Hydroxyapatite formation on cellulose cloth induced by citric acid

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Hydroxyapatite (HAp) formation on cellulose cloth with the aid of citric acid was investigated. The cellulose cloths were soaked in simulated body fluid (SBF) solutions (1.5 SBF) with ion concentrations 1.5 times that of SBF (1.0 SBF) with and without citric acid and carbonate containing HAp crystals were found to form only in the 1.5 SBF solution that contained citric acid. The results were explained in terms of hydrogen bonding of citric acid to the cellulose cloth and its chelating ability of calcium ions. Practical application may involve the inclusion of citric acid in the 1.5 SBF solution to promote formation of HAp on previously bioinert cellulose cloth.

1. Introduction

Simulated body fluid (1.0 SBF) was designed to test the bioactivity of artificial bone material *in-vitro* because its composition is very close to human blood plasma [1]. The SBF solution (1.5 SBF) with ion concentrations 1.5 times that of 1.0 SBF also has been used for coating of hydroxyapatite (HAp) on the surface of bioinert materials with artificially introduced surface functional groups that have an ability of inducing the HAp nucleation in SBF solutions. Such functional groups are, for example, silanol group [2–4], phosphite group [5–7], or sodium titanate hydrogel layer [8–11] introduced by treatment with $CaO\text{-}SiO_2$ glass powder, urea/H_3PO_4 solution, or strong alkaline solution (NaOH), respectively. The procedures for treatment, however, are so complicated or severe that the applications have been very limited.

In the present experiment, a simple coating method of HAp on bioinert cellulose cloth with the aid of citric acid in 1.5 SBF solution was examined. Since the citric acid has a strong chelating ability of calcium ions to its carboxylate groups, it is believed to induce HAp nucleation in 1.5 SBF solution [12]. Besides, citric acid exists in fresh wet bone in the form of citrate [13] and is involved in the citrate cycle in the body, it can mimic and offer gentle *in-vivo* conditions for synthesizing the HAp. Cellulose, made by polymerization of glucose, is a cheap and readily available material to employ, though no apatite forming ability in SBF solutions; it has one kind functional group (OH) which cannot directly induce HAp nucleation due to the absence of net charge [14]. Therefore, citric acid was used as a nucleating agent for HAp crystals on cellulose cloth. The hydroxyl group of citric acid may bond to the hydroxyl group in the cellulose through hydrogen bonding and can induce the HAp particles on it [15–18].

2. Experimental procedure

The SBF solution (1.5 SBF) which has 1.5 times higher ion concentrations than the SBF solution with ion concentrations close to human blood plasma, as shown in Table I was 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 ion exchanged distilled water. The solution was buffered at pH 7.4 with tris(hydroxymethyl) aminomethane ($(CH_2OH)_3CNH_2$) and 1 M hydrochloric acid (HCl) at 36.5 °C. Citric acid (Wako Pure Chem. Ind. Ltd, Osaka, Japan) was dissolved into the 1.5 SBF solution to give a concentration of 1 mM and pH was readjusted with tris(hydroxymethyl) aminomethane to 7.4.

Cellulose cloth (Bemcotlabo, Asahi Kasei, Osaka, Japan), which consists of 100% cellulose, lint free, and no HAp forming ability in SBF solution, was used in this experiment. Cellulose cloths with a dimension of $1 \times 1 \times 0.1\,cm^3$ were immersed in 30 ml of 1.5 SBF solution with and without citric acid and soaked for 1 week at 36.5 °C. Hereafter, the specimens obtained through the 1.5 SBF solutions with and without citric acid will be referred to as C- and B-specimens, respectively. After soaking, the specimens were removed

from the fluid and gently rinsed with ion exchanged distilled water and then dried at room temperature.

The surface microstructures, phases and functional groups of the cellulose cloths after soaking in 1.5 SBF solution were analyzed by scanning electron microscopy (SEM), thin-film X-ray diffractometry (XRD, Model RINT2000, Rigaku Co., Tokyo, Japan) with an angle of 1° to the direction of the incident X-ray, and diffuse reflectance of Fourier-transformed infrared (FT-IR) spectroscopy (Model Spectrum 2000, Perkin Elmer Co., Norwalk, U.S.A.). For IR spectroscopy measurements, the pulverized specimens were diluted with KBr powder by one tenth and the background noise was corrected with pure KBr data. The concentration changes of calcium in 1.5 SBF solutions before and after soaking were measured with inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Model UOPs-2S, MARK II, Kyotokoken Co., Kyoto, Japan).

