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Control of Bioresorption of Porous α-Tricalcium Phosphate by Coating with Silk Sericin

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Porous α-tricalcium phosphate (α-TCP) ceramics are attractive as a novel bioresorbable material in orthopedic field, since they can be easily fabricated through conventional sintering of β-TCP at high temperature. However higher solubility of α-TCP than β-TCP leads to complete dissolution before sufficient bone repair when implanted in bone defect. In this study, we attempted to control dissolution rate of the porous α-TCP by coating with sericin, a kind of silk protein. We fabricated α-TCP porous body with continuous pores of 10-50 μm in diameter. The α-TCP porous ceramics after coating with sericin showed higher chemical durability than those before coating in buffer solutions. They were then implanted in bone defect made at rabbit tibia. The porous α-TCP after the coating showed desirable bioresorption, where they were gradually dissolved according to bone repair. The prepared porous α-TCP modified with sericin is expected to be useful as a novel bone substitute by incorporating with drugs or osteoinductive factors.

Key words: Porous α-TCP, Bioresorption, Coating, Sericin, Chemical durability

1. INTRODUCTION

Tricalcium phosphate (TCP, $Ca_3(PO_4)_2$) is a typical bioresorbable ceramic, which has been already subjected to clinical use as bone filler [1-4]. Its porous body is gradually dissolved repairing the bony defects. In addition, the interconnected pores enable the ingrowth of bony tissue leading to substitution by newly regenerated bone. Porous α-TCP can be easily synthesized using conventional sintering process, since α-TCP is thermodynamically stable at temperatures higher than 1100°C. However, its solubility is much higher than that of β-TCP [5-6] and, therefore, α-TCP might be almost completely dissolved before the bony defect is sufficiently repaired. It was previously reported that the durability of porous α-TCP can be improved after coating with hydroxypropylcellulose [7]. This suggests that several kinds of organic polymers are available for the coatings.

In the present study, we selected sericin as a candidate of organic polymer. Sericin is a silk protein covering the surface of raw silk fibers, as shown in Fig. 1 [8]. We recently found that bone-like apatite can be deposited on sericin after immersion in a solution with 1.5 times inorganic ion concentrations of human blood plasma (1.5SBF) [9]. This indicates that coating of sericin brings not only improvement in the chemical durability of the α-TCP porous ceramics, but also provides the deposition of apatite due to dissolution of the ceramic. It is known that materials which can deposit bone-like apatite on their surfaces in body environment have a potential to directly bond to living bone, i.e. show bioactivity. On the basis of the results described above, novel bone substitutes exhibiting not only bioresorbability but also bioactivity, can be designed. We coated sericin on α-TCP porous bodies and examined effects on chemical durability and mechanical

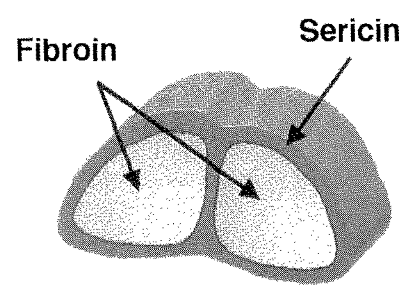

Fig. 1 Cross-sectional view of raw silk fiber.

properties of the ceramics. Bioresorption behavior was also investigated by *in vivo* implantation tests in rabbit tibiae.

2. MATERIALS AND METHODS

2.1 Preparation of porous ceramics

Commercially pure β-TCP powder and an equivalent mass of potato starch were dispersed in ultra pure water to form homogeneous slurries. Polyurethane sponges with 15 x 15 x 15 mm^3 in size were dipped in the slurries and subsequently dried at 60°C for 1 h (=hour). The sponges incorporated with the ceramics powder were then heated in air at 5 °C/min to 1000°C and kept at this temperature for 3 h to burn up the sponge. After cooling to room temperature, the ceramic bodies were heated at 5 °C/min to 1400 °C, kept at this temperature for 12 h and cooled to room temperature in the furnace. The resultant porous α-TCP ceramics were characterized using powder X-ray diffraction (XRD) and scanning electron microscopy (SEM).

2.2 Coating with sericin

The resultant porous α-TCP blocks of 1 g were soaked in a sericin solution that was extracted from raw silk fiber by degumming in water at 120°C for 30 min. They were then dried at 40°C for 24 h. The coating process was repeated five times. The sericin-coated α-TCP porous bodies were hereafter denoted as α-TCP/Sericin.

