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Antifouling activities of immobilized ferrocenyl glucose on glassy carbon surface

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ABSTRACT

In this article a new coating for antifouling applications has been developed. This coating has two distinct parts, one being a carbohydrate to render the surface polar and the other being a ferrocene which is electrically activatable to produce charges periodically. This material has been grafted onto carbon surfaces by oxidation of an amine group and has shown anti-bacterial activities.

Introduction.

Submerged surfaces in the marine environment are prone to microorganisms that quickly allow colonization by larger marine species responsible for the deterioration of the surface.¹ Prevention is generally done by coatings that can release active ingredients that are toxic to the

microorganisms. Among others copper-based,^{2,3} zinc-based biocides,^{4,5} and boosters⁶ can be caged and released gradually to avoid any colonization. In order to last in time, regular applications of the coating are necessary. They represent 90% of the coating used. The released products accumulate in the marine environment; they are not necessarily degraded and has side-effects on flora and fauna.⁷ It exists non-release coatings that can act either by preventing the approach of microorganisms or coatings that allow the release of microorganisms which attempt to bind on the surface.^{8,9} Surfaces can be highly hydrophobic or highly hydrophilic, both presenting advantages and inconvenience faces to fouling. For example, super hydrophilic surfaces will see a watery layer forming like a shield to prevent the adhesion of proteins. This is the case of materials with poly(ethyleneglycol) (PEG)¹⁰ or zwitterionic materials.¹¹ Carbohydrates also have a high polarity making them good candidates as antifouling materials. Colavita et al.^{12,13} have grafted on polyethersulfone membranes or simply on carbon, glucose, galactose and lactose with good antifouling properties. Cao et al.¹⁴ showed with alginic acid, hyaluronic acid and pectic acid immobilized on the surface a good activity against adhesion of *Ulva* spores and barnacle larvae. However, Kerchove et al.¹⁵ demonstrated the development of a bacterial biofilm on alginic acid which is hydrophilic. On the other hand, Matsunaga¹⁶ by grafting ferrocene onto a carbon surface showed an antibacterial activity by scanning a small range of electrical potential. Bressy et al.¹⁷ also confirmed this result by immobilizing ferrocene on polymethacrylate. This presents an alternative to the classical weapons against fouling. With this in mind, the association of ferrocene with a carbohydrate targeting antifouling properties has never been demonstrated. The association of two entities could be cooperative since one has very polar interactions that can preserve a protective aqueous layer while ferrocene is electrically switchable and can generate charges when the latter is solicited. This system would be active as protein-repellent on one hand and on the

other hand it would be possible to change the wettability, making the surface not very propitious to bacteria. Synthesis, electrochemical properties, grafting on surfaces and biological methodologies on ferrocene bound to glucose are described.

Experimental section

Material:

Ferrocene **6** was synthesized in 8 steps from glucose (see supp. info.). Compounds **5** and **6** are described below and **1**, **2**, **3** and **4** are described in the SI.

6-Deoxy-6-(4-ferrocenyl-1*H*-1,2,3-triazol-1-yl)-1-(4-nitrophenyl)- β -D-glucopyranose **5**

To a solution of 4-nitrophenyl 6-azido-6-deoxy- β -D-glucopyranose (3.8 mmol, 1 equiv) in a mixture of DMF:H₂O (20 mL, 3:1), ethynylferrocene (4.18 mmol, 1.1eq), sodium ascorbate (3 mmol, 0.8 equiv,) and CuSO₄.5H₂O (1.52 mmol, 0.4eq,) were added at room temperature. The reaction mixture was stirred at room temperature overnight. The mixture was poured into saturated NH₄Cl solution, and then extracted with EtOAc and washed with brine. The organic phase was concentrated under reduced pressure. The crude product was purified by flash column chromatography (EtOAc/EtOH, 100:0 to 95:5) to give an orange-brown powder in 69 % yield. Spectral data were consistent with the results reported in the literature.

6-Deoxy-6-(4-ferrocenyl-1*H*-1,2,3-triazol-1-yl)-1-(4-aminophenyl)- β -D-glucopyranose **6**

SnCl₂.2H₂O (1.89 g, 5eq, 8.39 mmol) was added to a solution of **5** (900 mg, 1.67 mmol) in ethyl acetate (40 ml) at room temperature. The reaction mixture was refluxed overnight. Then, the mixture was cooled to room temperature and poured into a saturated aqueous NaHCO₃. A white precipitate was formed and filtered. Then, the reaction mixture was extracted with ethyl acetate,

washed with water and brine. The organic phase was dried over anhydrous MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/EtOH, 100:0 to 90:10) to afford the final compound in 40 % yield as an orange solid. Spectral data were consistent with the results reported in the literature.

For the different immobilizations of compound **6**, 1mM solutions were prepared. In aqueous medium, it was dissolved in a solution of artificial seawater (36 g Sea-salt (Aldrich) in 100 ml ultrapure water). For electrodeposition, a 96-well microplate with 3 electrodes (ref DRP-96X110) associated with a connector (ref CONNECTOR96X) connected to a potentiostat (ref DRP-STAT8000P) was purchased from DROPSSENS. The 3 electrodes at the bottom of the wells are in carbon (C) for the working electrode (3 mm) and the counter-electrode and in silver for the reference electrode. Marine bacteria (TC8, *Pseudoalteromonas lipolytica*) were isolated in Toulon Bay (Mediterranean Sea, February 2008) from an artificial surface. This bacterial strain is Gram-negative and classified as hydrophilic bacteria. It was cultured on Vaatanen nine salts solution (VSSN) under agitation (120 rpm) at 20°C and harvested in the stationary phase for bacterial adhesion tests.¹⁸

Experimental procedures:

Electrodeposition of compound **6** in wells.

