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Hydroxyapatite coating of poly(2-hydroxyethyl methacrylate) hydrogel by biomimetic method

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The recently proposed biomimetic method has been performed on samples of poly(2-hydroxyethyl methacrylate) p(HEMA) hydrogel in order to improve their interface with bone. Following the biomimetic process it was possible to obtain a bioactive hydroxyapatite coating on the p(HEMA) substrates. FTIR analysis showed that characteristic peaks of p(HEMA) progressively disappear, owing to the formation of a surface coating. In fact new peaks appear corresponding to the ones of P-O stretching (1116 and 1035 cm^{-1}) and P-O bending (580 cm^{-1}) vibration modes, thus suggesting that the method is effective in promoting the formation of a surface phosphate layer. The formation of hydroxyapatite crystals is confirmed by SEM and EDS results. The adhesive strength was measured and turned out to be higher than the one reported for PMMA substrate. The experimental results suggest that, as reported in the literature for other supports, the silicate ions released from the glass in the first stage bind themselves to the polymeric support.

1. Introduction

A new class of biomaterials has been, recently, discovered [1, 2]; they are named bioactive ceramics because of their ability to bind to living bone. The contact with blood plasma, in fact, makes the surface to be covered with an hydroxyapatite layer on which the osteoblasts can proliferate. Recently it has been found [2–4] that bioactive glasses can help to form the same kind of apatite layer on the surface of other materials, both organic (Polyethyleneterephtalate, Polymethylmethacrylate, Polyethersulfone, Nylon 6, Polytetrafluoroethylene, Polyvinylalchol hydrogel) and inorganic (titanium metal, alumina ceramic, carbon cloth, silicon single crystal). In fact in the first stage of the method the substrate is soaked in a simulated body fluid (SBF) in contact with a bioactive glass. This stage is reported [2–4] to be essential to form hydroxyapatite nuclei that grow in the subsequent stage, when the support is soaked in a solution (1.5 SBF) with ion concentrations 1.5 times those of SBF.

Following this method it is expected that a dense and uniform layer of a bone-like apatite could be formed on various polymers. It is also known that poly(2-hydroxyethyl methacrylate) hydrogel is a promising material for orthopedic application as a stabilizing interface between bone and implant [5]. This is due to the forces that are generated on the swelling of the hydrogel when it is placed in a constrained space. The formation of an hydroxyapatite layer on the hydrogel coating could improve the bonding to living bone and lead to better stability of the interface by additional bioactive fixation.

In this paper the results of the application of the biomimetic method to poly(2-hydroxyethyl methacrylate) hydrogel are reported.

2. Experimental

2.1. Materials

2.1.1. Preparation of the glass

The glass of composition $MgO \cdot 7CaO \cdot P_2O_5 \cdot 5SiO_2 \cdot 0.056CaF_2$, which is the parent glass of glass-ceramics A-W, was prepared by melting pure reagent grade $MgCO_3$, $CaCO_3$, SiO_2, $NH_4H_2PO_4$ and CaF_2 in a platinum crucible at 1600 °C for 3 h. This glass, indicated as the G glass, is the one used by the researchers that proposed the biomimetic method [3, 4].

2.1.2. Preparation of solution

A simulated body fluid (SBF) with ion composition nearly equal to the blood plasma one and an aqueous solution (1.5 SBF) with ion concentration 1.5 times those of SBF were prepared by dissolving reagent grade NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$ and Na_2SO_4 in distilled water as reported by other researchers [3, 4]. They were buffered at pH 7 · 25 by using trishydroxymethilaminomethane

(Trizma base) and trishydroxymethilaminomethane-cloridric acid (trisma-HCl).

2.1.3. Preparation of poly(2-hydroxyethl methacrylate) substrate

Poly(2-hydroxyethyl methacrylate) or p(HEMA) films with thickness of 1.5 mm were prepared by adding 0.5 wt% ethylene dimethacrylate (EDMA) (Aldrich) as crosslinking agent and 0.1 wt% of 2, 2-azobisisobutyronitrile (AIBN) (Fluka Chemie) as initiator to 2-hydroxyethyl methacrylate (HEMA) (Aldrich) monomer; the reactive solution was poured into flat glass mold and cured at 80 °C for 2 h. After cooling, each sample was placed in distilled water at 37 °C for 48 h to remove any residual monomer.

2.2. Methods

2.2.1. Formation of hydroxyapatite layer

In order to apply the biomimetic method, samples of p(HEMA) were soaked, in contact with powdered (+150–300 μm) G glass in SBF. The amount of p(HEMA) and SBF were such to have a ratio of exposed surface to volume of SBF equal to $S/V = 10\,\text{mm}^2/\text{ml}$ SBF, as the one reported in the original papers on this method [3, 4]. Then the substrates were soaked in 1.5 SBF, whose volume was such to have the same S/V ratio. The compositional and structural changes occurring at the surface were followed by means of FTIR, SEM and EDS.

