

Bonding ability evaluation of bone cement on the cortical surface of rabbit's tibia

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Abstract A composite bone cement designated G2B1 that contains β tricalcium phosphate particles was developed as a bone substitute for percutaneous transpedicular vertebroplasty. In this study, both G2B1 and commercial PMMA bone cement (CMW1) were implanted into proximal tibiae of rabbits, and their bone-bonding strengths were evaluated at 4, 8, 12 and 16 weeks after implantation. Some of the specimens were evaluated histologically using Giemsa surface staining, contact microradiography (CMR) and scanning electron microscopy (SEM). Histological findings showed that G2B1 contacted bone directly without intervening soft tissue in the specimens at each time point, while there was always a soft tissue layer between CMW1 and bone. The bone-bonding strength of G2B1 was significantly higher than that of CMW1 at each time point, and significantly increased from 4 weeks to 8 and 12 weeks, while it decreased significantly from 12 weeks to 16 weeks. Bone remodeling of the cortex under the cement was observed especially for G2B1 and presumably influenced the bone bonding strength of the cement. The results indicate that G2B1 has bioactivity, and bone bonding strength of bioactive bone cements can be

estimated fairly with this experimental model in the short term.

1 Introduction

Since polymethylmethacrylate (PMMA) was first used for fixation of prostheses by Charnley [1], it has been widely used in orthopedics for prosthesis fixation and as a bone substitute. However, PMMA cannot bond to bone directly and an intervening fibrous tissue layer usually exists between the bone and the cement, which occasionally leads to aseptic loosening of prostheses [2, 3]. To overcome the disadvantages of PMMA, many types of bioactive bone cements have been developed since the eighties [4]. Among them, resin-based bone cements containing bioactive filler have especially been much concerned. To demonstrate bone-bonding ability, that is bioactivity, of bone cements, several experimental methods were developed and utilized in our laboratory [5–7]. Among them, Kamimura's method, in which cements in dough phase were placed in the metal frame fixed on medial aspect of rabbit tibiae and cured in situ [7], was unique and could be suitable for the evaluation of bioactivity of the bone cement. Recently a methacrylate-based composite bone cement containing β -TCP particles (designated G2B1) was developed and its biocompatibility and osteoconductivity were revealed to be excellent in the previous study [8]. As an osteoconductive cement, it was hypothesized that G2B1 had better bone-bonding ability than PMMA. The purpose of the present study was to evaluate bone–cement interface histologically and bone-bonding ability of G2B1 in vivo using modified Kamimura's experimental method.

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2 Materials and methods

2.1 Preparation of the cement

The powder of G2B1 was composed of methylmethacrylate-methyl acrylate copolymer, benzoyl peroxide in inert dicalcium phosphate base and β -TCP. The liquid of G2B1 was composed of methylmethacrylate monomer (MMA), urethane dimethacrylate monomer (UDMA), tetrahydrofurfuryl methacrylate monomer (THFMA), dimethyl-*p*-toluidine and hydroquinone. The powder content of G2B1 was 61.8 wt% and the final β -TCP content of G2B1 was 49.2 wt%. The powder and the liquid were supplied by Depuy International, UK. The powder contents were packed into bags and sterilized by gamma irradiation while the liquid was sterilized by filtration. Commercially available PMMA bone cement, CMW1 (Depuy) was used as a control. The details of the composition and the setting properties of G2B1 were described in the previous study [8].

