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The effect of deposition electrolyte on polypyrrole surface interaction with biological environment

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ABSTRACT

The effects of electrolyte type used in electrodeposition of polypyrrole (PPy) films on Ti6Al7Nb alloy was studied in order to design a titanium modified surface with enhanced antibacterial activity and better biocompatibility. Therefore, the polypyrrole coatings were synthesized by potentiostatic electrochemically technique from pyrrole and lithium perchlorate ($LiClO_4$) using aqueous and non-aqueous solutions. The both PPy films were characterized by electrochemical methods in Hank's Balanced Salt Solution (HBSS), and surface characterization by Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM) analysis, adhesion test and contact angle measurements. A correlation between the film stability and surface properties, synthesis parameters and the interaction with biological environment was established. The physical-chemical properties of the studied PPy films are in direct related with the doping level and have an important influence of the biocompatibility and antibacterial activity.

1. Introduction

The performance of implanted medical devices which is strictly depended on the material and its properties is crucial in current clinical applications [1]. Titanium and its alloys, mainly ternary alloys such as Ti-6Al-7Nb or Ti-6Al-4V, are widely used in medicine as materials for artificial hip or dental implants due to their unique mechanical, chemical and biological properties [2,3]. However, the insertion of any implant may lead to infections, taking into account that the bacteria adheres onto biomaterial surfaces and increases resistance to antibiotics [4]. Unmodified titanium is susceptible to bacterial infections, having a risk of plaque formation on titanium implants which might lead to implant failure, and such infections lead to devastating complications with high morbidity and treatment costs [5]. Consequently, in order to prevent attachment of bacteria on the surfaces of implants and to ensure their long-term clinical success, the development of various types of antimicrobial surfaces through specific treatments is necessary [6]. In this approach, in the literature were proposed various antibacterial coatings on titanium and titanium alloys [7] which include elaboration of various bioactive coatings [8,9], organic and inorganic antibacterial agents incorporated coatings and physically/chemically treated non-adhesive surface coatings [5,10] and more recently polymeric films at micro and nanolevel [11–13], such that bacterial adhesion could be reduced and/or the bacteria are killed upon contact with the surfaces [6].

It is well known that polymeric antimicrobial agents are non volatile, chemically stable, and do not permeate through the skin [14]. As a result, the application of polymers with antibacterial activities will be a major step toward a healthier living. Conducting polymers, such as Polyaniline (PAN), Polypyrrole (PPy), Poly O-Phenylenediamine (POPD), Poly (aminophenol) (PAP) have recently been paid a lot of attention for its application in biochemistry, biosensor, the immobilization of protein and enzyme [15] and effective antibacterial materials due to discoveries of bacteria new forms associated with frequent use of antibiotics [16].

Also, the growth of cells on conducting polymers showed a low cytotoxicity and good biocompatibility [17,18]. The biomedical applications of conducting polymers are extensive and include development of artificial muscles, controlled drug release and stimulation of nerve regeneration [19,20].

Polypyrrole, obtained either by chemical or electrochemical oxidation of pyrrole monomer [21], has attracted attention more than other conducting polymers due to their stability and important redox reversibility. PPy is easily deposited from aqueous and non-aqueous media [22], is adherent to many types of substrates, and is well-conducting and stable. Electrochemical synthesis of PPy is the most common method as it is simpler, quick and perfectly controllable and also, produces thin films with a thickness of few micrometers on an electrode surface [23], while a chemical oxidation yields a fine-grained material. However, to effectively

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use of PPy as a biomaterial coating, it is important to understand and reproducibly control the electrical properties, physical topography and surface chemistry of the polymer (hydrophilicity, surface roughness), and the biological effects of these properties on cells and on their antibacterial activity [24].

In fact biomedical coatings used to improve the tissue-biomaterial interface should be viewed more complex aiming at more features of these biometarials such as bioactivity and biocompatibility, anticorrosion and antiwear properties and antibacterial activity.

The quality of the resulting polymer films differ greatly in their characteristics on synthesis parameters such as nature and concentration of monomer and dopants, solvent, substrate [25,26], among other parameters.

One of the problems regarding conducting polymers is the adherence on the oxidizable metallic surfaces during electrodeposition, which determine the film detaching in time during immersion in different media [27]. The interaction of the conducting polymer coatings with the biological environment are in close relation with the electrostability and morphology of the PPy films which were found to depend on the doping anion and doping level [28–30].

In this study, we have synthesized PPy coatings on the Ti6Al7Nb alloys using aqueous and non-aqueous electrolytes in order to investigate the influence of electrolyte type on the PPy film doped with ClO^-_4. The important biomedical features such as cell adhesion and proliferation and antibacterial activity were studied and correlated with other PPy film characteristics such as doping process, surface roughness, morphology, wettability, conductivity.

The results of the present work should provide practical insight for biomedical applications on the relationships between synthesis parameters of polymeric coatings and their interaction with biological environment.

