Materials Science and Engineering C 42 (2014) 726–733

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Improved antibacterial behavior of titanium surface with torularhodin–polypyrrole film

Camelia Ungureanu [a], Simona Popescu [a], Gabriela Purcel [a], Vlad Tofan [c], Marian Popescu [a,b], Aurora Sălăgeanu [c], Cristian Pîrvu [a,*]

[a] University POLITEHNICA of Bucharest, 1-7 Polizu, 011061 Bucharest, Romania
[b] National Institute for Research and Development in Microtechnologies, 126A, Erou Iancu Nicolae Street, 077190 Bucharest, Romania
[c] "Cantacuzino" National Institute of Research-Development for Microbiology and Immunology, 103 Splaiul Independentei, Sector 5, 050096 Bucharest, Romania

ARTICLE INFO

Article history:
Received 16 September 2013
Received in revised form 17 May 2014
Accepted 15 June 2014
Available online 20 June 2014

Keywords:
Titanium
Polypyrrole
Torularhodin
Biomedical devices
Antimicrobial activity

ABSTRACT

The problem of microorganisms attaching and proliferating on implants and medical devices surfaces is still attracting interest in developing research on different coatings based on antibacterial agents. The aim of this work is centered on modifying titanium (Ti) based implants surfaces through incorporation of a natural compound with antimicrobial effect, torularhodin (T), by means of a polypyrrole (PPy) film. This *study* tested the potential *antimicrobial activity* of the new coating against a range of standard bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. The morphology, physical and electrochemical properties of the synthesized films were assessed by SEM, AFM, UV–Vis, FTIR and cyclic voltammetry. In addition, biocompatibility of this new coating was evaluated using L929 mouse fibroblast cells. The results showed that PPy–torularhodin composite film acts as a corrosion protective coating with antibacterial activity and it has no harmful effect on cell viability.

1. Introduction

There is an increasing demand for biomedical devices, and, moreover, clinicians are expressing their desire in improving these devices and implants via an antibacterial coating [1]. Microorganisms can colonize surfaces of synthetic materials, implants and other medical devices with the possibility of generating bacterial biofilms which are more difficult to combat than bacteria themselves, due to the ability of the colony to grow a protective extracellular bacterial polysaccharide layer. Bacterial pathogens including Gram positive (such as *Enterococcus, Staphylococcus*, etc.) and Gram negative (such as *Escherichia coli, Pseudomonas aeruginosa*, etc.) bacteria, can cause severe infections in humans. Most of these organisms have the ability to survive in severe conditions due to their adaptation to multiple environmental habitats [2]. Device-related infections (DRIs) caused by microorganisms attaching and proliferating on surfaces of implants and medical devices are a significant issue in implant surgery [3,4]. DRIs are not often detected at an early stage and not only cause serious pain to patients but also increase the medical cost [5]. A new strategy in reducing the occurrence of DRIs is to prevent the initial attachment of microorganisms to implant surfaces. Thus, there are a strong need and an increasing interest to prevent bacterial biofilm formation by applying different coatings, based on antibacterial agents that can be inappropriate for bacterial attachment to implants and biomedical devices surfaces [6,7].

Polymeric materials offer great flexibility for the design of medical devices [8]. However, only a few polymers are known to kill bacteria [9,10] or to prevent bacterial adherence [11]. These polymers are suitable for the fabrication of implants in terms of strength or flexibility. Polypyrrole (PPy) films deposited on titanium (Ti) substrates can be used to graft biologically active molecules [12,13] which accelerate the osseointegration process [14] and to protect the surface of the substrate against corrosion [15–17]. Ti is a material that exhibits long-lasting resistance to corrosion in physiological fluids due to instant formation of a protective and stable oxide film. In addition, it is biocompatible and it has suitable mechanical characteristics warranting its use as one of the most important materials in clinical applications [18]. However, integration of Ti-based materials has known failures due to possible partial dissolution of the protective titanium dioxide overlayer and to ion diffusion through the surrounding tissues [19]. For medical applications, PPy coatings are usually electrochemically generated on titanium substrate with the incorporation of various anionic species, also including negatively charged biomolecules, such as proteins and polysaccharides, to yield composite materials [20]. The preferred procedure to obtain PPy is the electrochemical synthesis due to the simplicity of the technique,