2.2 Animal experiment

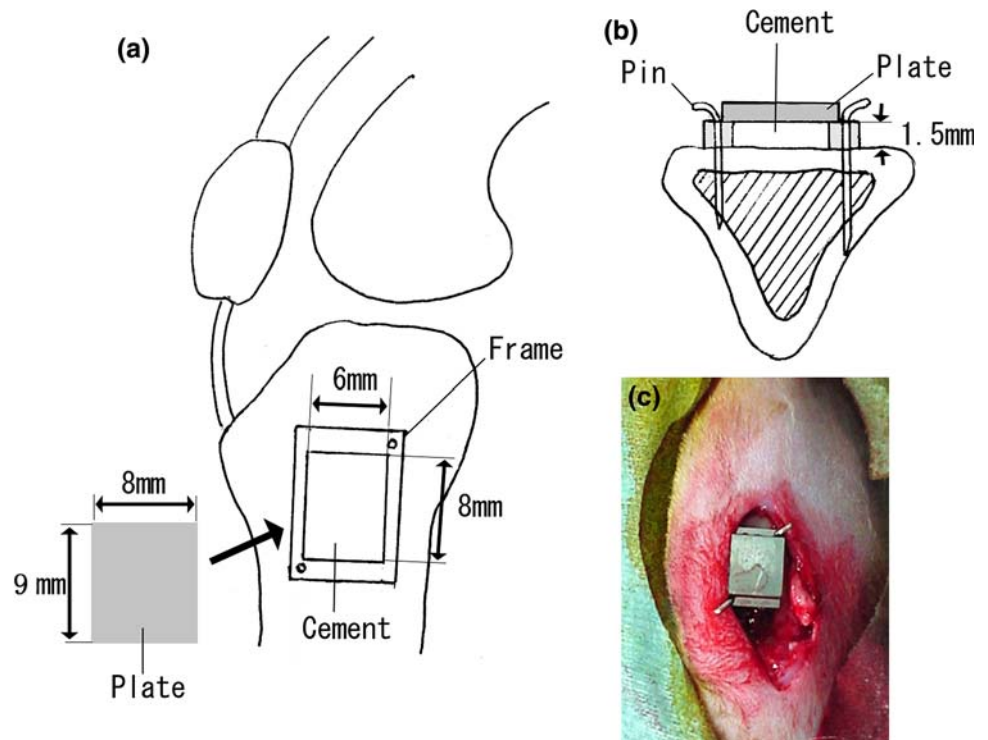
Mature male Japanese white rabbits weighing 3–3.5 kg were used for the implantation study. Under the guidelines for use of experimental animals set by Kyoto University, the animals were reared and the experiments carried out at the Institute of Laboratory Animals, Faculty of Medicine, Kyoto University. The rabbits were anesthetized by an intravenous injection of pentobarbital (50 mg/kg body

weight) and local administration of 0.25% lidocaine. Using sterile surgical techniques, a 2.5-cm-long skin incision was made on the antero-medial aspect of the proximal metaphysis of the tibia. An incision was made on the periosteum and the bone was separated from the periosteum by blunt dissection. Then the cortex of the tibia was exposed subperiosteally within an area of about 15×10 mm. It has been reported that this area is flat and the bonding strength was measured on its surface [9].

Stainless steel frames which were rectangular (inside measurement: 8×6 mm; outside measurement: 12×8 mm; thickness: 1.5 mm) with two holes, were fixed on the tibial cortex subperiosteally by two stainless steel pins (0.7 mm diameter). After lavage with physiological saline and wipe with gauze, the cement was placed manually in the frame in paste form, and covered by a stainless steel plate which was rectangular ($9 \times 8 \times 1$ mm) until cement polymerization was complete (Fig. 1a–c). After cleaning with sterile physiological saline, the wound was sutured in layers.

Both tibiae were operated on in this way with different cement types implanted in each leg of the rabbit, and a total of 40 rabbits (80 legs) was used for this implantation study. Ten rabbits were killed respectively by an intravenous injection of lethal doses of pentobarbital solution at 4, 8, 12, and 16 weeks after implantation. Eight groups (two materials and four time points) were defined, and one group consisted of 10 legs, two of which were used for histological examination and the remainder for bonding strength evaluation.

Fig. 1 Scheme showing the preparation of the cements on the proximal medial cortex of rabbit tibiae (**a**, **b**): (**a**): antero-medial view of the operated site; (**b**): axial view of the operated site), and a photograph of the operated site just after implantation (**c**)



2.3 Evaluation of bonding strength (detaching test)

The tibia was excised with the implant and cut transversely 3 mm proximally and distally to the implant (Fig. 2a). The bone tissue covering the margin of the implant was completely removed to leave the implant in contact with the bone only at its base. The pins and the plate were gently removed, and rectangular margin of the implanted cement was carefully drilled to the depth of approximately 1 mm with a steel bar (diameter: 0.4 mm) to remove the frame safely from the bone. Then the frame was carefully removed while the cement was held in situ with a clasp. Before the bonding strength test, the cement was attached to a metal jig using a cyanoacrylate adhesive. The jig was a rectangular block (5 × 5 × 14 mm) with holes at the lateral aspect allowing hooks to be connected. Metal hooks were attached to the cut tibia and the metal jig (Fig. 2b, c), which was then connected to an Instron-type mechanical testing machine (model-1323; Aikoh Engineering, Nagoya, Japan). The hooks were pulled perpendicularly to the bone–cement interface at a crosshead speed of 3.5 cm/min. The load required to detach the cement from the bone or to break the bone was measured.

