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Formation of calcium phosphate layer on ceramics with different reactivities

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Abstract

Biphasic ceramic samples of different biological reactivity are prepared by using hydroxyapatite (HAp) and tricalcium phosphate (TCP) in various ratios. Different parameters for sintering in an air atmosphere furnace were defined after dilatometric studies. An increased densification with decreased TCP content was observed. The sintered bodies were characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM). The dissolution kinetics and in vitro reactivity were investigated using simulated body fluid (SBF) at 37 °C for a maximum period of 3 weeks. The surfaces of the ceramics were analyzed by Fourier transform infrared spectroscopy (FTIR) and SEM in order to observe the formation of a calcium phosphate layer, which indicates the samples bioactivity. Dissolution in SBF demonstrated that layers with different kinetics on the samples surface were formed during the immersion period. The biphasic ceramics show bioactive behavior, even if the resorbable TCP is incorporated.

Keywords: Resorbability; Bioactivity; Hydroxyapatite; Biphasic ceramics

1. Introduction

Ceramic biomaterials consisting of calcium phosphates have been a successfully used alternative in different areas of biomedical applications, such as bone substitutes, ophthalmic implants, scaffolds for tissue engineering, and drug delivery system. Their biocompatibility properties in terms of bioactivity and solubility have already been reported in several studies [1–3].

When bioactive materials are implanted into the living body, interactions between the bone tissue and these materials only occur on their surface, with the bulk of the material remaining unchanged. It is generally known that TCP is more soluble than HAp at physiologic pH and also more susceptible to bioresorption. Therefore, biphasic calcium phosphate ceramics (BCP) of hydroxyapatite (HAp) and tricalcium phosphate (TCP) can develop an in situ formed porous structure after implantation into the living body by dissolution of the resorbable TCP phase of the interpenetrating TCP/HAp network. After dissolution only the bioactive HAp matrix remains [4,5].

The resorption rates of BCP in terms of their TCP/HAp ratio have been well discussed [6]. The biological behavior of these ceramics can be markedly influenced by the TCP phase content and affect the response of the implants. Thus, biphasic ceramics are suitable to combine stimulation of osteogenic activity with the resorbability during continuous remodeling [7].

Partial dissolution of the biphasic ceramic followed by an increase in the calcium and phosphate concentrations at the local environment is described to be very important for an excellent osteoconductivity and a tight chemical bond of the bioactive ceramic and the surrounding tissue with bone. A recent study demonstrated that HAp ceramics containing TCP induced better osseointegration than pure HAp ceramic when implanted into sheep femora [8]. The bioactive layer formation in ceramics of different resorbability is involved with a complex dissolution process [9]. Several factors such as stoichiometry, crystallinity, specific surface area, roughness, porosity and solubility are included into this process [10].

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A biological activity is characterized by the presence of a hydroxycarbonated apatite (HCA) layer. The mechanism of HCA formation on bioactive ceramics has been studied by various authors [11–14]. According to Kokubo et al. [12], an essential requirement for the in vivo bone ingrowth on a synthetic material is the formation of this bonelike apatite layer on the material surface. These materials of active surface are prone to in situ nucleation and crystal growth. Investigation of the in vitro reactivity by dissolution mechanism studies of calcium phosphate ceramics exposed to simulated body fluid (SBF) can provide relevant information concerning the precipitation mechanism of the bone like apatite layer [15,16].

The purpose of this study was to evaluate the effect of reactivity of calcium phosphate ceramics with different ratios of HAp and β-TCP, on the calcium phosphate layer formation by in vitro test in SBF, in order to investigate their dissolution/precipitation behavior.

2. Experimental

Biphasic calcium phosphates mixtures were prepared by using HAp (code: 20-2039 Strem Chemicals) and β-TCP (code: 21218-Fluka) with different HAp/TCP ratios, according to the description in the Table 1.

The powders were homogenized by turbula milling for 30 min, dried at 100 °C for 12 h, pulverized, and sieved (170 mesh). Each of the mixtures was uniaxially formed and subsequent pressed cold isostatically (200 MPa) into form cylindrical bodies with a diameter of 12 mm and a height of 7 mm. Dilatometric analysis of the samples were realized in a dilatometer (Netzsh-DIL 402E/7) in air atmosphere at 1250 °C with a holding time of 15 min. The heating and cooling rate was 10 °C/min. Aiming to reach comparable densities in the various compositions, the samples were sintered at different temperatures until they reached a high density without phase changes.

