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Dedicated to Professor Bogdan C. Simionescu on the occasion of his 65th anniversary

Ag-LOADED HYDROXYAPATITE COATINGS ON POLYURETHANE SURFACES BY BIOMIMETIC DEPOSITION

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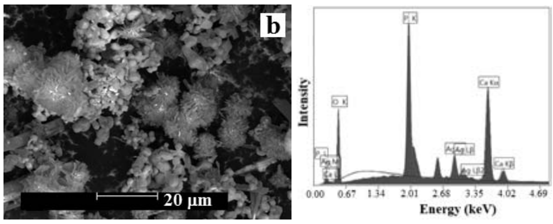
In the present paper a study on the possibility of obtaining Ag-loaded hydroxyapatite coatings on porous polyurethane surfaces by biomimetic deposition is reported. To achieve the formation of hydroxyapatite deposition on the surface of polyurethane and to incorporate the silver in the apatite layer, a supersaturated calcification solution containing silver ions was used. The samples were investigated by means of scanning electron microscopy (SEM) coupled with X-ray analysis (EDX), X-ray diffraction (XRD) and Fourier-transform infrared transmission spectroscopy (FT-IR). The hydroxyapatite/Ag coatings were evaluated for their antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The results obtained by SEM-EDX, XRD and FT-IR investigations revealed that porous polyurethane/hydroxyapatite scaffolds were formed, and the silver was incorporated into the apatite layer. The obtained data were also indicative of good antibacterial properties of the materials thus prepared.



INTRODUCTION

In the last years, many researches have been dedicated to the possibility of obtaining biomaterials that mimic natural bone tissue, to allow restoration of defects in the bones or teeth.[1-4] Most of these biomaterials are based on calcium phosphates, especially hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$).[5,6] The hydroxyapatite is chemically similar to the mineral component of bones and teeth in mammals and it is used as synthetic biomaterial. As a bioactive material, hydroxyapatite allows bone ingrowth and osseointegration when it is used in orthopedic, dental and maxillofacial applications.[7]

To be used in bone reconstruction, biomaterials have to show certain physical and chemical properties. They must be made in the form of matrices or scaffolds with three-dimensional structures exhibiting tailored porosity, pore size and interconnectivity.[8] From biological point of view, it is interesting to combine polymers and bioceramics to produce scaffolds for bone tissue engineering because natural bone is a composite materials based of a naturally polymer (collagen) and biological apatite.

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The fabrication of porous polymer-ceramic composite scaffolds involves a number of processing techniques such as: solvent casting (with and without particle leaching), thermally induced phase separation, microsphere sintering, inorganic coating, etc.[9-13] A commonly method used to obtain biocomposite materials is the coating of the polymeric scaffold with a thin layer of inorganic particles, especially with hydroxyapatite. These apatite coatings can be achieved by various methods such as: electrophoretic deposition, slurry dipping, biomimetic methods, etc.[14-18]

Silver, as its oxidation states (Ag^0, Ag^+, Ag^{2+}, Ag^{3+}), has much been recognized as having an inhibitory effect toward many bacterial strains and microorganisms present in medical processes.[19] The silver-based biomaterials have attracted much attention due to their perfect antibacterial activity and nontoxicity.[20,21]

This paper presents a study on the possibility of obtaining Ag-loaded hydroxyapatite coatings on porous polyurethane surfaces by biomimetic deposition. The obtained results have shown that porous polyurethane/hydroxyapatite scaffolds with an interconnected network were produced and silver is incorporated into apatite layer. The antibacterial activities of hydroxyapatite/Ag coatings were tested against *Staphylococcus aureus* and *Escherichia coli*.

EXPERIMENTAL

Procedure

The porous polyurethane scaffolds were obtained by the phase inversion method using polyurethane polymer, N,N-dimethylformamide as solvent, and deionized water as nonsolvent, as presented elsewhere.[22-24]

The supersaturated calcification solution (SCS) was prepared by dissolving in deionized water the following chemicals: $CaCl_2{\cdot}2H_2O$, $NaH_2PO_4{\cdot}H_2O$ and $NaHCO_3$ (supplied by Sigma-Aldrich, Germany). The ion concentrations of SCS solution are 4.0 $Mmol{\cdot}L^{-1}$ Na^+, 5.0 $Mmol{\cdot}L^{-1}$ Ca^{2+}, 10.0 $Mmol{\cdot}L^{-1}$ Cl^-, 2.5 $Mmol{\cdot}L^{-1}$ $(H_2PO_4)^-$, and 1.5 $Mmol{\cdot}L^{-1}$ $(HCO_3)^-$, as presented elsewhere.[25] The Ag-SCS solution was prepared by adding to the original SCS solution a desired amount of $AgNO_3$ (purchased from Sigma-Aldrich, Germany), so that the $AgNO_3$ concentration to be about $5{\cdot}10^{-2}$ M. All chemicals are reagent grade and used without further purification.