2.4 Micrographic examination

The specimens for histological examination were dehydrated through a graded ethanol series (70, 80, 90, 99, and

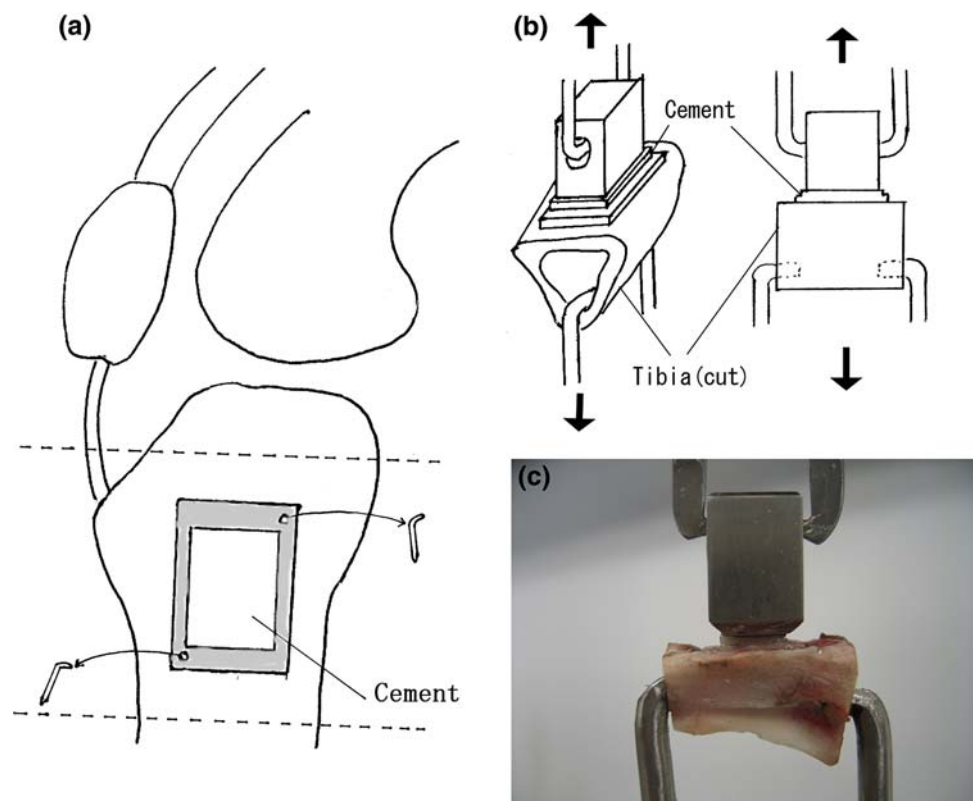
100 vol%) and embedded in epoxy resin (Epofix, Struers Co., Copenhagen, Denmark). With a band saw (BS-3000, EXAKT, Norderstedt, Germany), several sections were cut perpendicular to the axis of a tibia containing the cement, and were ground to a thickness of 60–80 and 100 μm using a diamond lap disk (Maruto Ltd., Tokyo, Japan) for Giemsa surface staining and contact microradiography, respectively. Additional sections (500 μm thick) were prepared for observation by a scanning electron microscope (S-4700, Hitachi Ltd., Tokyo, Japan). Some of those SEM specimens were analyzed by an energy-dispersive X-ray micro analyzer (EMAX-7000, HORIBA Ltd., Kyoto, Japan) attached to SEM (S-4700).

The cement specimens which were detached from the bone in the bonding strength test at each time interval, were dehydrated through a graded ethanol series (70, 80, 90, 99, and 100 vol%); then they were soaked in isopentyl acetate solution for 1 day and dried in critical point drying apparatus (HCP-2, Hitachi Ltd., Tokyo, Japan) for SEM observation.

