

# Bioactivity evaluation of new silver doped bone cement for prosthetic surgery

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Due to the broad antimicrobial spectrum of silver, the composites used as bone substitutes or for coating metallic implants can be doped with silver. Polymer-ceramic composites are widely applied in orthopaedics due to their biocompatibility and ability to support bone growth (osteoconductivity) and also due to bioactivity implied by self-assembling of a hydroxyapatite type layer on their surface. This *in vitro* study is focussed on surface microstructure changes in simulated body fluid and on antibacterial silver release from two different types of biocomposites doped with silver. SEM microscopy and ATR-FTIR spectroscopy were employed in order to evaluate the properties of the surface layer after immersion in simulated body fluid.

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## 1. Introduction

The prevention of infections disease represents nowadays a central need, especially for prosthetic surgery. Many nosocomial bacteria, in fact, show an increasing resistance towards antibiotics, causing serious infections that lead to prolonged times of hospitalization. The development of surfaces with low bacterial adhesion together with biocompatibility can represent a solution to prevent infections. Biocompatible composites can be used to realize bone substitutes as well as coated metallic devices; moreover such materials can be opportunely treated to enrich their surfaces with silver ions [1,6] thus providing a controlled ion release and consequently antibacterial efficacy. Silver is, in fact, a well-known broad-spectrum antimicrobial agent, so silver-doped composites can be produced to realize different devices, such as bone substitutes or coated metallic implants, with antibacterial action.

Porous materials have been used in surgical implant design to fabricate devices or augment soft or hard tissues, as coatings on prostheses to accommodate tissue ingrowth for biological fixation and as scaffolds to facilitate the regeneration of tissue. Polymer-ceramic composite are widely used in orthopaedics as suture materials and fixation devices due to their biocompatibility-ability to support bony growth (osteoconductive) and also bone bioactive (to form a calcium fosfat layer on its surface) [2]. The aim of the work is to compare the microstructure, biocompatibility and antimicrobial activity of two different types of acrylic bone cements after silver doping, from *in vitro* study in simulated body fluid using electrochemical measurements, SEM microscopy and ATR-FTIR spectroscopy in order to evaluate the properties of the surface layer.

## 2. Experimental

Silver doping procedure was performed with respect to different acrylic orthopedic cements, commercially available, the final biocomposites having the following composition: BIOLOS3<sup>®</sup>-Liquid total 16.4 g: methylmethacrylate (monomer) 84.4%, butylmethacrylate 13.2%, N:N dimethyl p- toluidine 2.4%, hidroquinone 20 ppm. Powder total 40 g: methylmethacrylate (copolymer) 87.3%, polymethyl metacrylate 2.7 %, barium sulphate 10%, AgNO<sub>3</sub> 20%. ANTIBIOTIC SIMPLEX<sup>®</sup>- Liquide total 20 ml: methyl methacrylate (monomer) 19.5 ml, N:N dimethyl p- toluidine 0.5 ml, hidroquinone 1.5 mg. Powder total 41 g: methyl methacrylate (copolymer) 30 g, polymethyl methacrylate 6 g, barium sulphate 4 g, erythromycin 0.5 g, colistin sulphomethate sodium 3 million I.U, AgNO<sub>3</sub> 8g. Samples from both materials were incubated for 14 days in SBF following the procedure describe by *Kokubo et al* [7]. Electrochemical measurement were performed in static conditions, without refreshing the fluid, using CONSORT C835 Multimeter equipped with Na<sup>+</sup>, Ca<sup>++</sup> and Ag<sup>+</sup> selective electrodes, during different time intervals. The FT-IR spectra of biocomposites were recorded in the region 4000-500 cm<sup>-1</sup> by a Bruker EQUINOX 55 spectrometer OPUS software, using an Attenuated Total Reflectance accessory with a scanning speed of 32 cm<sup>-1</sup> min<sup>-1</sup> and spectral width 2.0 cm<sup>-1</sup>. The internal reflection element was a ZnSe ATR plate (50 × 20 × 2 mm) with an aperture angle of 45°. Scanning Electron Microscope (SEM) images were taken with a JEOL- JSM 5510 LV microscope.