2. Material and methods

2.1. Samples preparation

As working electrodes Ti6Al7Nb samples with disk-shape, 1 cm diameters and 2 cm thickness were elaborated. The chemical composition of Ti6Al7Nb was as follows: 0.1% C, 5.88% Al, 6.65% Nb, 0.3% Fe, 0.05% N, 0.2% O, rest Ti. Their surface was polished before electrodeposition to a mirror finish with two kind of silica carbide paper from 320 to 4000 type and rinsed well in ultrasonic. All impurities were removed by immersing in ethyl alcohol and in distilled ultra sonnicated water at room temperature.

Pyrrole (Py) monomer was purchased from Aldrich, distilled and stored in the dark at −20 °C prior to use. The electrolytic medium of electropolymerization was prepared by dissolving 0.1 mol L^{-1} Py + 0.1 mol L^{-1} $LiClO_4$ (≥99%, Aldrich) in water (PPy/ClO_4^-/w) and 0.1 mol L^{-1} Py + 0.1 mol L^{-1} $LiClO_4$ in acetonitrile (ACN) (99%, Aldrich) (PPy/ClO_4^-/acn).

The PPy/ClO_4^- coatings were synthesized in both water and ACN solutions of monomer, maintaining the Ti6AlNb electrode under potentiostatic conditions for 500 s at 0.9 V versus Ag/AgCl [22,31].

The adhesion strength of obtained polymer films in both conditions was determined using a PosiTest Adhesion Tester, model AT-M, from DeFelsko corporation, following the ASTM D4541-02 procedure E [32].

2.2. Surface characterization

2.2.1. SEM characterization

SEM images were taking with a FEI Nova NanoSEM 630 scanning electron microscopy equipment with EDAX (emission in X-rays module for chemical constituents' distribution). The voltage of SEM analysis was 20 kV. The magnification of the images was between 3000 and 50,000.

2.2.2. AFM determinations

The roughness of the surface samples were evaluated with atomic force microscopy (AFM) from APE Research, Italia in contact mode. Data acquisition were performed with Gwyddion software.

2.2.3. Wettability

The contact angle between the liquid (distilled water) and tested materials has been measured using a Contact Angle Meter KSV Instruments CAM 100.

Each contact angle value is the average of minimum 10 measurements. The investigation was carried out with an accuracy of ±1° at a temperature of 25 °C.

2.3. Electrochemical behaviour of samples

The stability of PPy/ClO_4^-/w and PPy/ClO_4^-/acn coatings was evaluated from *Tafel plots and electrochemical impedance spectroscopy (EIS).* The electrochemical measurements were performed using a one compartment cell with three electrodes: Ti6Al7Nb/PPy as working electrode, platinum counter-electrode and Ag/AgCl, KCl reference electrode, connected to Autolab PGSTAT 302N potentiostat with general-purpose electrochemical system software. The tested electrochemical bioliquid was Hank's Balanced Salt Solution (HBSS) [33] containing (in g/L): 8 NaCl, 0.4 KCl, 0.35 $NaHCO_3$, 0.25 $NaH_2PO_4{\cdot}H_2O$, 0.06 $Na_2HPO_4{\cdot}2H_2O$, 0.19 $CaCl_2{\cdot}2H_2O$, 0.19 $MgCl_2$, 0.06 $MgSO_4{\cdot}7H_2O$, 1 glucose, at pH = 6.9.

(a) To substantiate the anticorrosion properties of conducting polymer films, the Tafel regions of cathodic and anodic polarization curves were extrapolated. Tafel plots were obtained by polarization with ±150 mV vs. Ag/AgCl toward electrode potential in anodic direction with a scan rate of 2 mV/s.
(b) The EIS measurements were conducted at open circuit potential in studied solution and in frequency range 0.01–10.000 Hz with amplitude by ±10 mV. EIS analyses were discussed in term of Bode representations.
(c) The Mott–Schottky measurements were performed from a start potential of −0.5 V to an end potential of 0.5 V with a step potential of 50 mV.

2.4. Evaluation of antibacterial activity of polymeric coatings

For antibacterial activity evaluation, two sterile samples PPy/ClO_4^-/w and PPy/ClO_4^-/acn and one Ti6Al7Nb as control were used.

Growth in *liquid* media was *evaluated* using *Escherichia coli* (ATCC 8738) and *Staphylococcus aureus* (ATCC 25923) strains. Bacteria strains were cultured in a tube containing Luria Bertani (LB) medium as reported recently by Ansari et al. [34] at 37 °C (Luria Bertani medium composition: peptone, 10 g/L; yeast extract 5 g/L and NaCl 5 g/L).

Sterile samples were incubated 18 h of test tubes containing 1 mL culture of a gram negative bacterium, *E. coli* respectively a gram positive bacterium, *S. aureus.*

The sterile medium was inoculated with 100 μL of bacteria strains (1%). Once obtained 10 mL of culture were placed over the samples. Optical density was determined after 18 hours of incubation. Incubation was performed in the incubator Laboshake Gerhardt at 150 rpm.

The bacterial growth was determined by measuring optical density for the four samples and control (bacteria culture without