Bone formation induced by calcium phosphate ceramics in soft tissue of dogs: a comparative study between porous α-TCP and β-TCP

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Two kinds of tri-calcium phosphate ceramics (Ca/P = 1.50), α-TCP and β-TCP, which has the same macrostructure and microstructure, but different phase composition, were implanted in dorsal muscles of dogs. The samples were retrieved at 30, 45 and 150 days, respectively, after implantation, and were analyzed histologically. There were critically different tissue responses between α-TCP ceramic and β-TCP ceramic. Higher cell populations were observed inside the pores of β-TCP than those of α-TCP, bone tissue was found in β-TCP at 45 and 150 days, but no bone formation could be detected in any α-TCP implants in this study. On the other hand, the bone tissue in β-TCP seemed to degenerate at 150 days. The results indicate that porous β-TCP can induce bone formation in soft tissues of dogs; while the rapid dissolution of the ceramic and the higher local Ca^{2+}, PO_4^{3-} concentration due to the rapid dissolution of α-TCP may resist bone formation in α-TCP and the less rapid dissolution of β-TCP may be detrimental to already formed bone in β-TCP.

1. Introduction

Different types of materials have been developed from calcium phosphates that are biocompatible and osteoconductive [1–4]. An ideal biomaterial for hard tissue repair should be biocompatible, osteoconductive, resorbable and osteoinductive [1, 2, 4, 5]; thus, in recent years, attention has been directed to the development of resorbable and osteoinductive biomaterials from calcium phosphates [2, 5–9]. Tricalcium phosphate ceramic (TCP), especially β-TCP ceramic, is an important calcium phosphate biomaterial, besides its profile of biocompatibility and osteoconductive capability, it has a higher resorption rate than hydroxyapatite ceramic (HA). Hence, it has sometimes been considered as a resorbable biomaterial [1–3, 10–16]. Due to its resorbability, TCP was also mixed with HA to make biphasic calcium phosphate ceramics (BCP, tricalcium phosphate/hydroxyapatite ceramic) with different resorption rates [17–22].

The resorption of calcium phosphate biomaterials has been the subject of many studies [13, 23–26]. It was suggested that the resorption rate of calcium phosphate biomaterials was related to their forms, chemical composition, structures including macropores and micropores, and both chemical dissolution and cell-mediated resorption were involved in the resorption process [13, 23–26]. Contrary to the extensive studies of factors affecting resorption rate of calcium phosphate biomaterials, few studies addressed the effects of resorption rate on tissue responses. It appeared that the resorption of calcium phosphate biomaterials is beneficial to bone formation [20, 27, 28] and that free Ca^{2+} could be considered as the origin of bioactivity [27, 29, 30]. This might, in fact be the case when the resorption rate of calcium phosphate biomaterials is within certain limits. Because too much dissolved Ca^{2+}, PO_4^{3-}, leading to a sharp change of the microenvironment, may disturb the activity of host cells and create adverse effects on tissues [17, 21].

As to the osteoinductive property of calcium phosphate biomaterials, it is generally thought that calcium phosphate biomaterials can stimulate bone formation, but can not induce bone formation [1–3, 11, 12, 31, 32]. However, in recent years, several types of calcium phosphate ceramics have been reported to induce bone formation in soft tissues of different animal models [33–45]. After observing the bone formation induced by HA ceramics and BCP in soft tissues of dogs [34, 39–45], we

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extended our investigation of calcium phosphate-induced osteogenesis to tricalcium phosphate ceramic, which was the first calcium phosphate biomaterial reported to be osteoconductive [12, 26], but has not been reported to be osteoinductive.

The purpose of this study was to investigate the osteoinductive property of TCP. However, the different tissue responses to α-TCP and β-TCP ceramics with different resorption rates [46–48], were used to answer the effects of resorption of calcium phosphate biomaterials on tissue responses.

2. Materials and methods

2.1. Ceramics

Apatite powder with a Ca/P ratio of 1.50 was wet-synthesized by the reaction of calcium nitrate and di-ammonium hydrogenphosphate in a basic ammonia condition. Green bodies were foamed by 5–10% H_2O_2 at the temperature of 70–80 °C and dried. Ceramics were obtained by sintering the green body at 1100 °C for 3 h and using a different cooling procedure for each ceramic. Natural cooling produced β-TCP, quenching produced α-TCP. The phase composition of the ceramics was identified by X-ray diffraction (XRD) (Fig. 1). The porous structures were observed under scanning electron microscopy (Philips SEM 525 connected with an X–ray energy dispersive detector). There were the same porous structures in both ceramics; they were porous with interconnected macropores (photos not shown) and micropores on the macropore surface (Fig. 2). Ceramic implants ($\phi 5 \times 6$ mm) were obtained from the ceramic bodies and cleaned with distilled water and then autoclaved at 121 °C for 30 min before implantation.

