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Short communication

Effect of micropores and citric acid on the bioactivity of phosphorylated chitosan/chitosan/hydroxyapatite composites

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Abstract

The phosphorylated chitosan/chitosan/hydroxyapatite (PCS/CS/HA) bio-composite was synthesized by the coprecipitation method, and the effect of micropores and citric acid on its bioactivity was studied via simulated body fluid (SBF). The PCS had been successfully prepared by introducing the phosphate groups into CS. After the aging process, the crystallinity of HA became higher. The CO_3^{2-} groups in PCS/CS/HA composite showed that the HA obtained in this study was carbonate apatite. After soaking in SBF for 21 days, the Ca/P molar ratios of unpolished PCS/CS/HA sheet increased from 1.28 to 1.53, which was closer to that of HA. In addition, the unpolished composite sheet soaked in SBF with citric acid presented a better bioactivity.

Keywords: Chitosan; Composites; Surfaces; Biomedical applications

1. Introduction

In recent years, considerable developments can be seen in the research field of the bone substitute materials. Among this field, the chitosan (CS) and hydroxyapatite (HA) composite is one of the most actively investigated biomaterials [1–3]. There has been a steady growth of interest in the CS due to its good biodegradability and biocompatibility [4,5]. Nevertheless, lack of bioactivity makes it difficult to use in bone tissue engineering. Recently, remarkable attention has been given to the phosphorylated chitosan (PCS) [6–9], which could exhibit metal chelating [10] and biocompatibility [11]. In addition, the HA displays high bioactivity [12], but hardness and brittleness [13]. Thus, a novel bone implantable biomaterial with better properties could be obtained by combining the PCS, CS and HA together.

The micropores on the surface of bone substitute materials can promote the adhesion and proliferation of osteoblasts. In addition, the PCS/CS/HA sheets studied here were formed by using citric acid which can chelate with calcium ions. Therefore, it is valuable to research the effect of micropores and citric acid on bioactivity of these bone implantable biomaterials. In this work, the bioactivity was investigated by using the PCS/CS/HA bio-composite with weight ratio of 30/30/40. The results showed that the micropores and citric acid were very important for the formation of apatite nuclei.

2. Materials and methods

2.1. Preparation of PCS/CS/HA composite sheets

The PCS was synthesized by the reaction of chitosan, orthophosphoric acid and urea in *N*,*N*-dimethylformamide [10]. The preparation procedure of HA powders were as follows. First, an 8.5% H_3PO_4 solution was added into the 5% ethanol solution of $Ca(OH)_2$, and the mixed solution was adjusted to pH 10. Then, the solution was stirred continuously for 24 h at room temperature. After it stood for another 24 h, the HA sample (denoted as HA24A) was obtained. In addition, the slurry obtained without aging process was denoted as HA24. The PCS/CS/HA powder

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was prepared through the coprecipitation method [14]. Finally, the PCS/CS/HA powder was formed into paste with the use of 20% solution of citric acid after grinding in a mortar. Then, the powder was pressed into sheets (thickness 2 mm and diameter 10 mm), and some of them were polished carefully with fine sand papers. The simulated body fluid (SBF) solution was self-made [15]. Another SBF with citric acid at a concentration of 1.5 mmol/L was denoted as c-SBF. All reagents used were of analytical grade.

2.2. Characterization

The surface morphology of the samples was studied by scanning electron microscopy (SEM, VEGAII SBH, TESCAN, Czech). The information on the elementary composition was determined by energy dispersive spectroscopy (EDS, GENESIS, EDAX) attached to the SEM. The phase structures of the HA24 and HA24A powders were measured by X-ray diffraction (XRD) on a Rigaku D/max-γB X-ray diffractometer with Ni-filtered CuKα radiation. The Fourier transform infrared (FT-IR) of the PCS/CS/HA composite was recorded with the KBr pellet method on a Nicolet 380 spectrometer at room temperature.

3. Results and discussion

Fig. 1a and b presented the surface morphology of CS and PCS. As observed in Fig. 1a, the CS had a quite smooth surface due to the nature of it. After the introducing of phosphate groups, the PCS presented a tight structure and rough surface morphology in Fig. 1b. The result was in accordance with the previous report [8]. The powder was examined by EDS to determine the change of phosphorus contents between the CS and PCS as showed in Fig. 1c and d. The EDS analysis in Fig. 1c indicated that the main elements of CS were C, O and N, but no P. However, the high content of phosphorus obviously appeared in the EDS of PCS in Fig. 1d. The reason of the existence of Au element was that the powder was coated with gold before the EDS. The EDS studies confirmed the success of phosphorylation.