3. Results and discussion

Fig. 1 shows the microstructures of B- and C-specimens obtained after soaking in 1.5 SBF solutions at 36.5 °C for 1 week. In the B-specimen, only fibers of the cellulose cloth were observed. In the C-specimen, however, many spherulites, which contain a large number of tiny crystals, deposited on the surface of cellulose cloth were observed. The microstructure of the HAp crystals was quite similar to that observed on the bioactive glass or glass-ceramics in 1.0 SBF solutions [19–22]. In the case of 1.0 SBF solution with the same amount of citric acid, however, no HAp formed on the surface of cellulose cloth. The differences of HAp forming abilities between 1.0 SBF and 1.5 SBF with citric acid may come from the degrees of supersaturation between the two solutions.

Fig. 2 shows the thin-film XRD results of B- and C-specimens. The broad peak due to the cellulose cloth was only observed in the 2θ range of 15° ∼ 35° in the B-specimen. However, several HAp peaks denoted by A were observed in the C-specimen. The broadness of apatite peaks may result from low crystallinity or small crystallite size of the HAp.

Fig. 3 shows the results of IR spectroscopy measurements on the B- and C-specimens. For the C-specimen, the stretching modes of $(PO_4)^{3-}$ ion were detected at 1048, 604 and 568 cm^{-1}. The stretching and out of plane modes of $(CO_3)^{2-}$ ion were also observed at 1419 and 878 cm^{-1}, respectively. It means that PO_4 sites of HAp structure, i.e. B-site, were partly substituted by carbonate ions. The HAp crystal, therefore, which formed on the cellulose cloth was carbonate containing hydroxyapatite.

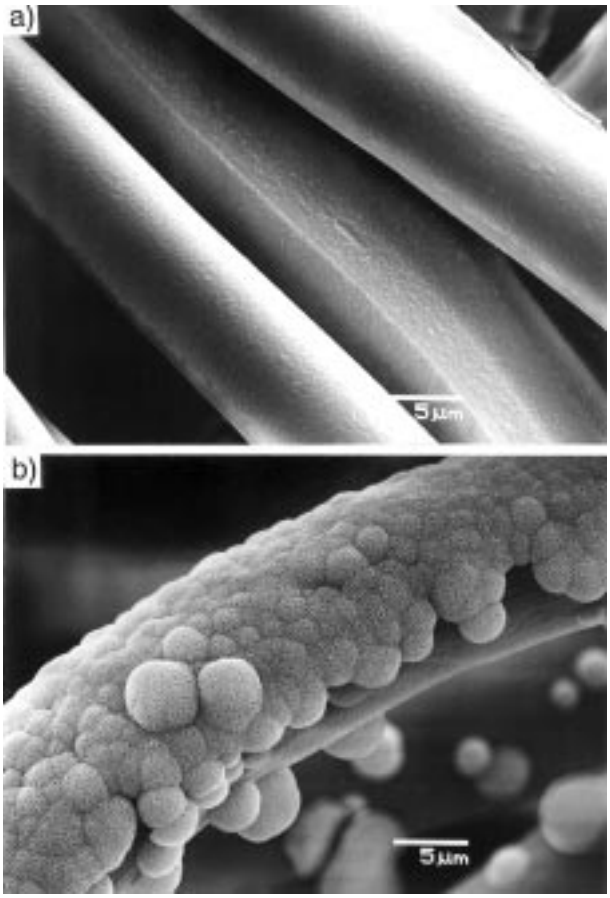

Figure 1 Microstructure of the (a) B- and (b) C-specimens soaked in 1.5 SBF solutions for 1 week at 36.5 °C.

However, HAp peaks were virtually absent from the spectrum of the B-specimen.

The elemental concentration changes of calcium in the 1.5 SBF solutions before and after soaking for 1 week at 36.5 °C were detected by ICP-AES. In the B- and C-specimens, the concentrations of calcium decreased by approximately 3% and 44% to 137.3 ± 1.1 and 79.1 ± 0.5 ppm, respectively, with respect to the original concentration of 141.9 ± 1.0 ppm before soaking. The appreciable decrease in calcium concentration of the C-specimen can therefore be attributed to the formation of HAp particles on the surface of the cellulose cloth. On the other hand, in the B-specimen, a small decrease of calcium concentration has occurred, which indicates that the formation of HAp is extremely slow without citric acid.