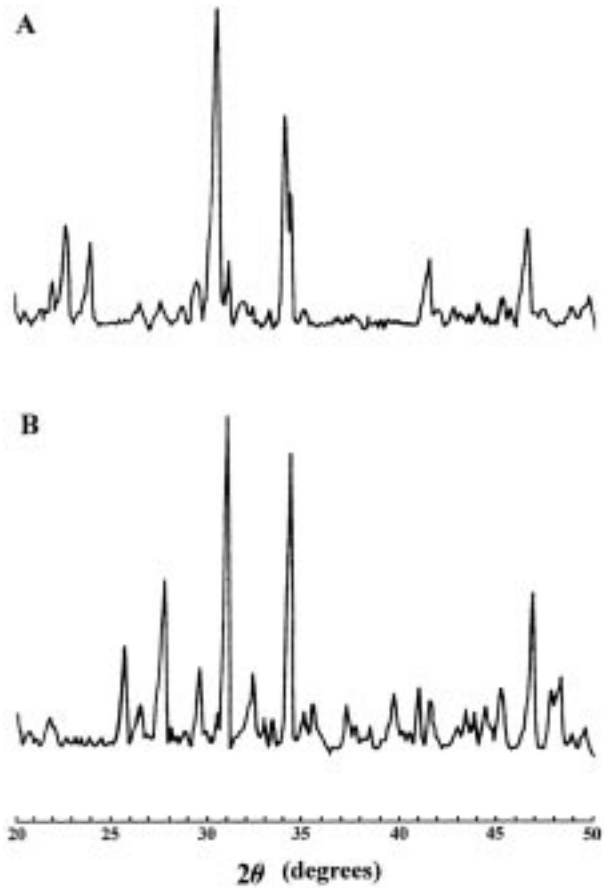

Figure 1 XRD patterns of α-TCP (A) and β-TCP (B).

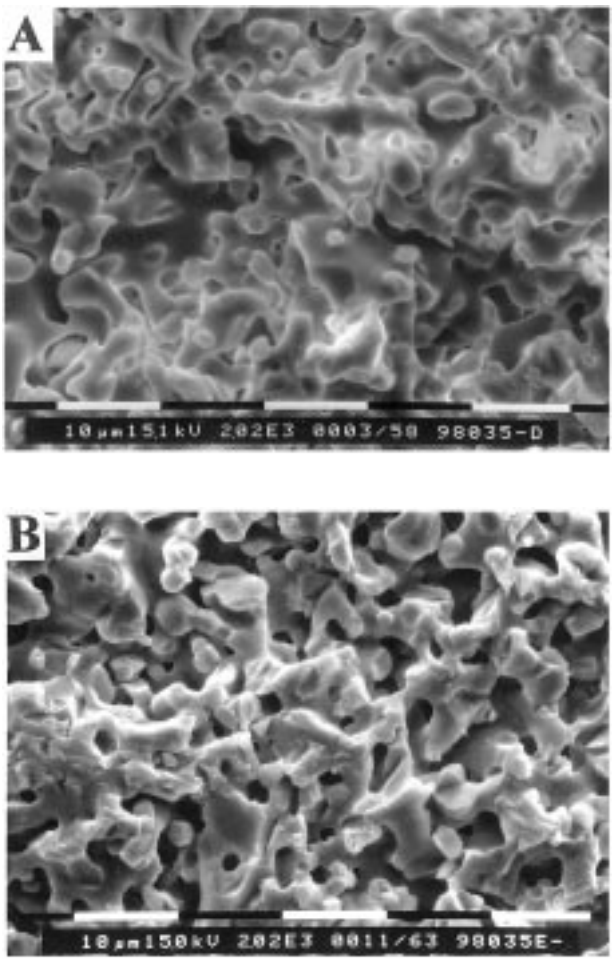

Figure 2 Microstructure of α-TCP (A) and β-TCP (B) under SEM.

2.2. Animal experiments

Surgery was conducted on six mature healthy dogs under general anaesthesia and sterile conditions. After anaesthetizing the animal by abdominal injection of pentobarbital sodium solution (30 mg/kg body weight), the hair of the back was shaved and the skin was sterilized. A longitudinal incision (10 cm in length) was made by scalpel at both the center and the middle of the back, and the muscle bundles of *longissimus dorsi* in both sides were disclosed by blunt separation. Two small longitudinal incisions were made in the muscle bundle of *longissimus dorsi* in each side. One ceramic rod was inserted into each incision and sealed by silk thread. Two α-TCP ceramic rods were implanted in one side and two β-TCP ceramic rods in the other side of each dog. After finishing the implantation, the skin was sutured and the animals were intramuscularly injected with penicillin for three d (one injection per day). Two dogs were sacrificed by overdose of pentobarbital sodium solution at each time period of 30, 45 and 150 d. Per material and per time period, a total of four implanted samples were collected with surrounding tissues and fixed in 10% buffered Formalin solution.

2.3. Histological observation

One half of the samples were decalcified in acid compounds (8.5 g sodium chloride, 100 ml Formalin, 70 ml 37% hydrochloric acid, 80 ml formic acid, 40 g aluminum chloride, 25 ml acetic acid glacial in 1000 ml) for 72 h, then dehydrated in series alcohol solutions and embedded in paraffin. Semi-thin sections were made and stained with hematoxylin and eosin (HE). The other samples were also dehydrated by series ethanol solutions, cleared by xylenes and embedded in methyl-methacrylate (MMA). Sections (20 μm) were made and stained with methylene blue and basic fuchsin. Some un-decalcified sections were coated with carbon and analyzed by back scattered electron scanning microscopy (BSE) and energy dispersive spectroscopy (EDX).

