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In vitro bioactivity investigation using FTIR, XRD and SEM/EDAX techniques of porous bioactive glasses prepared by sol-gel using PMMA beads as template

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Porous bioactive glasses were prepared by the sol-gel method using poly (methyl methacrylate) (PMMA) beads as organic template. The structure of PMMA beads and glasses prepared were investigated by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM) and energy dispersive x-ray analysis (EDAX),. In vitro bioactivity of these porous glasses was achieved by immersing them in a simulated body fluid (SBF) at 37 °C and pH 7.4 for a reaction time period of 14 days. These porous glasses are bioactive and may be used as biomaterials in tissue engineering applications.

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1. Introduction

Bioactive glasses are the oldest bioactive materials, first reported by Hench et al. in 1969 [1]. Due to the surface-active response of these types of materials, they have been accepted as bioactive (or surface-active) biomaterials and have found applications in middle ear, alveolar ridge maintenance implants and other non-load bearing conditions [2]. More recently, bioactive glass (e.g. composition 45S5 Bioglass) has been used to fabricate highly porous foam-like structures as scaffolds for use in bone tissue engineering [1]. Scaffolds need to be in a porous form in order to support high number of cells and allow vascularisation upon implantation. Moreover, the pores must be open and large enough so that cells can easily migrate through the scaffolds [3]. Porosity can be increased by adding polymer beads or other organic agents (porogens) such as gelatine, sucrose, that burn out during the sintering and leave pores [4]. For bioglass synthesis, sol–gel technique represents an improvement compared to the conventional melting technique, allowing the preparation of high-purity materials at low temperatures and in various shapes, such as powders and thin films. Moreover, sol–gel glasses have the advantages of a wider compositional range of the raw materials and a higher bioactivity, with respect to conventional glasses [5].

The aim of this work is the synthesis, characterization and the bioactivity study of porous bioactive glasses using the sol-gel method and PMMA beads as organic template. PMMA is a thermoplastic polymer having excellent biocompatibily.

2. Experimental

2.1 Preparation of PMMA beads

PMMA beads were prepared by free radical suspension polymerization. The raw materials: water, suspending agent carboxy methyl cellulose (CMC) (Fluka), monomer methyl methacrylate (MMA) (Fluka) and initiator benzoyl peroxide (BPO) (Merck) were added in a 1L three-necked reactor equipped with a reflux condenser, a mechanical stirrer and a temperature control system. Specifically, 600 ml of water was added into the reactor and then 1.2 g of CMC as suspending agent was dissolved. The MMA monomer was repeatedly washed with 5% (w/v) NaOH / aq. solution (Merck) and with distilled water in order to remove the inhibitor containing in the MMA. The monomer phase was prepared by dissolving of 0.6 g of BPO as initiator in 60 ml MMA and added into the reactor using a dropping funnel, after the temperature of the aq. solution of CMC was reached 70 ^{0}C. The polymerization took place at 75 ^{0}C for 2 h and then at 80 ^{0}C for 1 h under strong stirring with 600 rpm, in order to maintain the organic phase in suspension. The polymerization was stopped by the addition of cold water into the reactor. The polymer was isolated by precipitation with 200 ml methanol (Merck) under stirring for 15 minutes. The polymer beads were washed several times with distilled water, sieved and dried at 60 ^{0}C for 24 h and was used as organic template in the next procedure concerning the synthesis of porous bioactive glasses by sol-gel.

2.2 Preparation of glasses by sol-gel using PMMA beads as organic template

The glasses were prepared using the sol-gel method according to the following steps: distilled water, HNO_3, tetraethyl orthosilicate $Si(OC_2H_5)_4$ (Fluka) and calcium nitrate tetrahydrate $Ca(NO_3)_2 4H_2O$ (Fluka) were mixed in proper amounts in order to achieve the proportion of SiO_2/CaO=50/50 mol/mol. Then, PMMA beads (sieved in size < 300 micrometers) were added in the derived sol. After 10 minutes of soaking, the impregnated PMMA which form close-packed beads were separated from the solution by a Buchner funnel. The PMMA beads were casted in a cylindrical container where the sol was allowed to gel for 1 day at room temperature and then the gel aged in a drying oven for 1 day at 70 °C. The aged gel was sintered by heat treatment in an electrical furnace at 700 °C for 1 h in air atmosphere, in order to obtain a stabilized glass. The structure of PMMA beads and glasses prepared were investigated by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM) and energy dispersive x-ray analysis (EDAX).