## 3. Results and discussion

Electrochemical measurements carried out on pure SBF (initial) and after 14 days incubation in SBF at 37 °C, of silver doped materials, reveal a considerable diminution of Ca<sup>2+</sup> concentration (two order of magnitude)

after 14 days incubation, for both types of biocomposites (Fig. 1a,b). By comparing the diagrams *a*) and *b*) one can observe that the silver release is facilitated by the BIOLOS 3<sup>®</sup>, which is an antibiotic free material, as the concentration of Ag<sup>+</sup> measured after five hours incubation is significantly greater (one order of magnitude) then released from ANTIBIOTIC SIMPLEX<sup>®</sup>. Concomitant, Na<sup>+</sup> content decrease one order of magnitude, as shown in diagram of Fig.1b. The SBF conductivity measured after ANTIBIOTIC SIMPLEX<sup>®</sup> incubation during this interval increase continuously from 16.5 mSv to 28.3 mSv, whereas the conductivity of SBF related to BIOLOS 3<sup>®</sup> exhibit a minimum value of 12 mSv after 5 days and a maximum of 15 mSv after 14 days incubation. The initial value of the SBF conductivity is 17.5 mSv.

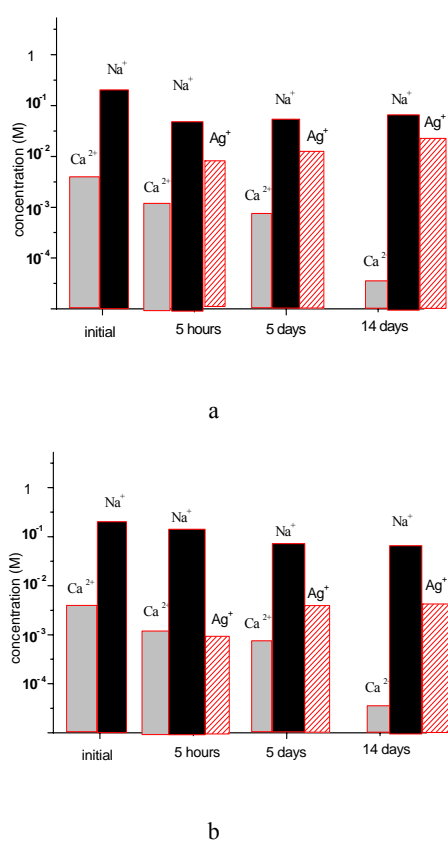


Fig. 1. Effect of SBF incubation on Ca<sup>2+</sup>, Na<sup>+</sup> and Ag<sup>+</sup> concentration, after different incubation times of silver doped ANTIBIOTIC SIMPLEX<sup>®</sup> (a) and silver doped BIOLOS<sup>®</sup> (b).

The silver release mechanism can be described as follow: In the presence of the sodium or calcium ions characteristic of body fluids, the antimicrobial agents ion exchange with these physiological cations. Long chain hydrophilic polymers incorporated in the coating adsorb water molecules and facilitate the ion exchange. Rather than exhibiting a huge “spike” of activity that diminishes rapidly over time - and that may be toxic to the surrounding tissues - the new biomaterials prevents bacterial adherence by its slow ion-exchange release of anti-microbial agents.

Prior to the doping procedure, the study was focused on scanning electron microscopic (SEM) analysis before

and after immersion in SBF, as the development of an active layer is expected [2,5], followed by infrared spectroscopic measurements on the graded layer structure of hydroxyapatite type developed after different periods of immersion in SBF and comparison with those of the native materials. ATR-FTIR spectroscopy is a non invasive method for monitoring the biomineralization at different times intervals, and is an advantageous method as it is very rapid, does not require sample preparation, is non-destructive and in the same time it provides information about the molecular structure of the polymers.