3. Results

Different tissue response was found in α-TCP and β-TCP implants. Only little loose fiber tissues could be found inside the pores of α-TCP ceramics, even the giant cells and macrophages were hardly observed at 30, 45 and 150 days (Fig. 3A,C,E) in the inner pores, but evident on the outer surface of the implants. No bone formation

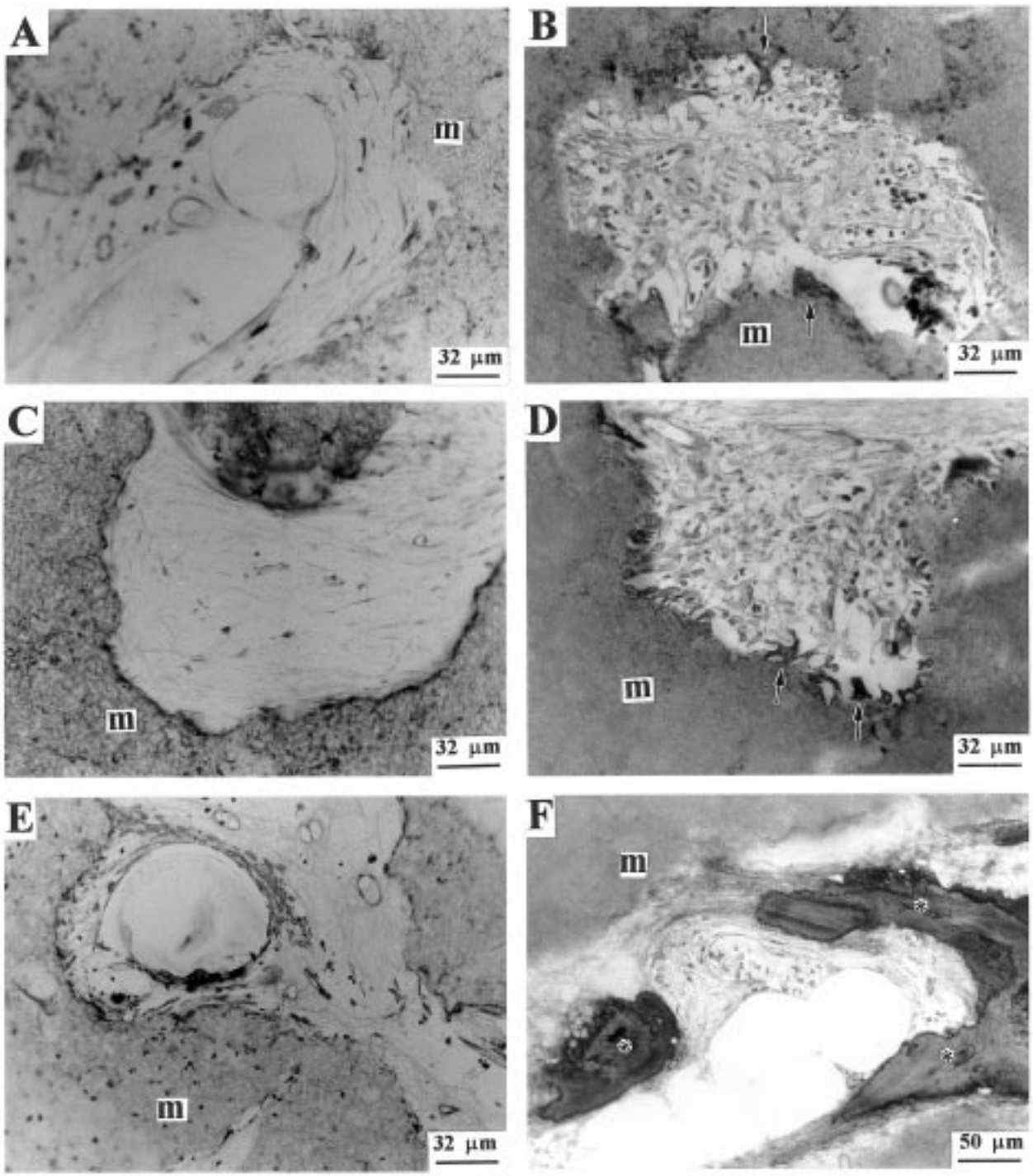

Figure 3 Tissue responses of α-TCP and β-TCP in muscles of dogs at different times. A, C, E: α-TCP; B, D, F: β-TCP; A, B: 30 d; C, D: 45 d; E, F: 150 d. *bone; arrow, giant cell; m, ceramic. Un-decalcified sections, methylene blue and basic fuchsin staining.

could be detected in any α-TCP implants at any experiment time in this study, while a clear dissolution marker could be observed in un-decalcified sections of α-TCP (Fig. 4). In β-TCP implants, a higher population of cells could be observed inside the pores at 30 d. Most cells are polymorphic cells, either aggregated to each other or attached on the pore surfaces. Giant cells were also found to adhere on the pore surface (Fig. 3B); at 45 d, the high populations of cells were still quite frequently found (Fig. 3D), while in some pores of some implants, bone formation occurred (Fig. 5). At 150 d, cells inside the pores were not active, and most of the pores were filled by bone-like tissues (Fig. 3F).