A solution of compound **6** at 1mM in ASW (36 g/L) was prepared and 20 equivalents of 1M NaOH solution was added. This solution was sonicated to ultrasounds to make compound **6** completely soluble. 200 µL of the solution were introduced in each well. **6** was grafted by cyclic voltammetry (5 cycles) from - 0.3 V to + 0.8 V at 100 mV/s. Then, the solution was removed from each well. All the wells were rinsed by ASW (three times). Fresh ASW was then placed back into

each well to study the grafting responses by cyclic voltammetry (10 cycles) from - 0.3 V to + 0.6 V, 100 mV/s. ASW was removed again to do the antiadhesion assay.

Antifouling test

Bacterial cultures were performed in VNSS, starting with an optical density (OD_{600nm}) equal to 0.1. This bacterial suspension was incubated at 20°C at 120 rpm. The growing was controlled every hour for 6 hours until reaching the stationary phase to enhance their adhesion on substrates. The bacterial suspension was centrifuged for 15 min. The VNSS was removed and replaced by ASW (Seasalt, Sigma Aldrich), with the same volume. This new suspension (bacteria+ASW) was diluted in ASW in order to reach an optical density (OD_{600nm}) equal to 0.3. 200 μ L of bacterial suspension (at $OD_{600nm} = 0.3$) were introduced in each wells of columns 8 and 9 noted WB, and free bacteria ASW solution in the well of columns 8 and 9 noted WoB. The plate was connected to the potentiostat and was incubated at 20°C at 120 rpm for 16 hours. Potential current scans were applied from -0.3 V to 0.6 V at 100 mV/s, for 16 hours, on one column. After 16 hours, wells were rinsed 3 times by NaCl (36 g/L). A 1 μ M Syto[®] 61 solution ($\lambda_{exc} = 620$ nm, $\lambda_{em} = 650$ nm) diluted in NaCl (36 g/L) was prepared without light. 200 μ L of Syto[®] 61 solution were introduced in each well (WB and WoB) and were incubated in the dark at 20°C at 120 rpm for 20 min. Then, the fluorescence intensity (FI) was measured by the Infinit 200pro microplate fluorescence reader (TECAN). In parallel to the adhesion test done on coatings, a polystyrene (PS) plate was prepared with the same procedure and was used as a reference. The percentage of adhesion for the PS reference was assumed to be 100%. The percentage of adhesion of TC8 on coatings was calculated as follows:

$$\% \text{ adhesion} = \frac{(FI \text{ with bacteria} - FI \text{ without bacteria})_{\text{coating}}}{(FI \text{ with bacteria} - FI \text{ without bacteria})_{PS}}$$