2.2.2. Infrared analysis

Fourier transform infrared (FTIR) transmittance spectra were recorded in the 400–120 cm^{-1} region using a Mattson 5020 system, equipped with a DTGS KBr (Deuterated Triglycine Sulphate with potassium bromide windows) detector, with a resolution of 2 cm^{-1} (20 scans). KBr pelletised disks containing 2 mg of sample and 200 mg KBr were made. The FTIR spectra have been elaborated by means of a Mattson software (FIRST Macros). FTIR was performed on powdered (+63–90 μm) samples soaked as such in SBF and 1.5 SBF.

2.2.3. Scanning electron microscopy

An electron microscope Cambridge Stereoscan 240 has been used, equipped with an energy dispersive analytical system (EDS) LINK AN 10000 in order to verify the morphology of the coated sample.

2.2.4. Measurement of the adhesive strength

In order to measure the adhesive strength of the hydroxyapatite layer, a pair of brass jigs with a base diameter of 6 mm were attached to both the apatite layers deposited on opposite outer surfaces of the p(HEMA) substrates with cyanoacrilate adhesive and left overnight for complete curing of the adhesive. Then the system jigs-p(HEMA) was fixed in appropriate grips of the Instrom Machine Mod. 2404 and tested in a tensile mode at $T = 23\,^{\circ}\text{C}$ and R. H. = 60%, at cross-head speed of 1 mm/min until fracture occurred between the apatite and the p(HEMA) substrate. The adhesive strength was measured on seven samples.

3. Results and discussion

In Fig. 1 the FTIR spectra are reported. Fig. 1a shows, for the sake of comparison, the spectrum of the not soaked p(HEMA). The effect of the application of the biomimetic method is shown in Fig. 1b and c, differing for the duration of the first stage. As can be seen the peaks characteristic of p(HEMA) progressively disappear, owing to the formation of a surface coating. In fact new peaks appear corresponding [6] to the ones of P-O stretching (1116 and 1035 cm^{-1}) and P-O bending (580 cm^{-1}) vibration modes, thus suggesting that the method is effective in promoting the formation of

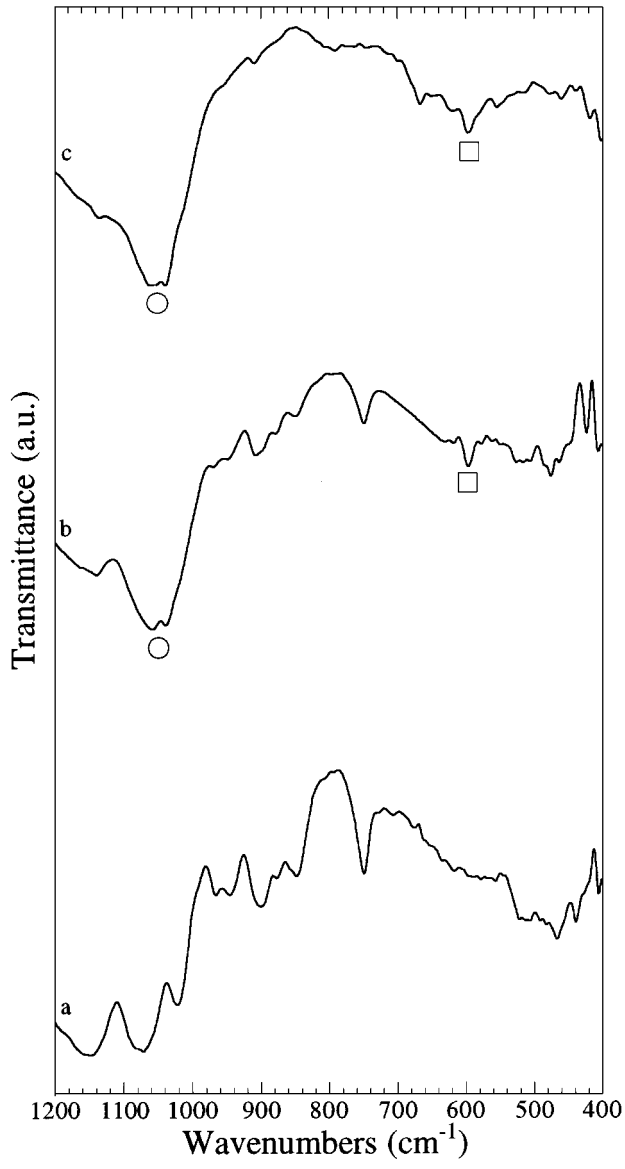

Figure 1 FTIR spectra of poly2-hydroxyethylmethacrylate p(HEMA) hydrogel after soaking in SBF and 1.5 SBF: (a) not soaked; (b) 3 h in SBF and 6 d in 1.5 SBF; (c) 6 h in SBF. and 6d in 1.5 SBF. (○) P-O stretching; (□) P-O bending.