From these results, it can be presumed that the formation of HAp on the non-bioactive cellulose cloth is critically dependent on the addition of citric acid in 1.5 SBF solution. For the explanation of HAp formation

TABLE I Ion concentrations of 1.0 SBF and 1.5 SBF solutions in comparison with those of human blood plasma

	Concentration (mM)							
	Na^+	K^+	Ca^{2+}	Mg^{2+}	HCO_3^-	Cl^-	HPO_4^{2-}	SO_4^{2-}
Blood plasma	142.0	5.0	2.5	1.5	27.0	103.0	1.0	0.5
1.0 SBF	142.0	5.0	2.5	1.5	4.2	148.0	1.0	0.5
1.5 SBF	213.0	7.5	3.8	2.3	6.3	223.0	1.5	0.75

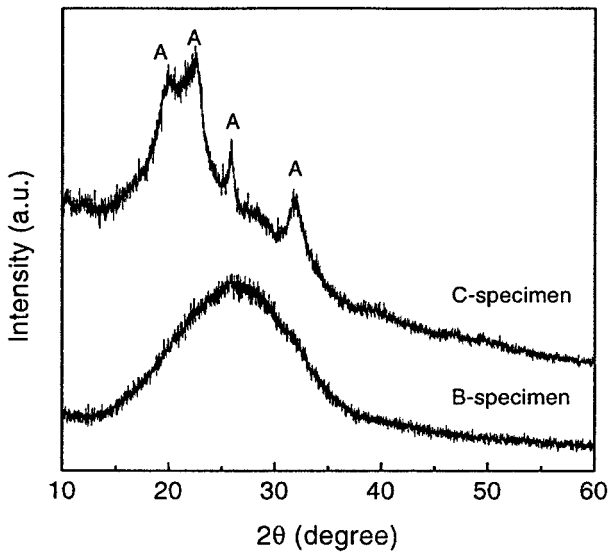

Figure 2 Thin-film XRD results for the specimens soaked in 1.5 SBF solutions for 1 week at 36.5 °C.

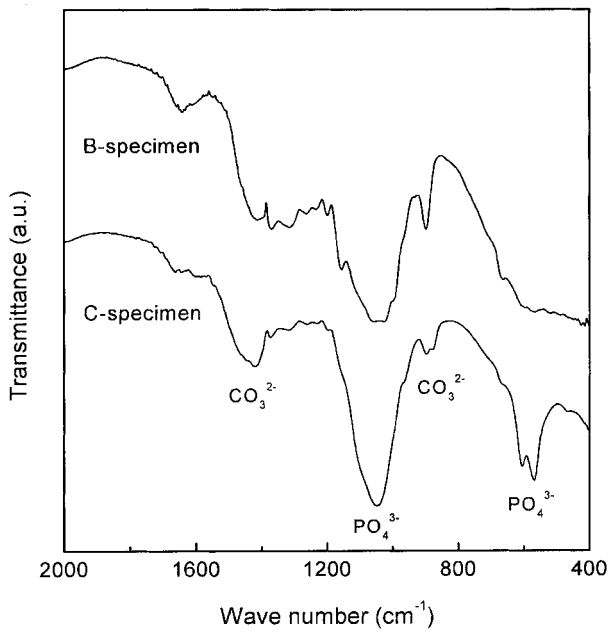

Figure 3 FT-IR transmission spectra for the specimens soaked in 1.5 SBF solutions for 1 week at 36.5 °C.

observed in this work, the nucleation model based on the chelation of calcium ions with citric acid is believed to be appropriate because calcium ion binding is believed to be an initial step for the formation of calcium phosphates [5, 23–26].

