JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS Vol. 8, No. 4, August 2006, p. 1520 - 1523

Microstructure and bioactivity of acrylic bone cements for prosthetic surgery

S. CAVALU*, V. SIMON[a]
University of Oradea, Faculty of Medicine and Pharmacy, Biophysics dept. 410068 Oradea, Romania,
[a]Babeş-Bolyai University, Faculty of Physics, 400084 Cluj-Napoca, Romania

Polymer-ceramic composites based on polymethyl methacrylate are widely used in orthopaedics as suture materials and fixation devices due to their biocompatibility and ability to support bony growth (osteoconductive) and also bone bioactive (to form a calcium phosphate layer on its surface). In this study are compared the microstructure, bioactivity and biocompatibility of two different types of biocomposites: BIOLOS3®and ANTIBIOTIC SIMPLEX®. They are investigated in vitro in simulated body fluid using electrochemical measurements, SEM microscopy and ATR-FTIR spectroscopy in order to evaluate the properties of the surface layer.

(Received June 7, 2006; accepted July 20, 2006)

Keywords: Biomaterials, Bioactivity, SEM, ATR-FTIR

1. Introduction

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. In spite of the great number of new biomaterials developed in last time, a gap remains to be filled since no synthetic material used until now presents characteristics close to the natural tissue, attending both biological aspects as well as mechanical requirements. Polymer-ceramic composites based on polymethyl metacrylate are widely used in orthopaedics as suture materials and fixation devices due to their biocompatibility and ability to support bony growth (osteoconductive) and also bone bioactive (to form a calcium phosphate layer on its surface) [1-5]. Through the pioneering efforts of J. Charnley in the 1960s, polymethyl methacrylate bone cement emerged as one of the premier synthetic biomaterials in contemporary orthopedics. Today, it is used throughout the world in roughly 50% of all total-hip arthroplasties and 80% of total-knee arthroplasties, aiding in the transfer of body weight from prostheses into the surrounding bone [6]. The use of antibiotic-loaded bone cement is a well-accepted adjunct in the treatment of infected joint arthroplasty and is gaining further application as a method of prophylaxis. The influence of antibiotic inclusion on cement mechanical properties, specifically fatigue, determines its resistance to crack formation and the long-term in vivo structural integrity of the cement. Several factors influence the choice of antibiotic to add to bone cement. The antibiotic must be able to withstand the exothermic temperature of polymerization, be available as a powder, have a low incidence of allergy, and be able to elute from the cement over an appropriate time period. Several bone cements containing antibiotics such as erythromycin, colistin, gentamicin and tobramycin have been commercially available in Europe for many years.

Our approach is to compare the microstructure, bioactivity and biocompatibility of two different types of polymethyl methacrylate based biocomposites: BIOLOS3®and ANTIBIOTIC SIMPLEX® (containing erythromycin and colistin) from in vitro study in simulated body fluid. It has been accepted that the biodegradation of polymeric composites in vivo is a non-enzymatic process and occurs by hydrolytic degradation [7]. Therefore in vitro degradation studies are relevant for estimating the degradation of scaffolds in vivo, in order to optimise the composition of the composite for a required degradation rate. The study is focused on scanning electron microscopic (SEM) analysis before and after immersion in SBF, as the development of an active layer is expected [2], 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-distructive and in the same time it provides information about the molecular structure of the polymers. Hemolysis tests and osmotic fragility of red blood cells were also performed, as the blood compatibility is dictated by the manner in wich the material surface interact with blood constituents (red blood cells, platelets, proteins) [8].