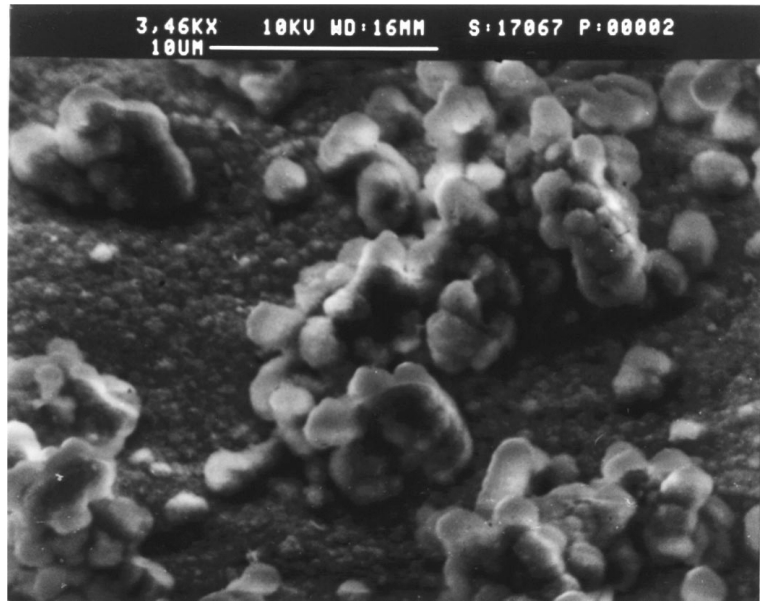

Figure 2 SEM micrograph of p(HEMA) after soaking for 4 days in SBF and 6 days in 1.5 SBF.

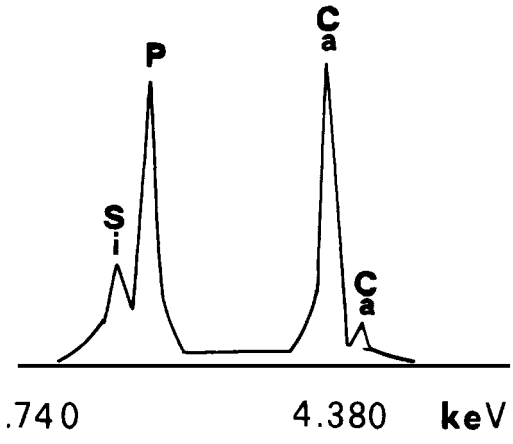

Figure 3 EDS spectrum of p(HEMA) after soaking for 4 days in SBF and 6 days in 1.5 SBF.

a surface phosphate layer. The results are also consistent with the findings reported in the literature that the apatite formed on the substrate increases in the density with increasing duration of the first stage, to form a continuous and uniform layer after a first stage whose duration should range [3] from 1 to 4 days depending on the nature of the substrate. The effect of so long times could not be studied, in our case, because of sintering of the mixed powders of hydrogel and G glass, that prevented to separate them after the first stage. This was certainly due to the formation of the phosphate rich layer on both p(HEMA) and G glass, which is known to be bioactive. However the results of Fig. 1 suggest that, in the studied case, 6 h is a time sufficiently long to have a good coating of the sample. The shortness of the time as compared to the ones reported in the literature (1–4 days) for the first stage, can be attributed, almost in part, to the greater reactivity of the fracture surfaces obtained in the grinding to (+63–90 μm) powder grains.

The effectiveness of the method is confirmed by SEM and EDS results. In Fig. 2 the SEM micrograph of a bulk sample soaked for 4 days in SBF, in contact to G glass, and 6 days in 1.5 SBF is shown. As can be seen the surface appears to be uniformly covered of globular crystals showing the same morphology than the ones reported to form in the case of hydroxyapatite coating on silica gel and on the surface of other supports [2–4]. The microprobe analysis is reported in Fig. 3 and shows the peaks of Ca, P and Si. Therefore the formation of hydroxyapatite crystals is confirmed. The presence of Si is coherent with the mechanism previously suggested [7]. In fact calcium and silicate ions are released from the surface of bioactive glasses owing to the reactions of cation exchange and hydrolysis occurring during soaking in SBF. The calcium ions increase the ionic activity product of the apatite in the narrow gap between the substrate and the glass particles. Otherwise it is believed [7] that the silicate ions bind themselves to the surface of the substrate and catalyze, there, the nucleation of apatite, in the same way as on the surface of the bioactive glasses [2].

The adhesive strength of the apatite layer on the dry p(HEMA) substrate was calculated to be 2.48 MPa. This value was higher than the one reported [4] for PMMA substrate (1.06 MPa). The higher value of the adhesive strength may be attributed to the higher amount of polar groups present in the polymeric structure of p(HEMA). The adhesive strength may be increased by surface modification of the substrate.

4. Conclusions

The experimental results suggest that the biomimetic method can be successfully used to obtain an hydroxyapatite coating on p(HEMA). The adhesive strength was measured and turned out to be higher than the one reported for PMMA substrate. The results well agree with the mechanism reported in the literature. The coating of hydroxyapatite on p(HEMA) hydrogel by biomimetic process can improve the interface hydrogel-bone and may make this system very interesting for repairing hard and soft tissue.

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