2.3 In vitro bioactivity study

In vitro bioactivity of these glasses (solid / SBF ratio is 1 mg/ml) was achieved by immersing them in an acellular simulated body fluid (SBF) proposed by Kokubo [6] with ion concentrations nearly equal to those of human blood plasma, at 37 °C and pH 7.4 for a reaction time period of 14 days. After this immersion in SBF, the surface of the bioactive glasses was studied using FTIR, XRD and SEM/EDAX techniques.

FTIR spectra were recorded using a Perkin Elmer Spectrum 2000, on discs prepared by mixing of the sample powder with KBr. XRD measurements were performed with a Siemens D5000 X-Ray Diffractometer by using a sample of the material as powder. The SEM-EDAX studies were carried out in a FEI Quanta 200 Scanning Electron Microscope (SEM).

3. Results and discussion

3.1 PMMA beads

The FTIR spectrum of the PMMA beads is shown in Fig.1.

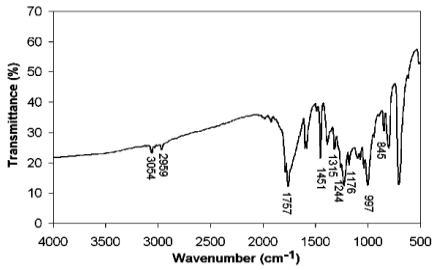
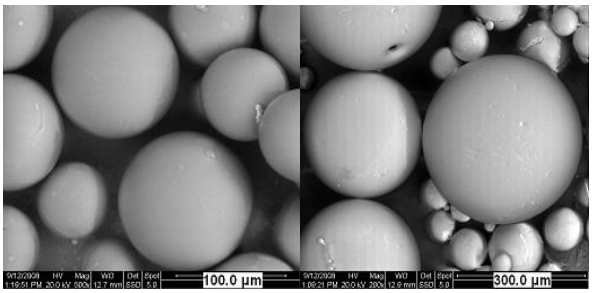


Fig.1. FTIR spectrum of PMMA beads.

According to literature, the characteristic vibration bands of PMMA appear at 1757 cm^{-1} ν(C=O) and 1451 cm^{-1} ν(C–O). The bands at 3054 and 2959 cm^{-1} correspond to the C–H stretching of the methyl group (CH_3) and the bands at 1315 and 1451 cm^{-1} are associated with C–H symmetric and asymmetric stretching modes, respectively. The 1244 cm^{-1} band is assigned to torsion of the methylene group (CH_2) and the 1176 cm^{-1} band corresponds to vibration of the ester group C–O, while C–C stretching bands are at 997 and 845 cm^{-1} [7, 8, 9].

SEM images of PMMA beads prepared by suspension polymerization are shown in Fig. 2 (a, b). As clearly seen, the PMMA beads have spherical form with diameter < 300 μm determined both from SEM images and proper sieves.



(a) (b)

Fig. 2. SEM images of PMMA beads (a) magnification 500x and (b) magnification 200x.

3.2 Bioactive glasses

Fig. 3 shows the FTIR spectra of the glass after aging and sintering, in order to obtain a stabilized glass.

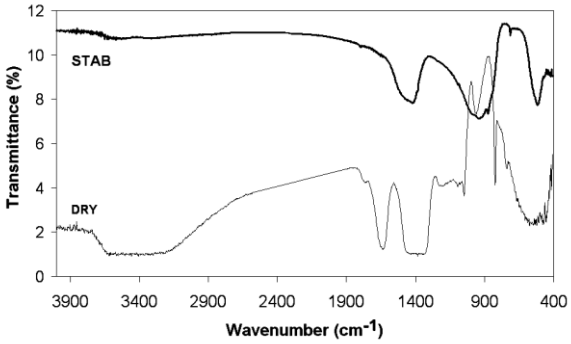


Fig. 3. FTIR spectra of the glass after aging at 70 $^0C/24h$ and sintering at 700 $^0C/1h$.