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. Then, the samples were rinsed with deionized water, followed by drying in air at 40 °C for 24 h. In order to prepare Ag-loaded hydroxyapatite coatings on porous polyurethane surfaces, polymeric samples were immersed in Ag-SCS solution at 37 °C, for certain period of time.

Characterization of samples

The morphology and chemical composition of samples were studied by scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDX) with QUANTA 200 3D microscope (FEI, Netherlands). Gold sputtering was used to make the coating surfaces conductive for the SEM investigations. The coating formed on polyurethane support was characterized by X-ray diffraction (XRD) with X'PERT PRO MRD diffractometer (PANalytical, Netherlands), operated with CuKα radiation. The FTIR spectra of all samples were recorded on a DIGILAB SCIMITAR-SERIES spectrophotometer with an attenuated total reflectance (ATR) accessory.

Evaluation of antibacterial activity

The spread plate method was used to analyse the antibacterial activity of hydroxyapatite/Ag coatings. The tests were performed according to the biological standard methodology. Two types of bacteria, *Escherichia coli* (Gram-negative) (ATCC 25922) and *Staphylococcus aureus* (Gram-positive) (ATCC 6538), were used in antibacterial experiments. The bacteria were counted through colony-forming units (CFUs) and bactericidal ratios (BR%) were calculated according to the following equation:

$$BR\% = \frac{CFUs_{(2)} - CFUs_{(1)}}{CFUs_{(2)}} \cdot 100 \quad (1)$$

where $CFUs_{(1)}$ is CFUs of experimental group, and $CFUs_{(2)}$ is CFUs of contrastive group.

RESULTS AND DISCUSSION

In recent years, numerous studies have been focused on the development of biocompatible, bioactive and antibacterial composite coatings on polymeric surfaces. The hydroxyapatite/Ag coatings present a special interest in biomaterial surface modification area in order to realize the double aims of bacterial inhibition and enhancement of osteoblast functions.

In this research, we have applied the biomimetic method to co-deposit simultaneously the hydroxyapatite and Ag on the porous polyurethane scaffold surface by means of polymeric sample treatment in supersaturated calcification solutions (SCS).

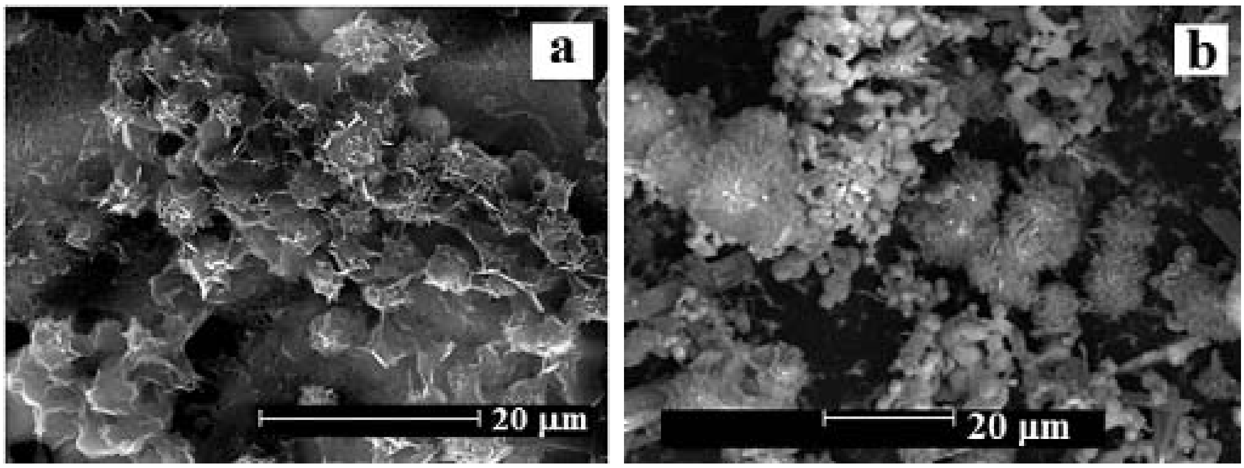
In previous studies, we have demonstrated that biomimetic treatment in SCS solution promoted the nucleation and growing rate of the hydroxyapatite coating on porous polyurethane surface.[25] In these solutions, where the degree of supersaturation of calcium and phosphate ions is high, some processes are developed. The main reaction involved in the formation of hydroxyapatite during the biomimetic process can be expressed as follows:

$$10Ca^{2+} + 6PO_4^{3-} + 2OH^- \rightarrow Ca_{10}(PO_4)_6(OH)_2 \downarrow \quad (2)$$

