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Swelling properties and bioactivity of silica gel/pHEMA nanocomposites

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Abstract A novel hydrogel based on 2-hydroxyethyl-methacrilate and SiO_2 nanoparticles was prepared. The filler was added at a concentration of 30% w/w of silica nanoparticles to the mass of polymer. The composite material was characterised as far as concerns swelling behaviour in comparison to pHEMA. Swelling ratio of modified pHEMA was higher. Bioactivity of both SiO_2 nanoparticles and the modified hydrogel was evaluated by soaking samples into a simulated body fluid (SBF). FT-IR spectroscopy, scanning electron microscopy (SEM) and energy dispersive system (EDS) results suggest silica nanoparticles keep bioactive in the polymer. SiO_2 filler in a p(HEMA) matrix makes the composite bioactive. Therefore, these composites can be used to make bioactive scaffold for bone engineering.

Introduction

Composite materials consisting of organic polymers and inorganic oxide particles are widely used for biomedical application [1–3].

Poly- (2-hydroxyethyl methacrylate) (pHEMA), a biocompatible hydrogel proposed as early as 1960 [4–6], is yet today an outstanding material in this field. It is used to make ophthalmic prostheses (contact or intraocular lenses), vascular prostheses, drug delivery and soft-tissue replacement [7].

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p-HEMA, as an hydrogel, can imbibe large amounts of water by swelling without dissolving, thus letting: tissue ingrowth; high permeability to small molecules to have highly purified networks; soft consistency, which minimizes mechanical frictional irritation to surrounding tissues; low interfacial tension between the hydrogel and the aqueous solution that can reduce proteins absorption to the gel; and a large number of morphologies. Three types of attachment to living tissues are possible: biological fixation, which is due to bone or tissue in growth; morphological fixation, linked to the forces that are generated on the swelling when the hydrogel is placed in a constrained space; bioactive fixation, that has the biomaterial bound to the living tissue, through an apatite layer [8, 9].

Bioactive fixation can be obtained by surface modification of pHEMA, this was performed in the past through the biomimetic method [10, 11].

A novel approach in designing innovative hydrogels by modification of these materials with the addition of nanoparticles is proposed in this work. Incorporating a dispersed inorganic filler in a polymeric material is a straightforward method to improve mechanical properties [12]. Moreover, the silica gel/pHEMA composite is bioactive and keeps swelling properties, thus assuring both morphological and bioactive fixation.

Experimental chemicals

2-hydroxyethyl methacrylate (HEMA), 2,20-azoisobutyronitrile (AIBN) and ethanol were supplied by Fluka, Milan, Italy. Both tetraethoxysilane (TEOS) and ammonia solution in ethyl alcohol were purchased by Sigma-Aldrich, Milan, Italy.

Polymers synthesis

Commercial HEMA contains about 200 ppm of hydroquinone monomethylether (IQ) as quencher and 0.4% of ethylene glycol dimethacrylate (EGDMA), a bifunctional agent, as contaminant. To eliminate these impurities, a purification step, based on silica gel adsorption chromatography (granulometry 0.040–0.073 mm), was carried out. Purification of HEMA monomer was confirmed by Gas Chromatography, using a Hewlett-Packard instrument Mod. 5971 A, equipped with a HP-5 column and a UV detector.

pHEMA was prepared by radical chain polymerisation using AIBN as initiator at a concentration of 0.1% w/w respect to the monomer. The mixture was degassed for about 15 min under nitrogen flow, loaded on sealed glass chambers equipped with a vulcanised silicon rubbers, and cured in a stove according to the following thermal program: 2 h at 60˚C, 4 h at 70˚C, and 1 h at 85˚C. After the polymerisation, the gels were extensively washed with sterile distilled water.

Silica nanoparticles synthesis

Silica gel nanoparticles were produced through sol-gel synthesis, using the Stober's method [13]. Tetraethylortosilicate (TEOS) and a 2M ammonia solution in ethyl alcohol were used as received. The appropriate amounts of TEOS and ammonia solution were mixed with distilled water at room temperature. The concentrations of the obtained solution were 1.7 M NH_3, 0.270 M TEOS and 5.00 M H_2O. It was kept under magnetic stirring for two hours. Particles were centrifuged to wash out ammonia and then dried at room temperature for 48 hours.

