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Influence of surface microstructure on the reaction of the active ceramics *in vivo*

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When porosity and macro-pore size differ in the same ceramic, the mode of bone regeneration and the degradation of ceramics *in vivo* is said to be different. However, the reactions *in vivo* of ceramics that have a different microstructure with the same porosity and the same macro-pore size, are not so far known. In this study, two kinds of β-tricalcium phosphate (TCP) that had different microstructures but the same porosity and macro-pore size, were manufactured. These TCP were implanted in the distal femurs of 20 mature male rabbits, and their respective areas of ceramics and of regenerated bone were measured after 4, 12 and 24 wk. In both TCPs, the regenerated bone similarly decreased from 4–24 wk in a different way. The area of ceramics in one of these TCPs significantly decreased gradually throughout the observation period. On the other hand, the other TCP showed no marked decrease during the same period. This suggested a possibility that the difference in microstructure has a large effect on the reaction of the ceramics in the bone.

1. Introduction

Porous hydroxyapatite (HA) and β-tricalcium phosphate (TCP) show good tissue tolerance with no immunological reaction and no toxic reaction [1–4]. They have been used as bone-graft substitutes not only in experimental models [5, 6] but also in clinical applications [7, 8]. When porosity and pore size are different in these ceramics, bone regeneration and the degeneration of ceramics *in vivo* are occasionally different [9, 10]. However, the effects of the different microstructures on the *in vivo* reaction are not known.

Two kinds of TCP which had the different microstructures with the same porosity and pore size were manufactured by different methods. This study was conducted to analyse the effects of the different microstructures on *in vivo* reactions to TCP implanted in the bone.

2. Materials and methods

One of the two β-TCPs was prepared by the Mitsubishi Material Co. Ltd, by sintering at 900 °C from β-TCP powder. The properties of material were as follows: compressive strength, 12.5 ± 4.0 MPa measured using a material testing machine (Shimadzu, REH-100, Tokyo) with a crosshead speed of 0.02 cm min^{-1}; porosity 60% ± 5% (measured by the Archimedean method using water); diameter of macro-pores: 150–400 μm; diameter of micro-pores, 0.2–0.5 μm measured by mercury intrusion porosimetry (Carlo Elba Series 220). The specificsurface area was found to be 10.0 m^2 g^{-1} by the Brunauer–Emmett–Teller (BET) method. The gross shape was a cylinder of 5 mm diameter and 5 mm diameter and 5 mm length (this material was termed TCP-1).

The cylinderical porous HA provided by the Mitsubishi Material Co. Ltd, 5 mm diameter and 5 mm length, was soaked in diammonium hydrogen phosphate solution and then the HA was sintered at 900 °C for 3 h [11]. The material thus manufactured was fully crystallized 100 wt % TCP (TCP-2) revealed by X-ray diffraction analysis (Rigaku, RAD-RC, Tokyo). Nothing except TCP was found on the surface of the material by infrared spectroscopy. The properties of TCP-2

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were as follows: porosity, 57% ± 5%; macro-pore size, 150–400 μm, micro-pores, nil. The specific surface area was 2.0 $m^2 g^{-1}$. The slight decrease in porosity in comparison with the starting HA, resulted from the disappearance of the micro-pores by TCP formation. The specific surface area of TCP decreased from 10.0 $m^2 g^{-1}$ to 2.0 $m^2 g^{-1}$. The surfaces of these materials were examined by scanning electron microscopy (SEM, Hitachi, S530, Tokyo).

A column of each of the two materials was implanted into the distal femurs of each of 20 male rabbits, 5–6 mon old, and of a mean weight of 3.0 kg. The rabbits were anaesthetized by intravenous injection of 50 mg kg^{-1} Nembutal (pentobarbital) (Dynabot Co., Tokyo). A 5 mm drill hole was made in each of the bilateral condyles of each of the bilateral distal femurs, through the cortex and into the medullary canal, using a specially prepared trephine (5.2 mm outer diameter). The drill speed was 1500 r.p.m. The implants were inserted tightly into the holes. The two different ceramics were always implanted into the four holes of each animal equally. These materials were equally divided among 20 rabbits (Fig. 1).