2. Experimental

BIOLOS3® and ANTIBIOTIC SIMPLEX® are acrylic orthopedic cements, commercially available, having the following composition: BIOLOS3®-Liquide 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%. ANTIBIOTIC SIMPLEX®- 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 metacrylate 6 g, barium sulphate 4 g, erythromycin 0.5 g, colistin sulphomethate sodium 3 million I.U. Samples from both materials were incubated for 34 days in SBF following the procedure describe by *Kokubo et al* [9]. Electrochemical measurement were performed in static conditions, without refreshing the fluid, using CONSORT C835 Multimeter equipped with Na^+ and Ca^{++} selective electrodes, during different time intervals. Hemolysis tests were performed after one hour incubation of biomaterials in freshly blood, with tri-sodium citrate as anticoagulant. An amount of 50 µl of whole blood was added to 5 ml of different saline dilutions (0.2, 0.4, 0.6, 0.8 and 1% NaCl), then centrifugated. The supernatant was considered for spectrofotometric measurement using MATERTECH SP-8001 UV-VIS spectrophotometer, as the absorbance at 540 nm is a measure of hemoglobin released. Absorbance values were normalized with that of the control blood. The FT-IR spectra of biocomposites were recorded in the region 4000-500 cm^{-1} by a Bruker EQUINOX 55 spectrometer OPUS software, using an Attenuated Total Reflectance accessory with a scanning speed of 32 cm^{-1} min^{-1} and spectral width 2.0 cm^{-1}. 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 and after 14, 20 and 34 days incubation at 37 °C of materials in SBF reveal a considerable diminution of Ca^{2+} concentration after 14 days incubation, for both types of biocomposites, the Na^+ concentration being less affected during the times (Fig. 1). The exact values of concentrations are very similar for both materials. At the same time, the SBF conductivity shows a maximum value after 20 days: σ = 29.1 mS/cm and σ = 31.9 mS/cm, respectively, and a minimum value after 34 days: σ = 19.5 mS/cm and σ = 18.6 mS/cm, respectively, for the non-antibiotic and antibiotic-loaded bone cement. 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 [10,11]. In Fig. 2 (a, b, c, d) is displaied 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, cuasi-spherical as well as irregularly shaped aggregates, with an average size of 2.5 µm, grouped homogeneously, the distribution of the aggregates being densely on the ANTIBIOTIC SIMPLEX® surface.

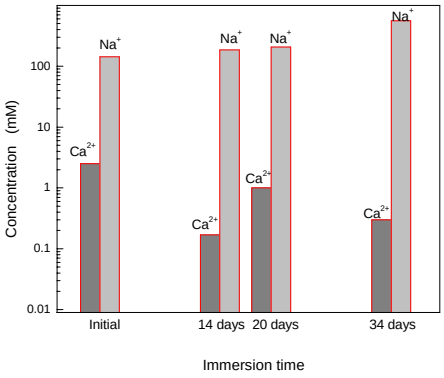
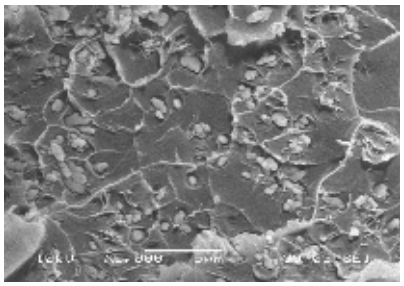
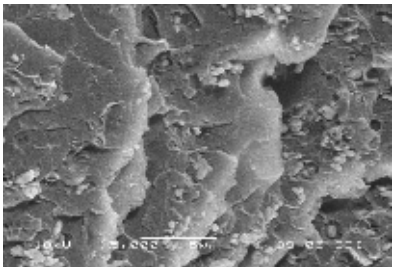


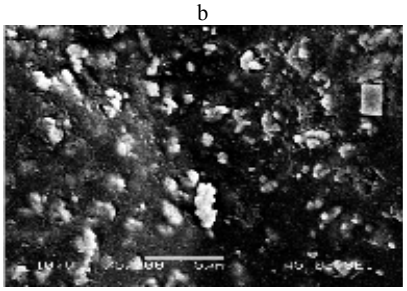
Fig. 1. Effect of SBF incubation on Ca^{2+} and Na^+ concentration, after different incubation times.