Nanocomposites synthesis

P(HEMA) polymer was mixed with methyl alcohol, when a homogeneus solution was obtained the silica gel nanoparticles were added at a concentration of 30% w/w of silica nanoparticles to the mass of polymer. The mixture was kept under magnetic stirring until the methyl alcohol completely evaporated. The obtained nanocomposites were washed with distilled water and then cured at 60˚C over night in a forced-air circulation oven. All samples appeared white and quite rigid.

Materials characterization

A scanning electron microscope Leica Stereoscan 440 was used, equipped with an energy dispersive analytical system (EDS) INCA Energy 200–Oxford Instruments.

Fourier transform infrared (FTIR) transmittance spectra were recorded in the 400–4000 cm^{-1} using a Nexus FT-IR spectrometer, operating etiher in transmission or using a single reflection attenuated total reflectance (ATR) accessory, with a resolution of 2 cm^{-1} and 20 scans.

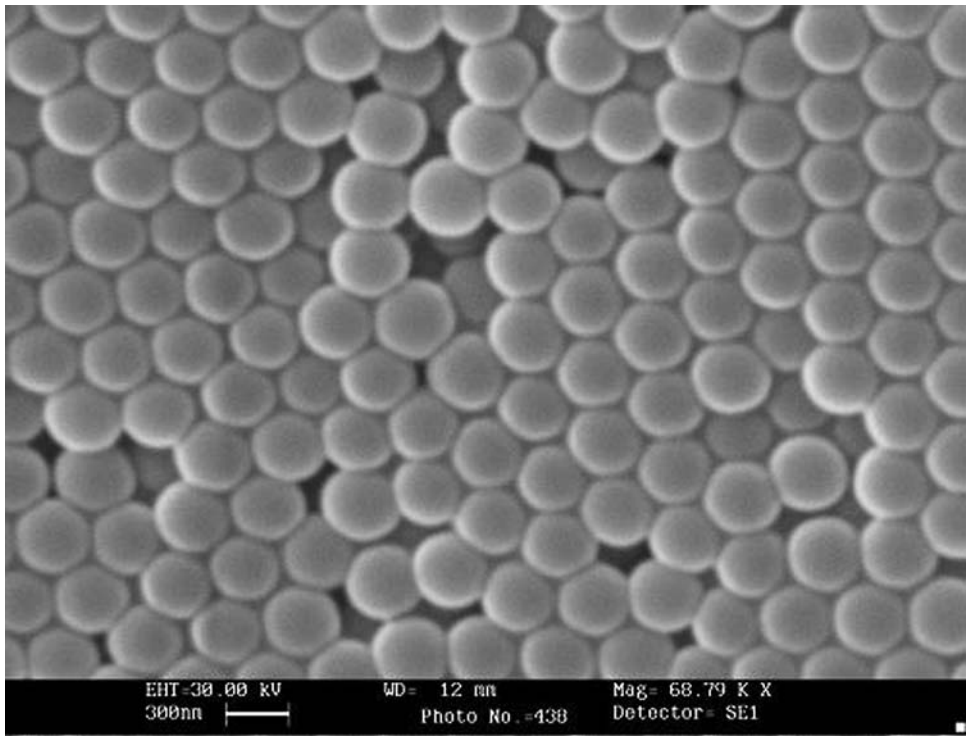


Fig. 1 SEM image of SiO_2 particles, 230 nm in size.

