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Dynamics of calcium and phosphorus ions at the interface of sol-gel hydroxyapatite with simulated body fluid

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Hydroxyapatite [$Ca_{10}(PO)_4(OH)_2$] type calcium phosphate powders were prepared by sol-gel method. By filtering and washing several times with deionized water samples with pH 6.5, 8 and 11 were obtained. In order to prove their bioactivity, the samples were soaked at 37 ^{0}C for several periods in simulated body fluid with almost equal ion concentrations to those of human blood plasma. Data on conductivity, calcium and phosphorus concentrations in SBF were used to estimate the dynamics of these cations on the hydroxyapatite interface with the simulated body fluid. The scanning electron microscopy evidences morphological changes on the sample surfaces after immersion in SBF.

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1. Introduction

Hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$, the main inorganic component of the hard tissues in bones, is a member of apatite family, including compounds with similar structure but not necessarily of identical composition. Biological apatites comprise the mineral phase of calcified tissues (enamel, dentin and bone). They differ from pure HA in stoichiometry, composition and crystallinity, as well as in other physical and mechanical properties.

Coatings of HA are used to improve the biocompatibility of dental and orthopedic metal implants [1].

The human bone is formed basically by an organic phase and other minerals. In the organic phase, the fibres of collagen serve as a matrix for the precipitation of HA, determining the organization of the crystals. The collagen gives the bone its elastic resistance. The mineral phase is mainly formed by HA. Hydroxyapatite that integrates the mineral phase of the bone is said to be non-stoichiometric and calcium-deficient with a relationship of $Ca/P < 1.67$ [2, 3].

Investigations in cell-free solution with compositions that are similar to human body fluid allow the determination of chemical and mineralogical changes of the implants under simulated physiological environments [4]. Simulated body fluid (SBF) with inorganic ion concentration nearly to those of human blood plasma, which was first introduced by Kokubo et al., is the most frequently used simulating solution for *in vitro* tests [5].

The objective of the current work is to study the effect of different pH values of sol-gel derived hydroxyapatite on the dynamics of calcium and phosphorus ions at the interface of hydroxyapatite with the simulated body fluid.

2. Experimental

HA powders were prepared by sol-gel method using calcium oxide (CaO) and phosphoric acid (H_3PO_4). The dried HA sol-gel was calcined in air atmosphere at 1000 ^{o}C, and then it was finely ground in agate mortar. Hydroxyapatite samples with pH 6.5, pH 8, and pH 11 were obtained by filtering and washing several times with deionized water.

In order to check the bioactivity of HA powder of different pH values, the samples were immersed in simulated body fluid (SBF) at 37 ^{0}C for 2, 7, 14, and 21 days in a programmable thermostat. The solutions were not renewed in all this time.

The SBF was prepared according to Kokubo's protocol [3] by dissolving in deionized water: NaCl, $NaHCO_3$, KCl, $K_2HPO_4{\cdot}3H_2O$, $MgCl_2{\cdot}6H_2O$, $CaCl_2$, HCl (1M), Na_2SO_4 and $NH_2C(CH_2OH)_3$. The solution was buffered at pH 7.38 at room temperature.

Ca^{2+} concentration, conductivity and pH value of SBF solution before and after immersion of samples for different periods were measured at room temperature with Jasco digital multimeter Consort C833.

Phosphorus and calcium ion concentration in SBF solution before and after 21 days immersions of samples were measured using inductively coupled plasma mass spectroscopy (ICP-MS).

The morphological changes occurred on the surface of HA samples immersed in SBF were evidenced by scanning electron microscopy (SEM).

3. Results and discussion

In vitro dissolution behavior of HA type materials depends on their composition, surface area, surface topography, micro- and macroporosities [2, 6]. This offer the possibility to produce HA coatings with the biodegradation rate desired for particular applications, by varying the composition and microstructure of the coating.