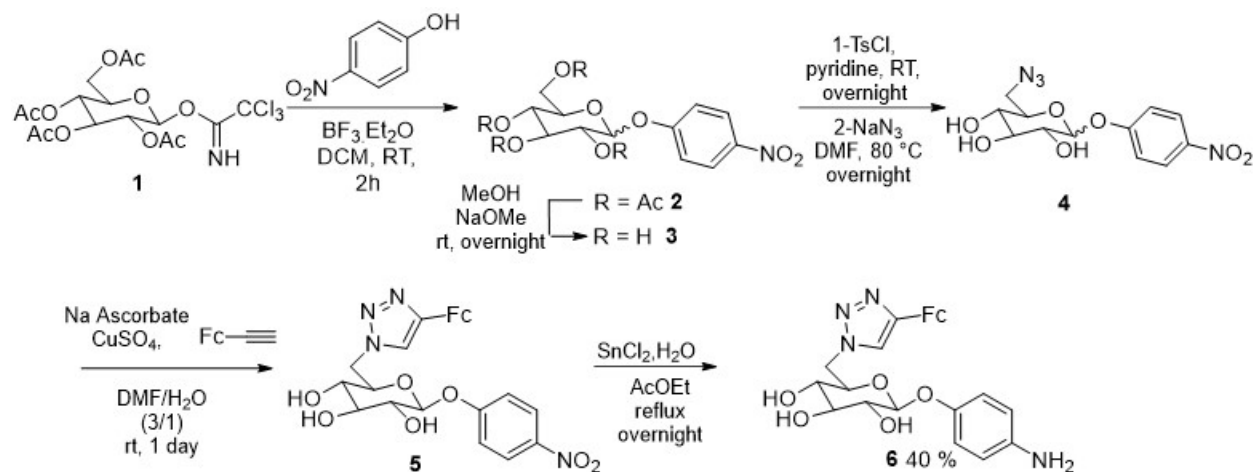
Toxicity assay

After 16h of incubation, 100 μL of solution from WB and WoB wells were kept and moved to a PS plate. In additional PS wells, 100 μL of a bacterial suspension at an $\text{OD}_{600\text{nm}}$ equal to 0.6 were introduced. In these later, ethanol at different concentrations (100 μL ; 0%, 5%, 10%, 20%, 40%, 70%, 100%) was introduced to lead to a bacterial suspension at $\text{OD} = 0.3$. The PS plate was incubated at 20 °C, 120 rpm for 1 hour. Then, 100 μL of a mixture Syto[®] 9 nucleic acid stain ($\lambda_{\text{exc}} = 470\text{nm}$, $\lambda_{\text{em}} = 515\text{nm}$) and propidium iodide (PI) ($\lambda_{\text{exc}} = 538\text{nm}$, $\lambda_{\text{em}} = 617\text{nm}$) in NaCl (36 g/L) were introduced in each well and the plate was incubated for 20 min at 20°C at 120rpm in dark. The PI shows the dead bacteria whereas the Syto[®] 9 shows the living and the dead bacteria. Their FI were measured by the Infinit 200pro microplate fluorescence reader TECAN. The ratio Syto[®] 9/PI allows to estimate the toxicity of the coating and to compare it to the toxicity of various % of ethanol. If this ratio is close to zero, the coating is considered as toxic.

Results

The immobilization of the targeted antifouling ferrocenyl glucose derivative by a covalent bond can be achieved in different ways, all using the amino group as surface anchoring functionality, such as the reduction of diazonium salts,¹⁹ or the spontaneous reaction of the amine derivatives with the surface²⁰ or by electrochemical oxidation of amine functions.^{21,22} The latter route is the most compatible with the ferrocene entity since the diazonium salt is not stable in the presence of ferrocene.^{23,24} Ferrocene is bound to glucose by 1,3-dipolar cycloaddition, the glucose azide having been prepared in 5 steps from glucose as shown in scheme 1.

Scheme 1: Synthesis of glucose clicked ferrocene.



The covalent immobilization of the ferrocenyl aminophenyl glucose derivative **6** was achieved by electrochemical oxidation in artificial sea water (ASW) which has more practical significance for antifouling applications than organic medium commonly used for surface modification. Indeed, acetonitrile/ $n\text{Bu}_4\text{NPF}_6$, usually used in electrochemistry could lead to entrapment of these derivatives in the coating. We have already shown that acetonitrile and $n\text{Bu}_4\text{NPF}_6$ can have some toxicity to bacteria (unpublished results). In order to really attribute the anti-fouling effect to the grafted material, it was better to put ourselves in the conditions closest to biological tests. Another reason to use ASW medium is technical, as the plates with the microwells do not support organic media. In this respect, it should be noted that electrografting process by oxidation of amines in aqueous medium are rarely reported in literature, especially in the natural seawater electrolyte for which no published data exist. Geneste et al.²⁵ showed the possibility of grafting TEMPO and nitrobenzylamine in both acidic and basic media. Other derivatives such as aminobenzylphosphonic acid,²⁶ aminobenzoic acid,²⁷ PAMAM dendrimers²⁸ and cyclodextrine derivatives²⁹ could be immobilized in glassy carbon electrode using aqueous medium.

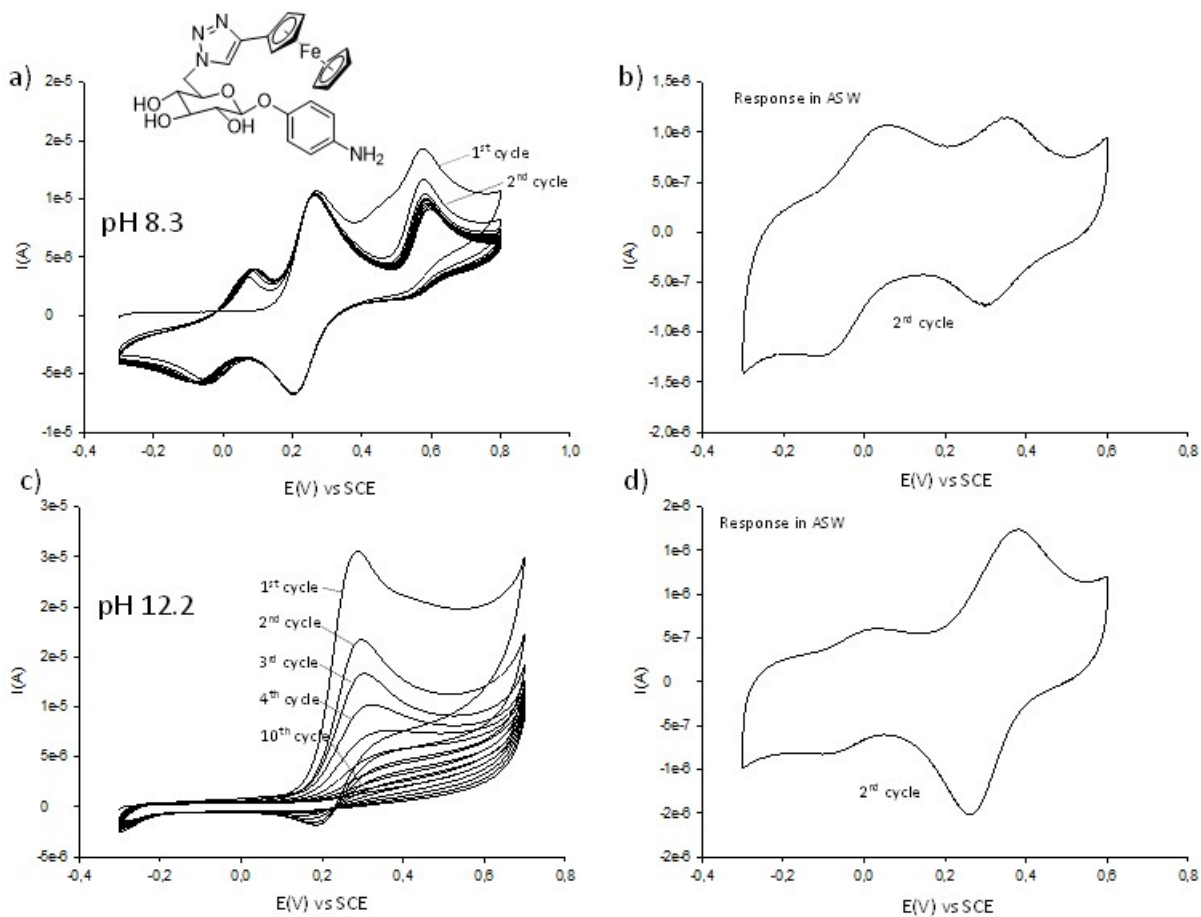
Cyclic voltammograms (CVs) presented in Figure 1 show that molecules essentially retain the redox characteristics of both ferrocene and amine groups, with features of a reversible system

