Materials Science and Engineering C 35 (2014) 36–42

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Hydroxyapatite-silver nanoparticles coatings on porous polyurethane scaffold

Gabriela Ciobanu *, Simona Ilisei, Constantin Luca

"Gheorghe Asachi" Technical University of Iasi, Faculty of Chemical Engineering and Environmental Protection, Prof. dr. docent Dimitrie Mangeron Rd., no. 63, 700050, Iasi, Romania

ARTICLE INFO

Article history:
Received 3 July 2013
Received in revised form 28 September 2013
Accepted 19 October 2013
Available online 31 October 2013

Keywords:
Polyurethane
Hydroxyapatite
Silver nanoparticles
Coating

ABSTRACT

The present paper is focused on a study regarding the possibility of obtaining hydroxyapatite-silver nanoparticle coatings on porous polyurethane scaffold. The method applied is based on a combined strategy involving hydroxyapatite biomimetic deposition on polyurethane surface using a Supersaturated Calcification Solution (SCS), combined with silver ions reduction and *in-situ* crystallization processes on hydroxyapatite-polyurethane surface by sample immersing in $AgNO_3$ solution. The morphology, composition and phase structure of the prepared samples were characterized by scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM-EDX), X-ray diffraction (XRD), UV-Vis spectroscopy and X-ray photoelectron spectroscopy (XPS) measurements. The data obtained show that a layer of hydroxyapatite was deposited on porous polyurethane support and the silver nanoparticles (average size 34.71 nm) were dispersed among and even on the hydroxyapatite crystals. Hydroxyapatite/polyurethane surface acts as a reducer and a stabilizing agent for silver ions. The surface plasmon resonance peak in UV-Vis absorption spectra showed an absorption maximum at 415 nm, indicating formation of silver nanoparticles. The hydroxyapatite-silver polyurethane scaffolds were tested against *Staphylococcus aureus* and *Escherichia coli* and the obtained data were indicative of good antibacterial properties of the materials.

1. Introduction

One of the most important applications of a biomaterial (polymeric or metallic) is the replacement of a damaged hard tissue. Implant surface properties are of key importance for initial tissue interactions, the acceleration of bone healing and osseointegration [1].

In recent times, the coating of implants has produced much interest for improving osseointegration and preventing adverse tissue reactions. The concept of multiple functionalities for surface coating of implants is still in the initial stage of development. These multifunctional coatings should be nontoxic, to mitigate possible adverse tissue responses including the foreign body reaction and implant infection, to be easily applied, efficient and cost-effective [2].

Much research has been directed to the coating of orthopedic and dental implants with porous layers to increase hard tissue integration *in vivo* [3]. The hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, is one of the materials able to form a direct and strong binding between the implant and bone tissue [4]. The synthetic form of hydroxyapatite has been widely investigated due to the similar chemical composition to the mineral matrix of bone, which is generally referred to as hydroxyapatite. The coatings with hydroxyapatite can be considered as bioactive because they promote a direct bone–implant contact without an intervening connective tissue layer leading to a proper biomechanical fixation of implants [5,6]. A diversity of coating methods including ion sputtering, laser ablation, plasma spraying, sol–gel, electrophoretic deposition, hydrothermal and biomimetic methods are usually applied to produce bioactive hydroxyapatite coatings over the solid implants [6–8].

Recent research efforts are focused on combining the best characteristics of both hydroxyapatite and polymers to create a material to be used for hard tissue replacement applications [9]. The polymer/hydroxyapatite composites have attractive features as candidates for novel bone substitutes because they may show bone-bonding capacity and mechanical performances derived from the organic substrate [10,11]. Using the biomimetic method, the hydroxyapatite can be added to synthetic polymers to develop biomimetic composites for bone regeneration. Consequently, these polymers/hydroxyapatite hybrids are expected to act as an artificial bone exhibiting bioactivity and mechanical performance similar to those of natural bone. The polyurethane polymer is widely engaged in numerous biomedical applications due to the excellent mechanical properties and high flexural endurance [12]. In addition to that, the hydroxyapatite/polyurethane composites are developed to enhance the mechanical and bioactive properties for bone repair or substitute [13,14].