Table 1
Composition of the mixtures with different HAp/TCP (wt.%) fractions

Code	H	7H	5H	T
(wt.%) HAp	100.0	75.0	50.0	–
(wt.%) TCP	–	25.0	50.0	100.0

The in vitro reactivity study was carried out by soaking the samples into SBF [12] solution at 37 °C for a maximum period of 3 weeks, maintaining an initial pH of 7.25. A surface area/volume ratio of 0.05 cm^{-1} was used. The solutions were renewed every 3 days. The surface samples were examined before and after immersion into SBF using scanning electron microscopy (SEM, Philips-XL 30) and Fourier Transform Infrared spectroscopy (FTIR, Perkin-Elmer Spectrum GX) in the mode of total reflection.

3. Results and discussion

Regarding to a previous work [17], we used the following sintering conditions: HAp was sintered at 1100 °C for 1 h, sample 7H was sintered at 1200 °C for 30 min,

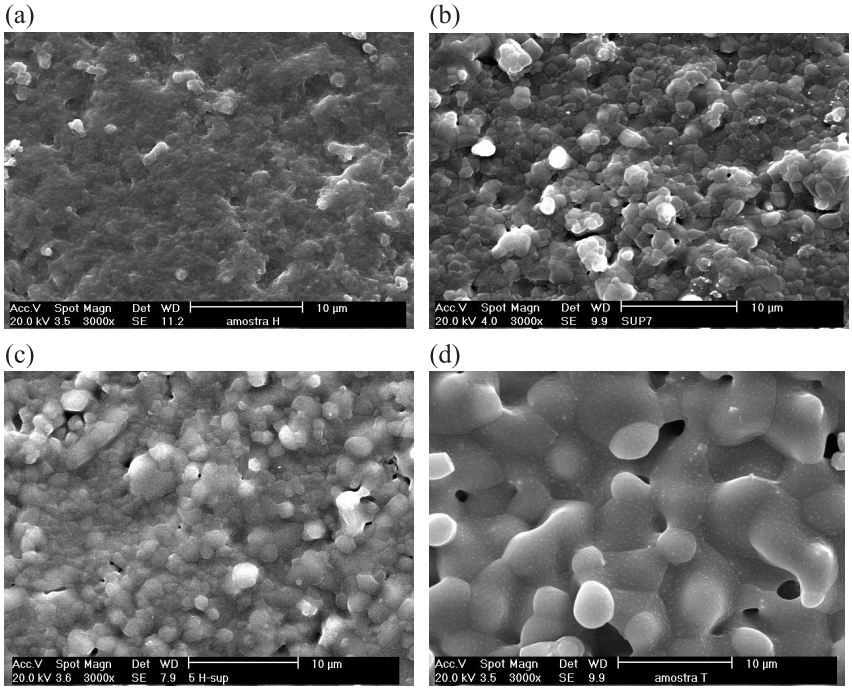

Fig. 1. SEM micrographs of sample surfaces after sintering; (a) H; (b) 7H; (c) 5H, and (d) T.

and TCP and sample 5H were sintered at 1250 °C for 15 min. Under these conditions, approximately 95% of the theoretical density was reached for all samples, without any phase changes [17].

Fig. 1 shows the microstructure of the sintered sample surfaces. A similar morphology between the BCP samples and an increase of the grain size and pores for TCP sample were observed.

Innumerous factors can contribute the dissolution/precipitation and crystallization of a calcium phosphate layer [10,18]. Intrinsic characteristics of the surface can supply a differentiated dissolution in some samples. The presence of defects is one of the parameters that can affect the dissolution rate. These defects can be defined in terms of (a) chemical heterogeneity or (b) surface roughness, acting as a site for dissolution [9,10,19]. Nanometric pores can also act as early sites for nucleation and growth of apatite crystals [10]. The surface roughness observed on TCP samples can assist an accelerated nucleation.

The layer formation as consequence of the dissolution and precipitation process of calcium phosphates was investigated by SEM. The micrographs of the ceramic surfaces after soaking in SBF for different time periods are presented in Fig. 2.

