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Mechanical, setting, and biological properties of bone cements containing micron-sized titania particles

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Abstract In this study, polymethylmethacrylate-based composite cements containing 40–55.6 wt% micron-sized titania (titanium oxide) particles were developed, and their mechanical, setting, and biological properties evaluated. Three types of composite cement containing 40, 50, and 55.6 wt% silanized titania were designated ST2-40c, ST2-50c, and ST2-56c, respectively. In animal experiments, ST2-50c and ST2-56c were implanted into rat tibiae and solidified in situ. An affinity index was used to evaluate osteoconductivity. Compressive and bending strength of ST2-56c was 147.7 ± 3.2 and 69.3 ± 7.4; those of the other cements exceeded 100 MPa and 50 MPa, respectively. The affinity indices of ST2-56c were 42.1 ± 12.9 at six weeks and 53.4 ± 16.6 at 12 weeks, and were significantly higher than for ST2-50c and a commercial PMMA bone cement within 12 weeks. Our data indicate that bone cement containing micron-sized titania particles can be applied to prosthesis fixation as well as vertebroplasty, and ST2-56c is a good candidate cement.

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Introduction

Since the 1990s, many types of bioactive bone cement have been developed to overcome the disadvantages of polymethylmethacrylate (PMMA) bone cement [1], especially its lack of bone-bonding ability, which occasionally leads to aseptic loosening of prostheses used for arthroplasty [2, 3]. However, the acceptable long-term clinical results of PMMA bone cement [4, 5], and concerns about the long-term stability of bioactive fillers in the cements have so far prevented bioactive bone cements being used for the fixation of prostheses in arthroplasty. Recently, anatase and rutile, crystal phases of titania, have been shown to have excellent in vitro apatite-forming ability and in vivo bioactivity [6–9]. Titania is stable in the body and does not degrade, so that bone cements containing bioactive titania filler can be stable in the body environment. Then a composite bone cement containing nanosized anatase-type titania particles was developed, and it was reported that certain compositions of the cement had good osteoconductivity [10]. However, some of the nanosized titania particles tended to aggregate in the cement. As a result, the cements containing nanosized titania particles did not reach the minimum bending strength required by the ISO 5833 standard (50 MPa), which is applied to acrylic resin cements used for prosthesis fixation, and could be applied clinically for vertebroplasty, but not for prosthesis fixation. One possible resolution of this problem was to increase the titania particle size. In this study, composite cements that contained micron-sized titania particles were developed. Preliminary PMMA cement candidates with different amounts of titania particles were examined for their mechanical properties and apatite forming ability in vitro [11], and two promising composites were used in an implantation study.

The purpose of the study was to evaluate the mechanical and setting properties, and osteoconductivity of cements containing micron-sized titania.

Materials and methods

Preparation of powders

Titania powder

Plate-like titania powder (Ishihara Sangyo Kaisha, Osaka, Japan) with an average particle size of 1.55 μm was used as supplied. The particle size distribution of the titania powder, which was determined using a laser diffraction analyzer (LA-910; Horiba, Kyoto, Japan), is shown in Fig. 1a. Powder X-ray diffraction of the particles revealed that the titania particles were composed of anatase and rutile phases (Fig. 1b). The weight ratio of anatase:rutile in the 1.55 μm titania powder was about one, based on the peak intensities of each diffraction pattern. The titania powder was mixed into three types of TiO_2-dispersed cements with 40, 50, and 55.6 wt% TiO_2, designated ST2-40c, ST2-50c, and ST2-56c, respectively.