2.3 Characterization

Structure of the specimens was characterized by powder X-ray diffraction and scanning electron microscopy (SEM). In order to evaluate chemical durability of the specimens, they were soaked in 30 mL of buffer solutions at pH 4, 6 or 7.25 at 36.5°C for 7 d (=days). Potassium hydrogen phthalate, 2-(N-morpholino) ethanesulfonic acid (MES) and tris(hydroxyethyl)aminomethane was used as buffer of pH 4, 6 and 7.25, respectively. Ca^{2+} concentration of the buffer solutions after soaking of the specimens was measured by a Ca^{2+} electrode. Compressive strength was quantitatively evaluated by an Instron-type material testing machine at cross-head speed of 20 mm/min.

2.4 Animal test

A hole of 4 mm in diameter was made at the medial aspect of the proximal metaphysis of the tibiae of Japanese white rabbits. The porous specimens 4 mm in diameter and 5 mm in length was then inserted into the hole. After 4 w (=weeks) implantation, the rabbits were sacrificed and the implanted specimens along with the bony tissues were extracted. The implants as well as surrounding bony tissues were observed by peripheral quantitative computed tomography (pQCT).

3. RESULTS

Figure 2 shows powder X-ray diffraction pattern of the porous ceramics. Peaks assigned to α-TCP were detected. This indicates that the prepared porous body was composed of only crystalline α-TCP.

Figure 3 shows SEM photographs of α-TCP and α-TCP/Sericin before and after coating with sericin. As-prepared porous body of α-TCP ceramic contained interconnected pores with diameter ranging from 10 to 50 μm. The morphology was almost the same even after the coating.

Figure 4 shows compressive strength of α-TCP and α-TCP/Sericin. Compressive strength of porous α-TCP increased after the coating.

Figure 5 shows SEM photographs of the inside of α-TCP and α-TCP/Sericin after soaking in buffer solution at pH 6 for 7 d. A lot of pits were observed on the

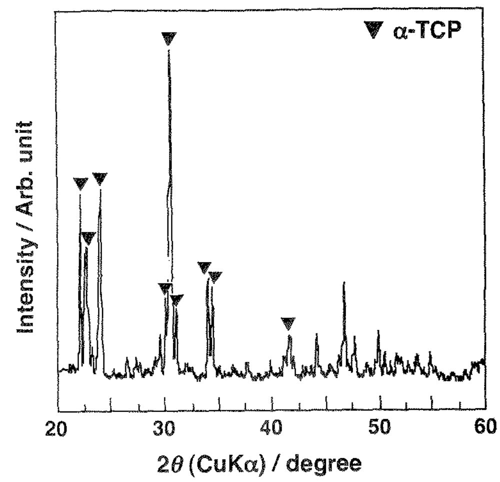

Fig 2 Powder X-ray diffraction pattern of the porous ceramics.

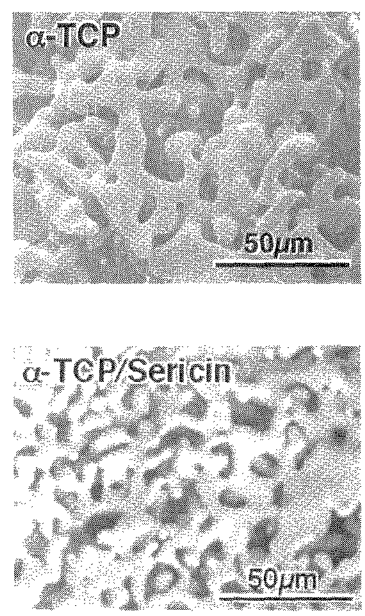

Fig. 3 SEM photographs of α-TCP and α-TCP/Sericin.

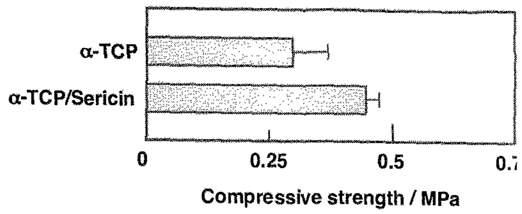

Fig. 4 Compressive strength of α-TCP and α-TCP/Sericin (n=5).

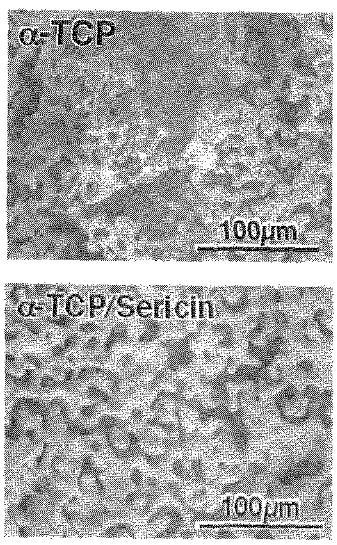

Fig. 5 SEM photographs of the inside of α-TCP and α-TCP/Sericin after soaking in buffer solution at pH 6 for 7 d.