* Corresponding author at: General Chemistry Department, Faculty of Applied Chemistry and Materials Science, University Politehnica of Bucharest, Romania, 1-7 Polizu, 011061 Bucharest, Romania. Tel.: +40 214023930; fax: +40 213111796.
E-mail addresses: c_pirvu@chim.pub.ro, c_pirvu@yahoo.com (C. Pîrvu).

http://dx.doi.org/10.1016/j.msec.2014.06.020
0928-4931/

the control over material thickness, geometry and location, the facility for doping during synthesis using appropriate dopant ions and the production of good quality films [13]. Current studies have shown that the PPy supports cell adhesion and growth of different cell types, including macrophage cells [12], endothelial cells [21], neurons and support cells (i.e glia, fibroblasts) associated with dorsal root ganglia (DRG) [22], primary neurons [23], keratinocytes [24], and mesenchymal stem cells [25]. A number of recent studies have shown the compatibility of PPy *in vitro*, using different cells and tissues and *in vivo* [22]. In order to enhance antibacterial properties of the polymer films, composite coatings based on PPy and polyethylene glycol (PEG) were successfully used on Ti alloy substrate [26].

Some natural compounds are emerging as promising new antibacterial agents [27] and they are used to obtain biocompatible composite coatings [28,29]. These compounds could be an alternative to antibiotics that have proved to possess limited effectiveness. It is costly and out of range for patients belonging to underdeveloped countries or communities and microorganisms develop resistance against antibiotics [30]. Also, antibiotics may be associated with adverse effects including immune suppression, and allergic reactions [31]. Torularhodin (T) is a carotenoid pigment with a terminal carboxylic group produced by the yeast *Rhodotorula rubra* and it is considered a powerful antioxidant and antimicrobial compound which can be included in food and drugs formulations [32]. Torularhodin exhibits antioxidant action by capturing active oxygen, it is effective in preventing formation of lipid peroxide or the oxidative injury in the living body [33,34]. Reactive oxygen species can attack various targets to exert antimicrobial activity, which helps to account for their versatility in mediating host defense against a broad range of pathogens [35]. Beside the formulation as a nutritional supplement other formulations of torularhodin are envisaged: the product can be used as natural dye or stabilizing agent for food, pharmaceuticals, cosmetics, for any final natural product susceptible to oxidation [36].

The main goal of this study is to perform the modification of Ti implant surfaces by incorporating torularhodin resulting thus a new PPy based composite coating with antibacterial activity. The microorganisms used in this study were selected due to their clinical importance in terms of Ti medical applications and association with the endodontic infection [37,38]. The presence of *Enterococcus faecalis* and *Staphylococcus aureus* in root canals has been reported in therapy-resistant periapical periodontitis [39–41]. Even though *E. coli and Bacillus subtilis* are not commonly found in root canals with necrotic pulp, some studies found these bacteria in root canals with periapical lesions [42–45]. A persisting endodontic infection with *P. aeruginosa* is also described together with a review of its presence in saliva and root canal infections by Ranta et al. [46].

2. Materials and methods

2.1. Preparation of substrate

PPy films were deposited on commercially pure Ti discs of 10 mm diameter and 1 mm thickness (99.6% purity - grade 2), Goodfellow Cambridge Ltd., UK). For electrochemical experiments the electrode area exposed to the solution was 40 mm^2. The surface of test specimens was polished with SiC paper to grade 4000 and then washed with a large amount of water followed by acetone and finally rinsed with distilled water and dried in air at room temperature.