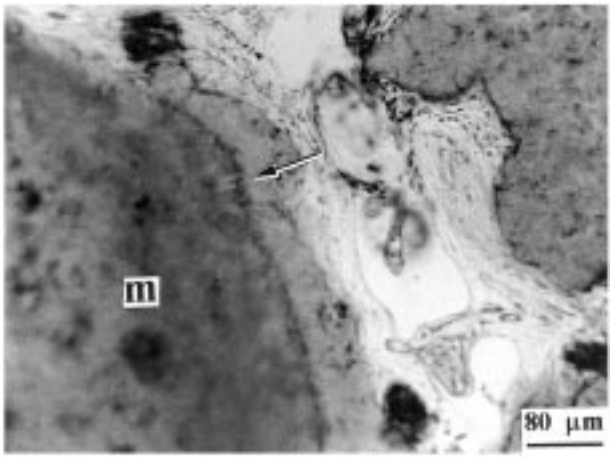

Figure 4 Obvious dissolution marker in α-TCP at 30 d post-operation. Un-decalcified section, methylene blue and basic fuchsin staining. m, ceramic; arrow, dissolution marker.

Bone formation could be found in some β-TCP implants (2/4 at 45 d; 4/4 at 150 d). The bone formation did not always start from the pore surface and towards the pore center (Fig. 5A,C), although direct contact between bone and ceramic surface was obvious at 45 d (Fig. 5A). The osteogenesis process in β-TCP (Fig. 5C) was similar to bone regeneration inside a narrow cortical bone defect in dog femur at 15 d after operation (Fig. 5D), in which osteogenic cells aggregated, produced bone matrix and ossified. At 45 d, the bone tissue inside the pores was normal bone tissue (Fig. 5B) but less mineralized (Fig. 5C). Osteoblast lineage and osteocytes were obvious (Fig. 5B). At 150 d, the bone tissues inside the pores of β-TCP implants were no longer normal; no bone marrow tissue, no bone remodeling process, no osteoblast lineage, even no obvious osteocytes could be observed, only a small amount of

