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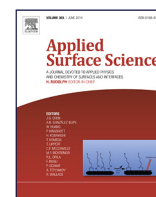

Washing-resistant surfactant coated surface is able to inhibit pathogenic bacteria adhesion

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ABSTRACT

Surface-active substances, which are able to organize themselves spontaneously on surfaces, triggering changes in the nature of the solid–liquid interface, are likely to influence microorganism adhesion and biofilm formation. Therefore, this study aimed to evaluate chemical non-ionic surfactants activity against pathogenic microbial biofilms and to cover biomaterial surfaces in order to obtain an anti-infective surface. After testing 11 different surfactants, Pluronic F127 was selected for further studies due to its non-biocidal properties and capability to inhibit up to 90% of biofilm formation of Gram-positive pathogen and its clinical isolates. The coating technique using direct impregnation on the surface showed important antibiofilm formation characteristics, even after extensive washes. Surface roughness and bacterial surface polarity does not influence the adhesion of *Staphylococcus epidermidis*, however, the material coated surface became extremely hydrophilic. The phenotype of *S. epidermidis* does not seem to have been affected by the contact with surfactant, reinforcing the evidence that a physical phenomenon is responsible for the activity. This paper presents a simple method of surface coating employing a synthetic surfactant to prevent *S. epidermidis* biofilm formation.

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1. Introduction

Biofilms are microbial aggregates which develop adhered to surfaces. The surface of an implantable medical device becomes an ideal environment for bacterial adhesion, and consequently biofilm formation process. Nowadays, it is well accepted in the scientific literature that the way of life adopted by most of the bacteria is in the biofilm form [1]. This way of life is ubiquitous in the environment, and plays a role in the pathogenesis of infectious processes [2]. About 80% of medical infections are associated to biofilm formation [3]. Such infections complicate the clinical course of patients, and create substantial economic and human costs [4,5]. It is estimated that 60–70% of medical infections are associated with some biomedical device [6]. These devices are conceptually known as an apparatus composed of a biomaterial, commonly polymeric materials such as poly(dimethyl siloxane), polyethylene, poly(methyl methacrylate), polyvinyl chloride, polystyrene, among others [7].

The abiotic surface of an implantable/inserted device such as cardiac valves, stents, vascular and urinary catheters, joint prostheses and others becomes ideal environment for providing bacterial adhesion, the first step of biofilm formation [7–9]. Once a bacterium is attached, a multi-step process starts, resulting in a microbial community embedded in a self-produced matrix, a biofilm [10,11]. Bacteria living in this complex way of life are notably more resistant to antibiotics and the host immune system [12,13]. *Staphylococcus* sp. and *Pseudomonas aeruginosa* are the most common pathogens associated with infections [14]. Staphylococci are currently the most common cause of nosocomial infections [15], whereas *P. aeruginosa*, an opportunistic pathogen, possessing many virulence factors, is the mostly responsible for infections on cystic fibrosis patients [16].

The surface chemistry and topography affect biological responses, and are of fundamental importance, especially when living systems (bacteria) encounter synthetic surfaces

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(biomaterial) [17,18]. In recent years, intensive efforts have been focused on improving the performance of existing surfaces, as the application of coatings, or surfaces architecture modification and/or alteration, procedures that advantageously ensure the same biomaterial bulk properties [19–21]. The ability of biofilms to adhere to surfaces led us to explore the use of surface-active substances, molecules inherently capable of position themselves in surfaces and interfaces.

Surfactants or surface active-compounds, both chemical and biological, are amphipathic compounds containing hydrophilic and hydrophobic moieties, which confer the capability of auto-organization between phases, reducing interfacial tensions and forming aggregates such as micelles [22]. This spontaneous process leads to a drastic change in the surface nature, directly affecting the adhesion of microorganisms [23]. Surfactants are classified according to their ionic properties in water (anionic, cationic, non-ionic, and amphoteric) and are used in a wide range of industrial applications, such as pharmaceutical, therapeutic and cosmetic products [24]. It is well established in the literature that poly(ethylene oxide) (PEO) coatings, are a promising method to avoid infections since these macromolecules have the capability to self-assemble on the material surface, juts outwards the PEO chains acting like a barrier between the microorganism and the surface [25]. This self-assemble phenomenon allows a comparison between PEO chains and lumps of a brush, thus PEO coatings are widely called as brush coatings.

Given the high levels of biofilm infection and the emergence of antibiotic-resistant bacteria combined with the slow progress in identifying new antimicrobial agents, combat strategies have inestimable clinical value. In this sense, the aim of this study was to search for antibiofilm activity of distinct nonionic surfactants containing PEO chains in order to obtain a surface covered less susceptible to bacterial adhesion.