centered at 0.23 V vs. ECS and an irreversible one-electron anodic wave located at 0.58 V vs. ECS, proving that in the time scale of the cyclic voltammetry, the amine cation radical is unstable and underwent an overall deprotonation/dimerization sequence. Importantly, the fate of the oxidation products of amine is pH dependent.³⁰ In ASW (pH 8.3), the irreversible oxidation of the amine is accompanied by a new reversible system appearing at around 0.03 V vs. ECS, attributable to an electroactive head-to-tail dimer as demonstrated by Bacon and Adams in aqueous media.³¹ When ASW was adjusted at pH 12 with NaOH 2M, a huge decrease in the current peak intensity of the oxidation of amine is obtained after the first cycle. A cathodic shift of amine signal is also observed, the amine oxidation seems to be facilitated in very basic medium, and the reversible system oxidation has become difficult to see.

These changes in the shape of the CVs as the pH increases demonstrate that it was possible to minimize the formation of the dimer to make grafting favourable. Indeed, at pH 12, the reversible oxidation peak at 0.03 V is less and less visible and the oxidation peak of the amine loses its intensity with each cycle indicating a progressive passivation of the electrode.³²

The cyclic voltammetry study in pure ASW of glassy carbon (GC) electrodes previously treated as in Figures 1a and 1b is also very instructive about the reactivity of the amino cation radical toward the GC electrode surface. At pH 8.3, the modified electrode shows two weak reversible systems with decreasing intensities upon the first CVs, which can be identified as a coupling product physisorbed onto the electrode surface. On the contrary, at pH 12, both the electrode passivation in ASW (figure 1c) containing 1 mM of **6** and the stable pair of reversible peaks at the same potential location than the one of the ferrocene/ferrocenium couple in pure ASW (figure 1d) are evidence for the robust grafting of the electrode surface. It should be noted that the recovery rate at pH 8.3 is $6.2 \cdot 10^{-11} \text{ mol/cm}^2$ and increases to $2.25 \cdot 10^{-10} \text{ mol/cm}^2$ at pH 12.2. Comparing these

results with the literature, it would appear that the layer formed on the surface of the electrode is fairly compact and is close to a monolayer ($4.4 \cdot 10^{-10}$ mol/cm² for a compact layer of ferrocene derivatives).³³



To make the covalent grafting possible, the carbon surface should be attacked by an aminyl radical produced by deprotonation of the amino cation radical.³⁴ With reference to the well-established reaction mechanism of the electrografting of amines, the stability of the cation radical governs the grafting efficiency of amines and a base is sometimes added in solution when the basic strength of the neutral amine is not sufficient to make the deprotonation of the exergonic amino cation radical.³⁵ This applies especially when electron-donating groups are present on the phenyl ring of aniline derivatives, the amino cation radical of which suffers from relative low acidity. As

examples, Amatore et al. demonstrate that, in DMF, neither the lutidine nor the collidine significantly deprotonates the cation radical of the p-anisidine,³⁶ while the neutral p-ethoxyphenazyl free radical was detected by ESR in water only at pH 11-12.³⁷ This is the reason why the pH of the amine solution needs to be adjusted at 12 to make the grafting possible. At pH 7.5-8, the anodic behavior of the amino-containing compound mostly involves cation radicals, yielding a dimerization product.

Figure 1. a) Grafting by cyclic voltammetry (10 CVs) from - 0.3 to + 0.8 V of **6** (1 mM in ASW); scan rate 100 mV/s; pH = 8.3. b) Response in a solution free of **6** in ASW by cyclic voltammetry from - 0.3 to + 0.6 V (representation of the 2nd cycle); scan rate: 100 mV/s. c) Grafting by cyclic voltammetry from - 0.3 to + 0.7 V of **6** (1 mM in ASW) at pH 12.2; scan rate 100 mV/s. d) Response in a solution free of **6** in ASW by cyclic voltammetry from - 0.3 to + 0.6 V (representation of the 2nd cycle); scan rate 100mV/s.

