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Antibacterial surfaces obtained through dopamine and fluorination functionalizations

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ABSTRACT

Self-polymerized dopamine was used to form a thin layer onto stainless steel (SS) and poly(ethylene terephthalate) (PET) sheets followed by covalent grafting of pentadecafluorooctanoyl chloride by esterification and amidation reactions. The surface functionalization was characterized at each step by contact angle measurements, X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). The anti-adhesive properties of native surfaces, polydopamine-coated surfaces and hydrophobic fluorinated surfaces were tested against Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive bacteria (*Listeria monocytogenes* and *Staphylococcus aureus*). The results reveal an inhibition of bacteria growth towards Gram-negative bacteria on fluorinated surfaces. This work proposes a novel method to easily fluorinate in two steps both metallic and organic surfaces using "universal" polydopamine coating as a key step.

1. Introduction

Microorganisms can colonize a wide variety of surfaces including food packaging and medical devices, this colonization leading to biofilm formation. Biofilms result from the accumulation of organic molecules, microorganisms and metabolites and are ubiquitous on surfaces submerged in aqueous environments [1]. A reduction of surface contamination could be obtained through a well-adapted choice in the materials to use or through modifying them by tailored surface treatments [2,3]. In the past years, there has been much interest in developing methods to modify the surface properties of different materials. Recently, we have published different methods to modify poly(ethylene terephthalate) surfaces as films and fibers. The techniques used were plasma treatment [4,5], covalently grafting of biomolecules by reductive amination [6], "grafting from" by controlled radical polymerization [7] and grafting of UV-reactive molecule [8].

Stainless steel (SS) is widely used in the food processing and medical device industries. Despite good cleanability, both bacteria and organic residues adsorb easily (bioadhesion) on the SS surfaces [9]. In addition, poly(ethylene terephthalate) (PET) is one of the most important polymeric materials and find applications in textile industry, packaging, high strength fibers, filtration membrane, cosmetics, biomedical fields and others. Stainless steel and PET are intrinsically incapable of preventing bioadhesion and therefore surface modification is necessary [9]. Thus, various types of modification methods have been developed to enhance the antibacterial properties of metallic and organic surfaces [10]. For example, surface modification with hydrated polymer chains (such as poly(ethylene glycol), polysaccharides, phospholipids, polyvinyl pyrrolidone, ...) provides a steric barrier that minimizes non-specific interactions with organic matter and living bacteria [9]. Other studies report the use of silver nanoparticles loaded on material surfaces for their bactericidal ability [11]. Another strategy that has emerged as potential means of inhibiting the biofilm formation involves the utilization of low-surface energy polymeric coating (non-stick surface to bacteria and other colonizing micro-organisms) [12,13]. One challenge for the preparation of antibacterial coating is to develop methods able to be applied to different types of substrates materials *via* simple and inexpensive strategies.

Recently, it has been shown that 3,4-dihydroxyphenylalanine, an amino acid in secreted mussel adhesive proteins, is responsible for the strong adhesion on many supports. Bio-inspired

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coating prepared by polymerization of dopamine (2-(3,4-dihydroxyphenyl)ethylamine) has attracted much attention due to the strong adhesion to almost all types of surfaces [14–18].

In this study, we examined a two-step coating process to enhance the antibacterial properties of stainless steel and PET surfaces. In the first step, dopamine was polymerized on the surfaces forming an organic coating. In the second step, the polydopamine coating was used to graft a fluorinated compound. These new surfaces were characterized using complementary techniques of analysis and imaging (such as X-ray photoelectron spectroscopy, contact angle measurements and atomic force microscopy). Finally, the impact of these surfaces modifications on the adhesion of different kinds of pathogenic bacteria was evaluated.

2. Experimental part

2.1. Materials and reagents

AISI 316 grade annealed stainless steel sheets (FE240300) with a thickness of 250 μm were purchased from Goodfellow. PET films Melinex© OD with a thickness of 125 μm were kindly provided by DuPont Teijin films. Stainless steel plates and PET films were cut into pieces of 10 mm × 20 mm. Stainless steel and PET surfaces were cleaned with a mixture of acetone and absolute ethanol (50/50%, v/v) for 1 h, and then dried under argon. Dopamine hydrochloride and pentadecafluorooctanoyl chloride (98%) were purchased from Aldrich and used without further purification. Triethylamine and toluene were distilled over drying agent (NaOH and CaH_2, respectively) prior to use. Deionized water was obtained with a Milli-Q water purification system.

