Apatite-forming ability of alginate fibers treated with calcium hydroxide solution

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Calcium alginate fibers were prepared by extruding an aqueous sodium alginate solution into an aqueous calcium chloride solution. The fibers were treated with a saturated aqueous calcium hydroxide solution for various periods and their apatite-forming ability was examined in a simulated body fluid (SBF). The calcium alginate fibers were treated with the aqueous calcium hydroxide solution for periods longer than five days formed apatite on their surfaces in SBF, and their apatite-forming ability improved with increasing calcium hydroxide treatment time. The amount of calcium ions released from the fibers also increased with increasing calcium hydroxide treatment time, resulting in acceleration of nucleation and growth of apatite on the fiber surfaces. The resultant apatite–alginate fiber composite is expected to be useful as a flexible bioactive bone-repairing material.

1. Introduction

Ceramics such as Bioglass® [1], sintered hydroxyapatite [2], and glass–ceramic A-W [3] spontaneously form bone-like apatite on their surfaces in the living body, and bond to surrounding bone through this apatite layer. These ceramics are already clinically used as bone grafts. They are, however, brittle and have poor fracture toughness. Recently, metals such as titanium [4], tantalum [5], and their alloys have also been shown to form bone-like apatite on their surfaces in the living body and to bond to the surrounding bone through the apatite layer, when they had been previously subjected to alkali- and heat-treatments. These metals are being clinically used as a bone substitute under load-bearing conditions, as they have high fracture toughness. Their elastic moduli are, however, much higher than those of human cortical bones.

Natural bone is a composite in which nano-sized bone minerals are deposited on organic collagen fibers that are fabricated into a complex 3-D structure. Living cells occupy only 1% of the bone volume. Such a composite is expected to be obtained by a biomimetic process, without the aid of cells, in which synthetic organic fibers are fabricated into a 3-D structure analogous to that of the collagen in the natural bone, modified on their surfaces with functional groups effective for apatite nucleation, then soaked in a simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma, which is highly supersaturated with respect to apatite.

Tanahashi *et al*. [6] made the first attempt to obtain such a composite by a biomimetic process. They aimed at producing Si–OH groups, which are effective for apatite nucleation, by exposing organic polymers to $CaO–SiO_2$-based glass particles in SBF. They succeeded in inducing

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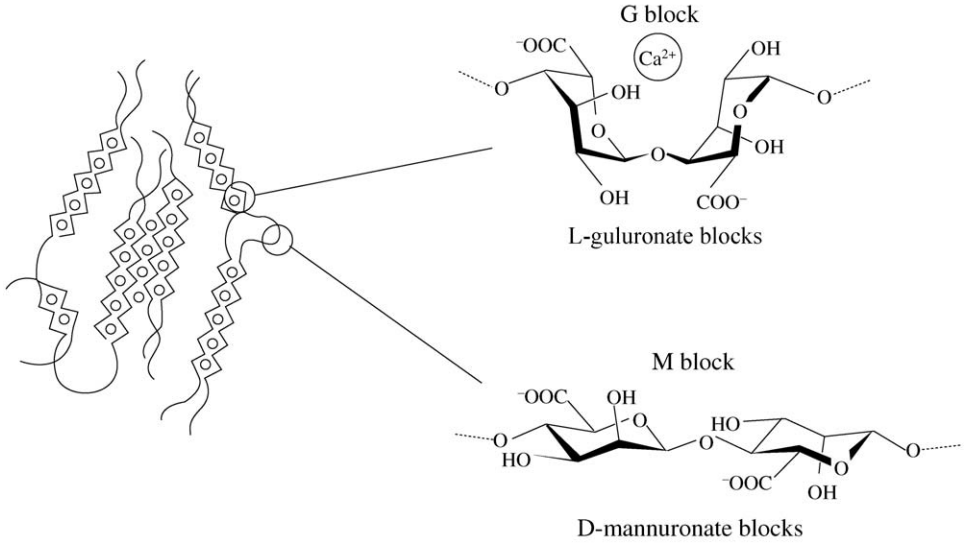

Figure 1 Schematic representation of calcium alginate gel.

apatite formation, but only on the surfaces exposed to the glass particles.