2.5 Statistical analysis

Values were expressed as means and the standard deviations (SD), and values for G2B1 and CMW1 at each time interval were compared using one-way analysis of variance with Fisher's PLSD post hoc statistical test.

Fig. 2 Schema showing the preparation of the specimens for the detaching test (**a**, **b**) and a photograph of the specimen in the detaching test (**c**). **a** The tibia is cut along the dotted line after killing the rabbit. **b** After attaching the metal jig, the detaching test is performed



P values smaller than 0.05 were considered statistically significant.

3 Results

No rabbits died during the implantation study.

3.1 Histological evaluation

Giemsa surface staining showed that there was almost no inflammatory reaction on G2B1 and CMW1. G2B1 contacted bone directly without intervening soft tissue in the specimens at each time interval, while there was always a soft tissue layer between CMW1 and bone (Fig. 3a–c). Bony tissue expanding on G2B1 was sometimes separated from the cortical bone surface of the medial tibia via intervening fibrous tissue or body fluid at 4 weeks (Fig. 3a). However at 8 weeks the gap between the cortical bone surface and G2B1 came to be partially filled up with bony tissue and it could not be distinguished at 12 and 16 weeks (Fig. 3b).

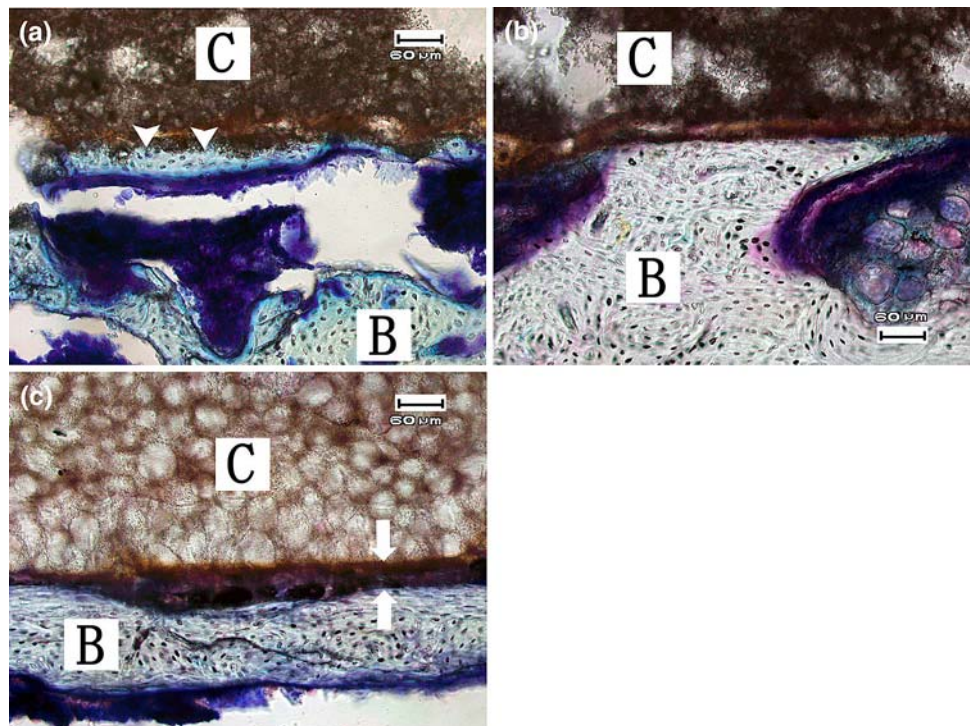
Contact microradiography revealed that the medial cortex of the tibia on G2B1 became porous bony tissue as 8 weeks (Fig. 4a). In addition, the area of bony tissue directly beneath G2B1 became smaller and more concentrated on the outer margin of the implanted G2B1 as the implantation period became longer. On the other hand, the

area of bony tissue directly beneath CMW1 changed less than G2B1 in size at 12 weeks (Fig. 4b).