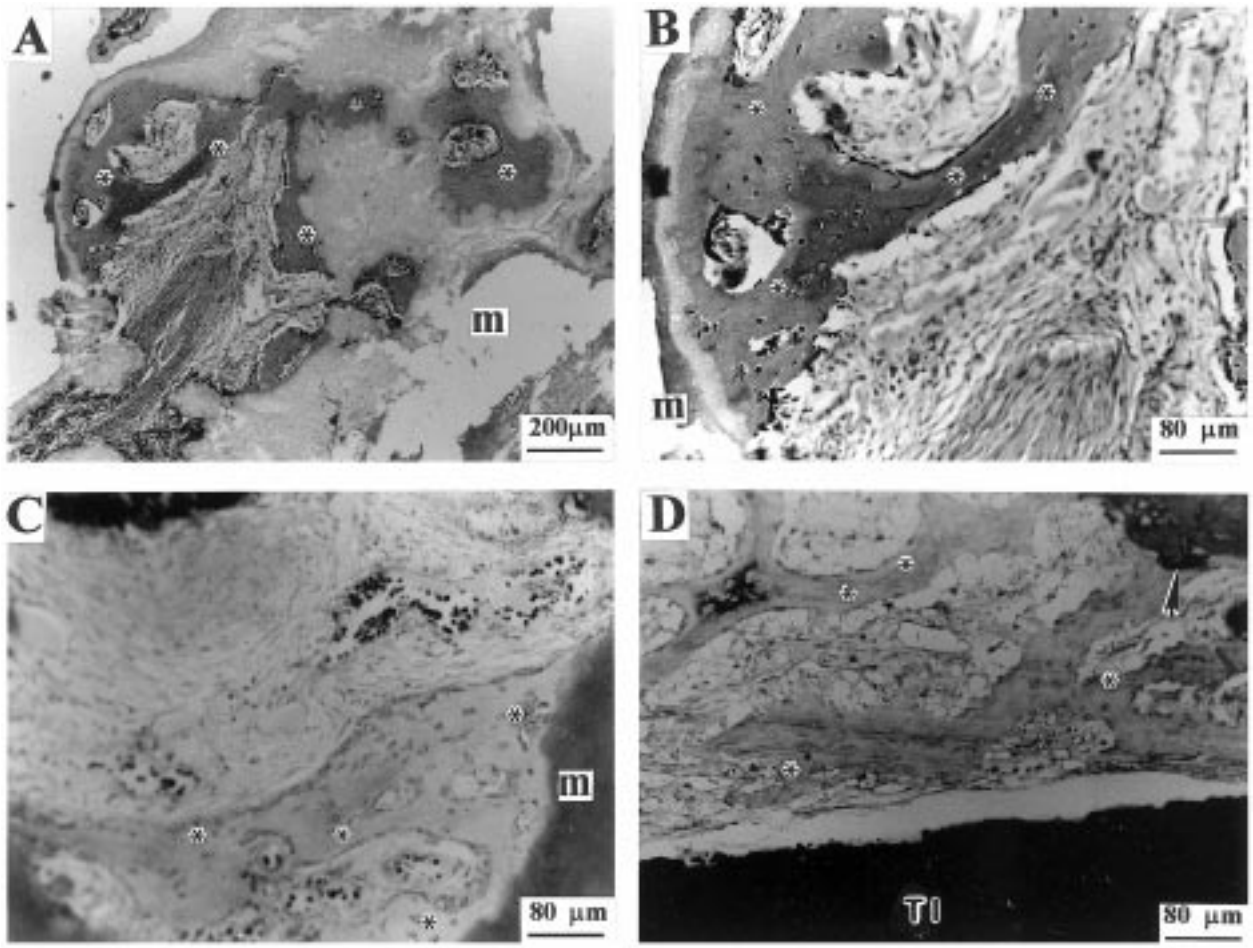

Figure 5 Bone formation in β-TCP at 45 d. A, overview of bone formation in β-TCP at low magnification, decalcified section, HE staining; m: ceramic ghost; * bone. B, details of bone in A at higher magnification, decalcified section, HE staining; m: ceramic ghost; *, bone. C, bone tissue shown in un-decalcified section, methylene blue and basic fuchsin staining; m, ceramic; * bone. D, Bone regeneration in a narrow defect of dog's femur bone cortex. Arrowhead, old bone; *, newly formed bone; Ti, Ti64A1V implants. This result was taken from an other experiment in which the Ti6Al4V (Ti) implant (5 × 5 × 6 mm in size) was implanted in artificial defects of dog's femur bone, bone regenerated in the narrow defects between host bone and implant at 15 d post-operation. Un-decalcified section, methylene blue and basic fuchsin staining. Note the same pattern of bone formation between C and D.

calcified tissue with less osteocytes could be found in the center of pores (Fig. 6A,B). The tissues between the pore surface and calcified tissue seemed to be demineralized bone (Fig. 6A). Direct bonding between bone and β-TCP ceramic was hardly observed at 150 d (Fig. 6B).

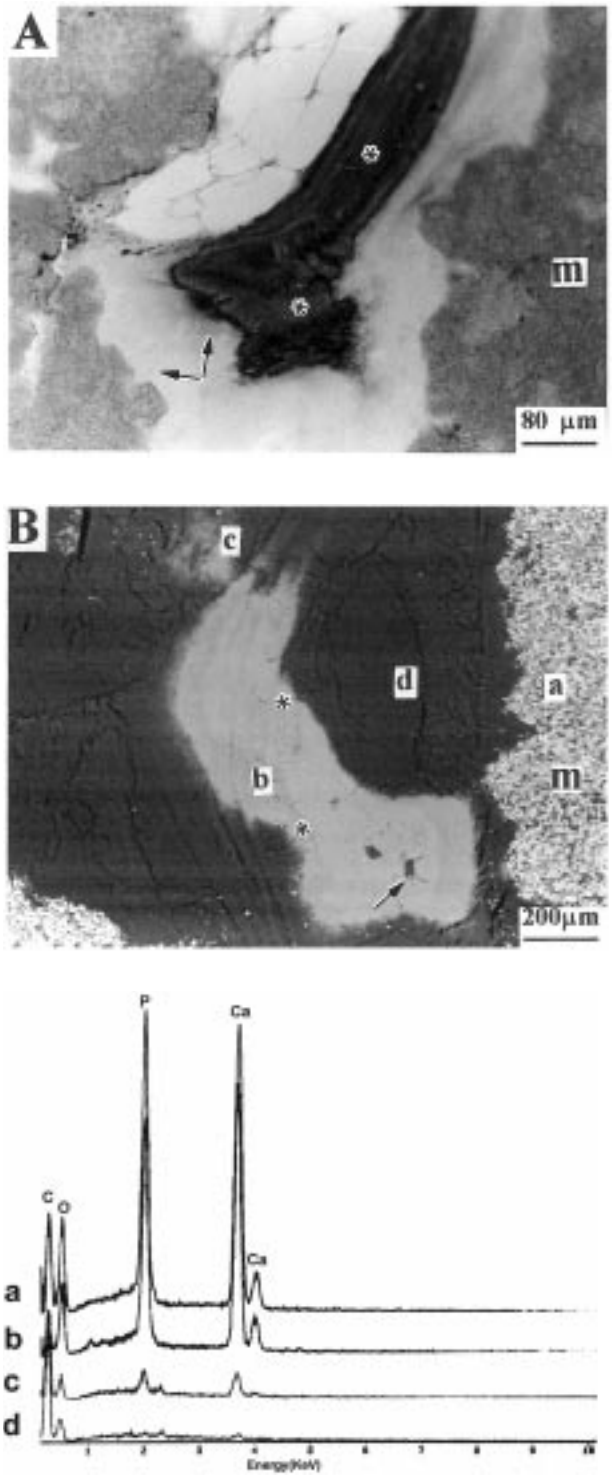

Figure 6 one degeneration in β-TCP at 150 d post-operation. A, undecalcified section, methylene blue and basic fuchsin staining. Note: bone tissue in the center of the pore and the demineralized area between pore surface and the bone tissue (arrow). B, BSE observation, bone tissue with some osteocyte lacunas (arrow) in the center of the pore. C, EDX analysis of four sites in B, a, ceramic; b, bone; c, demineralizing zone; d, soft tissue. m: ceramic; * bone; arrow, osteocyte lacuna.