A very important problem of using implants is the occurrence of bacterial infections when placed within the human body. This shortcoming can be overcome by modifying the implant surfaces by means of antibacterial coatings while maintaining the good biocompatibility. Among the different antimicrobial agents, silver has been the most extensively studied, being used since ancient times against infections and for preventing the spoilage. The antibacterial, antifungal and antiviral properties of silver ions, silver compounds and silver nanoparticles

* Corresponding author. Tel.: +40 741025163.
E-mail address: gciobanu03@yahoo.co.uk (G. Ciobanu).

0928-4931/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.msec.2013.10.024

have been extensively studied [15]. The silver was found to be non-toxic to human organism in low concentrations. It has also strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities. The microorganisms do not develop as much resistance against silver as the antibiotics do since the silver attacks a broad range of targets in the microbes [16].

The silver incorporation into hydroxyapatite coatings is an alternative that can provide good antibacterial properties of these coatings. There are several methods to introduce silver into hydroxyapatite coatings, such as electrochemical deposition, laser deposition, plasma spraying, ion beam-assisted deposition, magnetron sputtering, micro-arc oxidation and sol-gel technology [17–20].

Currently there are no studies on biocomposites consisting of hydroxyapatite-silver coating on polyurethane polymer. Consequently, in this paper a combined strategy involving biomimetic approach and silver reduction process has been advanced to deposit a hydroxyapatite/silver layer on polyurethane scaffolds able to regenerate the natural bone and mitigate implant infection. The bactericidal activity of the hydroxyapatite/silver/polyurethane scaffolds against *Escherichia coli* and *Staphylococcus aureus* bacteria was investigated and the inhibition ratio evaluated as the antimicrobial efficiency.

2. Experimental

2.1. Materials and methods

The porous polyurethane scaffolds were prepared by phase inversion method described in previous works [21,22]. The used materials were: polyurethane polymer (Institute of Macromolecular Chemistry "P. Poni" of Iasi, Romania), N,N-dimethylformamide (DMF, Sigma Aldrich, Germany) as a solvent, and deionized water as a nonsolvent. The polyurethane films obtained were about 100–500 μm thickness.

The supersaturated calcification solution (SCS) was prepared by dissolving the following chemicals in deionized water: $CaCl_2 \cdot 2H_2O$, $NaH_2PO_4 \cdot H_2O$ and $NaHCO_3$ (Sigma-Aldrich, Germany). The ion concentrations of SCS solution were of: 4.0 mmol/L Na^+, 5.0 mmol/L Ca^{2+}, 10.0 mmol/L Cl^-, 2.5 mmol/L $(H_2PO_4)^-$, and 1.5 mmol/L $(HCO_3)^-$.

The biomimetic method applied to coating polyurethane surface with hydroxyapatite layer consists in immersion of polymeric samples in SCS solution at 37 °C, for certain period of time (1–7 days), as presented elsewhere [23]. Then, in order to obtain a silver coating, the polymeric samples coated with hydroxyapatite layer were rapidly immersed in 50 mL of freshly prepared 0.5 M $AgNO_3$ (Sigma-Aldrich, Germany) solution with pH = 6.5 at 22 °C, for certain period of time (1–2 days). Finally, the samples were taken off and carefully rinsed with deionized water followed by drying in air at 60 °C for 24 h.

All chemicals used in this study were of reagent grade and used with no other purifications.

2.2. Samples characterization

The morphology and chemical composition of sample surface were elucidated by scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDX), with QUANTA 200 3D Dual Beam scanning electron microscope (FEI Co., USA). For the SEM-EDX investigations, gold sputtering was used to create a conductive coating surface.

