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Polymer/metal nanocomposite coating with antimicrobial activity against hospital isolated pathogen

D. Carvalho[a], T. Sousa[b], P.V. Morais[b], A.P. Piedade[a,*]

[a] CEMUC—Department of Mechanical Engineering, University of Coimbra, Rua Luís Reis Santos, 3030-788 Coimbra, Portugal
[b] CEMUC—Department of Life Sciences, University of Coimbra, 3001-401 Coimbra, Portugal

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ABSTRACT

Nosocomial infections are considered an important problem in healthcare systems and are responsible for a high percentage of morbidity. Among the pathogenic microorganisms responsible for this situation *Pseudomonas aeruginosa* (*P. aeruginosa*) is consider one of the most hazardous also due to the fact that antibiotic resistant and multi-resistant organisms begin to emerge as the prevalent strains. In this work the surface of poly(tetrafluoroethylene) (PTFE) was modified by the deposition of PTFE thin films with and without silver. The hydrophobic characteristics of PTFE were attenuated by the co-deposition of PTFE and poly(amide) (PA) with and without silver. The results show that this hospital isolated bacteria is able to degrade PTFE as bulk material as well as some of the developed thin films. However, the combination of both polymer and metal induced the formation of a nanocomposite structure with antimicrobial properties against *P. aeruginosa*, assessed in three different biotic tests.

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1. Introduction

Nosocomial infections are a public health problem which contributes to the overload of healthcare systems [1–3]. These infections are often associated with surgical interventions for the insertion of invasive medical devices such as joint prosthesis, heart valves, vascular catheters and ligaments [4–6], and result from the capacity of a bacterial strain to colonize the implant and to form a biofilm [7,8]. In fact it is estimated that over 65% of nosocomial infections and 80% of all microbial infections are biofilm associated [9]. When the infection reaches the point of biofilm formation its control by pharmaceuticals drugs is generally very complicated and hardly effective [10], resulting in most cases in the removal of the infected implant, with the consequent economic and health implications for the healthcare system and patient, respectively. For these reasons, it has been recognize that the most effective way to prevent biofilm formation is the suppression of the initial irreversible bacterial adhesion to the surfaces rather than the chemical- or drug-based treatments after bacterial colonization [11].

Within the microorganisms that are listed as responsible for hospital-acquired infections *Pseudomonas aeruginosa* (*P. aeruginosa*), an opportunistic bacterium, is considered one of the most harmful pathogens commonly implied in the infections of indwelling medical devices and a frequent cause of pathogen in general healthcare associated infections and morbidity [12,13]. This gram-negative microorganism is widely spread in hospital environments and has the capacity to rapidly develop a biofilm in several surfaces, and most strains are now resistant to conventional antibiotics [13–16]. Therefore, it seems of great interest the development of biocompatible materials that prevent the colonization of *P. aeruginosa*.

Considering the area of invasive polymeric devices poly(tetrafluoroethylene) (PTFE) is widely used in the production of several medical indwelling devices such as cardiovascular grafts, ligaments, catheters and spacers, just to name a few applications [17,18]. This polymer is characterized by its chemical stability, with a high degree of structural order, high thermal resistance and excellent mechanical strength [19,20]. In biomedicine, PTFE is able to respond positively to the peculiar conditions of the human body, such as changes in pH, high mechanical stresses and interactions between different types of tissues, being considered biocompatible, chemically and biologically inert [19,21]. However, given the degree of infectivity of *P. aeruginosa*, even the materials traditionally considered biologically inert, such as PTFE, cannot be excluded of colonization by this bacterial strain.

Considering the extensive use of PTFE as a biomaterial there have been made several approaches regarding the surface modification of PTFE in order to confer it the so desired antimicrobial

* Corresponding author.
E-mail address: ana.piedade@dem.uc.pt (A.P. Piedade).

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Table 1
Designation and deposition parameters of the deposited thin films.

Designation	Deposition density (W mm^{-2})		Ag foils	Discharge gas/Pressure(Pa)	Deposition time (s)	Gas in the cooling stage
	PTFE	PA				
PTFE0	3.2×10^{-2}	–	no	Ar/0.7	900	none
PTFE0_Ag	3.2×10^{-2}	–	yes	Ar/0.7	900	none
PTFE1	3.2×10^{-2}	–	no	Ar + air/0.6 + 0.1	900	air (0.1 Pa)
PTFE1_Ag	3.2×10^{-2}	–	yes	Ar + air/0.6 + 0.1	900	air (0.1 Pa)
PTFE2	3.2×10^{-2}	7.6×10^{-3}	no	Ar/0.7	900 + 300	none
PTFE2_Ag	3.2×10^{-2}	7.6×10^{-3}	yes	Ar/0.7	900 + 300	none