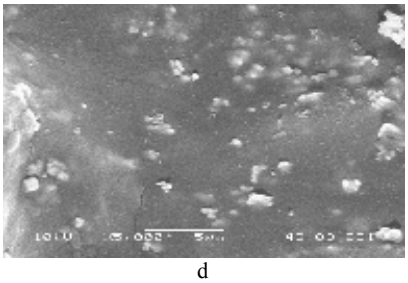
a



b



c



d

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

The surface layer structure has been investigated by ATR- FTIR technique after 14, 20 and 34 days incubation of both materials and the corresponding spectra are displayed in Figs. 3 and 4, 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 cm^{-1} to 2840 cm^{-1} are due to CH_2 asymmetric and symmetric stretching, a sharp and intense peak at 1726 $cm^{-1}$is due to the presence of ester carbonyl group stretching vibration, a broad band at 1438 cm^{-1} due to C-H bending and the peaks in the range 1260-900 cm^{-1} are assigned to O-C-O, C-CH_3 stretching and C-COO vibrations [12, 13]. The features of this spectrum are very well preserved (Fig. 3) after 14 and 20 days incubation in SBF. After 34 days immersion of ANTIBIOTIC SIMPLEX® in SBF, the major modifications are observed in the range 3200-2800 cm^{-1}, 1600-1540 cm^{-1} and 1150-1035 cm^{-1}. Typical calcium phosphates band is observed at 1035 cm^{-1} assigned to v_3 PO_4^{3-} streching, suggesting the presence of newly formed bone, and according to the literature, the sharpness of the phosphate band demonstrate increasing mineralisation [15-18]. Other bands of interest in Fig. 3 are the intense band at 3180 cm^{-1}assigned to OH stretching and those at 1630 and 1544 cm^{-1} corresponding to OH deformation, demonstrating that after 34 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 HPO_4^{2-} ions and an increase of the calcium content of the mineral phase. Simultaneously OH^- ions are included in the structure and water is excluded [17].

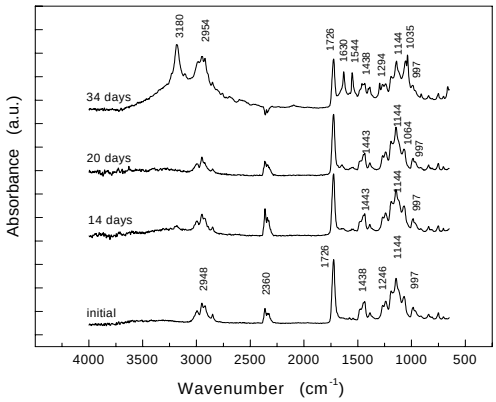


Fig. 3. ATR- FTIR spectra of ANTIBIOTIC SIMPLEX® after different time intervals of SBF incubation.

In Fig. 4 a similar behavior is emphasized. The calcium phosphate band arises at the same wavenumber, 1035 cm^{-1}, but the intensity is weaker compared to corresponding band in Fig. 3, indicating that mineralization at BIOLOS3® surface after 34 days is diminished in comparison with ANTIBIOTIC SIMPLEX®. Other interesting feature in Fig. 4 is related to the water uptake. After 14 and 34 days the corresponding spectra emphasized a strong band at 3180 cm^{-1} and two medium bands at 1630 and 1544 cm^{-1}, which are completely lacking in the corresponding spectrum after 20 days. This result suggests that the dinamics of water uptake in time is very different with respect to different types of methyl methacrylate biocomposites.

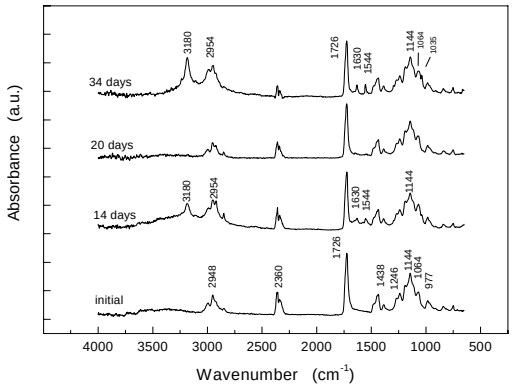


Fig. 4. ATR- FTIR spectra of BIOLOS3® after different time intervals of SBF incubation.

Hemolysis tests and osmotic fragility of red blood cells were also performed, as the blood compatibility is dictated by the manner in wich the material surface interact with blood constituents (red blood cells, platelets, proteins) [8]. Both biomaterials were incubated in blood for one hour, then the blood was centifugated. Spectrophotometric measurement of supernatant in different saline concentrations were performed at 540 nm to quantify the hemoglobin released in order to estimate the extent of red cell lysis. The absorbance of blood samples (following exposure to both biomaterials) were normalised with that of the control blood as shown in Fig. 5. One can observe from this figure that the absorbance values corresponding to antibiotic-loaded bone cement are closer to the control blood suggesting a good biocompatibility from this point of view.