To test the antifouling properties of this compound, a protocol that we have already tested on polymer materials was used.^{17,38} The equipment consists of a microplate with 96 wells at the bottom of which are three electrodes (WE, CE, Ref; see figure 2 for illustration), a configuration identical to what can be done in a conventional electrochemical cell. This microplate is placed on a connector which is connected to a potentiostat. The potentiostat allows to apply the same potential per column (as shown in yellow in figure 2), however not all columns can be solicited at the same time. The working electrodes of the same column can be functionalized under the conditions described. The different voltamperograms recorded (corresponding to wells a to h)

indicate that each of the wells is covered by glucose-bound ferrocene. The responses are then recorded in a **6**-free ASW solution and confirm the deposit.

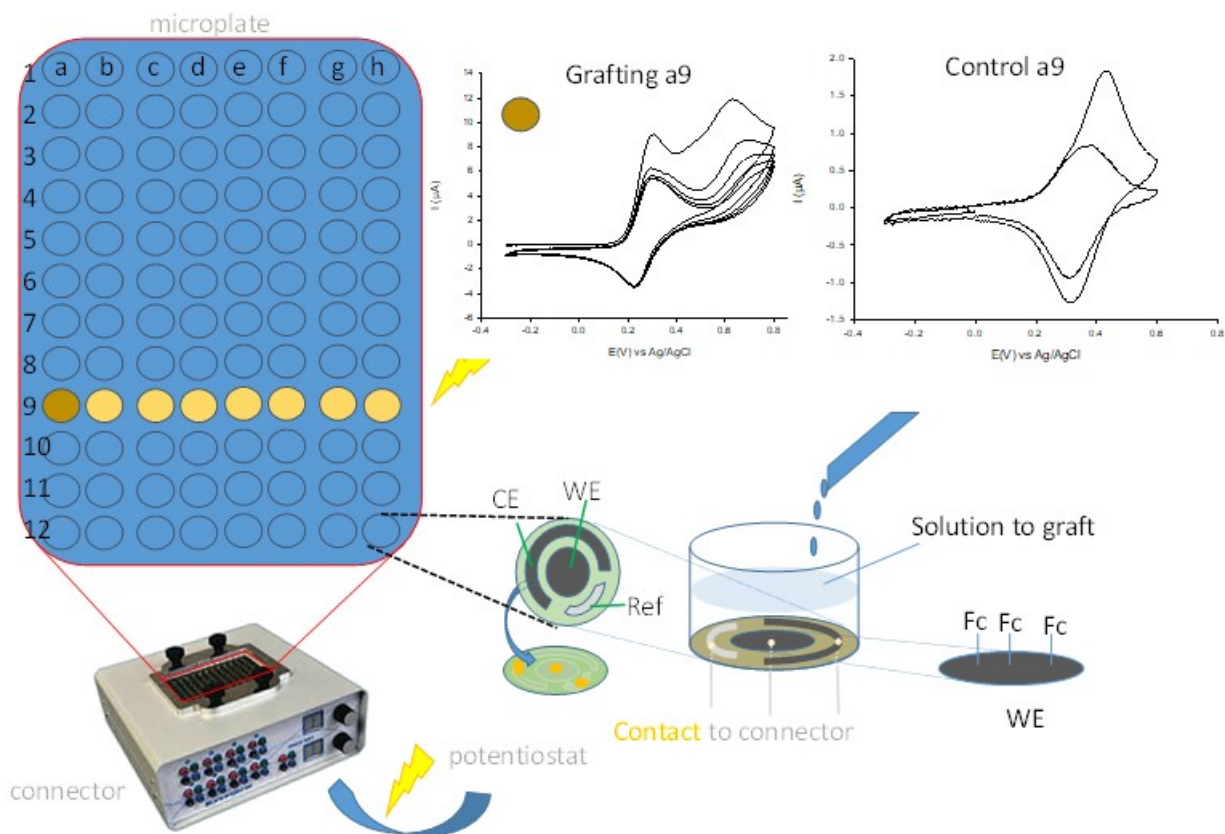


Figure 2. a) Illustration of ferrocene derivatives grafted in a column of wells; CE: counter electrode in C, WE: working electrode in C, Ref: reference electrode in Ag. b) First series of Voltammogram of well 9a: grafting sweeping from -0.3 to + 0.8 V of **6** (1mM in ASW) at pH 10.3 (grafting in column 9 of wells b to h in SI); 5 CVs; scan rate: 100 mV/s. Second voltammogram: control of well 9a sweeping from -0.3 to +0.6 V in ASW free of **6** (control in column 9 of wells b to h in SI); scan rate: 100 mV/s; 10 CVs (1st and 10th CV).