Oyane *et al*. [7, 8] recently showed that nanoapatite-organic fiber composites can be prepared in SBF by a biomimetic process if organic polymer surfaces are modified with a $CaO–SiO_2$ phase or an anatase-type TiO_2 phase by a sol–gel process.

On the other hand, Kawashita *et al*. [9] showed that polymeric gels containing carboxyl groups, such as carboxymethylated chitin gel and gellan gum gel, form apatite on their surfaces in SBF if they have been previously subjected to treatment with a $Ca(OH)_2$ solution [9].

In our study, calcium alginate fibers were prepared, and subjected to $Ca(OH)_2$ solution treatment for various periods. The apatite-forming ability of these treated fibers was examined in SBF, to reveal fundamental conditions for obtaining nanoapatite–organic fibre composites with mechanical and physiological properties analogous to those of natural bones.

2. Experimental

2.1. Preparation of calcium alginate fibers

A total of 4 g of sodium alginate (Kimitsu Chemical Industries Co. Ltd., Tokyo, Japan), with a 0.9 molar ratio of D-mannuronate (M) blocks to L-guluronate (G) blocks, both of which are shown in Fig. 1, was dissolved in 80 ml of distilled water in a polystyrene bottle and left to stand for one day. A total of 15 g of calcium chloride (Nacalai Tesque, Kyoto, Japan) was dissolved in 500 ml of distilled water. 300 ml of this aqueous calcium chloride solution was mixed with 300 ml of methanol.

A total of 40 ml of the aqueous sodium alginate solution was extruded into 500 ml of the calcium chloride aqueous solution through a nozzle with 50 holes of 0.1 mm diameter under a pressure of 0.7 kgf cm^{-2}, then passed through 500 ml of the calcium chloride methanol solution and wound using two rollers rotating at a rate of 5.9 m min^{-1} and 6.2 m min^{-1}, respectively [10], as shown in Fig. 2. The fibers obtained were kept in 1 wt % aqueous calcium chloride solution.

2.2. Treatment with $Ca(OH)_2$ solution

The prepared fibers were cut into 20 mm lengths with a steel knife. A bundle of fibers $20 \times 10 \times 1\ mm^3$ was prepared and washed with distilled water. About 1 g of $Ca(OH)_2$ (Wako Pure Chemicals Industries Ltd., Osaka, Japan) was dissolved in 300 ml of distilled water, stirred for 1 h at room temperature under a nitrogen atmosphere, and then left to stand for 1 h. The clear top layer of the

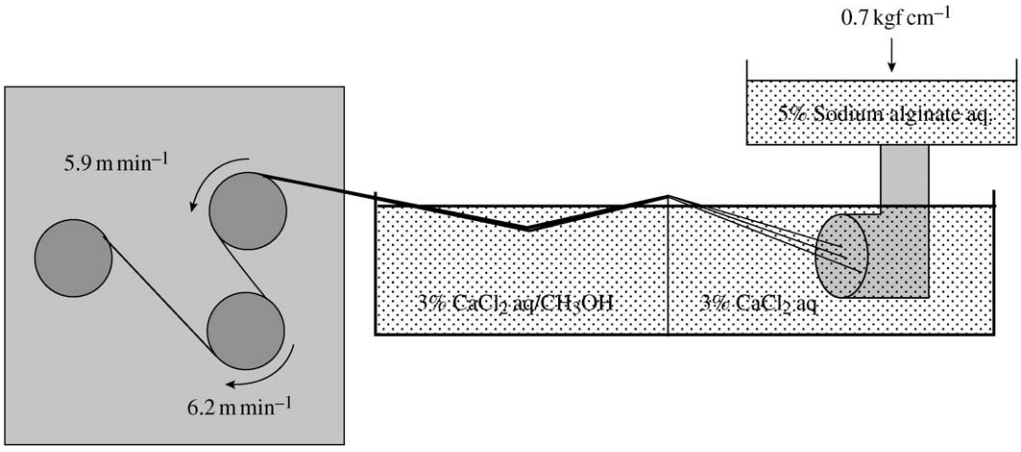

Figure 2 Spinning of an alginate fiber.