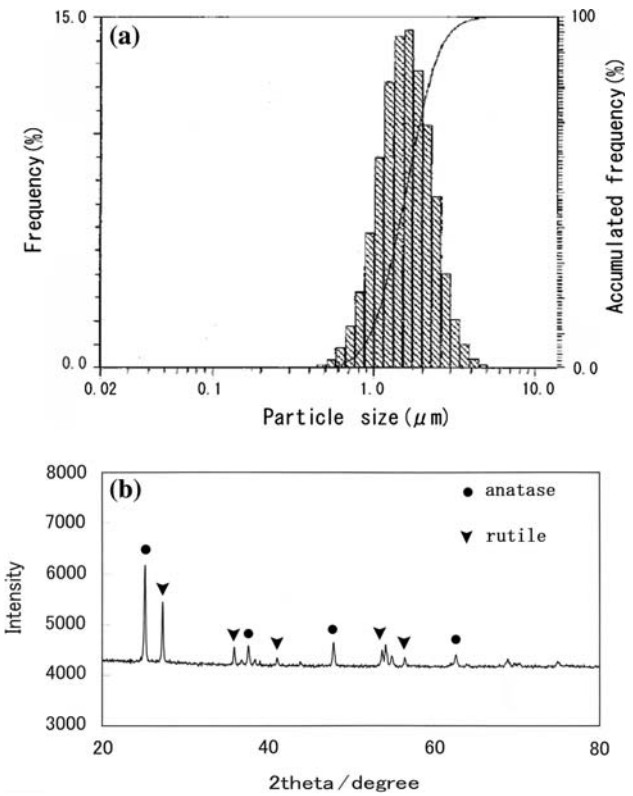

Fig. 1 (**a**) Titania powder particle size distribution. (**b**) Powder X-ray diffraction data for titania powder

Titania powders were treated with a silane-coupling agent as follows: 1.1 g of 3-methacryloxypropyltrimethoxysilane (Shin-Etsu Chemical Co., Tokyo), 1.6 g of ethanol and 0.2 g of deionized water were mixed on a magnetic stirrer for 10 min. The solution containing the silane-coupling agent was added to 110 g of the TiO_2 powder and mixed in a shaker mixer (TURBULA T2F, W. A. Bachofen AG Co., Basel, Switzerland) at 25 °C for 1 h. The rotation speed was 96 rpm. After mixing, the mixtures were dried and heated at 130 °C for 5 min.

Polymethylmethacrylate powder

Spherical PMMA powder, synthesized by suspension polymerization [12], with an average molecular weight of 270,000 Da and an average particle size of 5 μm (standard deviation: 2 μm) [13] was used.

Preparation of the liquid

Liquid methacrylate (MMA) monomer (Wako Pure Chemical Industries, Osaka, Japan) was used.

Cement preparation

Four types of cement, designated ST2-40c, ST2-50c, ST2-56c, and PMMAc, were prepared. PMMAc was a commercially available PMMA-based bone cement (Osteobond; Zimmer, Warsaw, IN, USA) and was used as a control material. The composition of each TiO_2-containing cement is shown in Table 1. As an initiator, benzoyl peroxide (Nacalai Tesque, Kyoto, Japan) was added to the powder at 4.0 wt% of the monomer, and as an accelerator, *N,N*-dimethyl-*p*-toluidine (Kanto Chemical Co. Inc., Tokyo, Japan) was dissolved in the liquid to 2.0 wt% of the monomer. Each cement was prepared by mixing the powder with the liquid for 1 min.

Mechanical testing

The compressive strength, bending strength, and bending modulus of ST2-40c, ST2-50c, and ST2-56c were mea-

Table 1 Composition of PMMA-based cements containing titania powders

Cement	Powders[a] (wt%)		Liquid[b] (wt%) MMA
	Titania	PMMA	
ST2-40c	40	20	40
ST2-50c	50	16.7	33.3
ST2-56c	55.6	14.6	29.6

[a] Benzoyl peroxide was added at 4 wt% of the MMA

[b] *N,N*-Dimethyl-*p*-toluidine was added at 2 wt% of the MMA

sured using five prehardened cement specimens for each mechanical test. For mechanical bending analysis, four-point bending testing was performed with rectangular specimens sized to 70 mm × 20 mm × 5 mm. For compressive mechanical analysis, prehardened cylindrical cement specimens, 6 mm in diameter and 12 mm in length, were prepared. The tests were carried out according to ISO 5833, with a Model 5582 testing machine (Instron Corporation, Canton, MA, USA); the test conditions were previously described in detail [10].

Some of the bending specimens were prepared for observation with a scanning electron microscope (SEM, S-4700; Hitachi, Tokyo, Japan), and the fracture surfaces were analyzed to determine the microstructure of the cements.

Setting of the cements

The cement pastes were mixed for 1 min and cast in a cylindrical mold made of polytetrafluoroethylene (inner diameter 60 mm, inner depth 20 mm). The temperature change during the setting reaction was measured using an infrared thermometer under ambient conditions of 23 °C and 54–65% humidity. By plotting the time and temperature, the setting time of each cement was determined according to ISO 5833.

Animal experiments

Eight-week-old male Wistar rats weighing 180–230 g were used for the implantation study. The animals were reared and the experiments carried out at the Institute of Laboratory Animals, Faculty of Medicine, Kyoto University, under the institutional guidelines for use of experimental animals set by Kyoto University.