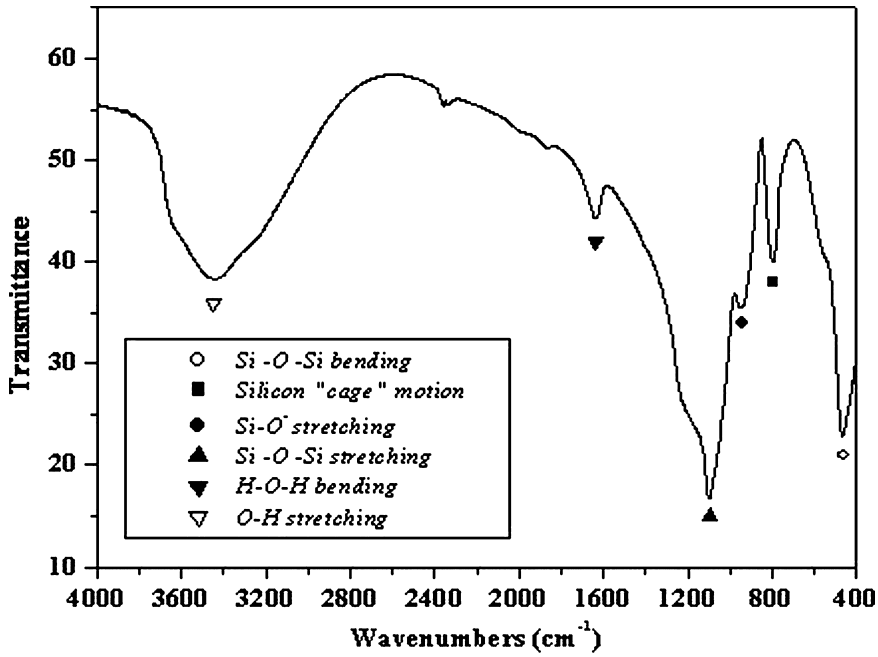


Fig. 2 FT-IR spectrum of silica nanoparticles.

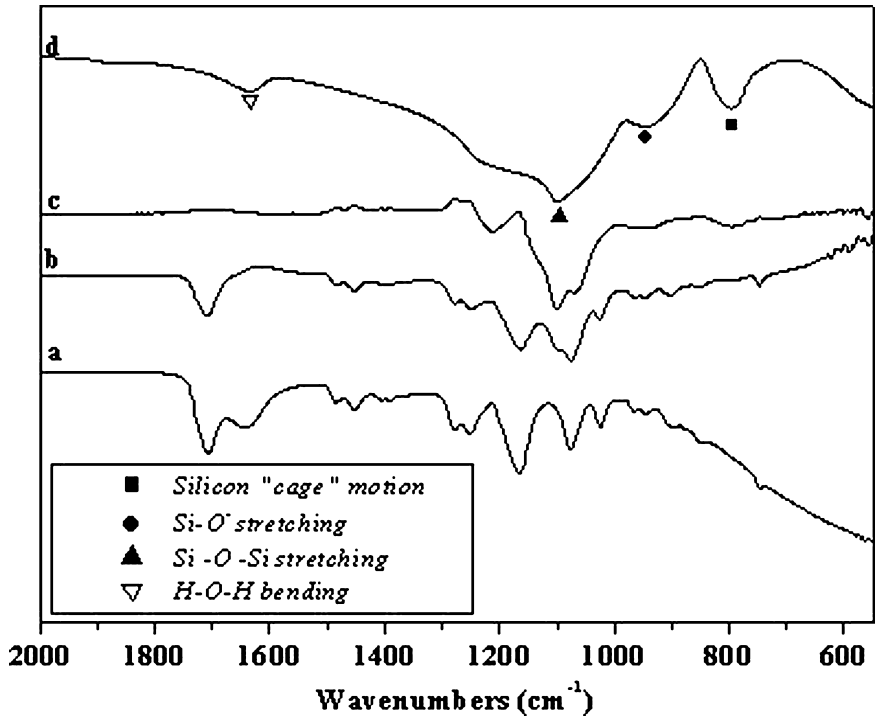


Fig. 3 FT-IR spectra of: pHEMA (a); pHEMA+SiO_2 (b); pHEMA+SiO_2 after subtraction of pHEMA spectrum (c); silica nanoparticles (d).

Results and discussion

Typical nanoparticles morphology is shown by SEM micrograph in Fig. 1. As can be seen a narrow size distribution was obtained and the average particles size was 230 nm.

Figure 2 reports an FT-IR spectrum of silica nanoparticles. The peak at 1100 cm^{-1} can be attributed to Si-O-Si stretching vibration modes in SiO_4 units, that at 470 cm^{-1} is usually assigned to Si-O-Si bending, whereas the peak at 800 cm^{-1} is assigned to Si-O-Si bond vibration between two adjacent tetrahedra [14, 15], occurring in silica gel [16]. The peak at 950 cm^{-1} can be attributed to Si-O terminal non-bridging vibration [17, 18].

