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The design of superhydrophobic stainless steel surfaces by controlling nanostructures: A key parameter to reduce the implantation of pathogenic bacteria

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ABSTRACT

Reducing bacterial adhesion on substrates is fundamental for various industries. In this work, new superhydrophobic surfaces are created by electrodeposition of hydrophobic polymers (PEDOT-F_4 or PEDOT-H_8) on stainless steel with controlled topographical features, especially at a nano-scale. Results show that anti-bioadhesive and anti-biofilm properties require the control of the surface topographical features, and should be associated with a low adhesion of water onto the surface (Cassie-Baxter state) with limited crevice features at the scale of bacterial cells (nano-scale structures).

1. Introduction

Stainless steel is a material with mechanical and chemical properties compatible with a large range of use in many fields. In medical facilities, most of surgery tools, operating tables but also common objects such as handrails, handles and cutlery are made of stainless steel. In the industry, it can be used for storage tanks, pipes, cutting tools, tables among many other examples.

When it is in contact with a biological environment, stainless steel can be contaminated by different types of microorganisms present in its vicinity such as bacteria, fungi, yeasts or even viruses. This surface biocontamination could involve pathogenic bacteria and therefore be the beginning of public health issues when occurring in critical environments [1–4]. Among these harmful pathogens, *Listeria monocytogenes*, a foodborne pathogen responsible for listeriosis, was accountable for multiple recent outbreaks in Europe and the USA involving numerous deaths (http://www.cdc.gov/listeria/outbreaks/caramel-apples-12-14/index.html, http://www.efsa.europa.eu/fr/topics/topic/listeria.html). *Pseudomonas aeruginosa* is another example of dangerous pathogens since it is responsible for most of the nosocomial infections in hospitals and healthcare facilities and is associated with some of the highest tolerances to antibiotic treatments [5].

In the light of such threats, the control of the stainless steel surface biocontamination appears to be primordial for numerous industries. Nowadays, biocontaminations are handled with the use of chemical compounds [6,7], but their efficiency may be limited by *i*) new legislations concerning toxic chemicals (European Chemicals Agency) or *ii*) the outbreak of bacterial resistance to these compounds [8]. As an example, *P. aeruginosa* is known to develop resistance to quaternary ammonium by selective pressure mutations. Resistance can also be the result of the physiological changes that could occur when bacteria organize in biofilms on a surface. Alternatives to these cleaning procedures have been proposed, among which the prevention of adhesion and biofilm growth by physical or chemical modifications of the surface.

Superhydrophobic surfaces are remarkable candidates for this purpose. With a very low affinity to water associated with a micro- and/or nanostructured topography, these surfaces were shown on numerous occasions to efficiently prevent surface contamination by microorganisms. At least three different strategies are used to reduce biocontaminations using superhydrophobic materials. The first strategy consists in the incorporation of biocide agents in order to kill bacteria. The biocides are for example Ag^+ or highly reactive species such as hydroxyl radicals, hydrogen peroxide and superoxide produced by the photocatalysis of TiO_2 [6,7,9,10]. However, this strategy should be avoided because bacteria develop resistance against these compounds.

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The second strategy consists in killing bacteria in contact to surface structures. Indeed, the presence of surface structures can induce the mechanical rupture of cell membranes. The group of Ivanova was the first to demonstrate that the presence of nanopillars on superhydrophobic Cicada wings can kill various kinds of bacteria but especially Gram-negative cells [11,12]. Similar effects were found on superhydrophobic Gecko skins, which are made of densely packed nanohairs [13]. Hasan et al. also fabricated silicon nanohairs by deep reactive ion etching and found that these substrates can not only kill Gram-negative cells but also Gram-positive ones [14].

Finally, the last strategy consists in the reduction of bioadhesion. Indeed, the presence of air inside the roughness of superhydrophobic surfaces can reduce, in certain conditions, the bacterial adhesion. It was first shown that a superhydrophobic surface can reduce protein adsorption [15–19]. More precisely, it was shown that proteins can be detached from a superhydrophobic surface with a nano-scale roughness much easier than with a micro-scale roughness [15]. However, if testing protein or cell adhesion may provide information and strategies to reduce the bacterial adhesion, it should be noticed that bacteria are extremely complex microorganisms having various surface chemistry, hydrophobicity, cell membranes, surface charges, shapes, and hardness and can also have pili and flagella to modify their adhesion [20–30]. In a recent work, superhydrophobic polystyrene, polycarbonate, and polyethylene efficiently reduced the adhesion of *Escherichia coli* [20]. Similarly, Privett et al. [21] showed that superhydrophobic coatings made of fluorinated silica nanoparticles decreased the biocontamination of stainless steel by *Staphylococcus aureus* and *P. aeruginosa* by a factor 100. This effect depends on multiple parameters such as the surface free energy, the surface charges or the surface topography. Roughness of superhydrophobic surfaces can be associated with either an increase or a decrease of bacterial adhesion and it has been suggested that the shift between the two aftermaths depends on topographical characteristics. Depending on how the surface is structured, bacteria can be trapped inside "anchoring zones" such as crevices, trenches or pits at the surface [22]; or on the contrary, be easily detached when the contact surfaces between the substrate and the bacterial cells are kept minimal [23] for instance when air is trapped in the structures of the substrate [24] or as mentioned by Marmur, when the solid surface area that is exposed to the liquid is low [31]. Therefore, understanding how the structure of superhydrophobic surface impacts the bacterial adhesion is crucial to produce efficient anti-bioadhesive surfaces.