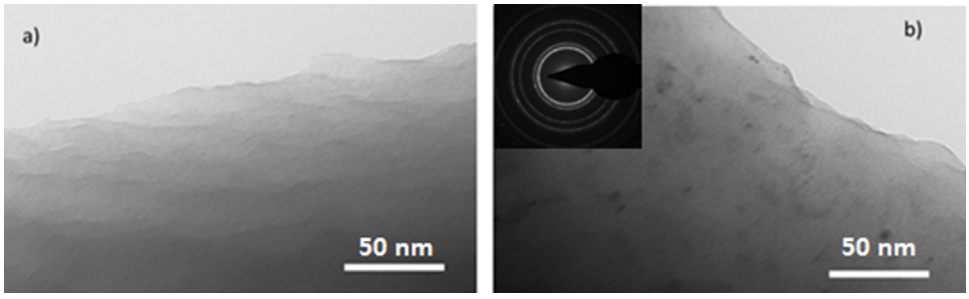

Fig. 1. TEM bright field images of PTFE0 (a) and PTFE0_Ag with corresponding electron diffraction pattern (b).

properties. These include the use of covalent and non-covalent attached antibiotics [22–25], enzymes [26], bacteriophages [27] or by incorporating PTFE with silver using different technologies [28–33]. Silver is an inert material in its metallic state and as a bulk material. However, silver ions (Ag^+) are reported to present antimicrobial properties concomitantly with low values of toxicity to eukaryotic cells [34,35]. The antimicrobial activity of Ag^+ it is not fully elucidated but some studies indicate that it is due to its interaction with the sulfhydryl groups present on the surface of microorganisms, specifically by replacing the hydrogen atoms. This modification inhibits respiration and electron transfer, leading to the collapse of the electro protomotriz force, resulting in cell death [35]. Silver toxicity depends on the production and availability of Ag^+, therefore to obtain an extended antimicrobial effect, the silver ions must be released slowly and continuously [36]. The use of silver nanoparticles has been widely reported as antimicrobial agent. Due to its nanometric dimensions several action mechanisms have been reported which also includes the formation of Ag^+. In fact, due to their high surface area to volume ratio a more favorable kinetic for the formation of silver ions must be considered. In fact, it has been reported that silver nanoparticles suspended in pure water dissolute up to 90% of their initial weight in the ionic form, Ag^+ [37].

Thereby, the objective of this work is to modify the surface of PTFE not by the conventional graft polymerization methodology [38] but by the deposition of PTFE and of PTFE/poly(amide) (PA) thin films deposited by radio-frequency (rf) magnetron sputtering, with and without the incorporation of Ag, and to assess their resistance and antibacterial properties against hospital isolated *P. aeruginosa*. The studied strategy allows a perfect chemical compatibility between the bulk PTFE and the coating thus avoiding the presence of interfaces which are often responsible for the failure of the modification surface strategy.

2. Experimental

2.1. Deposition technique

All reagents and materials were purchased from commercial sources and were used as received, unless otherwise stated. Thin films were deposited, in a non-reactive mode using Argon as the discharge gas, using a radiofrequency (r.f.) sputtering equipment, Edwards Coating System E306A, equipped with two power supplies of 1000 and one of 500 W, branched to the two assisted magnetron targets and substrate holder, respectively. The parameters used in the deposition were: 10^{-4} Pa ultimate vacuum pressure; 3.2×10^{-2} W mm^{-2} and 7.6×10^{-3} W mm^{-2} discharge power densities for PTFE and PA targets, respectively; 0.7 Pa total discharge pressure; and 900 s (PTFE) and 300 s (PA) deposition times. For the PTFE/PA thin films the coatings consist of two successive deposits: first PTFE and then PA. Both polymeric targets (99.9% purity from Goodfellow, UK) with 100 mm in diameter and 5 mm thickness were used. For the thin films doped with silver, four 10×10 mm metallic foils were placed over the PTFE target in the area of higher erosion. After the deposition the cooling stage was performed either in vacuum or in the presence of atmospheric air at the pressure of 0.1 Pa.

2.2. Characterization techniques

2.2.1. Chemical characterization

The XPS (X-ray photoelectron spectroscopy) analyses were performed in a VG–ESCLAB 250iXL spectrometer. The pressure in the analysis chamber was kept below 5×10^{-8} Pa and the analysis were performed using monochromatic radiation Al-Kα ($h\nu$ = 1486.92 eV). The photoelectrons were collected with an angle of 90° with respect to the surface of samples. The energy step was of 20 eV for the survey spectra and of 0.05 eV for the high-resolution spectra. The chemical compositions were obtained using the sensitivity factor of the Scofield library. The XPS was also used for the in-depth analysis of some of the thin films.

2.2.2. Surface characterization

The wettability characteristics of thin films were assessed by measuring the static contact angle of the surfaces with 10 μL of distilled and deionized water, and formamide in a DataPhysics QCA-20 143 apparatus. For each sample, triplicates were used and a minimum of seven measurements was taken, after allowing the system (air–water–surface) to reach equilibrium, and the average value calculated. The contact angle values were also used to determine the surface tension γ_s as the sum of its polar, γ_s^p, and dispersive, γ_s^d, components according to previously described procedure [39].

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