The surface morphology of HA24 and HA24A powders was illustrated by SEM images in Fig. 2a and b. Compared with HA24 powder, the HA24A had a more complete crystal structure. Meanwhile, the particles of HA24A were well-distributed after aging for 24 h. The XRD patterns and the reaction process for preparation of HA were presented in Fig. 2c. The HA powders before and after aging process both

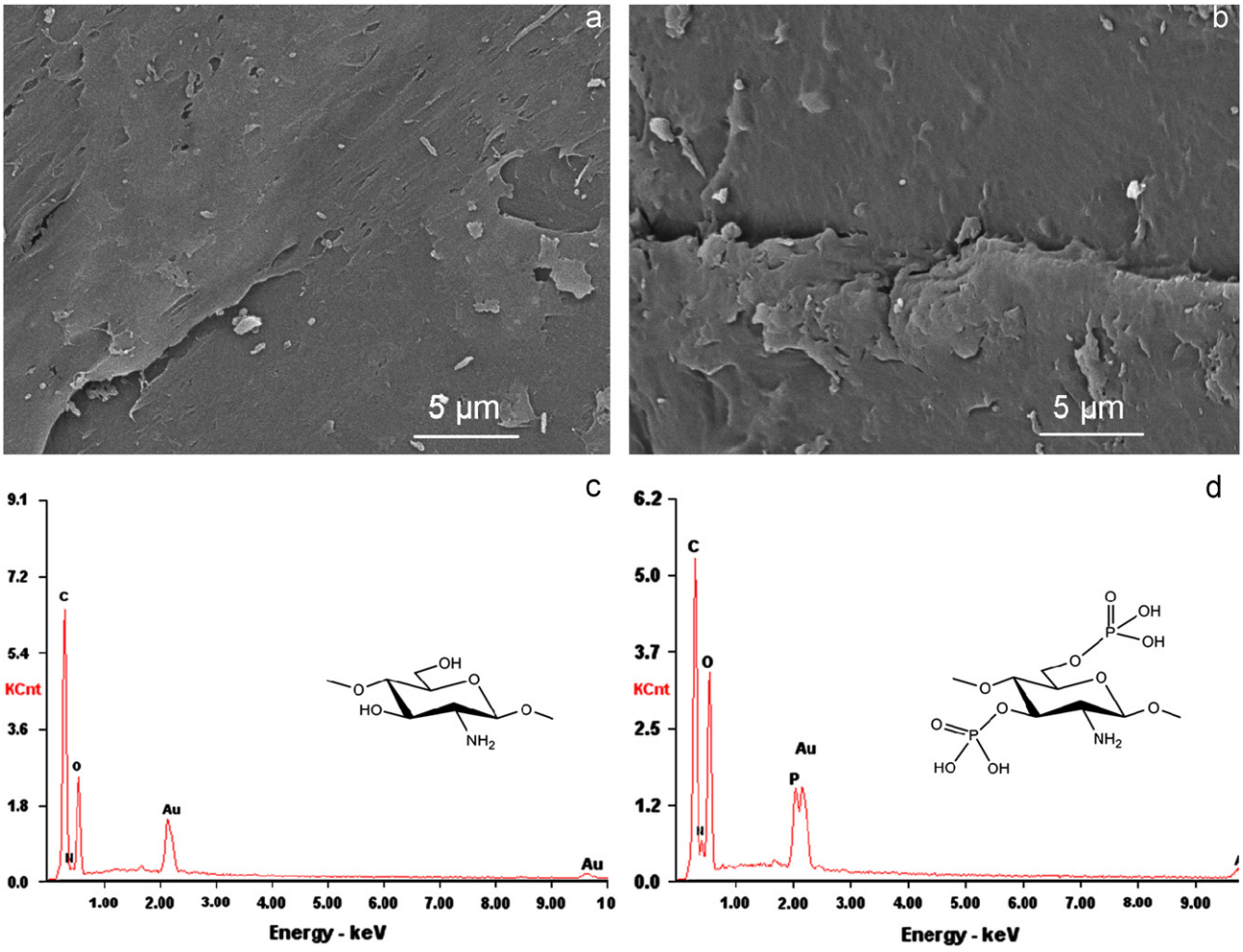

Fig. 1. SEM images and EDS spectra of (a) and (c) CS, and (b) and (d) PCS.