Various processes and strategies can be used to induce the formation of surface structures. The electrodeposition of conducting polymers is a process allowing an easy control of the surface topography [32–40]. Surface morphologies (fibers, flower-like structures, cauliflower-like structures, sheets…) and roughness are highly dependent on electrochemical parameters (electrolyte, solvent, deposition method, for example) and monomer structure.

Here, we report the evaluation of the anti-bioadhesive properties of superhydrophobic surfaces obtained by electropolymerization of hydrophobic monomers on stainless steel but especially for formation of superhydrophobic with a nano-scale roughness. The monomers selected for this work are derived from 3,4-ethylenedioxythiophene, containing either a fluorocarbon (EDOT-F_4) or hydrocarbon (EDOT-H_8) chain and chosen for their capacity to induce, in controlled conditions, the growth of nanofibers.

The anti-bioadhesive properties of the resulting polymer surfaces were investigated using *P. aeruginosa* and *L. monocytogenes*, two pathogenic bacterial strains involved in health issues from medical environments and the food industries. Their adhesion to these surfaces was compared to those to stainless steel and also to smooth coatings of the same polymers in order to evaluate the effect of the surface structures/roughness and chemistry.

2. Materials and methods

2.1. Electrodeposition procedure

All chemical products were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). The monomers EDOT-F_4 or EDOT-H_8 were synthesized using a procedure reported in the literature [40]. AISI 316 stainless steel substrates (FE240300/34) were purchased from Goodfellow (Huntingdon, United Kingdom). The stainless steel substrates were ultrasonically cleaned with ethanol and dried. All experiments were recorded using a PGSTAT 100 Autolab potentiostat (Metrohm, Villebon sur Yvette, France). An electrochemical glass was used for the experiments. Stainless steel substrates, platinum plates and saturated calomel electrodes were used as working, counter-electrode and reference electrode, respectively. All electrolytic solutions were degassed under argon before use.

The procedure consisted in a two-step electrodeposition process. The first step was an electrodeposition of a thin layer of polypyrrole in order to both increase the coating adherence but also decrease the monomer oxidation potential on stainless steel. For that purpose, an aqueous solution containing pyrrole (0.25 M) and oxalic acid (0.08 M) was introduced in the electrochemical cell. The thin films were grown at constant potential ($E = 0.77$ V *vs* SCE) and with a deposition charge ($Qs = 5$ mC cm^{-2}). Then, the film was cleaned and dried.

For the second step, the monomer (0.01 M of EDOT-F_4 or EDOT-H_8) was introduced in an anhydrous acetonitrile solution containing tetrabutylammonium perchlorate (Bu_4NClO_4 0.1 M) for EDOT-F_4 and tetrabutylammonium hexafluorophosphate (Bu_4NPF_6 0.1 M) for EDOT-H_8. The electrolytes were chosen in order to have a better control of the surface structures at a nanoscale with a nanofibrous morphology and also to reach superhydrophobic properties. The electrodeposition was performed at constant potential ($E = 1.45$ V *vs* SCE for EDOT-F_4 and 1.40 V for EDOT-H_8) and using different deposition charge (*Qs*) in order to investigate the effect of the polymer growth on the anti-bioadhesive properties.

In order to estimate the influence of the presence of surface structures on the anti-bioadhesive properties, smooth polymers were also produced using an extremely low *Qs* (1 mC cm^{-2}).

2.2. Surface characterization

Contact angles were measured using a DSA30 goniometer (Krüss, Villebon sur Yvette, France). The water apparent contact angles (θ_{water}) were taken by measuring the angle tangent to the triple point between a water droplet (2 μL) and the substrate. The sliding contact angles were measured by the tilted-drop method. Here, a water droplet (6 μL) was placed on the substrate and the substrate was inclined until the droplet moves. The maximum inclination angle is called sliding angle (α). Scanning electron microscopy (SEM) images were obtained using a 6700F microscope (JEOL) after metallization. The mean (*Ra*) surface roughness was realized with a Wyko NT 1100 optical microscope (Bruker). The measurements were performed with High Mag Phase Shift Interference (PSI) working mode, the 0.5× field of view and the 50× objective.

2.3. Bacterial strain cultures and preparation of cell suspensions

P. aeruginosa PAO1 and L. monocytogenes CIP 103574 were used in this study. Luria-Bertani Broth (LBB, Biorad, Marnes-la-Coquette, France) adjusted to pH 7.4 was used as the medium for P. aeruginosa cultures while Tryptone Soy Broth (TSB, Biorad, Marnes-la-Coquette, France) was used for the L. monocytogenes cultures. The strains were stored (−20 °C) in their specific medium containing glycerol (20% w/v). In order to obtain bacterial suspensions in early stationary phase, frozen stocks of strains were subcultured three times in medium (37 °C) with vigorous orbital shaking (180 RPM). For all assays, bacterial cultures were washed three times by centrifugations (7000 ×g, 10 min, 4 °C) with a NaCl medium (150 mM). Bacterial pellets were resuspended in