Citric acid ($C_6H_8O_7$), which has a hydroxyl and three carboxyl groups in its molecule, may adhere to the cellulose surface through hydrogen bonding between two hydroxyl groups in citric acid and cellulose cloth, respectively, and it is ionized to the $(C_6H_5O_7)^{3-}$ (*cit*) form [27] by the loss of hydrogen ions ($pK_3 = 6.40$) in 1.5 SBF solution (pH = 7.4), according to the following reaction:

$$\begin{array}{l} \mathrm{CH_2COOH} \\ | \\ \mathrm{HO{-}C{-}COOH} \\ | \\ \mathrm{CH_2COOH} \end{array} \longrightarrow \begin{array}{l} \mathrm{CH_2COO^-} \\ | \\ \mathrm{HO{-}C{-}COO^-} \\ | \\ \mathrm{CH_2COO^-} \end{array} + 3\mathrm{H^+} \qquad (1)$$

Negatively charged carboxylate headgroups will bind calcium ions [28] in 1.5 SBF solution and form a Ca-*cit* complex at the cellulose fiber surface. Then, a cluster of critical size can be formed by adsorbing calcium, phosphate ions and other citric acids on the Ca-*cit* complex three-dimensionally and the clusters may act as nuclei for the crystal growth of HAp as schematically shown in Fig. 4. After nucleation, HAp crystals can grow spontaneously, because the 1.5 SBF solution is already supersaturated with respect to the HAp [29]. In the B-specimen, therefore, HAp embryos of the critical size could not be formed and the growth of nuclei could not be energetically favored due to the absence of calcium binding site. In the C-specimen, however, large clusters of HAp could be formed with the aid of citric acid and these HAp clusters may act as the nucleation site for further growth of HAp crystals in the 1.5SBF solution.

The results suggest that HAp nucleation on the non-bioactive cellulose cloth can be obtained with the aid of citric acid in 1.5 SBF solution. The bonding strength, however, between HAp particles and cellulose cloth may not be strong enough because they might be bonded through weak hydrogen bonding. The growth mechanism of HAp crystals on the cellulose cloth, however, was a biomimetic, the environment of synthesis was very

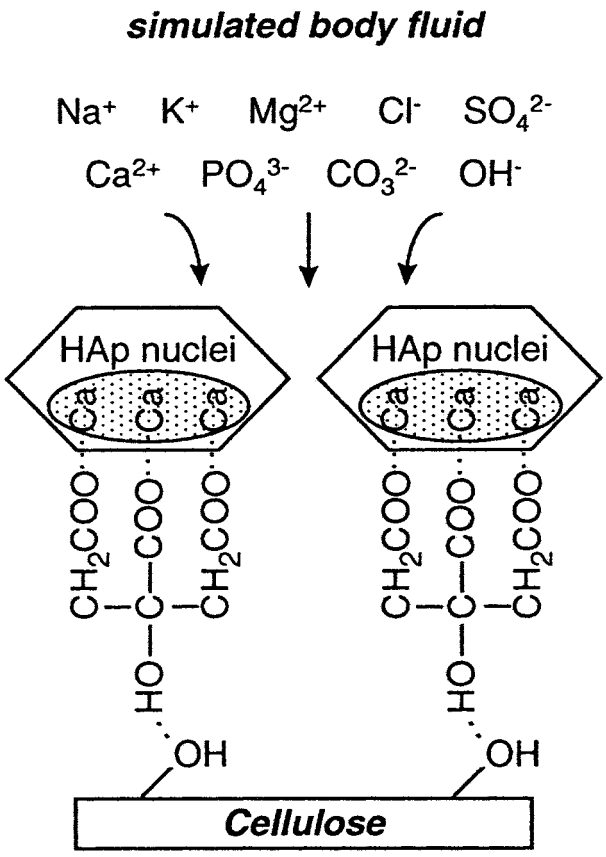

Figure 4 Schematic illustration for the formation of HAp embryo with the aid of citric acid in SBF.

gentle, and the procedure was simple compared with other methods to coat the HAp on bioinert materials. This method is believed to be applicable to other polysaccharides, such as chitin, which is biodegradable and non-toxic *in-vivo*.

4. Conclusions

This investigation has shown that HAp nucleation on cellulose cloth can be made in 1.5 SBF solution with the aid of citric acid. When cellulose cloth was soaked in 1.5 SBF solution without citric acid, HAp crystal growth was absent. When cellulose cloth was soaked in 1.5 SBF solution with 1 mM of citric acid, however, HAp crystals formed. The results therefore suggest that citric acid has a nucleating ability and can accelerate the nucleation of HAp crystals on non-bioactive cellulose cloth.

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