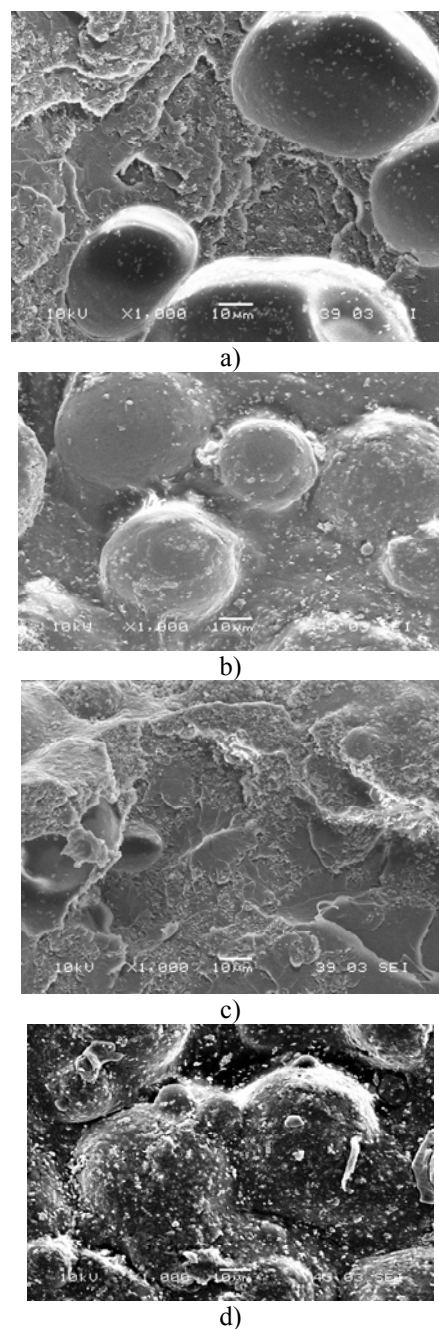


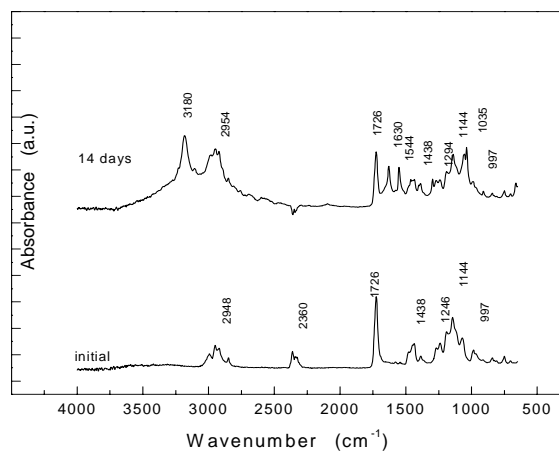
Fig. 2. Morphology of hydroxyapatite-like crystals at BIOLOS 3<sup>®</sup> surface before (a) and after 14 days incubation (b) compared to ANTIBIOTIC SIMPLEX<sup>®</sup> surface in the same conditions (c and d, respectively).

The SEM images show a nucleation process of calcium containing crystals, the formation of this layer in the early stages being considered indicative of the bioactivity of the materials [8,9]. In Fig. 2 (a, b, c, d) is displayed comparatively the surface morphology of both biocomposites, before and after 14 days incubation in SBF, in the same conditions. The SEM images show the formation of hydroxyapatite-like crystals, quasi-spherical as well as irregularly shaped aggregates, with an average size of 2.5  $\mu\text{m}$ , grouped homogeneously, the distribution of the aggregates being densely on the ANTIBIOTIC SIMPLEX<sup>®</sup> surface.