Figure 3 gives FTIR spectra for the pure pHEMA (Fig. 3a) and the composite material, filled with 30 wt % of silica. Figure 3b show a marked increase of the band below 1100 cm^{-1}. This is due to the overlapping of pHEMA groups with Si-O-Si stretching (1100 cm^{-1}), thus confirming the presence

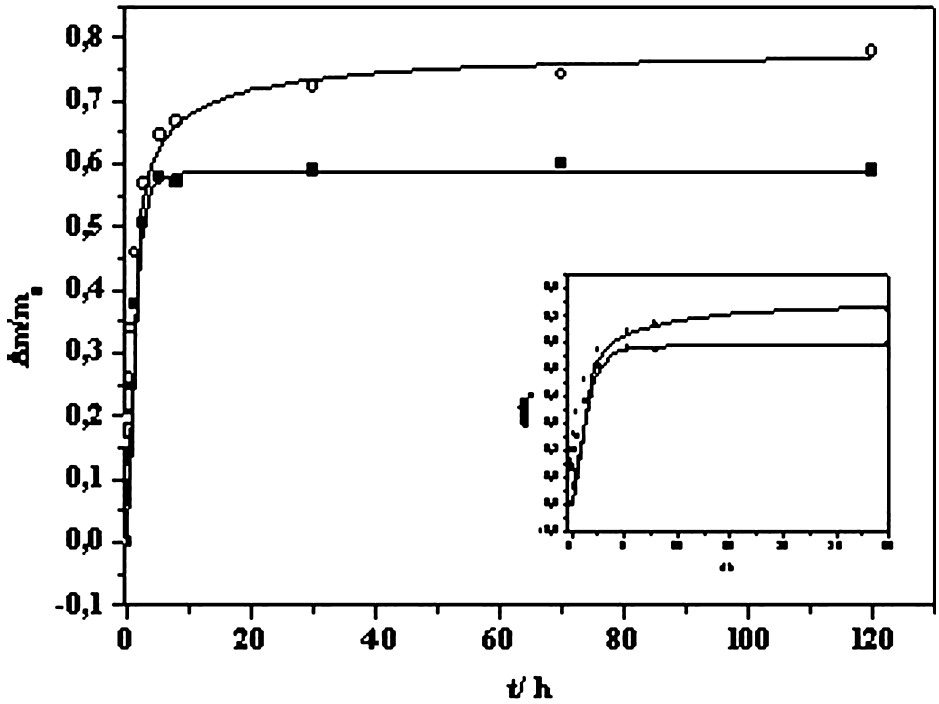


Fig. 4 Swelling kinetics: wt% increase vs soaking time in distilled water; ■) pHEMA; ●) pHEMA +SiO_2.

Table 1 Ion concentration (mM) of simulated body fluid (SBF) and blood plasma.

Ion	Simulated fluid	Blood plasma
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	147.8	103.0
HCO_3^-	4.2	27.0
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5

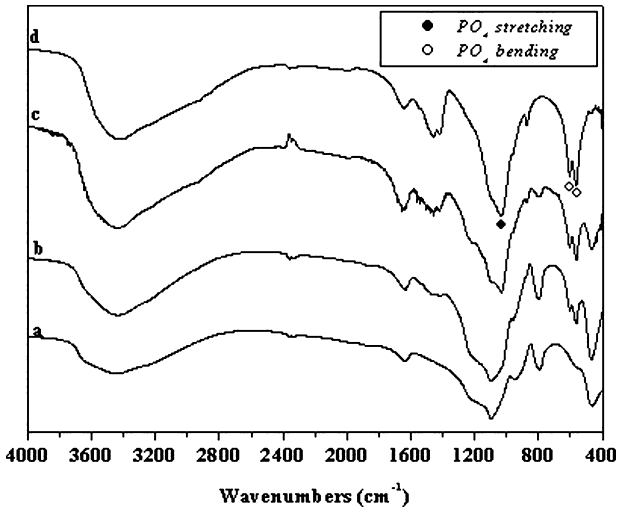


Fig. 5 FT-IR spectra of SiO_2 nanoparticles: (a) as-prepared; (b) after 4 days soaking in SBF; (c) after 7 days soaking in SBF, (d) after 14 days soaking in SBF.