solution was passed through a filter with a pore size of 0.45 μm in diameter (MILLEX®-HV, Millipore Corporation). The bundle of the calcium alginate fibers was immersed into 50 ml of this $Ca(OH)_2$ saturated solution and left to stand for various periods of time in a polystyrene box filled with nitrogen gas.

2.3. Soaking in SBF

The bundles of calcium alginate fibers treated with $Ca(OH)_2$ solution for various periods were soaked in 40 ml of SBF [11] with ion concentrations of Na^+ 142, K^+ 5.0, Mg^{2+} 1.5, Ca^{2+} 2.5, Cl^- 148.8, HCO_3^- 4.2, HPO_4^{2-} 1.0, and SO_4^{2-} 0.5 mM, which are nearly equal to those of human blood plasma, with pH 7.40 at 36.5 °C. After various periods, the fiber bundle was removed from the SBF, soaked in 40 ml of distilled water for one day and dried at room temperature.

2.4. Analysis of fiber and SBF

The structure of the fibers described in Section 2.1 was analyzed by Fourier-transform infrared attenuated total reflection spectroscopy (FT-IR ATR; Magna 860, Nicolet Instrument Co., Madison, WI, USA). Incident angle was 45°. Zinc selenide was used as an internal reflection element.

The surface structure of the fiber bundles soaked in SBF after $Ca(OH)_2$ treatment was analyzed by thin-film X-ray diffraction (TF-XRD; RINT2500, Rigaku Co., Tokyo, Japan) and field-emission scanning electron microscopy (FE-SEM; S-4700, Hitachi Ltd., Tokyo, Japan) with an attached energy dispersive X-ray spectroscope (EDX; EMAX-7000, Horiba Ltd., Kyoto, Japan).

Variations in the element concentrations of the SBF, caused by soaking the fiber bundle, were measured using inductively coupled plasma atomic emission spectroscopy (ICP; SPS-1500VR, Seiko Instruments Inc., Chiba, Japan).

3. Results and discussion

3.1. Structure of fibers

Calcium alginate fibers of about 5 μm in diameter and of uniform length were successfully prepared by the method described in Section 2.1.

Fig. 3 shows an FT-IR ATR spectrum of the fibers. One broad absorption band at around 3300 cm^{-1} and two sharp absorptions at around 1680 and 1400 cm^{-1} are observed. The former absorption band is ascribed to vibrations of OH bonds and the latter two absorption bands to asymmetric and symmetric stretching vibrations of carboxylate ions [12], indicating that the alginate fibers obtained contain a considerable amount of carboxylate ions.

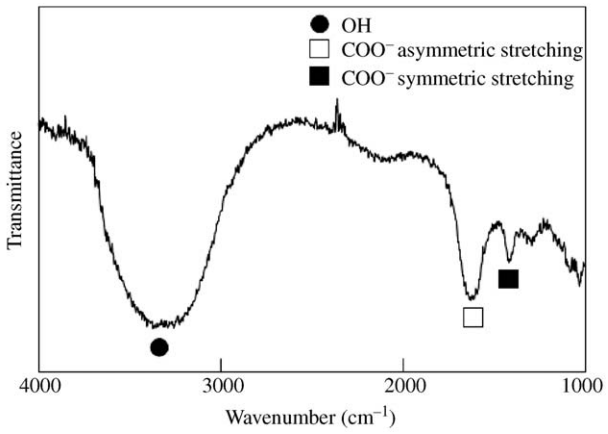

Figure 3 FT-IR ATR spectrum of the surface of calcium alginate fibers.