2. Experimental

2.1. Surface-active compounds and material

Synthetic chemical surfactants were supplied by Sigma–Aldrich Brazil: Span® 40, Span® 60, Span® 80; Tween® 20, Tween® 40, Tween® 60, Tween® 80; Pluronic® F127, Pluronic® F68; Tyloxapol®, Triton X®-100. As material model, polystyrene 96-well microplates (COSTAR, 3599) and PERMANOX™ slides (NUNC, USA), a polystyrene modified polymer acetone resistant, were used.

2.2. Bacterial strains and culturing

All strains, *Staphylococcus epidermidis* ATCC 35984, *Pseudomonas aeruginosa* ATCC 27853 and 11 *S. epidermidis* clinical isolates were grown aerobically at 37 °C for 24 h on Mueller Hinton agar (OXOID, England) plates from frozen stocks. A bacterial suspension was set in sterile sodium chloride 0.9% to a final concentration of 3×10^8 colony-forming unit per mL (CFU/mL) following McFarland standard was prepared (in practical terms, 80 μL = OD_{600} 0.090 ± 0.03). The clinical strains assayed (50, 73, 92, 113, 117, 122, 124a, 183b, 196, 228 and 229) were isolated from central venous catheters of patients of a Hospital in Porto Alegre between January 2008 and May 2009 [15].

2.3. Antibiotic, antibiofilm formation and biofilms eradication assays

In a 96-well microplate, optical density at 600 nm (OD_{600}) and crystal violet dye method were employed to measure bacterial growth and biofilm formation, respectively [26]. Briefly, in each well was added 80 μL of the surfactant solution, 80 μL of the bacterial suspension and 40 μL of tryptone soya broth (TSB) (OXOID, England). A control without surfactant treatment was carried out for microbial growth control and biofilm formation, the 100%. Non-inoculated TSB was included as a contamination standard. Before and after the incubation period at 37 °C the absorbance in 600 nm was measured to quantify the bacterial growth. For biofilm formation, after the incubation period of 24 h for Staphylococci and 6 h for Pseudomonas, the well content was removed and each well was washed three times with saline to remove planktonic cells, followed by a one hour biofilm fixation process at 60 °C. After that, 200 μL of crystal violet dye was added at each well for 15 min. To ensure removal of dye excess a washing process in running water was performed. The stained biofilm or adherent cells were extracted with 200 μL of dimethylsulfoxide 99.9% and then, the absorbance was measured in 570 nm.

The ability of surfactants to eradicate already formed biofilms was measured by adding 120 μL of TSB and 80 μL of inoculum to a polystyrene plate. After 24 h of *Staphylococcus epidermidis* biofilm formation at 37 °C in aerobic atmosphere, the supernatant was carefully removed in a laminar flow to prevent the disruption of the biofilm formed in the well. After, 120 μL of TSB and 80 μL surfactant solutions (0.5%) or water (control) were added. After 24 h incubation, the eradication ability of the test surfactant was estimated according to the crystal violet staining assay. All assays were performed in six replicates and biological triplicates.

2.4. Impregnation methodologies and washing assays

2.4.1. Surface activation by ionic plasma discharge followed by impregnation

Surface activation attempts were made in the polystyrene surface by an argon plasma discharge. The process was carried out in a cylindrical plasma-reactor made of stainless steel, equipped with a direct current power supply, a vacuum system and a gas reservoir. In order to minimize thermal effects on the sample, the sample holder was kept at a value two thirds below the glass transition temperature of polystyrene (95 °C) throughout the treatment period.

Polystyrene microplates were inserted in the vacuum chamber, which was pressurized to a base pressure of 2×10^{-2} mBar. Radio frequency source was kept at 13.56 MHz with a pressure of 0.6 mBar argon atmosphere (purity >99.999%), to generate the plasma ion. It was estimated that the atoms reach the surface with a power of 100 W for a 2 min treatment period. After depressurization of the chamber, samples were immediately subjected to impregnation with Pluronic F127 solutions. The controls for the plasma treatment were conducted exposing polystyrene samples only to argon gas.

Immediately after plasma treatment, the surface was covered with aqueous surfactant solution and left at 60 °C for 24 h. To ensure the stability of the impregnation process, the microplate wells were washed 150 times. The washing procedure was realized manually using a micropipette under laminar flow, with ultrapure sterile water as washing liquid.

2.4.2. Direct impregnation of the surface

Polystyrene 96-well microplates were directly impregnated (covered) with two different solutions containing Pluronic F127. The surface was treated for 12 h at room temperature with Pluronic F127 on laminar flow in: (1) aqueous solution and (2) ethanolic solution. Since ethanol is a volatile solvent, after its evaporation occurs a surfactant deposition and the surface becomes dry. To ensure the impregnation process stability of both surfaces, microplate wells were washed according to a sequence of 100 washing steps with two different liquids, MilliQ sterile water and

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