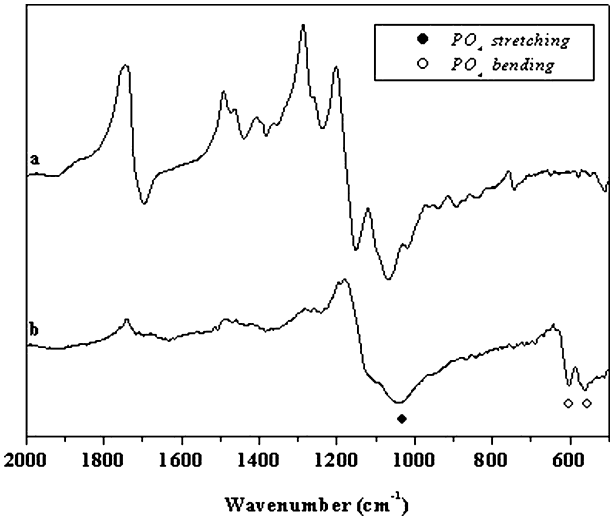


Fig. 6 FT-IR spectra of p(HEMA)/ SiO_2 nanoparticles (a) as-prepared; (b) after 24 days soaking in SBF.

of silica phase. Moreover, spectrum 3c, resulting from the subtraction of pHEMA spectrum (3a) from spectrum 3b, has the main band in the same range as silica gel nanoparticles (Fig. 3d). This result, combined with the white appearance of all samples, suggest that composite is made of two phases: a pHEMA rich phase and a silica rich one and not of a single organic-inorganic copolymer phase [19]. Covalent bonds might take place at organic-inorganic interfaces by heterocondensation reactions of HEMA hydroxyl groups and silanols [19–21]. Unfortunately, the presence of interfacial covalent bonds (Si-O-C bonds) are very difficult to probe

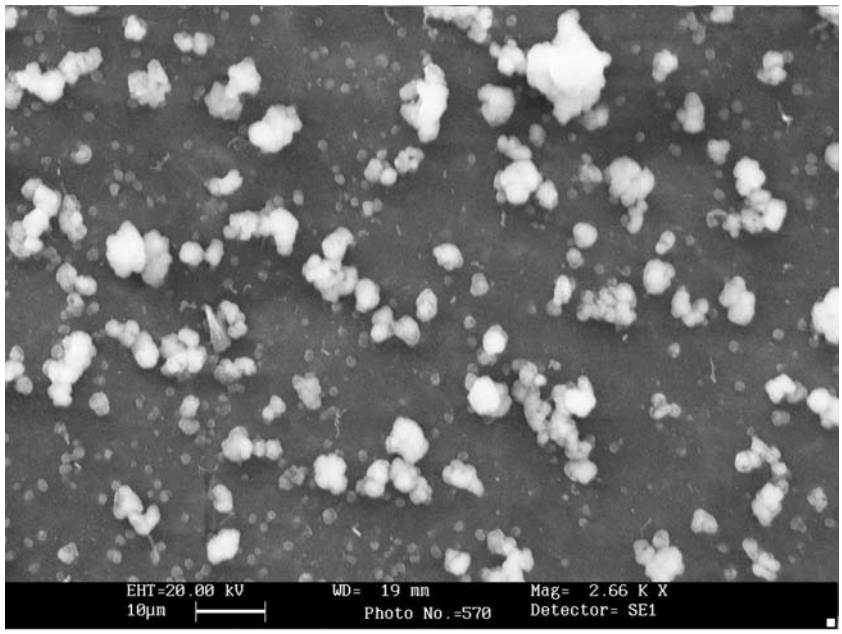


Fig. 7 SEM micrograph (a) and EDS spectrum (b) of PHEMA/SiO_2 nanocomposite after 14 days soaking in SBF.

by FT-IR since the spectrum region around 1000 cm^{-1} is very complex. Anyway results in literature seem to indicate that no extensive covalent interfacial bonding has taken place in this nanocomposite samples [19].

A further characterization on the composite materials, in comparison to pHEMA, was obtained undertaking swelling experiments. The dry polymers, were placed into distilled water and the water uptake was followed by gravimetric measurements at different times. Swelling kinetics of pHEMA and the composite are reported in Fig. 4. Almost all materials reached saturation. Moreover, silica/pHEMA composites show higher equilibrium swelling than pHEMA. The swelling ratio of hydrogels depends on their free volume, degree of the chain flexibility, and cross-link density and hydrophilicity [20]. The increase of swelling ratio cannot be due to the release of silica nanoparticles into the surrounding medium, since the weight of the dry samples after soaking did not change. The change in the swelling ratio must be attributed to the presence of hydrated silica in the composite, making it more hydrophilic. Thus, an increase of swelling ratio can be expected [11].