In the SCS solution containing silver ions (denoted Ag-SCS), subsequently to hydroxyapatite formation on the surface of polyurethane porous scaffold, the Ag crystals were achieved by reducing aqueous Ag^+ ions by the electron transfer ($Ag^+ + 1e^- \rightarrow Ag^0$) on the surface of hydroxyapatite. The hydroxyl groups on the surface of hydroxyapatite in this process acted both as a reducing and a binding agent, as mentioned in literature.[26]

The hydroxyapatite or Ag-loaded hydroxyapatite coatings formed on polyurethane surface after biomimetic treatment in SCS or Ag-SCS solutions were examined by SEM-EDX, XRD and FTIR methods.

The surface morphology of the Ag-loaded hydroxyapatite coatings formed on polyurethane surface after biomimetic treatment was made evident by SEM micrographs, comparatively with the simple hydroxyapatite coatings (Fig. 1). The surface morphology of the polyurethane sample soaked 120 h in SCS solution proved that the polyurethane surface to be completely covered by a dense and uniform hydroxyapatite layer (Fig. 1a). The plate-like shape apatite crystals clustered together to form regularly spherical particles on the surface of the porous polyurethane scaffold. These aggregates have an average diameter between 5 and 10 µm (Fig. 1a). In the case of the Ag-loaded hydroxyapatite/polyurethane samples, in addition to the apatite crystals, silver crystals in small and large granular shapes were observed (Fig. 1b,c). XRD analysis of Ag-loaded hydroxyapatite coatings confirms the presence of Ag in hydroxyapatite crystalline coatings (figure not shown). The SEM-EDX analysis also confirmed the presence of Ca, P, and Ag in Ag-loaded hydroxyapatite coatings (Fig. 2). The Ca/P molar ratio of apatite crystals was of 1.68, which corresponds to the stoichiometric hydroxyapatite.[27]



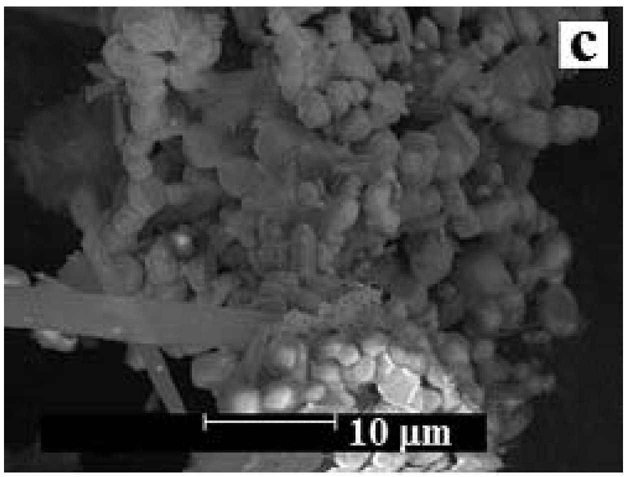


Fig. 1 – SEM micrographs of the hydroxyapatite (a) and Ag-loaded hydroxyapatite (b,c) coatings on polyurethane surface after soaking in biomimetic solutions for 120 h, at 37 °C.