The advantage of the equipment used allows a greater number of replicates to be made during anti-adhesion tests, which are difficult to realize on conventional electrodes. The antifouling tests

were carried out with marine TC8 bacteria. In the example in figure 3, columns 8 and 9 were functionalized with **6** by amine oxidation. Columns 8 and 9 have been seeded on wells a, b, c and d with bacteria inoculum while wells e, f, g and h are free of bacteria. The test is carried out over 16 h with column 8 left without electrical stress while column 9 is subjected to an electrical sweep of - 0.3 to + 0.6 V for the duration of the experiment. After adding the Syto[®] 61 dye, it is possible to quantify the bacterial adhesion (by fluorescence intensity) of TC8 strain to the Fc-grafted surface, compare to a polystyrene (PS) plate, used as a positive reference (adhesion = 100%). It can also be noted that the bare carbon undergoes colonization similar to polystyrene, and in previous studies we had also shown that the electrical stress of the bare carbon did not prevent bacteria from attaching to it. The results were similar to an electrically unstressed electrode. The modified wells without any solicitation (column 8) show less colonization than the polystyrene wells (figure 3b, 61 % \pm 6, see table SI-1), indicating that the coating used has properties that are less favourable to the adhesion of TC8. The solicitation of ferrocene (change from neutral to oxidized to neutral) (column 9) has a significant effect on bacterial adhesion (figure 3b, 32 % \pm 5, see table SI-1) and validates the fact that the change in surface state complicates bacterial colonization. Bacteria need a sufficiently large space with an electrically homogeneous surface to establish a close bond with the surface. If the bacteria is bothered by an untimely appearance of surface charges, the links created could be weak and reversible, preventing any definitive colonization.

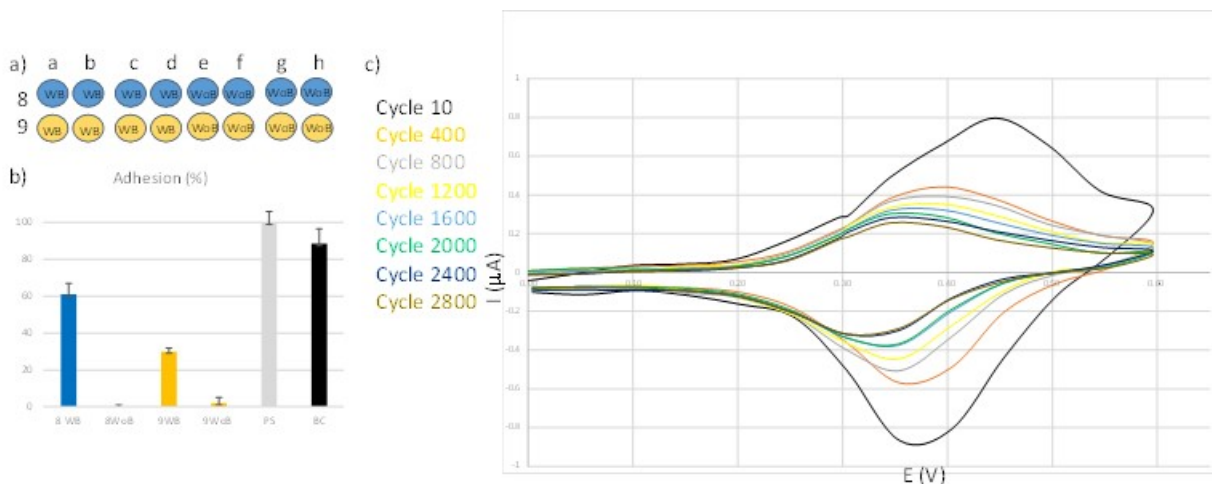


Figure 3. a) Columns 8 and 9 of a microplate; in blue: non solicited; in orange: solicited; WB: with bacteria and WoB: without bacteria. b) Percentage of TC8 adhesion on Fc-grafted wells, PS and carbon (C) c) Ageing tests in one solicited well: sweeping from -0.3 to + 0.6 V in ASW with a scan rate of 100 mV/s. Black: 10th cycle; orange: 400th cycle; grey: 800th cycle; yellow: 1200th cycle; blue: 1600th cycle; green: 2000th cycle; dark blue: 2400th cycle; brown: 2800th cycle.

A live/dead test was also performed on columns 8 and 9 to establish the toxicity of the coatings. The degree of toxicity of coatings is evaluated by comparison with the toxicity of ethanol (see the table SI-2 for the test results). Syto[®] 9 and Propidium Iodide (PI) dyes were used to stain all the cells and dead cells, respectively, in each well, after 1 hour of incubation. The ratio of the two fluorescent dyes intensities indicated that the toxicity of the coatings is comparable to the toxicity of 5 % ethanol solution, toxicity that cannot be detected.

An ageing test via ferrocene signal monitoring in electrochemistry allowed us to estimate the condition of the layers in each well (figure 3c). There is a decrease in the signal of the ferrocene, so the recovery rate is lower but the layer obtained seems to be stabilized till 7 hours of ageing.

The coatings were oxidized and reduced during 16 h of incubation. Then, the polarity of the layer was controlled by measuring the water contact angle at time $t = 0$ and after 16 hours of sollicitation. The measured polarity remains unchanged after the stress and angles of 30 to 40° could be measured indicating a rather polar coating. The contact angle of the bare carbon was measured around 140 °. Thus we can show that the carbon has been modified and that ageing only slightly alters the layer on the C electrode.