The rats were operated on under general anesthesia induced by intraperitoneal injection of sodium 5-ethyl-5-(1-methylbutyl) barbiturate (Nembutal [pentobarbital]; Dainippon Pharmaceutical Company, Osaka, Japan) at 40 mg/kg of body weight. Cortical bone defects measuring 2 mm × 7 mm were created in the medial aspect of the proximal metaphyses of both tibiae, and the bone marrow was curetted. The intramedullary canals of both bone defects were irrigated with physiological saline, and paste-form cement was inserted manually and allowed to cure in situ for evaluation of osteoconductivity [10, 14, 15]. Twelve rats (24 legs) were used for the evaluation of osteoconductivity, with ST2-50c and ST2-56c each being used in 12 legs. Half the rats in each subgroup were killed at six and 12 weeks after the operation.

To confirm the high radiopacity of ST2-56c, another operation was performed using an additional rat. After a hole had been made in the intercondylar space of the distal femur, and the intramedullary canal of the total femur was curetted and irrigated with physiological saline, ST2-56c and PMMAc in liquid phase were inserted into each of the bilateral canals using a syringe fitted with an 18-gauge needle, and this was allowed to cure in situ. One day after the operation, the rat was killed and an X-ray radiograph of the femurs was taken.

Micrographic examination

Specimens were dehydrated through a graded series of ethanol (70, 80, 90, 99, and 100 vol%) and embedded in epoxy resin (Epofix, Struers Co., Copenhagen, Denmark). Thin sections (100 or 500 μm thick) were cut with a band saw (BS-3000; Exakt, Norderstedt, Germany) perpendicular to the axis of the tibias containing the cement. Four sections could be typically made from each leg. The third section (100 μm thick) from the most distal portion of each leg was ground to a thickness of 60–80 μm using a grinding–sliding machine (Microgrinding MG-4000; Exakt) for Giemsa surface staining. The second section (100 μm thick) from each leg was prepared for contact microradiography. The first and fourth sections (500 μm thick) from each leg were polished with diamond paper and coated with a thin layer of carbon for observation by SEM (S-4700, Hitachi, Tokyo, Japan). Some of those specimens were analyzed using an energy-dispersive X-ray microanalyzer (EMAX-7000; Horiba, Kyoto, Japan) attached to the SEM (SEM–EDX). To evaluate osteoconductivity, affinity indices (%) for each subgroup were calculated as previously described [10, 14, 15].

Statistical analysis

Values were expressed as means and standard deviations (SD). Values of mechanical properties for each cement and the affinity indices for each cement at each time interval were compared using one-way analysis of variance. Subsequently, possible differences were investigated using Fisher's PLSD post hoc statistical test using StatView (version 5.0) for Windows. A P value less than 0.01 was considered statistically significant.

Results

Mechanical properties

The results of the mechanical property measurement and their statistical analyses are shown in Table 2. The ultimate compressive strength, flexural strength, and flexural modulus increased as the titania content of the cement increased. SEM revealed that titania particles were

Table 2 Mechanical properties of ST2-40c, ST2-50c, ST2-56c, and PMMAc (means ± SD, $n = 5$)

	Compressive strength (MPa)	Bending strength (MPa)	Bending modulus (GPa)
ST2-40c	106.1 ± 5.5*	54.3 ± 6.6	3.10 ± 0.47
ST2-50c	127.9 ± 6.4*	57.8 ± 4.1	3.88 ± 0.46
ST2-56c	147.7 ± 3.2*	69.3 ± 7.4**	4.07 ± 0.83
PMMAc	87.9 ± 2.7*	59.4 ± 7.8	1.56 ± 0.28***

The values for PMMAc were derived from our previous study[10]

* All pairs were significantly different

** Significantly different to ST2-40c

*** Significantly different to all the other cements

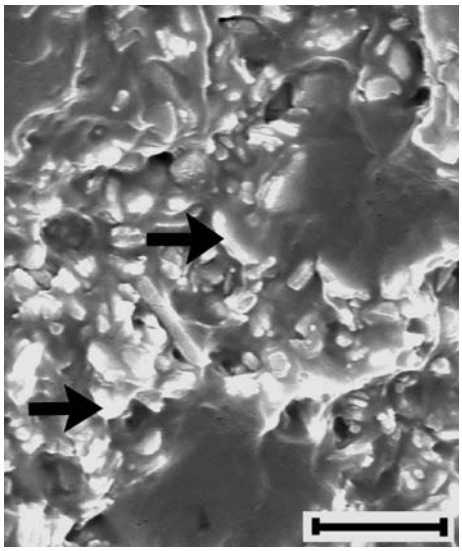

Fig. 2 Scanning electron micrograph of the fracture surface of ST2-56c. Arrows indicate titania particles. Bar = 3 μm