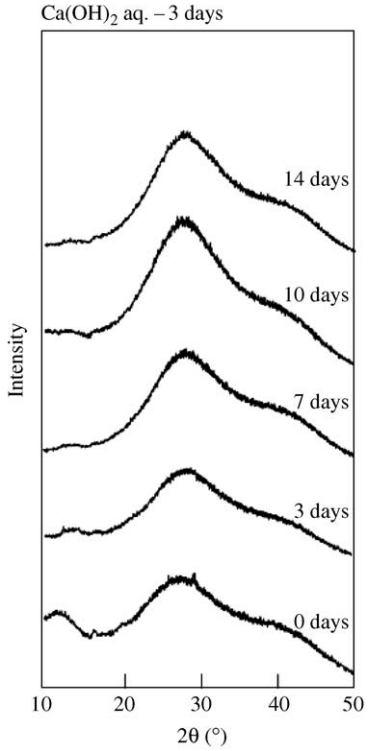

Figure 4 TF-XRD patterns of the surfaces of alginate fibers soaked in SBF for various periods after $Ca(OH)_2$ treatment for three days.

3.2. Apatite deposition on fibers

Figs. 4–6 show TF-XRD patterns of the alginate fibers treated with $Ca(OH)_2$ for different periods, then soaked in SBF for various periods. It can be seen from Figs. 4–6 that apatite is deposited on the alginate fibers in SBF within 14 days, when they had been previously treated with the $Ca(OH)_2$ solution for periods longer than five days, and that the induction time for apatite nucleation decreases with the increasing time of $Ca(OH)_2$ pretreatment. Fibers treated with the $Ca(OH)_2$ solution for periods shorter than three days did not form apatite on their surfaces within 14 days in SBF.

Figs. 7–9 show SEM photographs of alginate fibers treated with $Ca(OH)_2$ for different periods, then soaked in SBF for various periods. It can be seen from Figs. 7–9 that the surfaces of the alginate fibers are uniformly covered with a deposit within 14 days in SBF, when they had been previously treated with the $Ca(OH)_2$ solution for periods longer than five days. EDX analysis detected the presence of calcium and phosphorus in the deposit.

Fig. 10 shows variations in the calcium and phosphorus concentrations of the SBF caused by soaking the $Ca(OH)_2$ treated alginate fibers in the SBF. It can be seen from Fig. 10 that fibers treated with the $Ca(OH)_2$ aqueous solution for longer times show larger increases in the calcium ion concentration of the SBF in the early

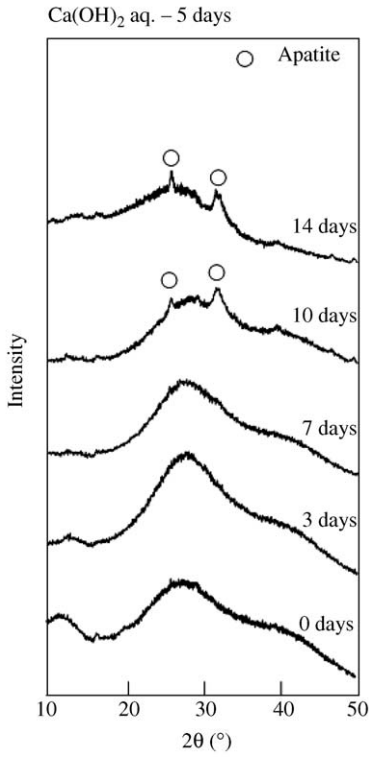

Figure 5 TF-XRD patterns of the surfaces of alginate fibers soaked in SBF for various periods after $Ca(OH)_2$ treatment for five days.