The rabbits were sacrificed by anaesthetic overdose after periods of 4, 12 and 24 wk (Table I). The implant specimens were retrieved, dehydrated in a 70% solution of ethanol, and embedded in methyl methacrylate. The specimens were cut into sections 150–200 μm thick, perpendicular to the long axis of the cylinders by a diamond-blade saw. The thickness of the sections was then further reduced to 95–105 μm by hand polishing, finally producing 6–8 slices of each specimen. Contact micro-radiography was taken on each slice and the areas of ceramic material, soft tissue, and regenerated bone were measured by a computer image analyser (Luzex 3U, Nicon Company, Tokyo).

The area of ceramics was calculated as follows: the mean of an area of ceramics for one columnar material was calculated from several (6–8) slices made from one column. A representative (mean plus S.D.) of the material at a certain material and at a certain period was calculated from the means mentioned above. The area of regenerated bone was also calculated in the same manner.

The unpaired Wilcoxon's *t*-test was used for statistical analysis, with significance taken at the 5% level. These sections obtained were then polished manually to a thickness of 40–50 μm and stained with toluidine blue for histological observation.

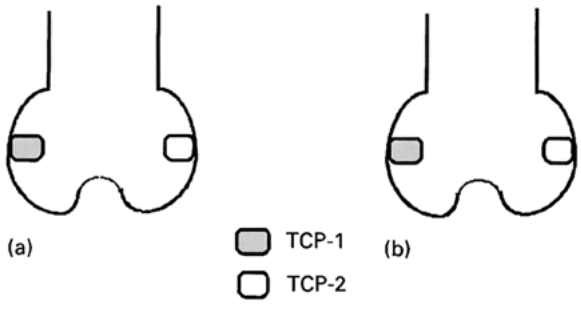

Figure 1 Method of implantation. (a) Right femur, (b) left femur.

TABLE I Number of columns implanted in 20 rabbits

Material	4 wk	12 wk	24 wk
TCP-1	6	6	6
TCP-2	6	9	6

3. Results

3.1. Microstructure of the material in the SEM

SEM revealed a sharp contrast of the two materials in surface structure. The surface of TCP-1 showed many micro-pores, less than 1 μm diameter, all over the surface (Fig. 2a). The surface of TCP-2, in contrast, was very smooth with a few round cavities, 1–3 μm diameter (Fig. 2b).

3.2. Quantitative analysis

3.2.1. Area of ceramics (Fig. 3)

The area of the ceramics before implantation was 13.7 ± 0.1 mm^2 in TCP-1 and 13.1 ± 0.6 mm^2 in TCP-2. The area of these two ceramics was not significantly different.

After implantation, the area of ceramics in TCP-1 was 13.1 ± 0.7 (4 wk), 10.6 ± 0.9 (12 wk), 9.6 ± 0.9 (24 wk) mm^2, and that in TCP-2 was 13.8 ± 0.5 (4 wk), 12.8 ± 1.7 (12 wk), and 12.8 ± 0.9 (24 wk) mm^2.

The area of ceramics in TCP-1 gradually and significantly decreased throughout the observation period,