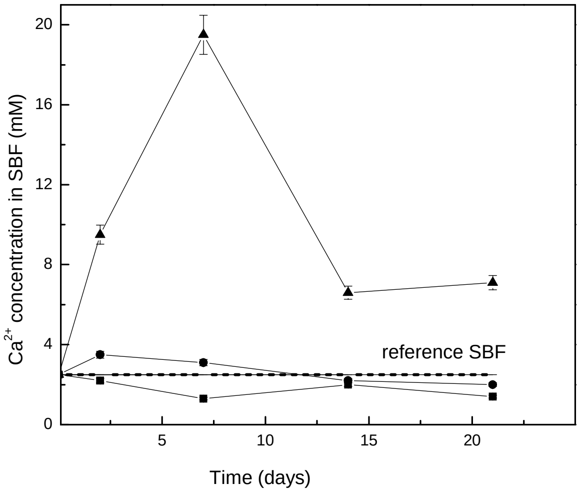


Fig. 1. Soaking time dependence of Ca^{2+} concentration in SBF solution (■ HA with pH 6.5, ● HA with pH 8, ▲ HA with pH 11).

The variation of Ca^{2+} concentration in SBF solution containing the investigated HA powders is shown in Fig. 1 as a function of soaking time. The increase followed by decrease of the Ca^{2+} ion concentration indicate dissolution and precipitation of apatite from the solution to the surface of the HA [2]. The Ca^{2+} ion concentration in SBF after 2 days increases for HA with pH 8 and pH 11, and decreases for HA with pH 6.5. The increase of Ca^{2+} ion concentration in the SBF solution confirms the biodissolution of HA with pH 8 and pH 11.

Fig. 2 shows the pH variation of SBF as function of soaking time. The pH value of the SBF solution contain the HA sample with pH 11 steady increases, while for the HA samples with pH 6.5 and 8 the pH of the SBF solution wherein they are immersed first decreases and only after two days exceeds the reference SBF pH value.

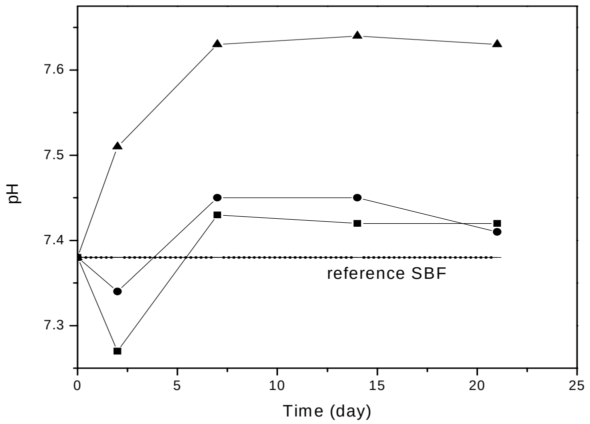


Fig. 2. Soaking time dependence of pH of SBF solution (■ HA with pH 6.5, ● HA with pH 8, ▲ HA with pH 11).

The increase in pH is considered to contribute to the partial dissolution of the outmost layer and the subsequent apatite precipitation [7].

Fig. 3 illustrates the conductivity variation of SBF as function of soaking time. After 7 days the conductivity reaches a maximum for all three HA samples.

