Osteoconductivity of hydrothermally synthesized beta-tricalcium phosphate composed of rod-shaped particles under mechanical unloading

Yoshinori Gonda[1, 2, a], Koji Ioku[3, b], Takatoshi Okuda[2, c], Yasuaki Shibata[1, d], Masanobu Kamitakahara[3, e], Giichiro Kawachi[4, f], Ikuho Yonezawa[2, g], Hisashi Kurosawa[2, h] and Tohru Ikeda[1, i]

[1]Division of Oral Pathology and Bone Metabolism, Department of Developmental and Reconstructive Medicine, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8588, Japan

[2]Department of Orthopedic Surgery, School of Medicine, Juntendo University, 2-1-1 Hongo, Tokyo 113-8421, Japan

[3]Graduate School of Environmental Studies, Tohoku University, 6-6-20Aramaki, Aoba-ku, Sendai 980-8579, Japan

[4]Department of Crystalline Materials Science, Graduate School of Engineering, Nagoya University, Furo-cyo, Chikusa-ku, Nagoya 464-8603, Japan

[a]gondayoshinori@mac.com, [b]ioku@mail.kankyo.tohoku.ac.jp, [c]o-kun@med.juntendo.ac.jp, [d]siva@nagasaki-u.ac.jp, [e]kamitaka@mail.kankyo.tohoku.ac.jp, [f]kawachi@apchem.nagoya-u.ac.jp, [g]yoza@med.juntendo.ac.jp, [h]kuro@med.juntendo.ac.jp, [i]tohrupth@net.nagasaki-u.ac.jp

Keywords: Beta tricalcium phosphate, Bone graft, Unloading

Abstract. Spherical beta-tricalcium phosphate (β-TCP) granules synthesized using a unique dropping slurry method expressed good osteoconductivity with prominent bone apposition and bioresorbability when implanted into the rat femur (Gonda et al., *Key Eng. Mater.* 361-363:1013-1016, 2008). The spherical β-TCP granules were implanted into the bone defect created in the distal end of the right femur of each 8-week-old female Wistar rat. To analyze performance of the spherical β-TCP granules as bone substitute in the bone with reduction in osteogenic potential, the right sciatic neurectomy was performed after implantation and the right hind limb was kept unloaded for 2 weeks before euthanization. Four weeks after implantation, some spherical β-TCP granules with resorption in part were surrounded by newly formed bone. Eight and 12 weeks after implantation, most of the residual β-TCP granules were embedded in newly formed bone, and total volume of the implant and newly formed bone was more than the other portions of the bone or the bone of control animals. Osteoclast activity in the implanted area was also higher than the other portions of the bone or the bone of control animals. Replacement of the intraosseous residual β-TCP granules for bone progressed at 12 weeks after implantation compared to those at 8 weeks after implantation. These data suggested that the spherical β-TCP granules stimulated osteogenesis and osteoclast activity of the unloaded bone.

Introduction

Beta-tricalcium phosphate (β-TCP) has been used clinically as a bioresorbable bone substitute. In addition to the porosity and the structure of micro and macro pores, microstructure of the particles composing the material has also been shown to be associated with the bioresorbability [1]. We have shown that the cylindrical block of β-TCP composed of unique rod-shaped particles behaved differently from the same-shaped block of conventional β-TCP composed of non rod-shaped particles when implanted into rabbit femurs, and proposed the hypothesis that bioresorbability of the bone substitute affected the metabolism of the subsequently formed bone tissue [2]. Previously, we also have developed spherical granules of β-TCP using a new unique method [3]. After implantation into rat femurs, both spherical β-TCP granules composed of rod-shaped particles produced by hydrothermal method and β-TCP granules composed of non rod-shaped particles revealed good osteoconductivity. Total volume of the newly formed bone and implant was similar between animals implanted β-TCP composed of rod-shaped particles and animals implanted β-TCP composed of non rod-shaped particles, but the volume of newly formed bone/total volume of newly formed bone and implant was higher in the animals implanted β-TCP granules composed of rod-shaped particles than the animals implanted β-TCP granules composed of non rod-shaped particles [4]. In this study, we analyzed osteoconductivity and bioresorbability of spherical β-TCP granules composed of rod-shaped particles in the bone with reduction in osteogenic potential induced by mechanical unloading [5].