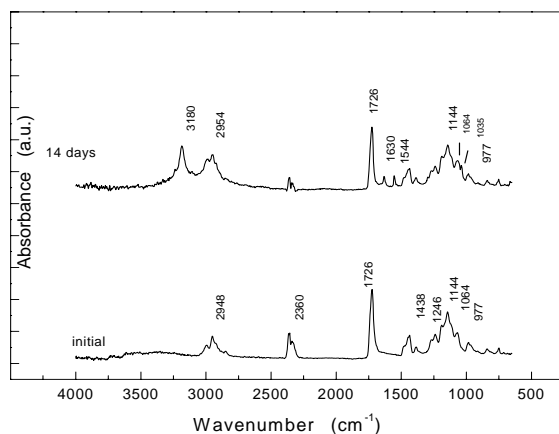
The surface layer structure has been investigated by ATR- FTIR technique after 14 days incubation of both materials and the corresponding spectra are displayed in Figures 3 (a,b) along with the reference spectrum recorded before the immersion in SBF. The reference spectrum indicate the details of functional groups in methyl methacrylate. The peaks from 2994  $\text{cm}^{-1}$  to 2840  $\text{cm}^{-1}$  are due to  $\text{CH}_2$  asymmetric and symmetric stretching, a sharp and intense peak at 1726  $\text{cm}^{-1}$  is due to the presence of ester carbonyl group stretching vibration, a broad band at 1438  $\text{cm}^{-1}$  due to C-H bending and the peaks in the range 1260-900  $\text{cm}^{-1}$  are assigned to O-C-O, C- $\text{CH}_3$  stretching and C-COO vibrations [10,11]. The features of this spectrum are very well preserved after 14 days incubation in SBF. The major modifications are observed in the range 3200-2800  $\text{cm}^{-1}$ , 1600-1540  $\text{cm}^{-1}$  and 1150-1035  $\text{cm}^{-1}$ . Typical calcium phosphates band is observed at 1035  $\text{cm}^{-1}$  assigned to  $\nu_3 \text{PO}_4^{3-}$  stretching, suggesting the presence of newly formed bone, and according to the literature, the sharpness of the phosphate band demonstrate increasing mineralisation [12-15]. Other bands of interest in Fig. 3 are the intense band at 3180  $\text{cm}^{-1}$  assigned to OH stretching and those at 1630 and 1544  $\text{cm}^{-1}$  corresponding to OH deformation, demonstrating that after 14 days incubation, the antibiotic-loaded bone cement absorbs a considerable amount of water. We assume that the ions belonging to the surface layer show a relatively high mobility. Thus, as already reported, the cations as well as the anions can be readily and reversibly exchanged. The hydrated layer is not stable and it is progressively replaced by apatite during ageing in an aqueous media (maturation). The mechanism of this transformation is not yet well known but it involves a decrease of the amount of  $\text{HPO}_4^{2-}$  ions and an increase of the calcium content of the mineral phase. Simultaneously  $\text{OH}^-$  ions are included in the structure and water is excluded [14,15].

The calcium phosphate band arises at the same wavenumber, 1035  $\text{cm}^{-1}$ , in both cases, but the intensity of this band indicates that mineralization at BIOLOS3<sup>®</sup> surface after 14 days is diminished in comparison with ANTIBIOTIC SIMPLEX<sup>®</sup>. On the other hands, the dynamics of water uptake in time is very different with

respect to the different types of methyl methacrylate biocomposites.



a)



b)

Fig. 3. ATR- FTIR spectra of ANTIBIOTIC SIMPLEX<sup>®</sup> (a) and BIOLOS3<sup>®</sup> (b) before and after 14 days incubation in SBF.

#### 4. Conclusions

Two different types of silver doped methyl methacrylate based biocomposites are compared in vitro, in simulated body fluid, with respect to their microstructure and bioactivity. Electrochemical measurements reveal a considerable diminution of  $\text{Ca}^{2+}$  content in SBF during the first two weeks of incubation. The antibiotic-free bone cement is capable to release more  $\text{Ag}^+$  after five hours incubation, as compared to the antibiotic loaded one. SEM images demonstrate that the mineralization process after 14 days incubation is more intense on the surface of antibiotic containing biomaterial. This result is confirmed by ATR-FTIR data which evidence calcium phosphate band characteristic for hydroxyapatite-type compounds and at the same time by the water uptake during the incubation time.

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