2.2. Torularhodin biosynthesis

Optimization experiments for culture growth and torularhodin biosynthesis, including the extraction and quantification of pigments were described in previous studies [47–49].

2.3. Film deposition

Pyrrole was used as purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Polymerization of pyrrole on the Ti substrate was performed by cyclic voltametry between 0 and 1.1 V vs. Ag/AgCl, 5 cycles, with a sweep rate of 50 mV/s, from an electrolyte solution containing 0.2 M oxalic acid and 0.2 M Py monomer [16,17]. For PPy–torularhodin film deposition, torularhodin was added in the Py polymerization electrolyte solution in a volumetric ratio PPy:torularhodin 10:1. The electrochemical process was carried out in one compartment cell with three electrodes configuration: a platinum counter electrode, an Ag/AgCl reference electrode and the Ti sample as working electrode. An Autolab PGSTAT 302N was used for electrochemical experiments including electrodeposition and characterization. The data was collected with NOVA 1.9 software. After electrodeposition, the sample with a PPy film was removed from the polymerization medium, rinsed with distilled water and dried in air.

2.4. Methods for coating characterization

All electrochemical characterizations were made in an aqueous buffer testing solution composed of NaCl 8.74 g/L, $NaHCO_3$ 0.35 g/L, $Na_2HPO_4 \cdot 12H_2O$ 0.06 g/L and NaH_2PO_4 0.06 g/L at pH 6.7. The substances were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA).

2.4.1. Electrochemical characterization

EIS measurements were made on a logarithmic distribution range between 100 kHz and 10 mHz, using a small excitation amplitude of 10 mV. The EIS fitting was performed using NOVA 1.9 software. In order to appreciate the anticorrosion properties, the Tafel regions of cathodic and anodic polarization curves were extrapolated with Autolab equipment. Tafel plots were obtained by polarization with ± 150 mV vs. OCP toward electrode potential in anodic direction with a scan rate of 2 mV/s. The main electrochemical parameters were computed based on the Tafel plots: i_{cor} (corrosion current density), R_p (polarization resistance), E_{cor} (corrosion potential), and v_{cor} (corrosion rate).

2.4.2. Surface characterization

Surface analysis was performed with an atomic force microscope (AFM) from APE Research, Italy. The microscopy data were processed with Gwyddion 2.9 software. For SEM imaging we employed a FEI Nova NanoSEM 630 FEG-SEM (SEM with Field Emission Gun) with ultra-high resolution characterization at high and low voltage in high vacuum. The characterization process was carried out at 20 kV in high vacuum and the magnification of images was between 3000 and 50,000×. The contact angle of a drop of water with the film surface was measured with a Contact Angle Meter—KSV Instruments CAM 100 equipment. The hydrophilic/hydrophobic balance of synthesized films was evaluated by measuring the static contact angle of a drop of water deposited on the film surface. Each contact angle value is the mean value from 5 measurements. The investigation was carried out at 25 °C with an accuracy of $\pm 1°$. For FT-IR spectroscopy, the Brucker VERTEX 70 instrument equipped with a Harrick MVP2 diamond ATR device was used. The UV–VIS analysis for torularhodin, PPy and PPy–torularhodin films was achieved with a Spectrophotometer UV–VIS-NIR Shimadzu UV-3600. All spectra were recorded in the wavenumber range from 4000 to 600 cm^{-1}.

2.4.3. Evaluation of antibacterial activity

Antibacterial activity of samples was tested against Gram positive and Gram negative bacteria from American Type Culture Collection (ATCC). In the present study, a total of five bacterial strains were used for the assay. Three of them were Gram positive, i.e. *B. subtilis* (ATCC 6633), *S. aureus* (ATCC 6538), and *E. faecalis* (ATCC 29212) and two were Gram negative namely *E. coli* (ATCC 8738) and *P. aeruginosa* (ATCC 9027). The bacterial strains were cultivated in tubes containing