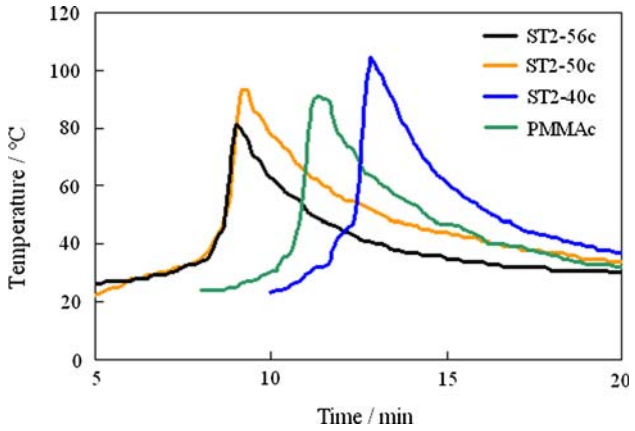

Fig. 3 Heat evolution curves for the setting reactions of ST2-50c, ST2-56c, PMMAc, and ST2-40c

uniformly dispersed and interacted well with the PMMA, and aggregates of titania particles could not be seen in the fracture surfaces of each cement (Fig. 2).

Setting time and peak temperature

The results of the temperature plotting of the cements are shown in Fig. 3. The setting times were 12 min 40 s for ST2-40c, 9 min 0 s for ST2-50c, 8 min 50 s for ST2-56c, and 11 min 0 s for PMMAc. The peak temperatures were 104 °C for ST2-40c, 93 °C for ST2-50c, 81 °C for ST2-56c, and 91 °C for PMMAc. The setting time of the cements containing titania particles reduced and the peak temperature decreased as the titania filler content increased.

Radiopacity

The ST2-56c in the femur was much more radiopaque than the PMMAc (Fig. 4). Both the ST2-56c and PMMAc were injected through an 18-gauge needle without problems, although ST2-56c was more easily injected.

Evaluation of the bone–cement interface

Giemsa surface staining indicated that there was typically no inflammatory reaction around ST2-50c and ST2-56c (Fig. 5a, b). The intervening soft tissue layer between cement and bone was more often seen around ST2-50c than ST2-56c at each time interval. For ST2-56c, no significant change in appearance could be seen with Giemsa surface staining between the 6- and 12-week specimens. On the other hand, for ST2-50c, there appeared to be less intervening soft tissue layer between the cement and bone in the 12-week specimens than in the 6-week specimens.

Low magnification SEM revealed that ST2-56c was in direct contact with bone over large areas within six weeks, whereas ST2-50c was in contact with bone in only small areas (Fig. 6a, b). In the 12-week specimens, both ST2-56c and ST2-50c were in direct contact with bone over large areas (Fig. 6c, d). Both ST2-50c and ST2-56c showed a marginal white line 30–60 μm wide at each time interval,

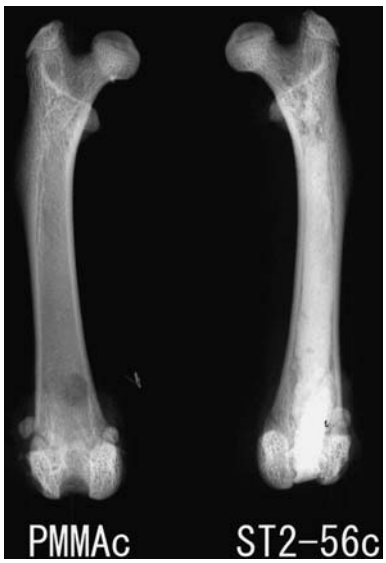

Fig. 4 X-ray radiograph of bilateral femurs of a rat one day after the operation

regardless of whether they were in contact with bone. These findings were also revealed by contact microradiography, in which each cement appeared to be in direct contact with bone over larger areas than in the SEM observation (Fig. 7a, b).

Backscattered SEM at high magnification revealed that both ST2-56c and ST2-50c were in direct contact with bone within six weeks, but a thin intervening soft tissue layer less than 10 μm thick was often observed between ST2-50c and the bone (Fig. 8a, b). It also showed that ST2-50c and ST2-56c were in contact with bone via a white line, which was demonstrated by SEM–EDX analyses to be a Ti-rich layer (Fig. 8c). An increase in the intensity of calcium was also detected along the outer margin of this white line (Fig. 8c).