By increasing the β-TCP/HAp ratio of the samples, the dissolution rate can be controlled due to the degradability of

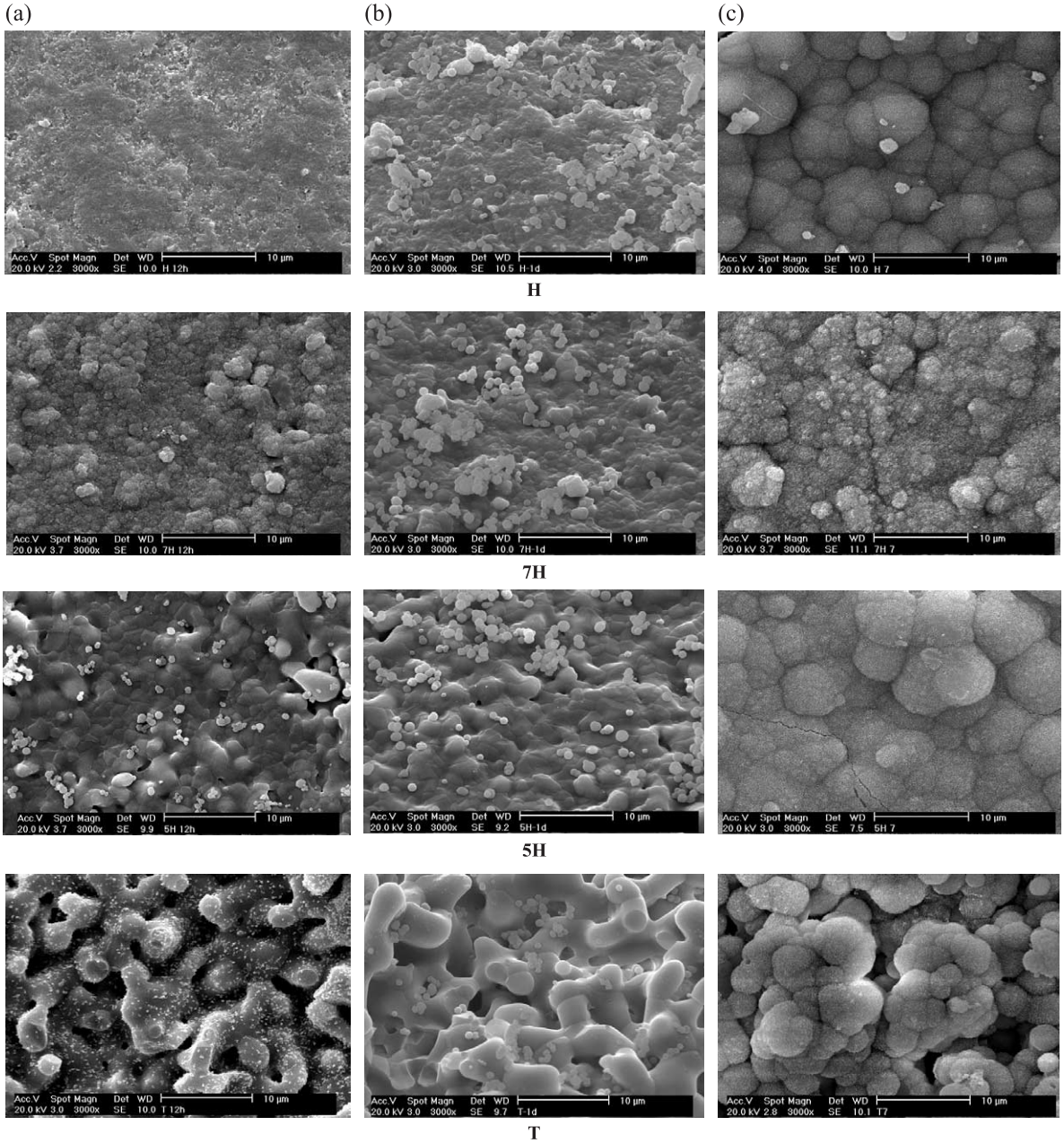

Fig. 2. SEM of the samples surfaces after exposure to SBF for: (a) 12 h, (b) 24 h, and (c) 3 weeks.

the β-TCP phase. For high concentration of HAp in the samples, the dissolution process slows down. In a previous study [20], it was shown that SBF solutions of the 5H and 7H samples exhibited more variations in pH than the others. An increased weight gain was observed for TCP and 5H samples. This behavior can be explained by the higher solubility of these samples due to the presence of the TCP phase. Thus, the supersaturation of the solutions is increased and the subsequent precipitation is initiated.