2.2. Surface functionalization of AISI 316 stainless steel and PET by PDA

The clean stainless steel surfaces were activated by immersion into a piranha solution (H_2SO_4/H_2O_2 = 75/25%, v/v) for 30 min and rinsed with large amount of deionized water. The activated stainless steel surfaces (named SS-OH) were immersed in aqueous dopamine solution (1 mg mL^{-1} with pH adjusted to 11 using 1 mol L^{-1} NaOH) without stirring.

After cleaning with organic solvents (mixture of ethanol and acetone 50/50%, v/v, immersion during 1 h) followed by rinsing with deionized water, PET surfaces were directly immersed in aqueous dopamine solution (1 mg mL^{-1} with pH adjusted to 11 by using 1 mol L^{-1} NaOH solution) without stirring.

Dopamine treatment was performed at room temperature in the dark for 48 h. At the end of reaction, the substrates (named SS-PDA and PET-PDA) were removed from reaction mixture and rinsed with large amount of deionized water, followed by rinsing with acetone and drying under argon flow. The surfaces were stored in a vacuum desiccator under reduced pressure before being subjected to characterization or fluorination step.

2.3. Covalent immobilization of pentadecafluorooctanoyl chloride

The SS-PDA and PET-PDA substrates were placed in reduced volume reactors and degassed for 20 min with argon. Then, 4 mL of toluene and 1 mL of triethylamine were added followed by the drop wise addition of pentadecafluorooctanoyl chloride (0.8 mL, 3.22×10^{-3} mol). The reaction was allowed to proceed under argon atmosphere at room temperature with continuous stirring (orbital shaker at 100 rpm) for different reaction times (15 min, 4 and 24 h). The surfaces were rinsed with copious amount of methanol and acetone during 24 h and dried under argon flow. The surfaces were stored in a vacuum desiccator under reduced pressure before being subjected to bacterial tests.

Table 1
Energy characteristics (mJ/m²) of pure liquids used for contact angle measurements.

Liquid	δ_L	δ_L^{LW}	δ_L^{AB}	δ_L^+	δ_L^-
Water, H_2O	72.8	19.9	51.0	25.5	25.5
Diiodomethane, CH_2I_2	50.8	50.8	0	0	0
Ethylene glycol, C_2H_6O	48.0	29.0	19.0	1.9	47.0
Formamide, CH_3NO	58.1	39.0	19.1	2.3	39.6

2.4. Surfaces characterizations

2.4.1. Apparatus

Contact angle measurements were carried out by the Young-Laplace method using a DS100 Krüss goniometer with four liquids of known surface properties, i.e. high purity water (Millipore, milliQ), diiodomethane, ethylene glycol and formamide (supplied by Aldrich). The contact angle was measured within 10 s of placing the drop (3 μL) on the surfaces and an average of six measurements was reported.

XPS measurements were performed on a K-Alpha spectrometer from ThermoFisher, equipped with a monochromated X-ray Source (Al Kα, 1486.6 eV). For all measurements a spot size of 400 μm was employed. The hemispherical analyzer was operated in CAE (Constant Analyzer Energy) mode, with a pass energy of 200 eV and a step of 1 eV for the acquisition of surveys spectra, and a pass energy of 50 eV and a step of 0.1 eV for the acquisition of high resolution spectra. A "dual beam" flood gun was used to neutralize the charge build-up. The spectra obtained were treated by means of the "Avantage" software provided by ThermoFisher. A Shirley type background subtraction was used and the peak areas were normalized using the Scofield sensitivity factors in the calculation of elemental compositions.

For atomic force microscopy (AFM), tapping mode topography and phase imaging was accomplished using a di Innova AFM Bruker with NanoDrive v8.02 software. Tapping mode images were acquired using silicon tips from Nanosensors (PPP NCSTR) with a resonance frequency ranging between 76 and 263 kHz. Images were processed using WsXM software, freely available on internet.

2.5. Determination of energetic characteristics of surfaces

The approach of Good, Van Oss and Chaudhury (acid–base theory) was used to calculate the surface free energy [19,20]. The surface energy δ_i is seen as the sum of a Lifshitz–van der Waals apolar component δ_i^{LW} and a Lewis acid–base polar component δ_i^{AB}:

$$\delta_i = \delta_i^{LW} + \delta_i^{AB} \quad (1)$$

The acid–base polar component δ_i^{AB} can be further subdivided by using specific terms for an electron donor (δ_i^+) and an electron acceptor (δ_i^-) subcomponent:

$$\delta_i^{AB} = 2(\delta_i^+ \delta_i^-)^{1/2} \quad (2)$$

The surface energy components of a surface (δ_S^+, δ_S^- and δ_S^{LW}) were determined by performing contact angle measurements (Θ) with four liquids with known surface tension parameters (δ_L^+, δ_L^- and δ_L^{LW}). Using the Young equation, a relation between the