Evaluation of osteoconductivity

The affinity indices for all of the cements at six and 12 weeks, and the statistical comparisons, are shown in Fig. 9.

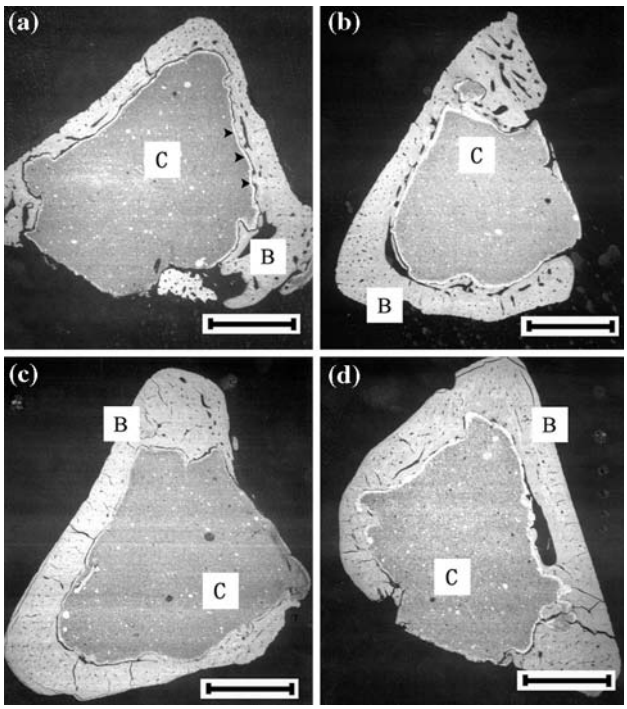

Fig. 6 Low magnification scanning electron micrographs of (**a**) ST2-50c and (**b**) ST2-56c in rat tibiae six weeks after implantation; (**c**) ST2-50c and (**d**) ST2-56c in rat tibiae 12 weeks after implantation. C, cement; B, bone; Arrowheads indicate the white line. Bar = 30 μm

Discussion

In preliminarily trials, it was attempted to prepare cements containing over 60 wt% micron-sized titania particles, but it was often difficult to effectively mix the powder and the liquid. Preliminary in vitro studies revealed that the apatite-forming ability of the composite cements increased with the content of titania particles. Because ST2-50c and ST2-56c were consistently made in a well-mixed form and were expected to have better osteoconductivity than ST2-40c, as judged from the in vitro studies, they were chosen for the animal study.

With the previously reported composite bone cement containing nanosized anatase-type titania particles, it was difficult to disperse the titania particles uniformly in the

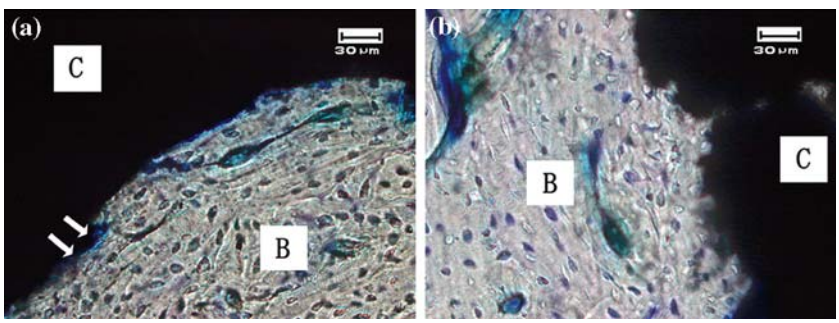

Fig. 5 Giemsa surface staining of (**a**) ST2-50c and (**b**) ST2-56c in rat tibiae 12 weeks after implantation. C, cement; B, bone; Arrows indicate intervening soft tissue. Bar = 30 μm

