness in the range of 100 nm to 1 µm (data not shown). Factors such as the roughness and thickness differences of *tris*-SBSi and *mono*-SBSi coatings may affect the antifouling properties. These considerations are undergoing further experimental evaluation.

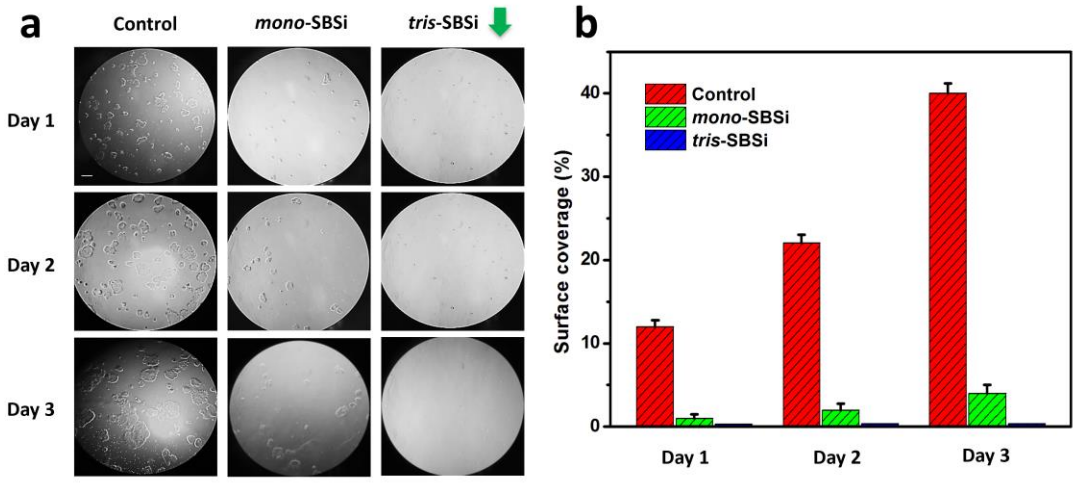


Figure 4. ***a***: microscopic analysis of grafting of human adherent cells (MCF-7 breast cancer cells) for 3 days on zwitterionic silica coated glasses (*mono*-SBSi, *tris*-SBSi) in comparison with the control glass. The green arrow points to the coverslips with less adhesive properties. Scale bar on control slide, 1 day: 100 µm. ***b***: Surface coverage of MCF-7 cells for 3 days on zwitterionic silica coated glasses (*mono*-SBSi, *tris*-SBSi) in comparison with the control glass. Errors bars indicate standard deviation for the different zones of the same surface.

With these highly promising results, we performed a second trial of antifouling experiment in seawater in order to evaluate the formation of biofilms. For this purpose, we placed these strips on new 6-well plates and immersed them in 5 mL of seawater for 8 days (**Figure 5**). After this time, we observed the glasses under the microscope. We could clearly observe that the surface coated samples show lower biofilm formation compared to the untreated glass substrate. Secondly, the *tris*-SBSI-coated sample was more efficient to avoid biofilm adhesion compared to the *mono*-SBSi coated sample. These results confirm our previous results on cancer cell adhesion and suggest that *tris*-SBSi could be a highly efficient candidate to prevent the formation of the biofilm responsible for attaching mollusks and small organisms to boat hulls.

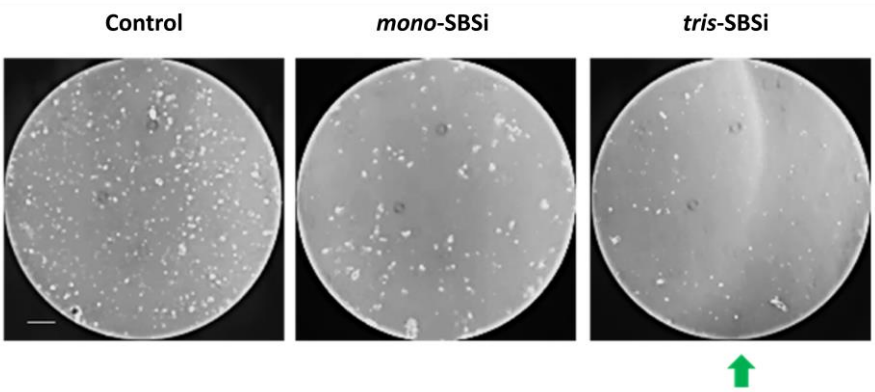


Figure 5. Microscopic analysis of grafting of microorganisms from seawater after 8 days on zwitterionic silica coated glasses (*mono*-SBSi, *tris*-SBSi) in comparison with the control glass. The green arrow points to the coating with less adhesive properties. Scale bar on control slide: 100 µm.

However, it was necessary to verify whether this anti-adhesive property was not in fact due to its inherent toxicity, which could in turn be harmful to the environment. For this purpose, we carried out a toxicological study on zebrafish embryos (**Figure 6**). First, glasses were placed in 6-well plates, each well containing 6 mL of water (glass with no antifouling coating was considered as a control and the well without glass was considered as negative control). At 5 hours post fertilization (hpf), embryos were examined under the microscope, and only embryos that developed normally were selected for the study. Thirty embryos were placed in each well and their development was observed. More precisely, embryos' development including chorionated, hatching, mortality and malformation were recorded at different time intervals (24, 48, 52, 57 and 72 hpf). Results of these observations were reported in bar graphs of **Figure 6a** where we can see that no significant differences were detected between conditions. No changes in the hatching rate was observed for coated coverslips in comparison to control as embryos started to hatch (dechorionated) from 52 hpf until 72 hpf. Also, no change in mortality neither death was detected in all conditions. The representative pictures of each step of embryos development were reported in **Figure 6b**. We can therefore conclude that the zwitterionic coating of the glass substrates do not display any toxicological effect on the development of zebrafish embryos.