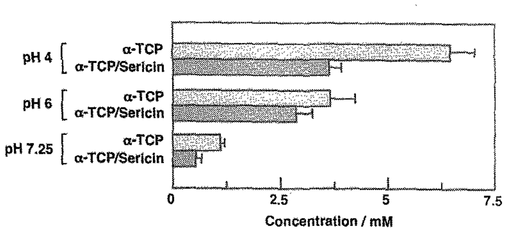

Fig. 6 Ca^{2+} concentration of the buffer solutions at various pH after soaking of α-TCP and α-TCP/Sericin for 7 d (n=5).

skeleton of porous α-TCP ceramic without modification, whereas amount of the pit remarkably decreased for α-TCP/Sericin. This indicates that the porous α-TCP/Sericin was less corroded than α-TCP in the buffer solution.

Figure 6 shows Ca^{2+} concentration of the buffer solutions at various pH after soaking of α-TCP and α-TCP/Sericin for 7 d. Release of Ca^{2+} into the buffer solutions corresponds to dissolution of porous α-TCP

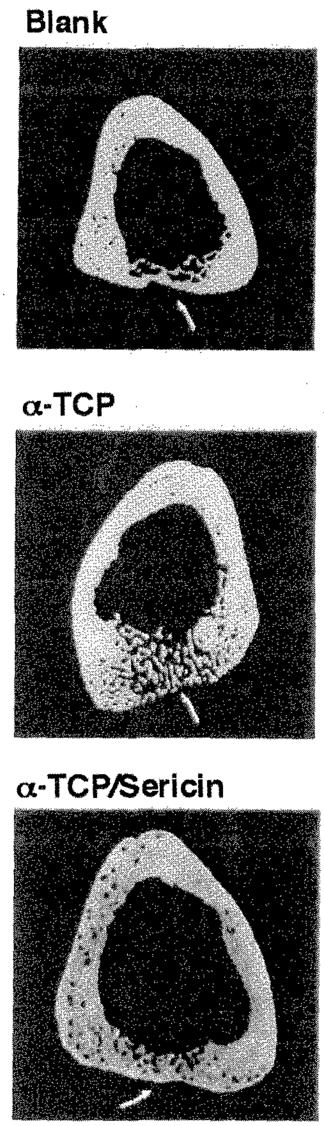

Fig. 7 pQCT images of rabbit tibiae after the implantation of α-TCP and α-TCP/Sericin for 4 w.

ceramic. The specimen coated with sericin showed lower Ca^{2+} concentration in buffer solutions with any pH, in comparison with α-TCP ceramic without the coating.

Figure 7 shows pQCT images of rabbit tibiae after the implantation of α-TCP and α-TCP/Sericin for 4 w. Blank means that the prepared bony defect was left without implantation as reference. Residual specimens were not observed under pQCT images. The images indicate that a higher degree of bone regeneration occurred in the tibiae implanted with α-TCP/Sericin than those implanted with α-TCP, though both specimens were completely dissolved after the implantation period. It is therefore supposed that sericin coated on α-TCP favors a slower dissolution of the ceramic phase supporting more desirable bone regeneration.

4. DISCUSSION

The present method easily produces porous α-TCP ceramic with interconnected pores ranging 10 to 50 μm in diameter by conventional heat treatment. Chemical durability of porous α-TCP ceramic can be improved by simple coating with sericin. Optimum behavior of bioresorption in bony defect for α-TCP/Sericin supports the results concerning the improved chemical durability (see Fig. 6). This reduced dissolution of porous α-TCP ceramic is attributed for the protective effect of the coating on the porous α-TCP against surrounding buffer solutions. Therefore the skeleton of α-TCP porous body was able to sufficiently play a role in scaffold for bone regeneration.

It should be noted that increased compressive strength by the coating shows that such a modification with sericin has another role in improving workability in clinical usage as well as chemical durability of the porous α-TCP ceramic (see Fig. 4). This improved mechanical property is quite important factor from a practical point of view.

5. CONCLUSION

Bioresorption of porous α-TCP ceramic was reduced by simple coating with silk sericin. The porous α-TCP ceramic coated with sericin showed more desirable behavior of bioresorption in rabbit tibia, where larger amount of bone regeneration was observed. This type of simple organic modification is expected to be applicable to other bioresorbable ceramics.

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