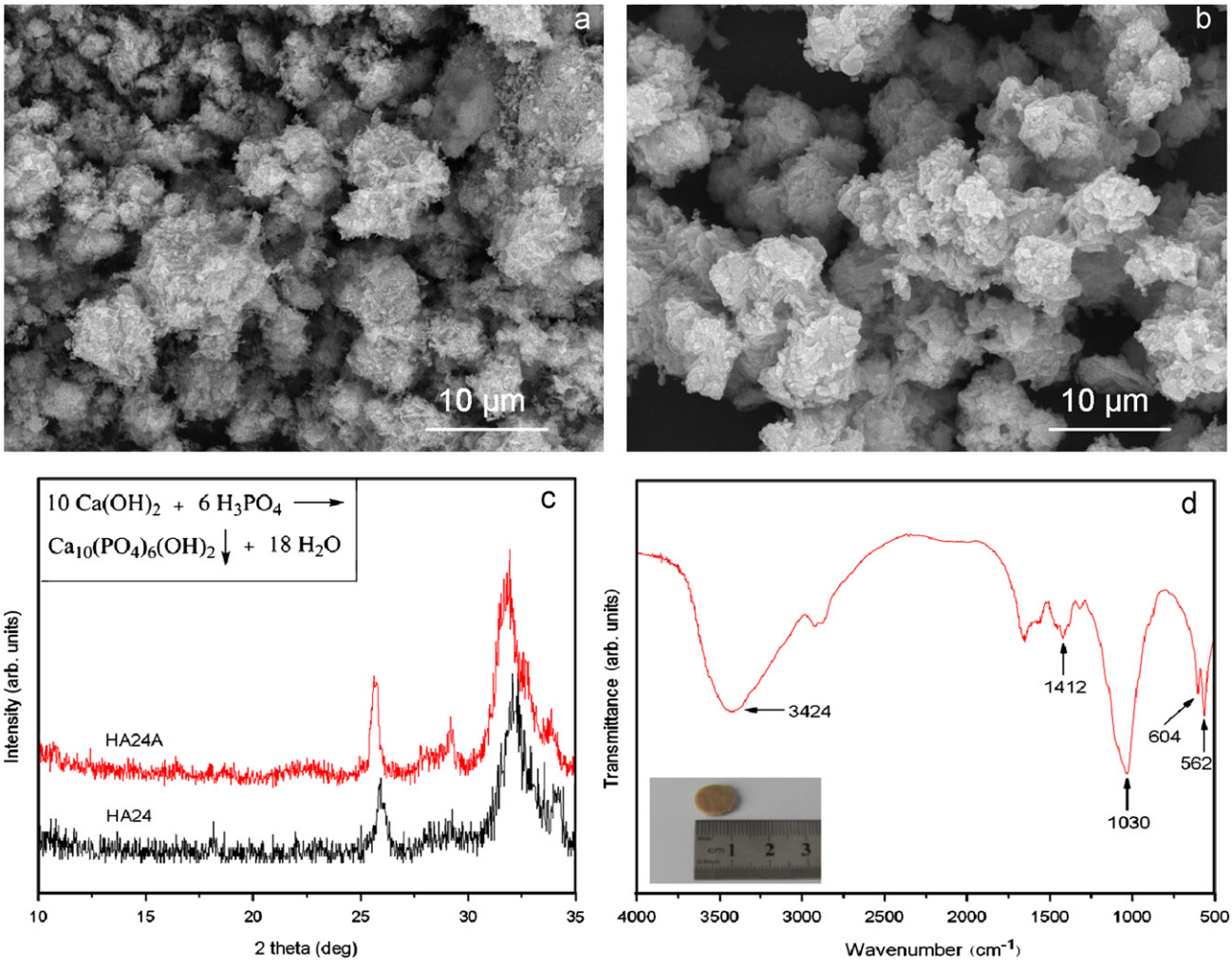

Fig. 2. SEM images of (a) HA24, and (b) HA24A powders. (c) XRD patterns of HA24 and HA24A (inset: preparation process of HA). (d) FT-IR spectrum of PCS/CS/HA composite with weight ratio of 30/30/40 (inset: photograph of the unpolished sheet).

showed the specific diffraction peaks at $2\theta = 26^\circ$ and 32° [13]. Compared to HA24, the characteristic peaks of HA24A were stronger, and the crystallinity was higher. The results demonstrated that the process of aging for 24 h was very important for the formation of HA crystal. The main reason was that the stirring and aging were the processes of nucleation and growing for HA, respectively. The FT-IR spectrum of the PCS/CS/HA powder with weight ratio of 30/30/40 was observed in Fig. 2d. As showed in FT-IR, the characteristic band at 3424 cm^{-1} was assigned to –OH of the PCS and CS. This FT-IR result was also observed by other researchers [8]. The peaks at 1030, 604 and 562 cm^{-1} were assigned to PO_4 functional groups in HA. Meanwhile, a characteristic band for CO_3^{2-} groups at 1412 cm^{-1} was easily observed, which indicated that the HA obtained was carbonate apatite crystal. The result was confirmed by Zhang et al. in chitosan/nano-hydroxyapatite composite [14]. As exhibited in the photograph of composite sheet, the color of PCS/CS/HA presented light yellow owing to the phosphorylated modification of CS.