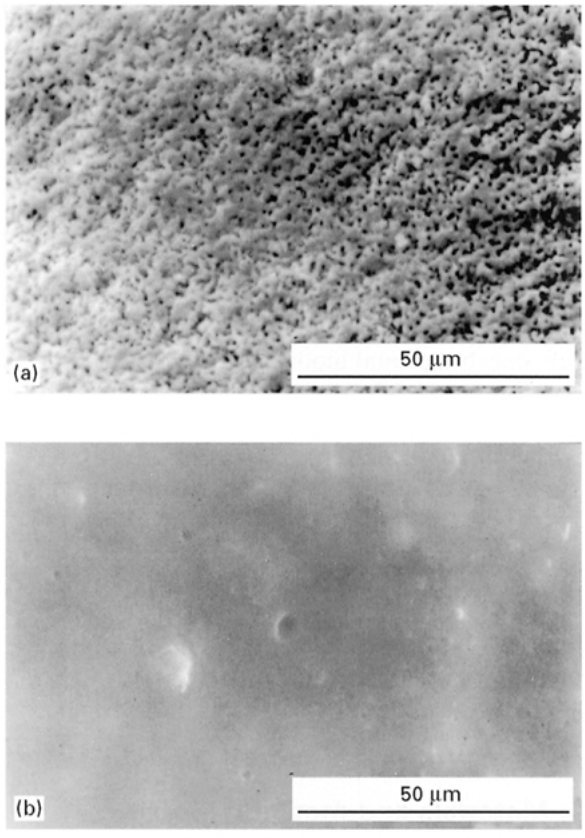

Figure 2 Scanning electron micrographs of the surface of (a) TCP-1 and (b) TCP-2. TCP-1 can be recognized to have many micro-pores, < 1 μm diameter all over the surface. The surface of TCP-2 is smooth except for some pits of diameter 3–5 μm.

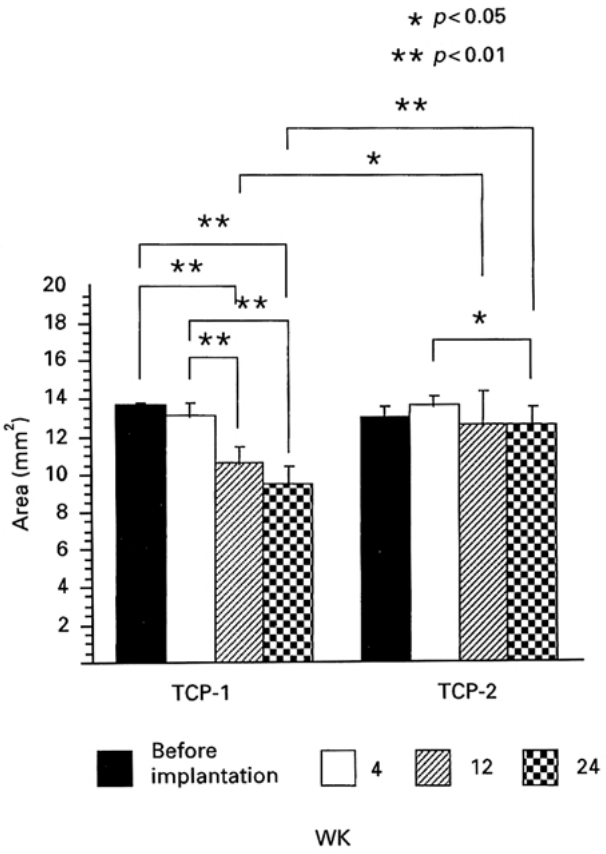

Figure 3 The area of ceramics.

and it was 70% of the starting area at 24 wk. In TCP-2, the area of ceramics did not change markedly during the period. The area at 24 wk was not significantly different from that at the start. TCP-1 showed significantly greater bioresorption than TCP-2 after 12 and 24 wk implantation.

3.2.2. Area of regenerated bone (Fig. 4)

The area of regenerated bone in TCP-1 was 1.31 ± 0.2 (4 wk), 0.75 ± 0.3 (12 wk) and 0.94 ± 0.3 (24 wk) mm^2 and that in TCP-2 was 1.5 ± 0.4 (4 wk), 1.25 ± 0.4 (12 wk), and 1.06 ± 0.2 (24 wk) mm^2.

The regenerated bone in TCP-1 decreased significantly at 12 wk in relation to that at 4 wk. On the other hand, TCP-2 showed a gradual decrease in regenerated bone after 4 wk but the decrease was not statistically significant.

3.3. Histology

In TCP-1 at 4 wk, a large amount of regenerated bone surrounded the ceramic cylinder and was attached to the surface, and many of the pores of the cylinder were infiltrated with the regenerated bone. The pores of ceramics in TCP-1 became larger compared with those in TCP-2 and some pores fused into larger ones (Fig. 5a). At 24 wk, the ceramic was so biodegraded that it was difficult to distinguish the original shape of the implant. The regenerated bone in TCP-1 markedly decreased and only a thin layer of bone was attached to the outer surface of the ceramics and on the inner walls of the pores (Fig. 5b).