Conclusion

The method we have developed makes it possible to covalently immobilize the substrate on the surface and avoid any release of active substances into the environment, as is the case with most coatings used on ship hulls, for example. On the other hand, amine immobilization for anti-bacterial effects is new since the most relevant work is that of P. Colavita showing an anti-protein effect after immobilization by reduction of diazonium salts. Thus, it has been shown that a glucose-bound ferrocene can be grafted by oxidation of an amine on the carbon surface in aqueous medium such as sea water. Functionalization was confirmed by cyclovoltamperometry and contact angle measurement and an ageing test ensured the perenity of the Fc-grafted layer on the electrode surface. The biological tests were carried out using a device linking a potentiostat to a microplate with 96 wells in which electrochemistry can be carried out. The coatings alone tend to have a marine antibacterial effect on the TC8 strain, an effect that becomes more pronounced if ferrocene is oxidized. Indeed, the developed system allows an electric cycling with a potential that allows to extract an electron from the ferrocene and transform it into ferrocenium. This charge will disappear in the return cycle. The to-and-fro of this charge has an additional effect on the layer deposited material on the prevention of bacteria adhesion.

ASSOCIATED CONTENT

Supporting Information. The following files are available free of charge.

Syntheses of **1** to **4**, Adhesion tests (table SI-1), Toxicity tests (table SI-2) and Voltamperograms of wells a9-h9.

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REFERENCES

1. Bixler, G. D.; Bhushan, B., Biofouling: lessons from nature. 2012, 370 (1967), 2381-2417.
2. Voulvoulis, N.; Scrimshaw, M. D.; Lester, J. N., Alternative antifouling biocides. 1999, 13 (3), 135-143.
3. Watermann, B.; Eklund, B., Can the input of biocides and polymeric substances from antifouling paints into the sea be reduced by the use of non-toxic hard coatings? Marine Pollution Bulletin 2019, 144, 146-151.
4. Omae, I., General Aspects of Tin-Free Antifouling Paints. Chemical Reviews 2003, 103 (9), 3431-3448.

5. Martins, S. E.; Fillmann, G.; Lillicrap, A.; Thomas, K. V., Review: ecotoxicity of organic and organo-metallic antifouling co-biocides and implications for environmental hazard and risk assessments in aquatic ecosystems. *Biofouling* 2018, 34 (1), 34-52.
6. Konstantinou, I. K.; Albanis, T. A., Worldwide occurrence and effects of antifouling paint booster biocides in the aquatic environment: a review. *Environment International* 2004, 30 (2), 235-248.
7. Amara, I.; Miled, W.; Slama, R. B.; Ladhari, N., Antifouling processes and toxicity effects of antifouling paints on marine environment. A review. *Environmental Toxicology and Pharmacology* 2018, 57, 115-130.
8. Lejars, M.; Margailan, A.; Bressy, C., Fouling Release Coatings: A Nontoxic Alternative to Biocidal Antifouling Coatings. *Chemical Reviews* 2012, 112 (8), 4347-4390.
9. Hu, P.; Xie, Q.; Ma, C.; Zhang, G., Silicone-Based Fouling-Release Coatings for Marine Antifouling. *Langmuir* 2020, 36 (9), 2170-2183.
10. Jeon, S. I.; Lee, J. H.; Andrade, J. D.; De Gennes, P. G., Protein—surface interactions in the presence of polyethylene oxide: I. Simplified theory. *Journal of Colloid and Interface Science* 1991, 142 (1), 149-158.
11. Chen, S.; Li, L.; Zhao, C.; Zheng, J., Surface hydration: Principles and applications toward low-fouling/nonfouling biomaterials. *Polymer* 2010, 51 (23), 5283-5293.
12. Zen, F.; Angione, M. D.; Behan, J. A.; Cullen, R. J.; Duff, T.; Vasconcelos, J. M.; Scanlan, E. M.; Colavita, P. E., Modulation of Protein Fouling and Interfacial Properties at Carbon

Surfaces via Immobilization of Glycans Using Aryldiazonium Chemistry. *Scientific Reports* 2016, 6 (1), 24840.

13. Angione, M. D.; Duff, T.; Bell, A. P.; Stamatina, S. N.; Fay, C.; Diamond, D.; Scanlan, E. M.; Colavita, P. E., Enhanced Antifouling Properties of Carbohydrate Coated Poly(ether sulfone) Membranes. *ACS Applied Materials & Interfaces* 2015, 7 (31), 17238-17246.

14. 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.

15. de Kerchove, A. J.; Elimelech, M., Calcium and Magnesium Cations Enhance the Adhesion of Motile and Nonmotile *Pseudomonas aeruginosa* on Alginate Films. *Langmuir* 2008, 24 (7), 3392-3399.

16. Okochi, M.; Matsunaga, T., Electrochemical sterilization of bacteria using a graphite electrode modified with adsorbed ferrocene. *Electrochimica Acta* 1997, 42 (20), 3247-3250.

17. Nguema Edzang, R. W.; Duong, T. H.; Briand, J.-F.; Lejars, M.; Raimundo, J.-M.; Bressy, C.; Brisset, H., Bacterial anti-adhesion activity based on the electrochemical properties of polymethacrylates bearing ferrocenyl pendant groups. *Biofouling* 2018, 34 (9), 1055-1063.

18. Camps, M.; Briand, J.-F.; Guentas-Dombrowsky, L.; Culioli, G.; Bazire, A.; Blache, Y., Antifouling activity of commercial biocides vs. natural and natural-derived products assessed by marine bacteria adhesion bioassay. *Marine Pollution Bulletin* 2011, 62 (5), 1032-1040.