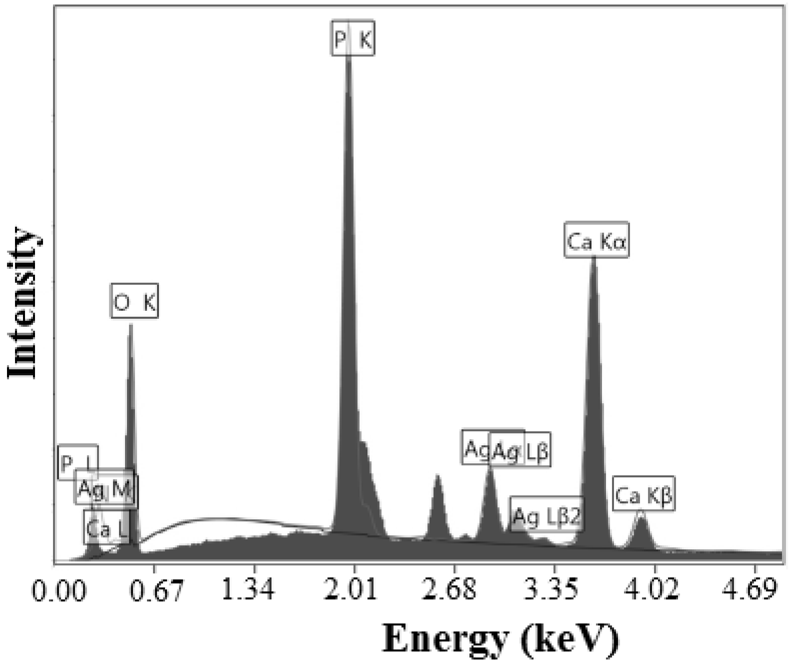


Fig. 2 – EDX spectrum of Ag-loaded hydroxyapatite coatings on polyurethane surface after soaking in Ag-SCS for 120 h, at 37 ^{o}C.

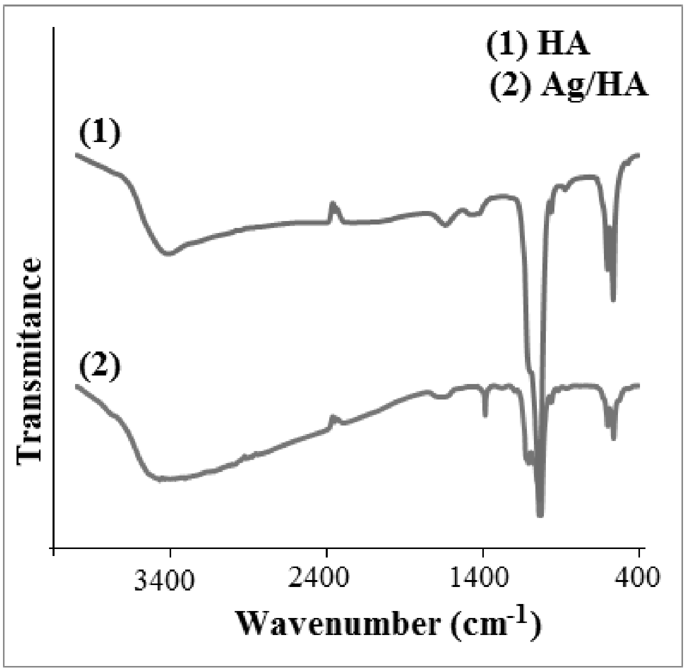


Fig. 3 – FT-IR spectra of hydroxyapatite and Ag-loaded hydroxyapatite coatings on polyurethane surface after soaking in biomimetic solutions for 120 h, at 37 ^{o}C.

Fig. 3 shows FTIR spectra of hydroxyapatite and Ag-loaded hydroxyapatite coatings within the spectral region 400-4000 cm^{-1}. These spectra revealed the presence of various vibrational modes corresponding to phosphates and hydroxyl groups. The bands in regions 1600 to 1700 cm^{-1} and 3200 to 3600 cm^{-1} correspond to H-O-H bands of water lattice. Band characteristics of the phosphate groups (PO_4^{3-}) in apatitic environment were observed at 561, 639, 607 and 958 cm^{-1}, as mentioned in literature.[26,27]

The antibacterial activities of hydroxyapatite/Ag coatings on porous polyurethane scaffolds were tested against

Staphylococcus aureus and *Escherichia coli*. After an incubation of 24 h of the bacteria on Ag-loaded hydroxyapatite surface, 92.5 % *Staphylococcus aureus* and more than 94.3 % *Escherichia coli* in the bacteria suspension were killed. Therefore, the conclusion can be drawn that the Ag-loaded hydroxyapatite/polyurethane scaffolds exhibited a strong antibacterial property.

Finally, we can say that the bone bonding properties of hydroxyapatite coatings on porous polyurethane scaffolds combined with the antibiotic effects of silver may offer a real means for removing the bacteria from the site of infection. Therefore, these biomaterials may have good applications in implantology.

CONCLUSIONS

In the present study an alternative method is advanced for preparing bioactive surfaces with bactericidal ability for biomedical devices. Ag-loaded hydroxyapatite coatings were obtained on porous polyurethane surfaces by biomimetic deposition. The obtained results have shown that hydroxyapatite layers were produced on porous polyurethane scaffolds with an interconnected network with silver incorporated in these apatite layers. The results also indicated that the as-prepared coatings have good antibacterial properties. These data suggest that Ag-loaded hydroxyapatite coatings on porous polyurethane scaffolds are promising materials with the antibacterial properties that might be used as implantable biomaterials.

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