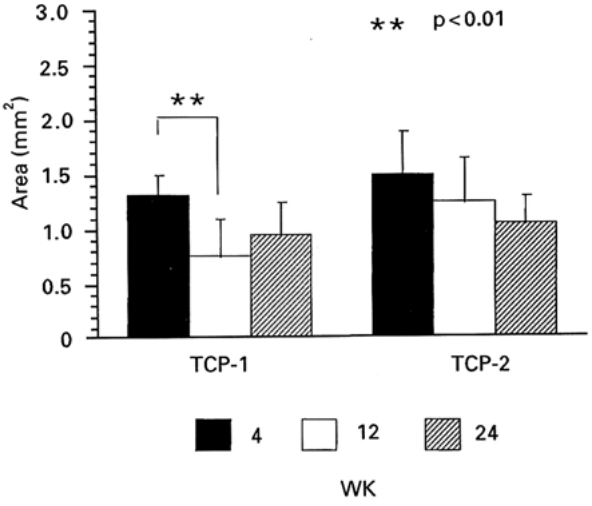

Figure 4 The area of regenerated bone.

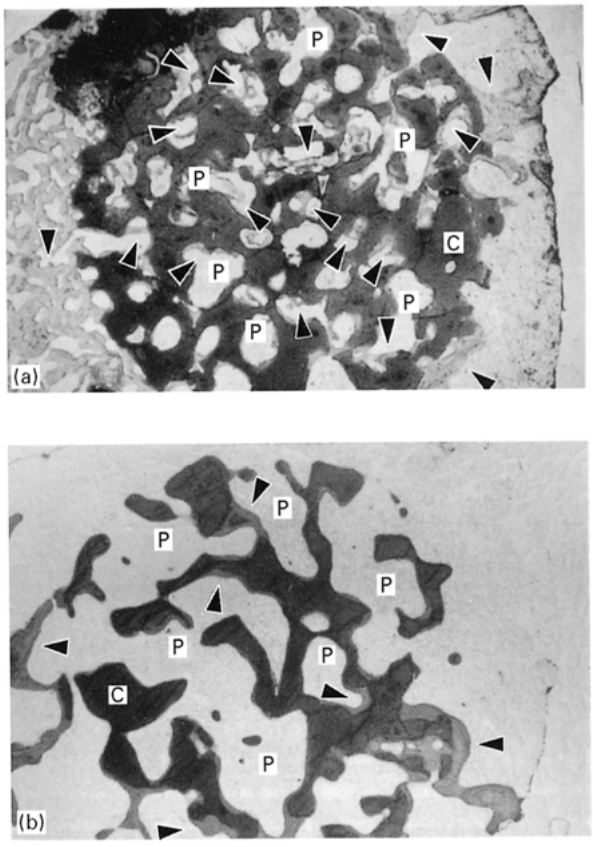

Figure 5 Photomicrographs of implanted TCP-1. (a) At 4 wk, note that large amount of regenerated bone (arrow heads) surrounds the ceramic cylinder (C) and is attached to the surface and many of the pores (P) of the cylinder are infiltrated with the regenerated bone. Note that some of the pores are enlarged and fused into larger ones. (b) At 24 wk, the ceramic is so biodegraded that it is difficult to distinguish the original shape of implant. Only a thin layer of the bone (arrow heads) is attached to the outer surface of the ceramic cylinder (C) and on the inner walls of the pores (P). ×5

In TCP-2, at 4 wk a large amount of regenerated bone surrounded the cylinders and many of the pores of the cylinder were infiltrated with the regenerated bone (Fig. 6a). The size of the pores in TCP-2 was smaller compared with that in TCP-1. At 24 wk some of the pores were enlarged as compared with these at 4 wk. The regenerated bone in TCP-2 also decreased