4. Discussion

The results of this study added another calcium phosphate ceramic, namely β-TCP ceramic, to the literature of calcium phosphate-induced osteogenesis.

The mechanism of osteoinduction of calcium phosphate biomaterials is not clear from the available literature. From the viewpoint of materials, the chemical composition, the geometry, the macropores and the micropores were considered as important factors for this kind of osteoinduction [33, 36, 40, 45]. In the present study, the materials were made at the same time by almost the same procedure; the only difference was the different cooling procedure after sintering. The structure, geometry and chemical composition (Ca/P = 1.50) of the implants were the same. The different tissue responses showed that process in calcium phosphate-induced osteogenesis is very complicated, even a difference in phase composition can result in a critical difference. With respect to bone formation: bone was found in β-TCP, but not in α-TCP.

The only difference between α-TCP and β-TCP was their different resorption rate. α-TCP has been demonstrated to be more resorbable than β-TCP [46–48], obvious dissolution marker could be found in histological sections of α-TCP samples (Fig. 4), but not in sections of β-TCP samples. The difference in tissue responses between them could be the result of their different resorption rates.

It has been suggested that calcium phosphate biomaterials were resorbed *in vivo* in two ways: chemical dissolution and cell-mediated degradation [1, 3, 21, 26]. The different tissue responses in this study between α-TCP and β-TCP could only be attributed to chemical dissolution, because giant cells and macrophages involved in cell–mediated resorption were hardly observed in the pores of α-TCP implants. Two factors in the dissolution of calcium phosphate biomaterials may affect the tissue responses. One was the local concentration of Ca^{2+} and PO_4^{3-} ions. A large amount of Ca^{2+} and PO_4^{3-} ions were produced in the rapid dissolution of α-TCP and their local concentration, especially inside the pores, could be too high for cells to survive [22, 49], even too high for giant cells and macrophages to survive. So, when giant cells and macrophages were considered as the indicator of inflammation reaction, α-TCP seemed to be much more biocompatible than β-TCP [47]. The second factor was the change of microenvironment during the dissolution. When TCP dissolved, the microenvironment was acidic, the cells may not tolerate such an acidic environment resulting from the rapid dissolution [17]. Therefore, only a little loose fiber tissue could be observed inside the pores of fast dissolving, and hence more acidic α-TCP.

Compared to α-TCP, higher population cells and normal bone formation at early time in β-TCP indicated that its mild dissolution was not detrimental to cells at early times and may stimulate bone formation. Evidence could be found in other studies. Normal bone could be formed at early time in TCP combined with bone marrow cells or BMP (bone morphogenetic protein) in soft tissues [8, 36, 50, 51]. Many studies also showed that mild dissolution made calcium phosphate biomaterials more active. Tricalcium phosphate/hydroxyapatite

ceramic (TCP/HA) with mild dissolution was more active in bone formation than pure hydroxyapatite [18, 28]; when sintered at lower temperature, HA ceramic was more active than that sintered at higher temperature due to the dissolution of the ceramic sintered at lower temperature [23, 47]; and at early time, more bone formation in TCP implants than in TCP/HA and HA were observed [28]. The reason may be that bone-like apatite surface formation was related to the dissolution rate and thereafter stimulated osteogenic precursor cells' proliferation, aggregation, differentiation, bone matrix formation and mineralization [20, 27]; and the dissolution may provide Ca^{2+}, PO_4^{3-} needed by bone formation.

However, at longer time, the effects of dissolution of β-TCP reversed, bone tissue seemed to degenerate at 150 d. These adverse effects of dissolution on bone were also shown in other reports. The bone-bonding rate decreased with increasing β-TCP in HA/β-TCP composites [22, 49]. TCP resorption in bone defects routinely was not accompanied by bone formation [1, 2], moreover, bone tissues decreased with time when porous β-TCP was implanted in osseous sites [12, 28, 52].

At longer time *in vivo*, β-TCP became more resorbable [26]; the circulation inside the implants decreased with the bone formation. So, Ca^{2+}, PO_4^{3-} ions accumulated in local sites and a high Ca^{2+}, PO_4^{3-} concentration was reached. The high Ca^{2+}, PO_4^{3-} concentration was detrimental to cells including osteoblast, osteocyte, macrophages and also not suitable for osteoclast resorption function [17, 21]. As a result, no bone remodeling and no bone marrow formation occurred in β-TCP implants. Furthermore, the acidic microenvironment caused by dissolution [17] made the formed bone demineralize. In this study, bone tissue did not bond to ceramic surfaces directly at 150 d. No bone remodeling resulted in no new bone formation; bone demineralization resulted in bone loss. Thus, the formed bone degenerated and decreased.

The bone degeneration in β-TCP did not mean that bone formation induced by calcium phosphates in general disappeared at last in an environment without stress. It was the case of one calcium phosphate ceramic in particular, namely β-TCP and it may be caused by its dissolution, because in the cases of HA and BCP, the bone tissues induced by calcium phosphates were normal mature bone at long time with bone remodeling process and sometimes bone marrow tissues. Damage of bone by rapid dissolution of calcium phosphate biomaterials was frequently found in materials with high resorption rate. When implanted as powder, the early-formed bone within HA powder disappeared at last; in the case of TCP ceramic, bone formed in it disappeared also when TCP was totally resorbed [32].