β-TCP exhibits a solubility product Kps of 2.83×10^{-30}, while Kps for HAp is 3.37×10^{-58}. Thus, the Ca^{+2} and PO_4^{-3} ions of TCP in nonequilibrium conditions tend to migrate more easily to the solid/liquid interface, decreasing its free energy [21,22]. The migration of these ions creates lacunae that can facilitate the liquid phase penetration and promote the nucleation, i.e., in SBF. The nucleation rate, in this case, is controlled by ionic diffusion [9,19]. The TCP surface presented high content of nucleus, but of little size. This behavior can be associated to the saturation state of the system that influences the nucleation and growth process [22]. In the precipitation and crystallization processes from aqueous solutions, a competition between nucleation and crystal growth phenomena occurs. These phenomena determine the size and morphology of the crystals in different saturation states [22]. This fact could explain the behavior of the TCP samples that probably exhibit a greater interfacial energy than other samples [22,23]. Its precipitates were unstable early, by pH sensibility and/or by the fact that a nucleus has not reached the critic size that is necessary for a stable nucleus formation, thus being dissolved and reprecipitated.

After 15 days of immersion the biphasic and bioactive ceramics showed a greater tendency for a homogeneous layer formation, with no differences between these samples concerning to the precipitated layer evolution.

Fig. 3 shows the infrared spectra before and after samples immersion into SBF for 3 weeks. The spectra reveal an amorphous state and a low thickness of the layer, resulting in the low definition of the spectra. In general, during in vitro experiments with SBF, the phases deposited on the surface are initially amorphous being subsequently crystallized to HCA [10,24,25].

The presence of characteristic bands for HAp with detectable peaks at wave numbers 561, 603, 962, 1033, and 1089 (cm^{-1}) regarding to phosphate groups and 631 and 3572 (cm^{-1}) regarding to the hydroxyl group [21,26].