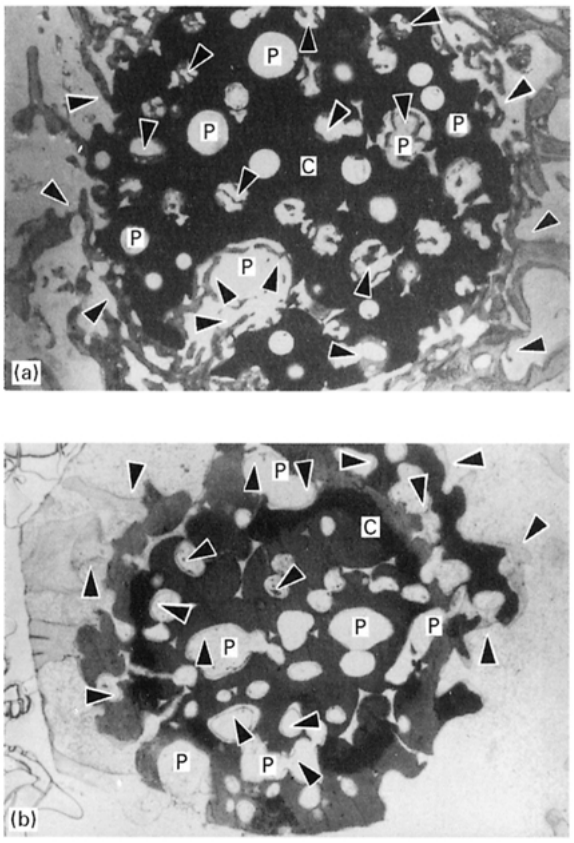

Figure 6 Photomicrographs of implanted TCP-2, (a) at 4 wk after implantation, a large amount of regenerated bone (arrow heads) surrounds the ceramic cylinder (C), is attached to the surface and infiltrated into the pores (P). (b) At 24 wk, only a thin layer of the bone (arrow heads) is attached to the outer surface of the ceramic cylinder (C) and on the inner walls of the pores (P). Note that some of the pores are enlarged and fuse into larger ones as compared with those at 4 wk. × 5

and became a thin layer on the outer surface of the ceramics cylinder and on the inner walls of the pores (Fig. 6b).

4. Discussion

Eggli *et al.* [9] reported that ceramics in the bone reacted differently according to the different pore sizes. Shimazaki and Mooney [10] also reported that the reaction of ceramics in the bone was different according to the porosity and pore size in HA. These studies deal with macro-pores of about 50–400 μm in size. Peelen *et al.* [12] reported the reaction of ceramics *in vivo* that had different micro- and macro-pores. However, their ceramics were a mixture of HA and TCP, and they were not pure. The microstructure and macrostructure of their implanted ceramics were not clear, and biological evaluation of the ceramics *in vivo* was not performed quantitatively. This study showed that, even if the porosity and the macro-pore size were the same, the biodegradation and the bone regeneration could be different in our material, according to the difference in the microstructures, especially micro-pores.

In general, as the sintering temperature rises, the crystallization of HA progresses and the grain size increases with reduction in surface area [13]. The dissolution of HA in an isotonic sodium chloride solution decreases as the sintering temperature rises [13]. This report suggests that it is possible to have a relationship between the dissolution and the surface area. The surface area of TCP-1 is much larger than that of TCP-2, with few micro-pores.

This might lead to wider contact of TCP-1 with the body fluid and to a more rapid chemical dissolution than in TCP-2. Another assumption about the more rapid biodegeneration of TCP-1 than of TCP-2 can be made in biological terms. Bioactive ceramics have been said to be biodegraded by macrophages and giant cells [14,15]. The ceramic is easily contacted by phagocytes resorbing active bioceramics as the surface area increases. In this sense, TCP-1 provided more favourable surface conditions for bioresorption than in smooth-surfaced TCP-2. Regarding the difference in bone regeneration between the two materials, it can be said that the rapid decrease of the ceramics as a scaffold for bone caused a decrease in regenerated bone at 12 wk in TCP-1.

This result means that when the reactions of ceramics implanted in the bone are compared, not only the porosity and the macro-pore size are prescribed, but the micro-pore should also be scrutinized.

We suggest it is possible that the difference in microstructure of the ceramics, largely influences the reaction of the ceramics in the bone. Micro-pores, as well as macro-pores, must be controlled for porous-material design, although the size of micro-pores is too small for cells.

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