Materials and Methods

Production of Spherical β-TCP Granules: Alpha-TCP powder was mixed and kneaded with gelatine, and dropped into stirring oil bath heated at 80^{o}C. Then, the bath was chilled on ice and formed spherical α-TCP granules. The granules were separated from the oil, rinsed, and served for hydrothermal treatment to produce spherical β-TCP granules composed of rod-shaped particles [6]. The size was ranged from 0.5 to 0.6 mm in diameter. The synthesized spherical β-TCP granules were analyzed by X-ray diffractometry, and no phase other than β-TCP was detected. Rod-shaped particles of spherical β-TCP granules were confirmed using a scanning electron microscope.

Animal Experiments: Eight-week-old female Wistar rats were anesthetized, a bone defect 2 mm in diameter and 3 mm in depth was created in distal end of the right femur of each animal, 30 mg of β-TCP granules were implanted and the wound portion was sutured. Operated animals without implantation of β-TCP granules were used as a control. The right sciatic neurectomy was performed 2 weeks before euthanization. Four, 8 and 12 weeks after implantation, animals were euthanized and operated bones were resected. Undecalcified bone tissue sections were made from the resected bones and analyzed histologically. Rearing of these animals and animal experiments were performed following the Guidelines of Animal Experimentation of Nagasaki University.

Results and Discussion

Right tibiae 2 weeks after sciatic neurectomy revealed growth retardation and decreased bone mass compared to the left tibiae without neurectomy, and unloading of the right limb was confirmed. In the operated right femur, regeneration of the non-critical sized bone defect in the unloaded bone was apparently delayed compared to the loaded bone (data not shown). Four weeks after implantation, newly formed bone appeared around the partially resorbed implant. Bone regeneration occurred from marginal region of the bone defect and implanted β-TCP granules in the marginal region of operated area were already surrounded by newly formed bone. In central region of the operated area, connective tissue still remained (Fig. 1A). Marginal region of the bone defect of control animals was partly regenerated, but most part of the defect remained and was filled with connective tissue (Fig. 1B). At 8 weeks after implantation, prominent bone formation was seen in and around the implant in contrast to the control (Fig. 1C, D). Greater numbers of tartrate-resistant acid phosphatase-positive cells were present around the implant than those in the other portions of the bone and in the control bone (data not shown). At 12 weeks after implantation, histological findings were similar to those at 8 weeks after implantation (Fig. 1E). Total volume of the implant and newly formed bone was much more than the other portions of the bone in the animals 8 weeks and 12 weeks after implantation. In the control bone, the created bone defect was mostly regenerated at 8 weeks after operation, the bone defect were mostly regenerated. At 12 weeks after operation the cancellous bone which was formed in the created bone defect was indistinguishable from surrounding slender cancellous bone under mechanical unloading (Fig. 1D, F). Under polarizing microscope, refringence of lamellar structure of bone enables to distinguish implanted β-TCP granules from bone tissue. As shown in Fig. 2A, considerable amount of β-TCP embedded in the bone was seen in the specimens 8 weeks after implantation. At 12 weeks after implantation, part of the embedded β-TCP granules was replaced by bone and newly formed bone was seen within the implanted granules (Fig. 2B). Total volume of newly formed bone in the animals implanted with β-TCP granules was significantly higher than that in control animals created the bone defect. These data suggested that the spherical β-TCP granules stimulated osteogenesis and osteoclast activity in the unloaded bone. In addition, it was also suggested that replacement of the intraosseous residual β-TCP granules for bone occurred in the suppressive osteogenetic conditionunder the mechanical unloading.