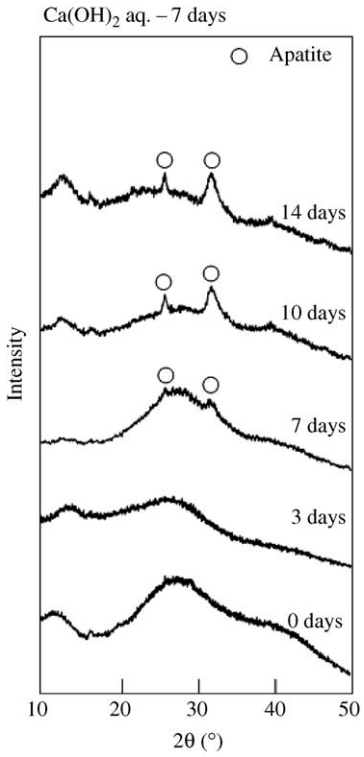

Figure 6 TF-XRD patterns of the surfaces of alginate fibers soaked in SBF for various periods after $Ca(OH)_2$ treatment for seven days.

stage. Subsequently, all the fibers show a decrease in the calcium and phosphorus concentrations. Fibres treated with the aqueous $Ca(OH)_2$ solution for longer times showed a larger decrease in the phosphorus concentration of the SBF.

These results indicate that the fibers treated with the aqueous $Ca(OH)_2$ solution for long periods release a large amount of calcium ions into the SBF. This release forms free carboxyl groups effective for apatite nucleation [13] on the surfaces of the fibers and accelerates apatite nucleation more effectively by increasing the ionic activity product of the apatite in SBF. Once the apatite nuclei are formed, they spontaneously grow by consuming the calcium and phosphate ions in the SBF, as shown in the decrease in the calcium and phosphorus concentrations of the SBF in Fig. 10.

The resultant apatite–alginate fiber composites are expected to be useful as a flexible bioactive bone repairing material.

4. Summary

Calcium alginate fibers of 5 μm in average diameter and of uniform length were prepared by extruding a sodium alginate solution into an aqueous calcium chloride solution. The fibers formed apatite on their surfaces within 14 days in SBF when they had been previously treated with an aqueous saturated $Ca(OH)_2$ solution for periods longer than five days. This apatite formation is interpreted in terms of the formation of carboxyl groups effective for apatite nucleation on surfaces of the fibers and the increase in the ionic activity product of the apatite in SBF, both of which arose from the release of calcium ions from the fibers.

Acknowledgments

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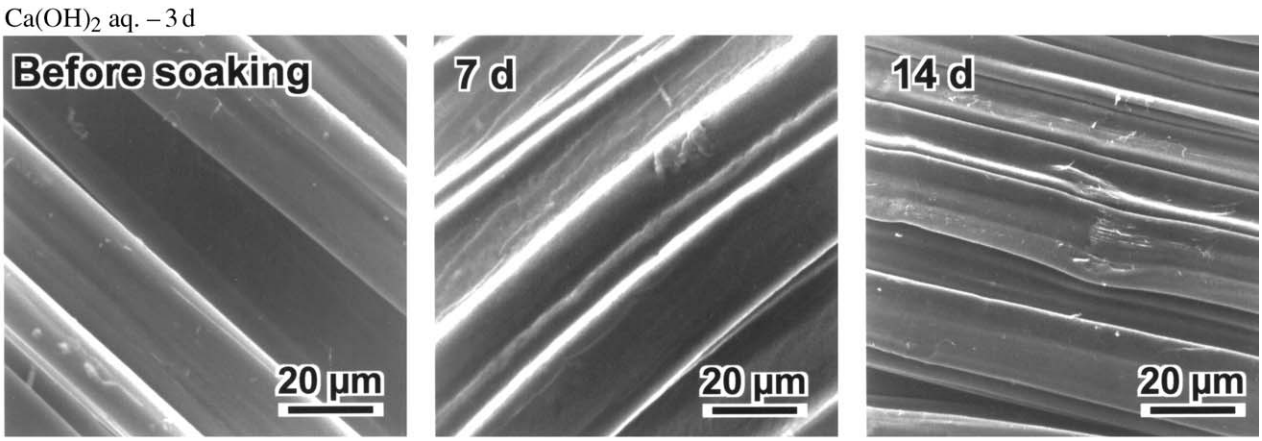

Figure 7 SEM photographs of the surfaces of alginate fibers soaked in SBF for various periods after $Ca(OH)_2$ treatment for three days.