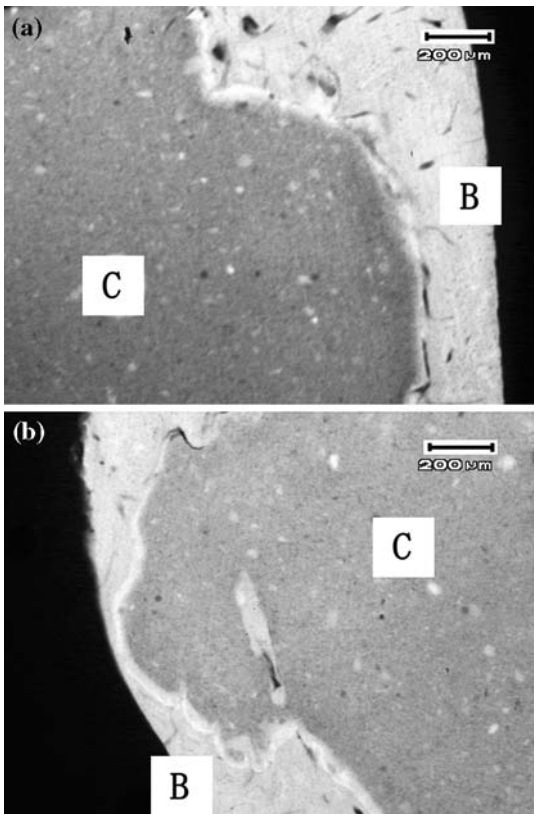

Fig. 7 Contact microradiography of (**a**) ST2-50c and (**b**) ST2-56c in rat tibiae 12 weeks after implantation. C, cement; B, bone. Bar = 200 μm

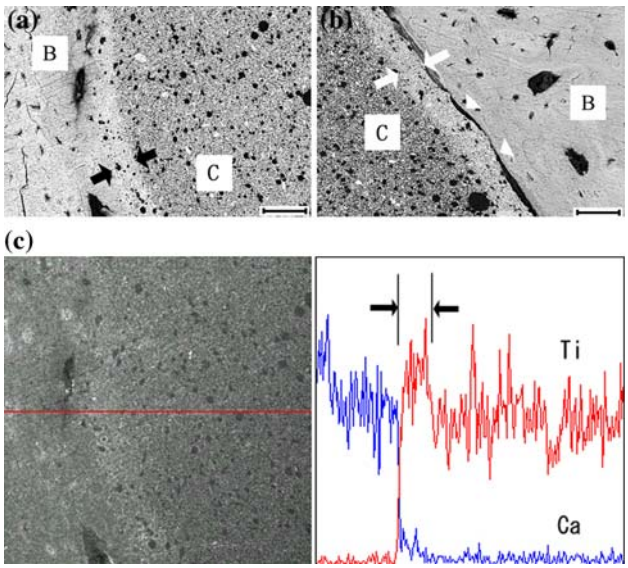

Fig. 8 Back-scattered scanning electron micrographs (SEM) of (**a**) ST2-56c and (**b**) ST2-50c in rat tibiae six weeks after implantation, and (**c**) SEM energy-dispersive X-ray (EDX) analysis of ST2-56c in the same site of (**a**). The white line is clearly visible in (**a**) and (**b**), and SEM–EDX analysis indicated that the white line is a Ti-rich layer. An increase of the intensity of the calcium peak was also detected along the outer margin of the white line. Arrowheads indicate thin intervening soft tissue. Between arrows = white line. C, cement; B, bone. Bar = 40 μm

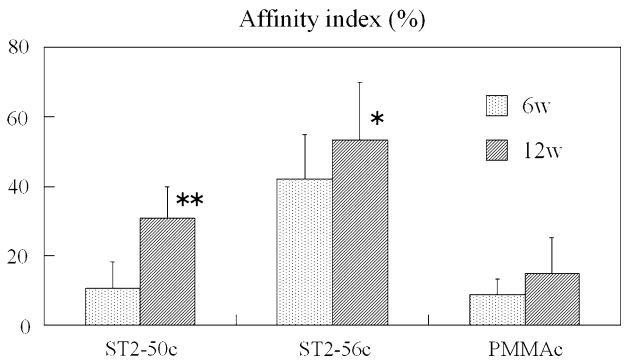

Fig. 9 Affinity indices (%) for ST2-50c, ST2-56c, and PMMAc in rat tibiae at six and 12 weeks after implantation (mean ± SD, n = 12). At six weeks, the value for ST2-50c was 10.6 ± 7.8 and 42.1 ± 12.9 for ST2-56c; and at 12 weeks, 30.8 ± 9.0 and 53.4 ± 16.6 respectively. The comparative affinity indices for PMMAc were 8.9 ± 4.4 and 14.9 ± 10.4 at six and 12 weeks, respectively [16]. *Significant compared with ST2-50c and PMMAc at six and 12 weeks. **Significant compared with PMMAc at 12 weeks

cement [10]. In contrast, micron-sized particles of mixed-phase anatase–rutile titania dispersed well in the cements, as was shown in this study. Indeed, it is difficult to simply compare the results of the experiments because there was little difference in the experimental conditions including the PMMA/MMA ratio and silanization of the powders. However, the difference in the particle size presumably influenced the degree of the particle dispersion.