Bioactivity was evaluated by soaking either the silica gel nanoparticles or nanocomposite samples into a simulated body fluid (SBF) with ion concentrations, reported in Table 1, nearly equal to human blood plasma. SBF solution was buffered at pH = 7.25; temperature was kept at 37°C during soaking [8, 9]. Figure 5 shows FT-IR spectra of silica gel nanoparticles soaked in SBF for various periods. After soaking in SBF, a new peak appears at 1035 cm^{-1}, which superimposes to Si-O-Si stretching mode at 1100 cm^{-1}. It can be assigned to the P-O stretching vibration mode of PO_4 units. Similarly, the 600 and 560 cm^{-1} peaks can be assigned to P-O bending vibration in a PO_4 unit [14, 15]. Moreover, the peak at 800 cm^{-1} decreases with soaking time in SBF. Thus, experimental results suggest that silica particles are uniformly covered by an apatite layer within 7 days. This apatite is considered to be a carbonate-containing hydroxyapatite, since it gives an infrared absorption peak at 1400 cm^{-1}, which is assigned to CO_3^{2-} groups.

Figure 6 show FT-IR spectra of pHEMA/SiO_2 nanocomposite as-prepared (Fig. 6a) and after 24 days soaking in SBF (Fig. 6b). As can be seen the characteristic peaks