According to Fig. 3 and based on the literature, the peaks at 3413 cm^{-1} and 1643 cm^{-1} are attributed to O-H stretching of molecular adsorbed water with hydrogen bonds or to isolated -OH and to the H-O-H bending vibration of molecular water, respectively [10, 11]. The peak at 1392 cm^{-1} is assigned to the vibration of ionic $(NO_3)^-$. The peak at 1050 cm^{-1} is attributed to the symmetric stretching vibration of the Si-O-Si bonds [10, 11]. The peak at 968 cm^{-1} corresponds to the Si-O-Ca bonds containing non-bridging oxygen [10]. The peak at 823 cm^{-1} corresponds to the stretching mode of the O-Si-O

bond [10, 11, 12]. The aged gel show a peak at 480 cm^{-1} which is assigned to the bending modes of the Si-O-Si and O-Si-O bonds [10, 12].

After the sintering at 700 ^{0}C, the stabilized glass shows the peaks of Si-O-Si and O-Si-O (bending modes), O-Si-O (stretching modes) and Si-O-Si (symmetric stretching modes). According to the literature, the peak at 1430 cm^{-1} corresponds to $(CO_3)^{2-}$ groups [11]. The presence of carbonate is attributed to a carbonation process of the material due to the atmospheric CO_2 as a consequence of the high calcium content [11]. The peak at 1392 cm^{-1} due to the vibration of ionic $(NO_3)^-$ is disappeared [10, 11, 12].

3.3 In vitro bioactivity study

According to Fig. 4 and based on the literature, the stabilized glass after 14 days immersion in SBF shows CO_3^{2-} (peaks at 1430-1480 and 873 cm^{-1}) assigned to carbonate group and amorphous phosphate, PO_4^{-3} (peaks at 1060 and 578 cm^{-1}) assigned to the (P-O) vibrational mode [12].

The XRD diffractograms of the stabilized glass before and after 14 days immersion in SBF are shown in Figure 5.

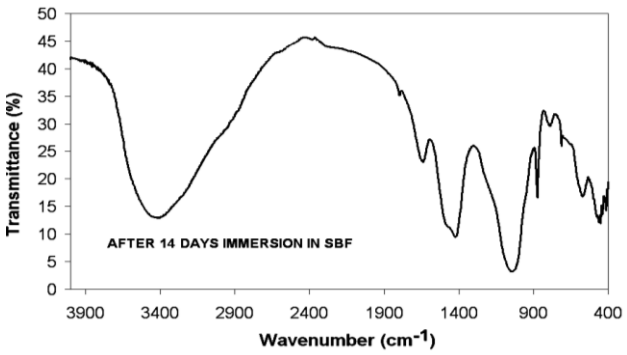


Fig. 4. FTIR spectrum of the stabilized glass after 14 days immersion in SBF.

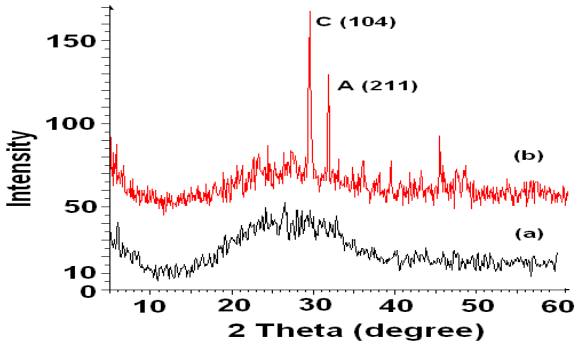
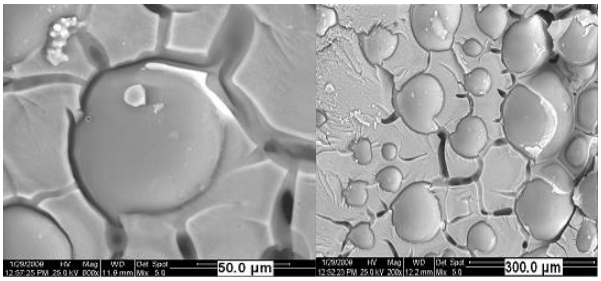


Fig. 5. XRD diffractograms of the stabilized glass (a) before and (b) after 14 days immersion in SBF.

According to the XRD results, the diffractogram of the stabilized glass at 700 ^{0}C, Fig. 5(a), confirm its amorphous state as indicative of its internal disorder and glassy nature. After 14 days immersion of the stabilized glass in SBF solution, the XRD diffractogram, Fig. 5(b), indicates the formation of two phases: (211) of apatite and (104) calcite [11].