The compressive and bending strengths of ST2-50c were much higher than those previously reported for PMMA-based composite cements containing nanosized spherical titania particles at 50 wt%, the compressive and bending strengths of which were 91.8 ± 7.7 and 25.5 ± 9.5 MPa, respectively [10]. Shinzato et al. developed PMMA-based composite cements containing glass beads and reported that a decreasing trend in the bending strength was observed as the glass bead size increased [16]. Their results suggest that cements containing smaller-sized titania could have higher strength. However, our findings were not consistent with theirs, and were presumably influenced mainly by the difference in filler dispersion in our studies, where the micron-sized titania dispersed well, whereas the nanosized titania used in the previous study formed some aggregates in the cement [10]. In this study, there was an increasing trend in compressive strength, bending strength, and bending modulus with increasing titania filler content. Juhasz et al. investigated the effect of filler content on the mechanical properties of AW-glass ceramic–polyethylene composites, and noted that a stiffening effect was observed as the AW-glass ceramic filler content increased from 10 to 50 vol% [17]. Their results were consistent with ours. In this study,

the mechanical strengths of the cements containing micron-sized titania particles met the criteria required by the ISO 5833 standard.

The setting times of the cements containing titania particles in this study were reduced, and the peak temperature decreased, as titania filler content increased. Only ST2-56c exhibited a lower peak temperature than PMMAc, and that of ST2-50c was almost the same as that of PMMAc. This was probably because the weight ratio of PMMA/MMA was 1/2 in ST2-50c, whereas the weight ratio of powder/liquid monomer was about 2/1 in PMMAc, and the content of MMA that polymerizes with an exothermic reaction was almost the same in ST2-50c and PMMAc. As less exothermic setting reactions for bone cement are desirable, both ST2-50c and ST2-56c would be acceptable, but ST2-56c appeared to have lower peak-setting temperature properties and is therefore recommended.

Animal experiments revealed that both ST2-50c and ST2-56c were more osteoconductive than cements containing nanosized titania particles. According to a previous study, the affinity indices of cements containing nanosized titania particles were 20.9 ± 7.3% for cement containing 50 wt% titania particles and 31.3 ± 8.7% for cement containing 60 wt% titania particles at 12 weeks [10]. In the previous study [10] and in this study, high molecular weight PMMA powder was used because it showed low solubility in the MMA monomer during the polymerizing reaction [14]. As a result, bioactive fillers could be exposed at the cement surface without being covered by a layer of polymerized MMA [14]. Therefore, the amount of bioactive particles exposed on the cement surface, which must be proportional to cement bioactivity, was greatly influenced by the particle size of bioactive fillers. In this study, the micron size of the titania particles presumably had a beneficial effect on the osteoconductivity of ST2-50c and ST2-56c. However, ST2-56c was in direct contact with bone over larger areas than ST2-50c, at both six and 12 weeks after implantation into rat tibiae. As well as the larger amount of titania filler, presumably the decrease in toxic monomer content contributed to the higher affinity indices of ST2-56c compared with those of ST2-50c. Further research on bone-bonding strength is necessary to demonstrate the bioactivity of ST2-50c and ST2-56c.

The marginal white line seen on SEM was a Ti-rich layer, as revealed by SEM–EDX analyses (Fig. 8c) and has been similarly observed in cements containing nanosized titania particles [10]. In contrast, it has been reported that PMMA-based composite cement containing glass beads, AW-GC, or hydroxyapatite fillers developed by Shinzato et al. exhibited no such marginal line, although they used a similar PMMA/MMA system and bioactive fillers silanized with γ-methacryloxypropyltrimethoxy silane [14]. Therefore, the properties of the titania filler itself are suggested to contribute to the formation of the white line. Because a Ti-rich layer indicated bioactive titania gathered at the cement surface, it presumably contributes to the osteoconductivity of cements containing titania particles. SEM–EDX analyses also revealed an increase in the concentration of calcium along the outer margin of the white line, which suggests calcium ion transfer or bony tissue invasion into the cement margin. Although a small amount of unpolymerized MMA might leak out of the cement surface, SEM observations revealed no such cement degradation and that leakage was minimal. No toxic effects of the monomer were detected by Giemsa surface staining, and excellent osteointegration of ST2-56c and ST2-50c was seen.