Two aspects of calcium phosphate biomaterials were concentrated on in this study, osteoinduction and the effect of rapid dissolution on bone formation. They are important for porous calcium phosphate biomaterials. For dense calcium phosphate biomaterials, they are less important, because osteoinduction did not occur in the cases of dense calcium phosphate biomaterials [40] and the dense materials were much less soluble than porous ones [26]. Even if a rapid dissolution occurred in the cases of dense materials, the products of rapid dissolution could be buffered by body fluids and taken away immediately by circulation, so no high local Ca^{2+}, PO_4^{3-} concentration as in porous materials could be reached. While in porous materials, Ca^{2+} and PO_4^{3-} could be accumulated at local site and caused adverse effects inside the implants.

The biocompatibility of calcium phosphate biomaterials has been well addressed. The good biocompatibility of calcium phosphate biomaterials originated from the fact that Ca^{2+} and PO_4^{3-} are inorganic components of hard tissues. Previous studies indicated that the bioactivity of calcium phosphate biomaterials depends on their solubility [27] and that it is possible to develop resorbable calcium phosphate biomaterials [3, 53]. However, present results indicated that too rapid dissolution of calcium phosphate biomaterials should not be encouraged.

5. Conclusions

Calcium phosphates have been considered for medical use for almost a century, and at this moment calcium phosphate biomaterials are commercially available for hard tissue repair [1–3, 12, 20, 26, 32]. The increasing evidence of calcium phosphate-induced osteogenesis may promote the development and application of calcium phosphate biomaterials with intrinsic osteoinductive property.

Porous β-TCP ceramic can induce bone formation in soft tissues of dogs, but porous α-TCP ceramic can't. No bone formation in α-TCP may be resulted from its higher resorbability, accurately due to its rapid dissolution, the rapid dissolution of α-TCP also affected its cell-mediated resorption, no macrophages and giant cells survived in such a high local Ca^{2+}, PO_4^{3-} environment. In the case of β-TCP, the effect of degradation had two phases; at early time, the degradation was mild and stimulated bone formation, while at longer time, the rapid dissolution was detrimental to cells and bone tissues. Due to the complexity of calcium phosphate biomaterials, further optimization of calcium phosphate biomaterials is still necessary. The osteoinduction model may be useful in the optimization process.

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References

1. C. J. DAMIEN and J. R. PARSONS, *J. Appl. Biomater.* **2** (1991) 187.
2. J. O. HOLLINGER, J. BREKKE, E. GRUSKIN and D. LEE, *Clin. Orthop.* **324** (1996) 55.
3. J. O. HOLLINGER and G. C. BATTISTONE, *Clin. Orthop.* **207** (1986) 290.
4. M. J. YASZEMSKI, R. G. PAYNE, W. C. HAYES, R. LANGER and A. G. MIKOS, *Biomaterials* **17** (1996) 175.
5. H. OHGUSHI, M. OKUMURA, T. YOSHIKAWA, T. SENPUKU,