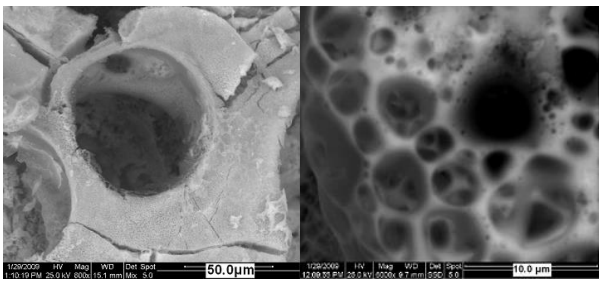
In Fig. 6, the aged gel structure, after aging at 70 ^{0}C for 24 h, contains PMMA beads which are used as organic template.



(a) (b)

Fig. 6. SEM images of aged gel structure after aging at 70 ^{0}C/24h (a) magnification 800x and (b) magnification 200x.

Fig. 7 shows the stabilized glass structure after heat treatment at 700 ^{0}C for 1h. Many porous between 5 and 100μm are formed into the glass due to the elimination of PMMA beads by combustion.



(a) (b)

Fig. 7. SEM images of stabilized glass structure after heat treatment at 700 ^{0}C/1h (a) magnification 800x and (b) magnification 6000x.

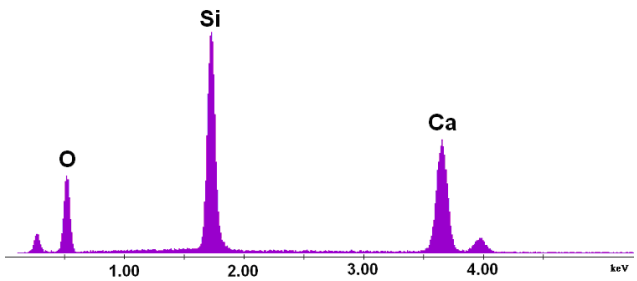
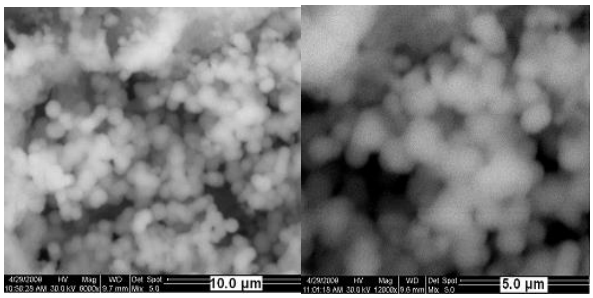


Fig. 8. EDAX spectrum of the stabilized glass before the immersion in SBF solution.

According to Fig. 8, the EDAX results reveal the presence of the Si, Ca and O elements.



(a) *(b)*

Fig. 9. SEM images of stabilized glass structure after 14 days immersion in SBF solution (a) magnification 6000x and (b) magnification 12000x.

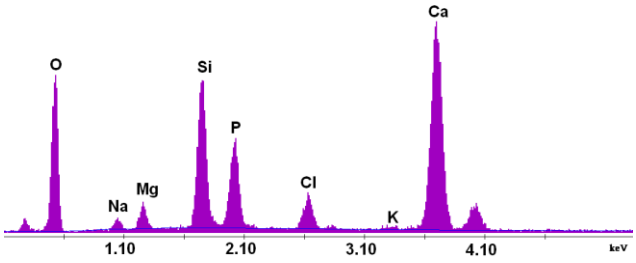


Fig. 10. EDAX spectrum of the stabilized glass after 14 days immersion in SBF solution.

According to Fig. 9 (a, b), the surface of porous bioglasses had been covered by distinct shaped aggregated particles. According to Figure 10, the EDAX results, beside Si, Ca and O elements, reveal the inclusion of phosphorous and other elements, such as Na, Cl, and K. The atomic ratio of Ca/P was 1.78, which is close to the theoretical value of 1.67 for hydroxyapatite / HA, $(Ca_{10}(PO_4)(OH)_2)$ [13].

4. Conclusions

The PMMA beads obtained by suspension polymerization of MMA have spherical form with diameter <300 µm. According to FTIR measurements, no traces of organic matter or nitrate groups were found in the stabilized glass. The glasses produced are amorphous, even after stabilization at 700 ^{0}C. These glasses have a macroporous network due to the elimination of PMMA beads by combustion, containing pores mainly between 5 and 100 µm. After immersion in SBF solution, the surface of porous bioglasses had been covered by hydroxyapatite confirmed by EDAX and XRD diffractograms.

Therefore, these porous calcium silicate glasses are bioactive and may be used as biomaterials in tissue engineering applications.

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