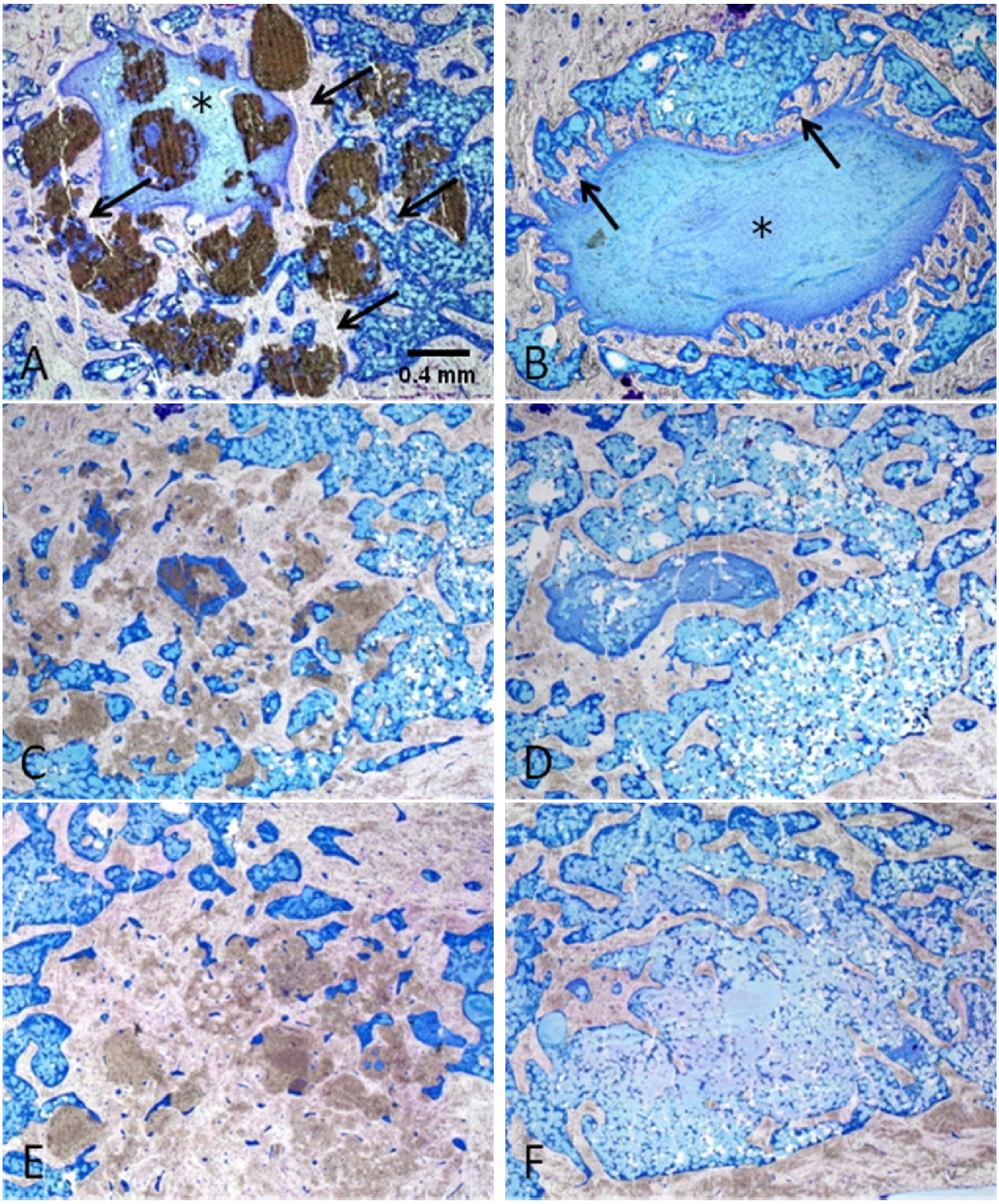

Fig. 1 Representative photomicrograph of specimens implanted with spherical β-TCP granules (A, C, E) and control specimens without implantation into the bone defects (B, D, F). Specimens 4 weeks (A, B), 8 weeks (C, D) and 12 weeks after operation (E, F) were stained with Giemsa's method. For all specimens, sciatic neurectomy was performed 2 weeks before sampling for histological analyses. Arrows indicate newly formed bone in marginal region. Asterisks (*) represent connective tissue in the bone defect.

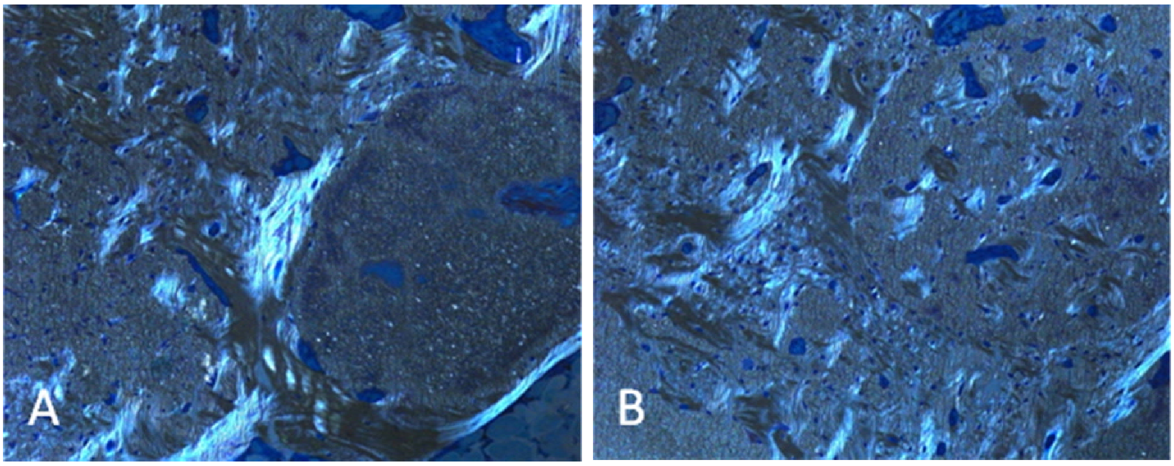

Fig. 2 Representative photomicrographs observed by a polarizing microscope. Specimens 8 weeks (A) and 12 weeks (B) after implantation of spherical β-TCP granules.

References

[1] H. Yokozeki, T. Hayashi, N. Nakagawa, H. Kurosawa, K. Shibuya and K. Ioku. *J. Mater. Sci. Mater. Med.* **9**:381-384. (1998)

[2] T. Okuda, K. Ioku, I. Yonezawa, H. Minagi, G. Kawachi, Y. Gonda, H. Murayama, Y. Shibata, S. Minami, S. Kamihira, H. Kurosawa and T. Ikeda. *Biomaterilas* **28**:2612-2621. (2007)

[3] K. Ioku, G. Kawachi, S. Sasaki, H. Fujimori, S. Goto. *J. Mater. Sci.* **41**:1341-1344. (2006)

[4] Y. Gonda, K. Ioku, T. Okuda, G. Kawachi, I. Yonezawa, H. Kurosawa, T. Ikeda. *Key Eng. Mater.* **361-363**:1013-1016. (2008)

[5] M. Weinreb, G. A. Rodan, D. D. Thompson. *Bone* **10**:187-194. (1989)

[6] K. Ioku, M. Kamitakahara, G. Kawachi, Y. Gonda, T. Okuda, I. Yonezawa, H. Kurosawa, T. Ikeda, *Key Eng. Mater.* **361-363**:989-992. (2008)