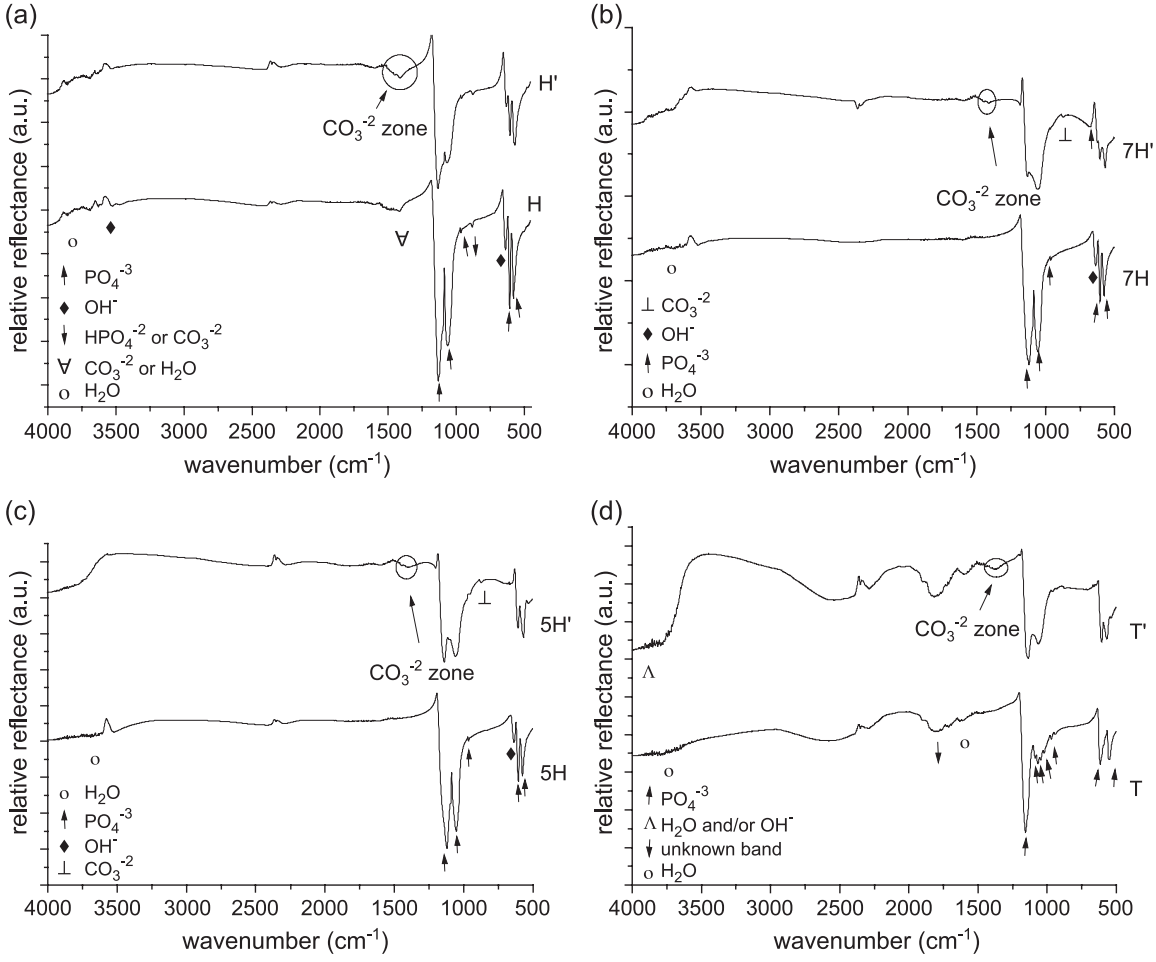

Fig. 3. FTIR spectra of samples surface: before and after (symbol ′) of immersion in SBF; (a) HAp, (b) 7H, (c) 5H, and (d) TCP.

The β-TCP can be identified by the large band at 900–1200 (cm^{-1}) and the absence of ν_4 PO_4 band could be shown at 600 (cm^{-1}), which is characteristic for the α-TCP phase and also for the hydroxyl group [10,21,26].

An unknown band (1800 cm^{-1}) for TCP samples and the presence of HPO_4^{-2} and CO_3^{-2} peaks in the HAp sample were detected. The CO_3^{-2} group (~1400 cm^{-1}) and HPO_4^{-2} (1214 cm^{-1}) [26,27] can be incorporated into the structure during the air sintering process or from SBF. The HAp and biphasic (5H and 7H) samples showed higher definition than the TCP spectra; this can be explained by a disordered disposition of globules that constitute the layer. The structure and characteristics of the HAp (sample) and precipitated HAp are similar. Thus, the necessary energy for nucleation and crystal growth of the HCA on a HAp surface is lower than the energy for crystallization on a TCP surface [28,29].

An increase of the H_2O band intensity (~3600 cm^{-1}) was observed for TCP and 5H samples. This behavior demonstrates the more hydrated layer formation due the higher content of TCP phase, which is more reactive in aqueous systems [10,21,25,27].

In biphasic samples, characteristic carbonate peaks were observed, indicating a greater in vitro reactivity than HAp and β-TCP samples, this is in good agreement with results in the literature, where the same behavior is reported [5,6,27]. The presence of CO_3^{-2} bands (~875 and 1400 cm^{-1}) suggests the substituted HAp formation of the type-A, occupying OH^- position and/or type-B, PO_4^{-3} position [10,21,30]. After 3 weeks of exposure, these samples behave more similar to HAp samples in concerning to the layer formation, confirming a superior bioactive character with a simultaneous degradability of these ceramics.

4. Conclusion

The results obtained in this study demonstrate that the evaluated ceramics HAp, TCP, and mixtures of both showed different reactivities in the presence of SBF. The kinetics of dissolution in BCP and TCP ceramics increase the velocity of a subsequent HCA precipitation due to an increased supersaturation after exposure into SBF. In comparison with pure hydroxyapatite and tricalcium phosphate, the biphasic ceramics presented higher reactivity in vitro.

The precipitation occurred only on the ceramic surface. The inner layers of the ceramic block remained intact. The morphology of the precipitates showed a globular aspect, being constituted of aggregates that are characteristic for precursor phases of the biologic apatite under these conditions.

The infrared spectra of the samples surfaces indicated the formation of an amorphous calcium phosphate layer. The apatitic precipitation may initially involve the maturation of a poorly crystallized apatite. The TCP surfaces showed a disordered orientation of globules resulting in nonuniform surfaces.

The carbonate reflection bands observed on the surface of the biphasic samples after immersion in SBF are indicative of the formation of hydroxycarbonate apatite (HCA) layer.

The samples with higher content of TCP showed the formation of a more hydrated layer than the others, corresponding to an increase in the water bands intensity.

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