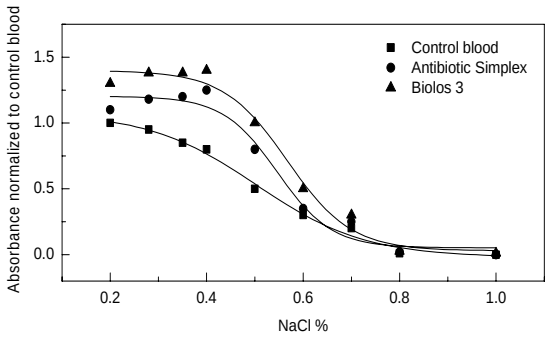


Fig. 5. Osmotic fragility of red blood cells following exposure to both type of biocomposites compared with the control blood (the lines are only guide to the eyes).

4. Conclusions

Two different types of methyl methacrylate based biocomposites are compared in vitro, in simulated body fluid, with respect to their microstructure, bioactivity and biocompatibility. Electrochemical measurements reveal considerabe fluctuation of Ca^{2+} content in SBF during the five weeks of incubation. SEM images demonstrate that the mineralization process after 14 days incubation is more intense on the surface of antibiotic containing sample. 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. Upon hemolysis tests and osmotic fragility of red blood cells and comparison of the results obtained in this study, the antibiotic containing cement outmatchs the properties of the orthopedic cement without antibiotic.

Aknowledgement

The authors would like to thank Prof. Dr. C. Crăciun for providing access to SEM analyses.

References

[1] L. L. Hench, J. Am. Ceram. Soc. **81**(7), 1705 (1998).
[2] A. E. Porter, N. Patel, J. N. Skeppre, S. M. Best, W. Bonfield, Biomaterials **24**, 4609 (2003).
[3] S. Verrier, J. J. Blaker, V. Maquet, L. L. Hench, A. R. Boccaccini, Biomaterials **25**, 3013 (2004).
[4] H. H. Lu, S. A. El-Amin, C. T. Laurencin, Bioenengineering Conference ASME 2001, BED-**50**, 693 (2001).
[5] M. M. Pereira, R. L. Orefice, H. S. Mansur, M. T. P. Lopes, R. M. Turchetti-Maia, A. C. Vasconcelos, Mat. Res. **6** (3) apr/june (2003).
[6] T. W. Bauer, Orthopedic Technology Review **5**(5) sept/oct. (2003).
[7] V. Maquet, A. R. Boccaccini, L. Pravata, I. Notinger, R. Jerome, Biomaterials **25**, 4185 (2004).
[8] K. Vijayanand, D. K. Pattanayak, T. R. Mohan, R. Bnerjee, Trends Biomat. Artif. Organs **18**(2), 73 (2005).
[9] T. Kokubo, S. Ito, Z. T. Huang, T. Hayashi, S. Sakka, T. Kitsugi, T. Yamamuro, J. Biomed. Mater. Res. **24**, 331 (1990).
[10] J. Vandiver, N. Patel, W. Bonfield, C. Ortiz, Key Engineering Materials **284**, 497 (2005).
[11] L. Pramatarova, E. Pecheva, R. Presker, M. T. Pham, M. F. Maitz, M. Stutzmann, Eur. Cells Mater. **9**, 9 (2005).
[12] A. Balamurugan, S. Kannan, V. Selvaraj, S. Rajeswari, Trends. Biomater. Artif. Organs **18**(1) 41 (2004).
[13] I. Notinger, J. J. Blaker, V. Maquet, L. L. Hench, A. Boccaccini, Asian J. Physics **15**(2), 221 (2006).
[15] S. Kale, S. Biermann, C. Edwards, C. Tarnowski, M. Morris, M. W. Long, Nature Biotech, **18**, 954 (2000).
[16] A. Tinti, P. Tadei, R. Simoni, C. Fagnano, Asian J. Physics **15**(2), 267 (2006).
[17] D. Eichert, H. Sfihi, M. Banu, S. Cazalbou, C. Combes, C. Rey, CIMTEC 2002 Proceedings of 10th Inter. Ceramics Congress, Florence, Italy (2002).
[18] P. Sutandar, D. J. Ahn, E. I. Franses, Macromolecules **27**, 7316 (1994).

*Corresponding author: scavalu@rdslink.ro