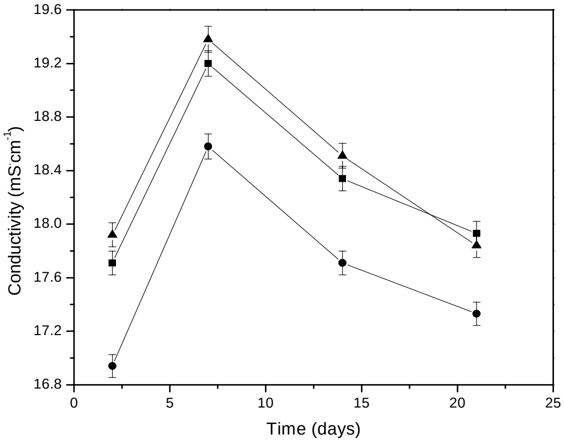
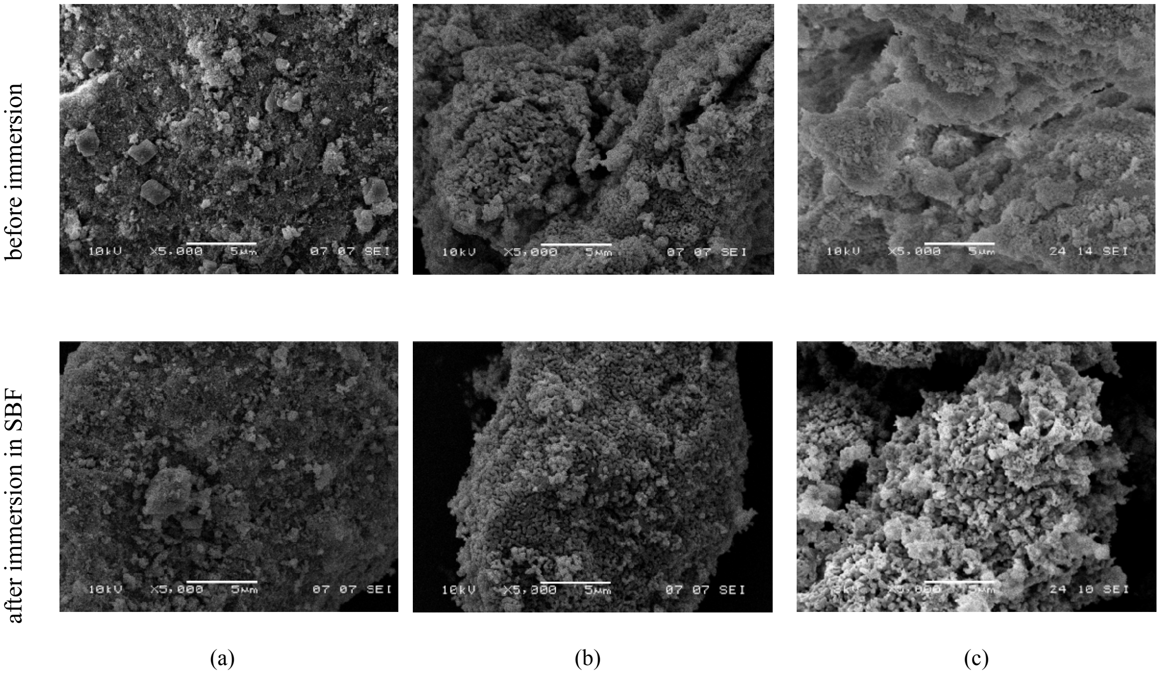


Fig. 3 Soaking time dependence of conductivity of SBF solution (■ HA with pH 6.5, ● HA with pH 8, ▲ HA with pH 11).

Data obtained by inductively coupled plasma mass spectroscopy (ICP-MS) regarding the phosphorus and calcium ion concentration in SBF solution, before and after 21 days immersions of HA, show that after 21 days the calcium concentration in SBF increases with the rise pH value and the phosphorus concentration decreases. The increase of Ca/P ratio in SBF reflects its lowering on HA surface below 1.67 which is the ideal ratio for HA (Table 1).



(a) (b) (c)

Fig. 4 SEM micrographs of HA with pH 6.5 (a), pH 8 (b) and pH 11 (c) before and after 21 days immersion in SBF.

Table 1. Calcium and phosphorus concentration and Ca/P ratio in reference SBF and in SBF with HA of different pH.

SBF	Ca (ppb)	P (ppb)	Ca/P
pure	27700	18100	1.53
with HA pH6.5	15400	360	4.27
with HA pH 8	22400	897	24.97
with HA pH11	96300	170	566.47

After the soaking, the surface was examined again using SEM morphological observation. Fig. 4 shows the SEM micrographs of the HA with different pH values before and after 21 days immersion in SBF. One remark a significant difference after SBF immersion of HA sample with pH 11, related to the apatite precipitation on sample surface.

4. Conclusions

The bioactivity of the prepared HA samples were proved by tests in SBF. The time dependence of Ca^{2+} ion concentration in SBF after immersion of HA samples indicates the dissolution of calcium and precipitation of apatite on the HA surface. After 7 days the conductivity of the SBF solution reaches a maximum for all three HA samples. The Ca/P ratio drastically increases in SBF solution. SEM images show a significant change after SBF immersion of HA sample with pH 11.

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