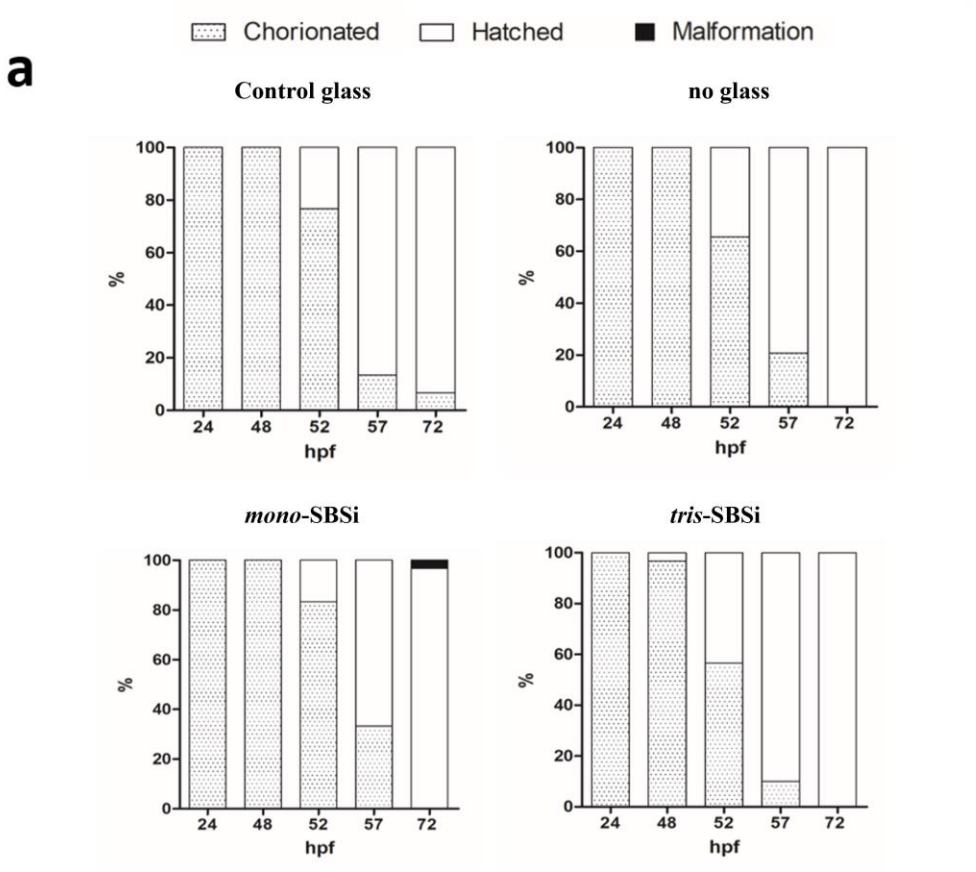


Figure 6. Study of the toxic effect of the antifouling coatings on Zebrafish embryos development. (a) Bar graphs are expressed as a percentage of chorionated, hatched and malformation (n=30). No dead embryos were observed during the experimentation period. (b) Evolution of studied embryos according to the number of hours post fertilization (hpf) with representative pictures of their morphology.

5 Conclusions

We report an effective antifouling coating strategy using two zwitterionic silica molecules: *mono*-SBSi and *tris*-SBSi. The two zwitterionic molecules have different numbers of anchoring hydrolysable silane substituents, which were served for their grafting the glass surface by silanization reactions to form organosilica layers. Zwitterionic silica coatings enhanced the surface hydrophilicity. Surface coating using the *tris*-SBSi precursor increased the surface roughness due to surface sol gel reactions. The antifouling property of both *mono*-SBSi and

tris-SBSi coatings were demonstrated by the adsorption of MCF-7 cell and micro-organisms of seawater. We found that the *tris*-SBSi coating exhibited excellent repellence against adsorption of MCF-7 cells and micro-organisms from seawater. This result can be attributed to the morphology of the sol-gel coating strongly that affects the anti-adhesion properties. Whereas the *mono*-SBSi coating significantly reduces the adhesion of cells and the formation of biofilm, the *tris* SBSi coating totally impedes these phenomena and highly efficiently protects the glass surface. In addition, zebrafish viability tests indicate that both coatings are non-toxic antifouling materials. This study highlights that the *tris*-SBSi coating is a new and highly efficient organosilicon material with a significant potential to resist fouling, which has the potential to be used in biomedical science and marine technologies. Our study highlights that besides the chemical constitution of the zwitterionic sulfobetaine group and its high hydrophilicity, other parameters such as surface roughness and layer thickness have an impact on the antifouling properties of zwitterionic coatings. The constitution of the precursor directly affects these parameters and is therefore of prime importance for the development of more efficient anti-adhesion coatings.

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