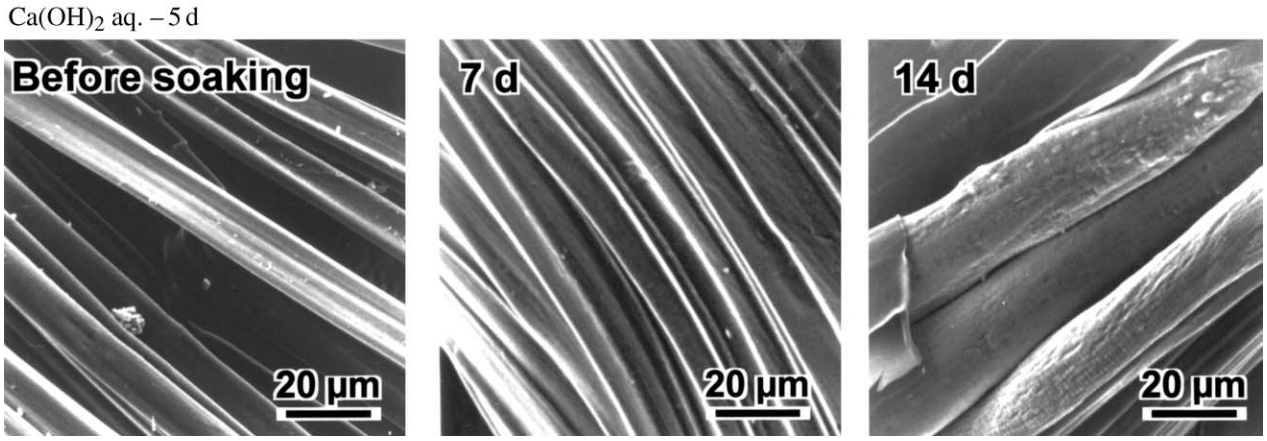

Figure 8 SEM photographs of the surfaces of alginate fibers soaked in SBF for various periods, after $Ca(OH)_2$ treatment for five days.

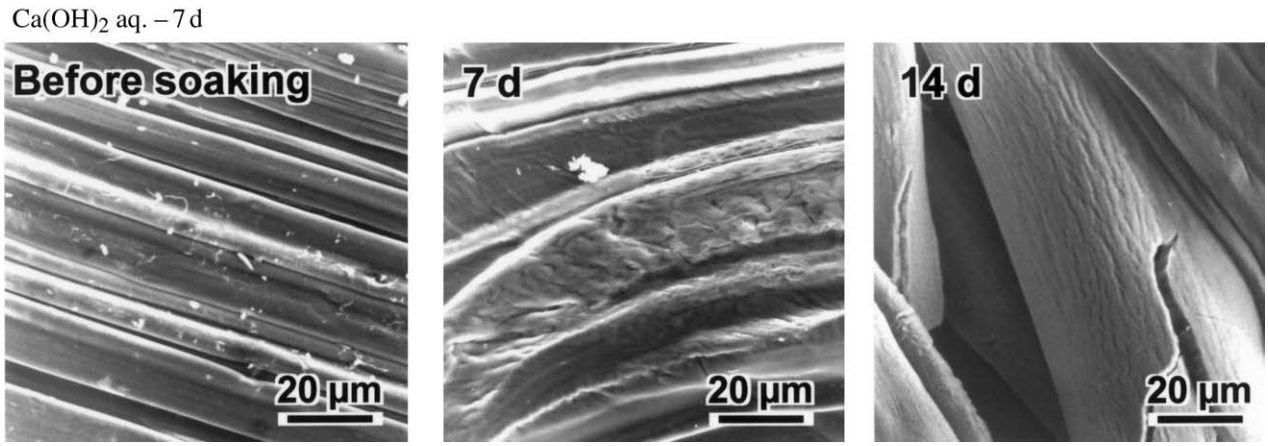

Figure 9 SEM photographs of the surfaces of alginate fibers soaked in SBF for various periods, after $Ca(OH)_2$ treatment for seven days.