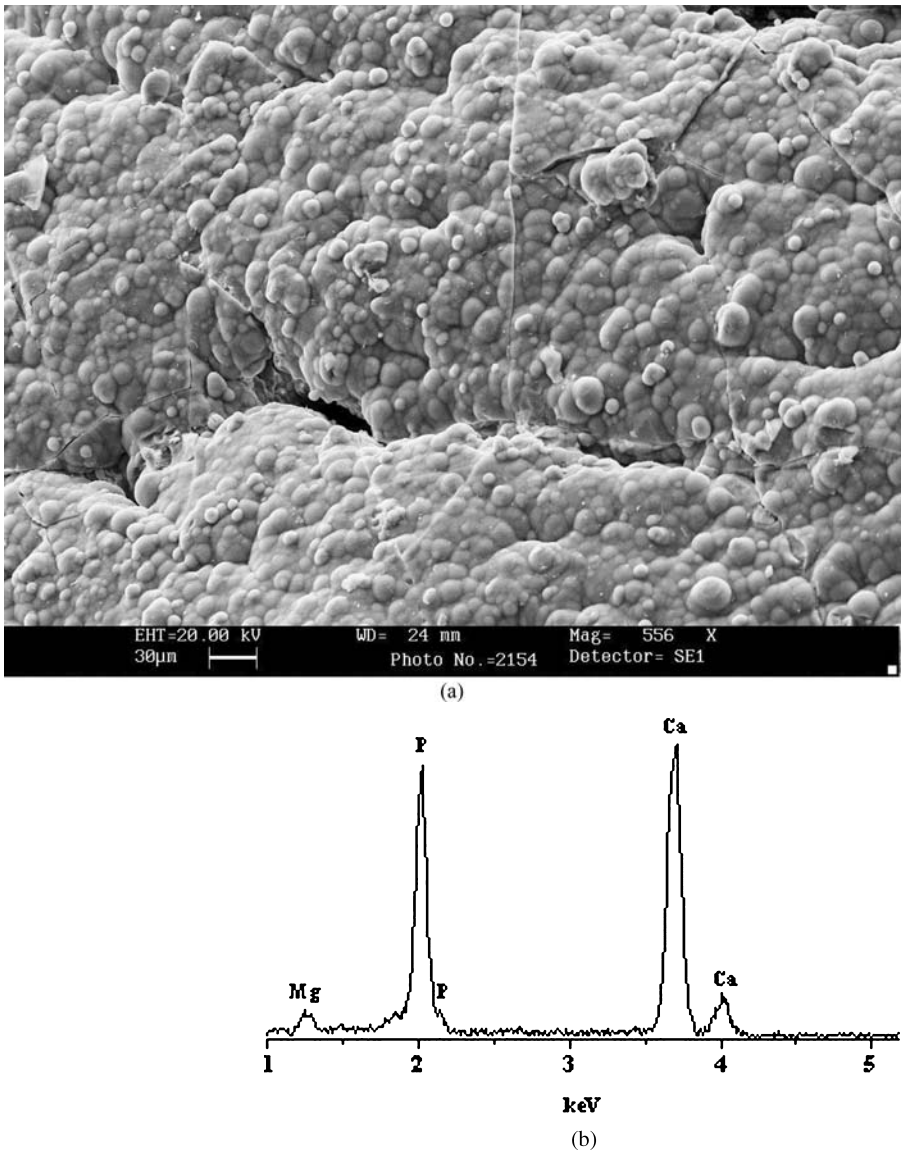


Fig. 8 SEM micrograph (a) and EDS spectrum (b) of PHEMA/SiO_2 nanocomposite after 24 days soaking in SBF.

of modified p(HEMA) disappear, owing to the formation of a surface coating. In fact, new peaks appear corresponding to P-O stretching (1035 cm^{-1}) and P-O bending (600 and 560 cm^{-1}) vibration mode [14, 15]. The deposition of an hydroxyapatite layer is confirmed by SEM and EDS results. Figure 7 shows a SEM micrograph (7a) and an EDS spectrum (7b) of a pHEMA/SiO_2 sample after 14 days soaking in SBF. As can be seen (Fig. 7a) some globular crystals appear on the surface. EDS results (Fig. 7b) confirm they are apatite crystals, as it shows Ca and P peaks. Figure 8 shows a SEM micrograph (8a) and an EDS spectrum (8b) of a pHEMA/SiO_2 sample after 24 days soaking in SBF. As can be seen, the surface is uniformly covered by globular apatite crystals, as confirmed by the EDS spectrum, which only shows Ca and P peaks. It is also known that when a glass is exposed to aqueous solutions three reactions can occur [23, 24]. First, modifier ions rapid exchange with H_3O^+ from the solution:

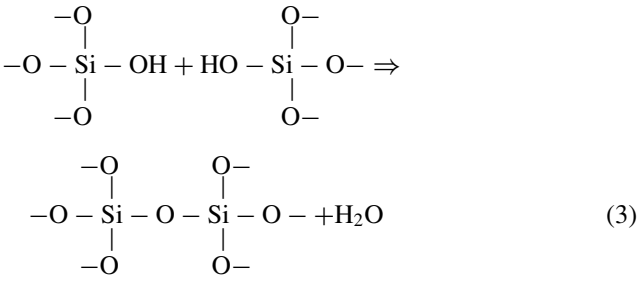
$$2(\equiv SiO^-)Ca^{2+} + H_2O \Rightarrow 2 \equiv SiOH + Ca^{2+} + OH^- \quad (1)$$

Second, some soluble silica is lost in the form of $Si(OH)_4$ to the solution, as a result of the breaking of Si-O-Si bonds and formation of Si-OH groups at the glass solution interface:

$$\equiv Si - O - Si\equiv + H_2O \Rightarrow 2\equiv Si - OH. \quad (2)$$

Then, condensation and repolymerization of a SiO_2 rich layer occur on the surface, which is depleted in alkalis and

alkaline-earth cations:



$$-O-Si(-O-)(-O-)-OH + HO-Si(-O-)(-O-)-O- \Rightarrow -O-Si(-O-)(-O-)-O-Si(-O-)(-O-)-O- + H_2O \quad (3)$$

Next, migration of Ca^{2+} and PO_4^{3-} ions from solution through the SiO_2 rich layer allows formation of a $CaO\text{-}P_2O_5$ rich film by precipitation from supersaturated solution. The $CaO\text{-}P_2O_5$ film, which is initially amorphous, crystallizes by incorporation of OH^- and/or CO_3^{2-} anions from solution to form a mixed hydroxyl-carbonate apatite layer [8, 9].

Experimental results suggest silica nanoparticles keep bioactive in the polymer and have the composite covered by a uniform apatite layer.

Therefore, SiO_2 filler in a p(HEMA) matrix makes the latter bioactive and is a valid alternative to the biomimetic method [10, 11].

Conclusions

This paper describes the synthesis and characterization of a novel composite material based on SiO_2 nanoparticles and pHEMA. In particular, silica nanoparticles improve swelling properties with respect to pHEMA. Moreover, SiO_2 nanoparticles proved bioactive and promote apatite formation on the surface of the modified hydrogel, when it is soaked in SBF. The obtained bioactive composites can be used to make bioactive scaffold for bone engineering.

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