K. INOUE, S. TAMAI and E. C. SHORS, *Bioceramics* **4** (1991) 213.
6. Y. YAMAZAKI, S. OIDA, Y. AKIMOTO and S. SHIODA, *Clin. Orthop.* **234** (1988) 240.
7. H. OHGUSHI, M. OKUMURA, T. YOSHIKAWA, K. INOUE, N. SENPUTU and S. TAMAI, *J. Biomed. Mater. Res.* **26** (1992) 885.
8. M. R. URIST, *Clin. Orthop.* **187** (1984) 277.
9. G. HOTZ and G. HERR, *Oral & Maxillofacial Surgery* **239** (1994) 413.
10. K. D. JOHNSON, K. E. FRIERSON, T. S. KELLER, C. COOK, R. SCHEINBERG, J. ZERWEKH, L. MEYERS and M. F. SCIADINI, *J. Orthop. Res.* **14** (1996) 351.
11. G. DACULSI and N. PASSUTI, *Bioceramics* **2** (1989) 3.
12. M. JARCHO, *Clin. Orthop.* **157** (1981) 260.
13. P. S. EGGLI, W. MULLER and R. K. SCHENK, *Clin. Orthop.* **232** (1988) 127.
14. A. A. DRIESSEN, C. P. A. T. KLEIN and K. DE GROOT, *Biomaterials* **3** (1982) 113.
15. C. P. A. T. KLEIN, P. PATKA and W. DEN HOLLANDER, *Biomaterials* **10** (1989) 59.
16. D. S. METSEGER and T. D. DRISKELL, *J. Am. Dent. Assoc.* **105** (1982) 1035.
17. T. SUZUKI, T. YAMAMOTO, M. TORIYAMA, K. NISHIZAWA, Y. YOKOGAWA, M. R. MUCALO, Y. KAWAMOTO, F. NATA and T. KAMEYAMA, *J. Biomed. Mater. Res.* **34** (1997) 507.
18. J. M. TOTH, W. M. HIRTHE, W. G. HUBBARD, W. A. BRANTLY and K. L. LYNCH, *J. Appl. Biomater.* **2** (1991) 37.
19. E. NERY, R. Z. LEGEROS, K. L. LYNCH and J. KALBFLEISCH, *J. Dent. Res.* **67** (1988) 178.
20. N. PASSUTI, G. DACULSI, J. M. ROGEZ, S. MARTIN and J. V. BAINEL, *Clin. Orthop.* **248** (1989) 169.
21. S. YAMADA, D. HEYMANN, J. M. BOULER and G. DACULSI, *Biomaterials* **18** (1997) 1037.
22. A. PIATTELLI, C. MANGANO, A. KRAJEWSKI, A. RAVAGLIOLI, R. MARTINETTI and M. FABBRI, *Bioceramics* **7** (1994) 177.
23. J. D. DE BRUIJN, Y. P. BOVELL and C. A. VAN BLITTERSWIJK, *Bioceramics* **7** (1994) 293.
24. K. S. TENHUISEN and P. W. BROWN, *J. Biomed. Mater. Res.* **36** (1997) 233.
25. S. KIMAKHE, D. HEYMANN, J. GUICHEUX, P. PILET, B. GIUMELLI and G. DACULSI, *J. Biomed. Mater. Res.* **40** (1998) 336.
26. K. DE GROOT, in ''Contemporary Biomaterials'', edited by J. W. Boretos and M. Eden (Noyes Publication, USA, 1984) pp. 477–492.
27. P. DUCHEYNE and J. M. CUCKLER, *Clin. Orthop.* **276** (1992) 102.
28. H. YOKOZEKI, H. KUROSAWA, K. SHIBUYA, K. IOKU, T. HAYASHI and H. KAWAHARA, *Bioceramics* **7** (1994) 171.
29. R. G. T. GEESINK, K. DE GROOT and C. P. A. T. KLEIN, *J. Bone & Joint Surg.* **70B** (1988) 17.
30. T. HANAWA, Y. KAMIURA, S. YAMAMOTO, T. KOHGO, A. AMEMIYA, H. UKAI, K. MURAKAMI and K. ASAOKA, *J. Biomed. Mater. Res.* **36** (1997) 131.
31. T. NAKAMURA, *Bioceramics* **9** (1996) 31.
32. J. F. OSBORN, in ''Biomaterials Degradation'', edited by M. A. Barbosa (Elsevier Science Publishers B.V., 1991) pp. 185–225.
33. U. RIPAMONTI, *Biomaterials* **17** (1996) 31.
34. Z. YANG, H. YUAN, W. TONG, P. ZOU, W. CHEN and X. ZHANG, *Biomaterials* **17** (1996) 2131.
35. U. RIPAMONTI, *J. Bone & Joint Surg.* **73A** (1991) 692.
36. H. YAMASAKI and H. SAKAI, *Biomaterials* **13** (1992) 308.
37. J. M. TOTH, K. L. LYNCH and D. A. HACKBARTH, *Bioceramics* **6** (1993) 9.
38. K. VARGERVIK, in ''Bone Grafts & Bone Subsitutes'', edited by M. B. Habal and A. H. Reddi (W. B. Saunders, USA, 1992) pp. 112–120.
39. Z. YANG, H. YUAN, P. ZOU, W. TONG, S. QU and X. ZHANG, *J. Mater. Sci.: Mater. Med.* **8** (1997) 697.
40. C. P. A. T. KLEIN, K. DE GROOT, W. CHEN, Y. LI and X. ZHANG, *Biomaterials* **15** (1994) 15.
41. H. YUAN, Y. LI, Z. YANG, J. FENG and X. ZHANG, *Biomed. Eng. Appl. Basis. Com.* **9** (1997) 274.
42. D. S. METSEGER and T. D. DRISKELL, *J. Am. Dent. Assoc.* **105** (1982) 1035.
43. J. D. DE BRUIJN, Personal Communication (1998).
44. H. YUAN, Z. YANG, P. ZOU, Y. LI and X. ZHANG, *Biomed. Eng. Appl. Bas. Com.* **9** (1997) 268.
45. H. YUAN, K. KURASHINA, J. D. DE BRUIJN, Y. LI, K. DE GROOT and X. ZHANG, *Biomaterials* **20** (1999) 1799.
46. R. Z. LEGEROS and J. P. LEGEROS, *Bioceramics* **9** (1996) 7.
47. H. OONISHI, S. KUSHITANI, H. IWAKI, K. SAKA, H. ONO, A. TAMURA, T. SUGIHARA, L. L. HENCH, J. WILSON and E. TSUJI, *Bioceramics* **8** (1995) 137.
48. J. R. SHARPE, R. L. SAMMONS and P. M. MARQUIS, *Biomaterials* **18** (1997) 471.
49. K. DONATH, *Bioceramics* **1** (1988) 96.
50. M. NEO, C. F. VOIGT, H. HERBST and U. M. GROSS, *J. Biomed. Mater. Res.* **39** (1998) 1.
51. M. NEO, H. HERBST, C. F. VOIGT and U. M. GROSS, *J. Biomed. Mater. Res.* **39** (1998) 71.
52. K. IOKU, S. GOTO, H. KUROSAWA, H. YOKOZEKI, T. HAYASHI and T. NAKAGAWA, *Bioceramics* **9** (1996) 201.
53. H. OHGUSHI, M. OKUMURA, S. TAMAI and E. C. SHORS, *Bioceramics* **2** (1989) 65.

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