19. Pinson, J.; Podvorica, F., Attachment of organic layers to conductive or semiconductive surfaces by reduction of diazonium salts. *Chemical Society Reviews* 2005, 34 (5), 429-439.

20. Gallardo, I.; Pinson, J.; Vilà, N., Spontaneous Attachment of Amines to Carbon and Metallic Surfaces. *The Journal of Physical Chemistry B* 2006, 110 (39), 19521-19529.
21. Barbier, B., Electrochemical Bonding of Amines to Carbon Fiber Surfaces Toward Improved Carbon-Epoxy Composites. *Journal of The Electrochemical Society* 1990, 137 (6), 1757.
22. Bélanger, D.; Pinson, J., Electrografting: a powerful method for surface modification. *Chemical Society Reviews* 2011, 40 (7), 3995-4048.
23. Berlin, A. A.; Liogonkii, B. I.; Parini, V. P., Polymers with conjugated double bonds and heteroatoms in the conjugated chain—XXVI. Polyazophenyleneferrocenes. *Polymer Science U.S.S.R.* 1963, 4 (5), 980-989.
24. Rosenblum, M.; Brawn, N.; Papenmeier, J.; Applebaum, M., Synthesis of ferrocenylacetylenes. *Journal of Organometallic Chemistry* 1966, 6 (2), 173-180.
25. Geneste, F.; Moinet, C., Electrochemically linking TEMPO to carbon via amine bridges. *New Journal of Chemistry* 2005, 29 (2), 269-271.
26. Yang, G.; Liu, B.; Dong, S., Covalent modification of glassy carbon electrode during electrochemical oxidation process of 4-aminobenzylphosphonic acid in aqueous solution. *Journal of Electroanalytical Chemistry* 2005, 585 (2), 301-305.
27. Yang, G.; Shen, Y.; Gang, M.; Chen, H.; Liu, B.; Dong, S., Copper hexacyanoferrate multilayer films on glassy carbon electrode modified with 4-aminobenzoic acid in aqueous solution. *Talanta* 2006, 68 (3), 741-747.

28. Kim, T. H.; Choi, H. S.; Go, B. R.; Kim, J., Modification of a glassy carbon surface with amine-terminated dendrimers and its application to electrocatalytic hydrazine oxidation. *Electrochemistry Communications* 2010, 12 (6), 788-791.
29. Silva, K.; Marco, J. F.; Yañez, C., Covalent Immobilization of Amino- β -Cyclodextrins on Glassy Carbon Electrode in Aqueous Media. *Journal of The Electrochemical Society* 2019, 166 (8), G75-G81.
30. Cauquis, G.; Delhomme, H.; Serve, D., Les propriétés électrochimiques des diphénylamines et de leurs produits d'oxydation en milieu organique—II. Propriétés des diaryl-5,10 dihydro-5,10 phénazines et des tétraarylhazines. *Electrochimica Acta* 1975, 20 (12), 1019-1026.
31. Bacon, J.; Adams, R. N., Anodic oxidations of aromatic amines. III. Substituted anilines in aqueous media. *Journal of the American Chemical Society* 1968, 90 (24), 6596-6599.
32. Peigneguy, F.; Dabos-Seignon, S.; Frère, P.; Bressy, C.; Gohier, F.; Cougnon, C., Preparation and study of a carbohydrate-functionalized carbon surface by electrochemical oxidation of 4-aminophenyl- β -D-glucopyranoside. *Electrochemistry Communications* 2018, 93, 175-179.
33. Leroux, Y. R.; Fei, H.; Noël, J.-M.; Roux, C.; Hapiot, P., Efficient Covalent Modification of a Carbon Surface: Use of a Silyl Protecting Group To Form an Active Monolayer. *Journal of the American Chemical Society* 2010, 132 (40), 14039-14041.

34. Adenier, A.; Chehimi, M. M.; Gallardo, I.; Pinson, J.; Vilà, N., Electrochemical Oxidation of Aliphatic Amines and Their Attachment to Carbon and Metal Surfaces. *Langmuir* 2004, 20 (19), 8243-8253.
35. Anex, C.; Touzé, E.; Curet, L.; Gohier, F.; Cougnon, C., Base-Assisted Electrografting of Aromatic Amines. 2019, 6 (19), 4963-4969.
36. Simon, P.; Farsang, G.; Amatore, C., Mechanistic investigation of the oxidation of p-anisidine in unbuffered DMF using fast scan rates at ultramicroelectrodes. *Journal of Electroanalytical Chemistry* 1997, 435 (1), 165-171.
37. Fischer, V.; Harman, L. S.; West, P. R.; Mason, R. P., Direct electron spin resonance detection of free radical intermediates during the peroxidase catalyzed oxidation of phenacetin metabolites. *Chemico-Biological Interactions* 1986, 60 (2), 115-127.
38. Brisset, H.; Briand, J.-F.; Barry-Martinet, R.; Duong, T. H.; Frère, P.; Gohier, F.; Leriche, P.; Bressy, C., 96X Screen-Printed Gold Electrode Platform to Evaluate Electroactive Polymers as Marine Antifouling Coatings. *Analytical Chemistry* 2018, 90 (8), 4978-4981.