In comparison to the surface of original polished sheet, there were no obvious changes in the morphology after soaking in SBF for 21 days, as indicated in Fig. 3a and b. In contrast, after soaking in SBF for 3 weeks, a large number of crystal particles appeared on the surface of unpolished composite sheet in Fig. 3d. The result could be ascribed to the micropores on unpolished sheet. The crystal nuclei of bone-like apatite were firstly formed on the surface of these micropores, which could hinder the ion diffusion of SBF and keep high ion concentrations of Ca and P near them. The information on elementary composition of the unpolished sheets was demonstrated by EDS analysis in Fig. 3e and f. After soaking in SBF for 21 days, the Ca/P molar ratios of sheet surface increased from 1.28 to 1.53.

Fig. 4 showed the SEM images and EDS spectrum of unpolished sheet soaked in c-SBF for 21 days. The micropores were completely covered by calcium phosphate, and the Ca/P molar ratio increased to 1.60 which was closer to that of HA. The unpolished sheet after soaking in c-SBF showed better bioactivity than that in SBF. The main reason was that one part of the carboxyl groups in citric acid could chelate with calcium ions, meanwhile, another part could crosslink with amino groups of PCS and CS.

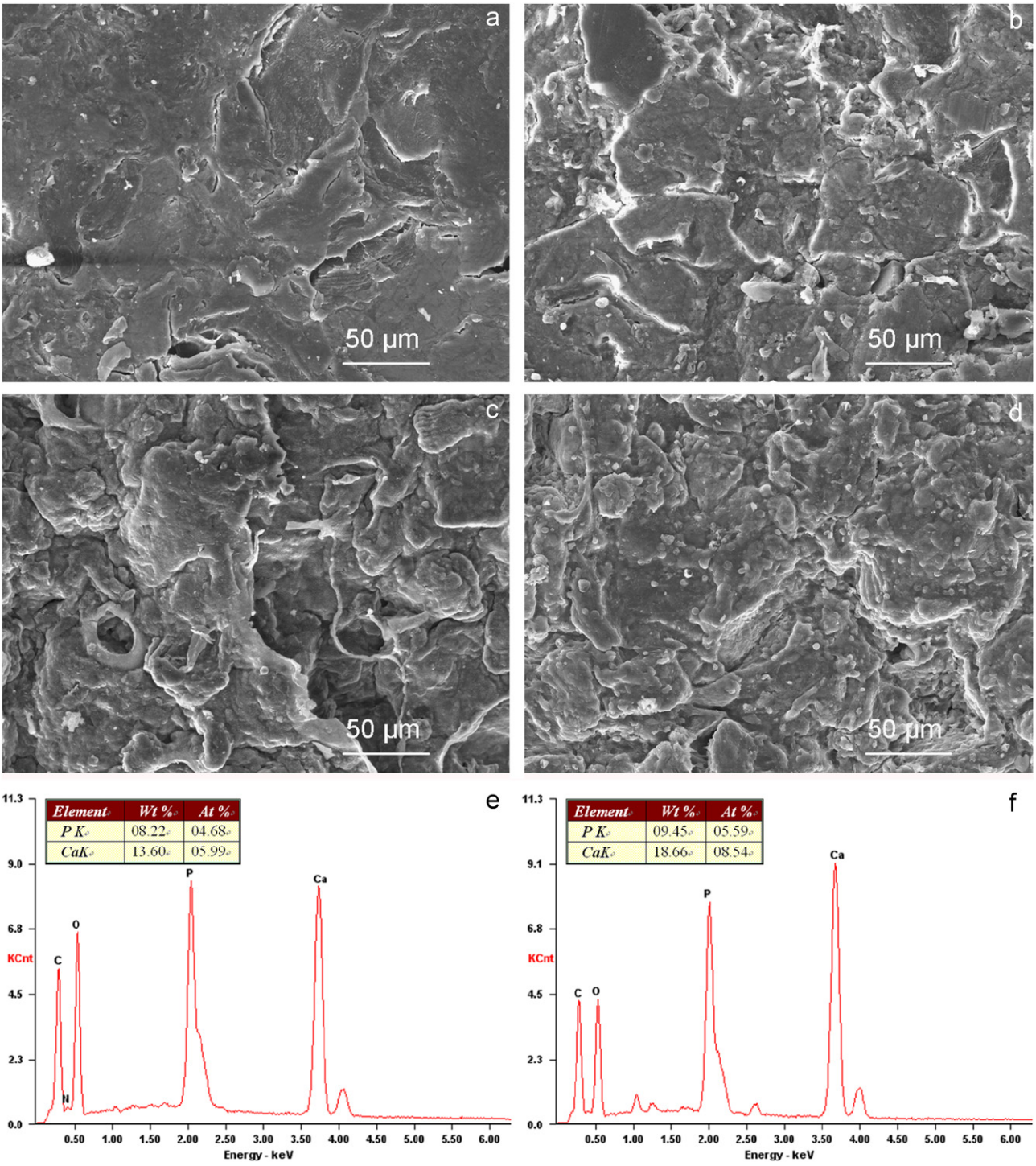

Fig. 3. SEM images of polished composite sheets after soaking in SBF for (a) 0 day, and (b) 21 days. SEM images and EDS spectra of unpolished sheets after soaking in SBF for (c) and (e) 0 day, and (d) and (f) 21 days.

4. Conclusions

In summary, the success of phosphorylation was confirmed by the change of elementary composition between the CS and PCS. The specific diffraction peaks at $2\theta = 26^\circ$ and 32° suggested that the HA was formed, and its crystallinity became higher after aging for 24 h. The unpolished PCS/CS/HA sheet exhibited a better bioactivity after soaking in SBF for 3 weeks. The main reason was that the micropores on unpolished sheet could keep high ion concentrations of Ca and P near them. Thus, the crystal nuclei of bone-like apatite were firstly formed on these micropores. Due to the chelating behavior of carboxyl and Ca^{2+}, the unpolished composite sheet

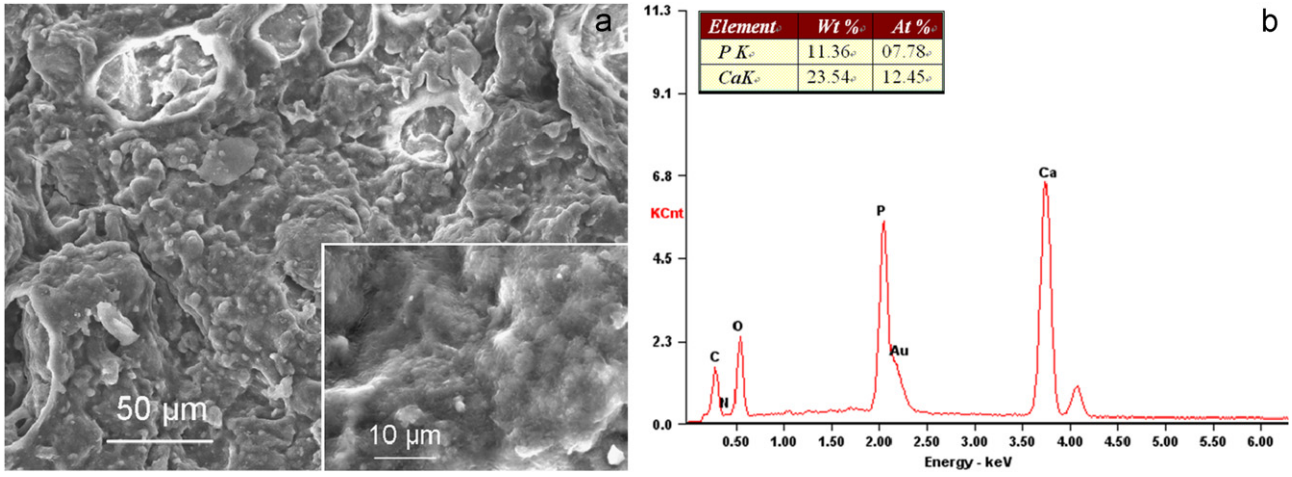

Fig. 4. SEM images and EDS spectrum of unpolished composite sheet after soaking in c-SBF for 21 days.

soaked in c-SBF possessed better bioactivity than that in SBF. The results illustrated that the micropores and citric acid were very important for the biological activity of bone substitute materials.

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