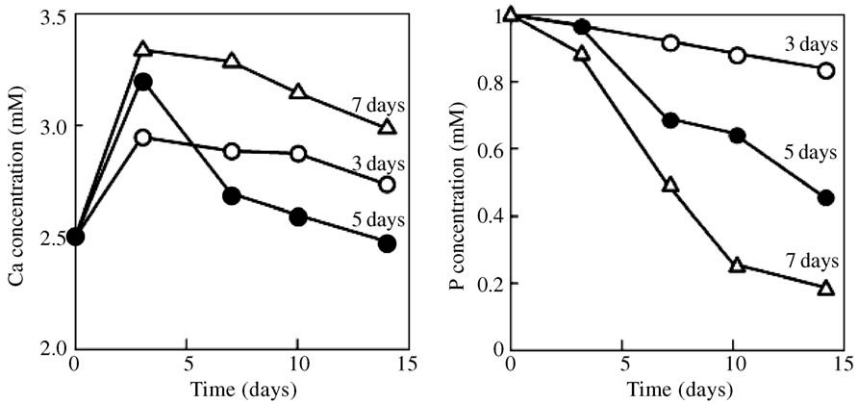

Figure 10 Variations in Ca and P concentrations of SBF after soaking of alginate fibers that had been treated with $Ca(OH)_2$ solution for different periods.

References

1. L. L. HENCH, R. J. SPLINTER, W. C. ALLEN and T. K. GREENLEE, *J. Biomed. Mater. Res. Symp.* **2** (1972) 117.
2. M. JARCHO, J. F. KAY, K. I. GUMAER, R. H. DOREMUS and H. P. DROBECK, *J. Bioeng.* **1** (1977) 79.
3. T. KOKUBO, M. SHIGEMATSU, Y. NAGASHIMA, M. TASHIRO, T. NAKAMURA, T. YAMAMURO and S. HIGASHI, *Bull. Inst. Chem. Res. Kyoto. Univ.* **60** (1982) 260.
4. H.-M. KIM, F. MIYAJI, T. KOKUBO and T. NAKAMURA, *J. Biomed. Mater. Res.* **32** (1996) 409.
5. T. MIYAZAKI, H.-M. KIM, F. MIYAJI, T. KOKUBO, H. KATO and T. NAKAMURA, *ibid.* **50** (2000) 35.
6. M. TANAHASHI, T. YAO, T. KOKUBO, M. MINODA, M. MIYAMOTO, T. NAKAMURA and T. YAMAMURO, *J. Amer. Ceram. Soc.* **77** (1994) 2805.
7. A. OYANE, M. KAWASHITA, K. NAKANISHI, T. KOKUBO, M. MINODA, T. MIYAMOTO and T. NAKAMURA, *Biomaterials* **24** (2003) 1729.
8. A. OYANE, M. KAWASHITA, T. KOKUBO, M. MINODA, T. MIYAMOTO and T. NAKAMURA, *J. Ceram. Soc. Japan* **110** (2002) 248.
9. M. KAWASHITA, M. NAKAO, M. MINODA, H.-M. KIM, T. BEPPU, T. MIYAMOTO, T. KOKUBO and T. NAKAMURA, *Biomaterials* **24** (2003) 2477.
10. H. TAMURA, Y. TSURUTA and S. TOKURA, *Sen-i Gakkai Preprints F* **151** (1998) 151.
11. T. KOKUBO, H. KUSHITANI, S. SAKKA, T. KITSUGI and T. YAMAMURO, *J. Biomed. Mater. Res.* **24** (1990) 721.
12. R. M. SILVERSTEIN, G. C. BASSLER and T. C. MORRILL, in ''Spectrometric Identification of Organic Compounds'', 4th edn, translated by S. Araki, Y. Masuko and O. Yamamoto (Tokyo Kagaku Dozin Co. Ltd., Tokyo, 1983) p. 90.
13. M. TANAHASHI and T. MATSUDA, *J. Biomed. Mater. Res.* **34** (1997) 305.

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