The coatings formed on polyurethane support were characterized by X-ray diffraction (XRD) with X'PERT PRO MRD X-ray diffractometer (PANalytical, Netherlands), using monochromatic CuKα radiation (λ = 0.15418 nm), operating at 40 kV and 50 mA, over a 2θ range from 2° to 70°. The average crystallite size (D) was calculated from XRD data using the Scherrer equation:

$$D = \frac{k \cdot \lambda}{B_{1/2} \cdot \cos\theta} \quad (1)$$

where D is the crystallite size (nm), λ is the wavelength of Cu Kα radiation (λ = 0.15418 nm), $B_{1/2}$ is the full width at half maximum intensity value for the diffraction peak under consideration (rad), θ is the diffraction angle of the corresponding reflection (°), and k is the broadening constant varying with crystal habit and chosen as 0.89 for the Ag particles. For quantitative determinations, the peak at $2\theta = 38.4°$ for (111) reflection was used to evaluate the crystallite size of Ag particles. This peak was chosen since it is well resolved and shows no interferences.

The surface analysis of samples was performed by X-ray photoelectron spectroscopy (XPS) using a PHI-5000 VersaProbe photoelectron spectrometer (Φ ULVAC-PHI, INC.) with a hemispherical energy analyser (0.85 eV binding energy resolution). A monochromatic Al Kα X-ray radiation ($h\nu$ = 1486.7 eV) was used as an excitation source.

The optical properties of the coatings were characterized by UV-Vis spectroscopy with a Shimadzu UV-2450 UV-Vis spectrophotometer (Shimadzu Co., Japan) with an integrating sphere attachment. The absorbance was recorded at a resolution of 0.5 nm at 300–800 nm.

2.3. Antibacterial activity test

The antibacterial test was performed by the LB broth method [19]. Two types of microorganisms, *Escherichia coli* as Gram-negative bacteria and *Staphylococcus aureus* as Gram-positive bacteria, were taken in antibacterial experiments. These bacteria were supplied by the collection of microorganisms and cell cultures of the microbiology laboratory of our institution. The bacteria were cultivated at 37 °C in a sterilized broth (peptone 10 g, glucose 20 g, agar 15 g, distilled water 1000 mL) in a rotary shaker at 100 rpm for 24 h. The concentration of all the bacterial cell suspensions was of about 10^4 colony-forming units (CFU)/mL.

With the LB broth method, the hydroxyapatite/silver/polyurethane samples (0.5 g) were put into the flasks containing 10 mL aqueous medium at 37 °C for 24 h. Then, 10 mL of the bacteria medium was added and the incubation was continued for another 24 h. The culture with pure broth served as control sample.

The bacterial concentrations in liquid cultures were determined by measuring the optical density as absorbance at 600 nm (OD_{600}) using a Shimadzu UV-2450 UV-Vis spectrophotometer (Shimadzu Co., Japan). The inhibition ratio based on OD_{600} data was evaluated as the antimicrobial efficiency, calculated according to the equation:

$$IR\% = 100 - \frac{A_t - A_o}{A_{con} - A_o} \times 100 \quad (2)$$

where A_o is the OD_{600} value of the bacterial broth medium before incubation; A_t and A_{con} are OD_{600} values of the bacterial broth medium for tested sample (containing the Ag nanoparticles) and the control sample respectively, after incubation for a certain period of time (24 h).

All the experiments were run in triplicate. The average and standard deviation were calculated. Statistical analysis was performed by using the ANOVA test of Microsoft Excel.

3. Results and discussion

The biomimetic method has been shown to be a good way to obtain hydroxyapatite coatings which are homogeneous and would be more favorable to biological interaction. Generally, a surface on which hydroxyapatite is precipitated in the biomimetic solution shows well biomaterial-bone in a living body [24]. By the biomimetic method, the hydroxyapatite coatings can be produced in aqueous solutions at physiological temperatures using artificial solutions with ionic concentrations similar to those of human blood plasma. In practice, the most widely used artificial biomimetic solutions are: Simulated Body Fluid (SBF) and Supersaturated Calcification Solution (SCS) [24,25].

The hydroxyapatite coatings can provide an osteoconductive and an osteoinductive approach for enhancement of the bone formation on