The overall results of this study indicate that PMMA bone cement containing micron-sized titania particles is a promising bone cement for prosthesis fixation as well as for vertebroplasty, but further research on long-term osteointegration and bone-bonding strength should be performed before clinical application.

Conclusions

Three types of PMMA-based composite cements containing 40–56 wt% micron-sized bioactive titania powder were prepared, and their mechanical, setting, and biological properties were evaluated. Compressive strength, bending strength, and bending modulus increased with increasing content of titania filler. The mechanical strengths met the criteria required in the ISO 5833 standard. The peak temperature during the setting reaction decreased as the amount of filler increased, and ST2-56c exhibited a lower peak temperature than commercial PMMA cement. Cements ST2-50c and ST2-56c were revealed to be biocompatible and osteoconductive. This was especially the case with ST2-56c, as it was in direct contact with bone over large areas within six weeks after implantation into rat tibiae, and showed significantly higher affinity indices than those of ST2-50c within 12 weeks. Overall, the data indicate that bone cement containing micron-sized titania particles can be applied to prosthesis fixation as well as vertebroplasty, and ST2-56c is a good candidate cement.

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References

1. S. M. KENNY and M. BUGGY, *J. Mater. Sci. Mater. Med.* **14** (2003) 923
2. M. A. FREEMAN, G. W. BRADLEY and P. A. REVEL, *J. Bone Joint Surg. Br.* **64** (1982) 489

3. S. R. GOLDRING, A. L. SCHILLER, M. ROELKE, C. M. ROURKE, D. A. O'NEIL and W. H. HARRIS, *J. Bone Joint Surg. Am.* **65** (1983) 575
4. F. M. KHAW, L. M. KIRK, R. W. MORRIS and P. J. GREGG, *J. Bone Joint Surg. Br.* **84** (2002) 658
5. J. J. CALLAGHAN, J. E. TEMPLETON, S. S. LIU, D. R. PEDERSEN, D. D. GOETZ, P. M. SULLIVAN and R. C. JOHNSTON, *J. Bone Joint Surg. Am.* **86-A** (2004) 690
6. M. UCHIDA, H. M. KIM, T. KOKUBO, S. FUJIBAYASHI and T. NAKAMURA, *J. Biomed. Mater. Res.* **64A** (2003) 164
7. M. UCHIDA, H. M. KIM, T. KOKUBO, S. FUJIBAYASHI and T. NAKAMURA, *J. Biomed. Mater. Res.* **63** (2002) 522
8. W. Q. YAN, T. NAKAMURA, M. KOBAYASHI, H. M. KIM, J. MIYAJI and T. KOKUBO, *J. Biomed. Mater. Res.* **37** (1997) 267
9. Y. T. SUL, *Biomaterials* **24** (2003) 3893
10. K. GOTO, J. TAMURA, S. SHINZATO, S. FUJIBAYASHI, M. HASHIMOTO, M. KAWASHITA, T. KOKUBO and T. NAKAMURA, *Biomaterials* **26** (2005) 6496
11. T. KOKUBO, S. ITO, Z. T. HUANG, T. HAYASHI, S. SAKKA, T. KITSUGI and T. YAMAMURO, *J. Biomed. Mater. Res.* **24** (1990) 331
12. S. SHINZATO, T. NAKAMURA, T. KOKUBO and Y. KITAMURA, *J. Biomed. Mater. Res.* **54** (2001) 491
13. T. NAKAMURA, H. KATO, Y. OKADA, S. SHINZATO, K. KAWANABE, J. TAMURA and T. KOKUBO, In *Bioceramics*, edited by S. Gianniniand A. Moroni (Bologna: Trans Tech, 2000), p. 661
14. S. SHINZATO, M. KOBAYASHI, W. F. MOUSA, M. KAMIMURA, M. NEO, Y. KITAMURA, T. KOKUBO and T. NAKAMURA, *J. Biomed. Mater. Res.* **51** (2000) 258
15. J. TAMURA, K. KAWANABE, T. YAMAMURO, T. NAKAMURA, T. KOKUBO, S. YOSHIHARA and T. SHIBUYA, *J. Biomed. Mater. Res.* **29** (1995) 551
16. S. SHINZATO, T. NAKAMURA, T. KOKUBO and Y. KITAMURA, *J. Biomed. Mater. Res.* **56** (2001) 452
17. J. A. JUHASZ, S. M. BEST, R. BROOKS, M. KAWASHITA, N. MIYATA, T. KOKUBO, T. NAKAMURA and W. BONFIELD, *Biomaterials* **25** (2004) 949