SEM observation at the cement–bone interface revealed that G2B1 contacted bone directly within 4 weeks, and as was revealed by Giemsa surface staining, bony tissue expanding on G2B1 was sometimes separated from the cortical bone surface at 4 weeks (Fig. 5a). At 8 weeks, a large part of the bony tissue expanding on G2B1 apposed to the cortical surface, and the border line between the original cortex and the newly formed bony tissue was sometimes observed (Fig. 5b). Remarkably fragmented β -TCP particles were often seen in the margin of the newly formed bony tissue at 8 weeks (Fig. 5b). At 12 and 16 weeks, the border between the original cortex and the newly formed bony tissue could not be distinguished, and fragmented β -TCP particles were only seen in the margin between G2B1 and bone. Bony tissue rarely intruded in to the surface of G2B1 in 4 weeks specimens, but the bony intrusions were often seen in 8 and 12 weeks specimens (Fig. 5b). At 16 weeks, the area where bony tissue directly contacted G2B1 were smaller than at 8 and 12 weeks. On the other hand, there was always a soft tissue layer between CMW1 and bone also in the SEM specimens (Fig. 5c).

SEM observation of the detached surfaces of G2B1 revealed that the lamellar structure could be seen in the tissue attached to the surface of G2B1 at each time interval (Fig. 6a, b, e). SEM–EDX analyses also demonstrated that the attached tissue included calcium and phosphate like

Fig. 3 Giemsa surface staining of G2B1 in rabbit tibiae **a** 4 and **b** 16 weeks after implantation; CMW1 in rabbit tibiae **c** 16 weeks after implantation. **a** Arrowheads indicate bony tissue expanding on G2B1. **c** Between arrows indicate intervening fibrous tissue. *C* cement; *B* bone. Bar = 60 μ m



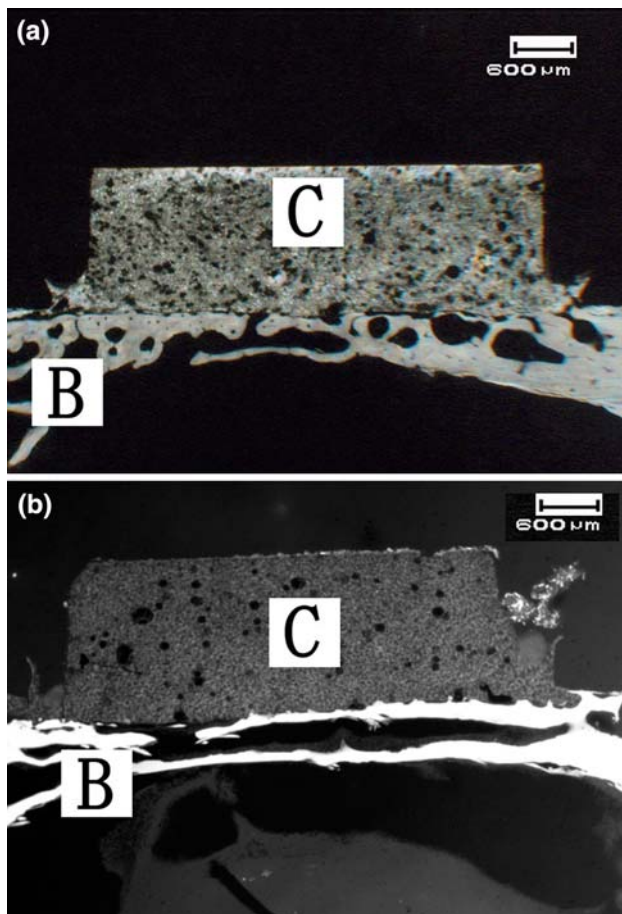


Fig. 4 **a** CMR image of G2B1 in rabbit tibiae 8 weeks after implantation; **b** CMW1 in rabbit tibiae 12 weeks after implantation. C cement; B bone. Bar = 600 µm

G2B1 (Fig. 6c, d), and indicated that the attached tissue was certainly bone. The attached bony tissue was small at 4 weeks, but it became large at 8, 12, and 16 weeks. Most of the detached specimens of G2B1 had more or less attachments of bony or fibrous tissues macroscopically, while no bony or fibrous tissue could be found on the detached surfaces of CMW1 specimens so that SEM observation was not performed for them.

3.2 Evaluation of bone-bonding strength

In detaching test, all the cement specimens of CMW1 were detached from the confronting bone at each time interval, while all the cement specimens of G2B1 were detached from the confronting bone at 4, 8, and 12 weeks; however some specimens of G2B1 were detached from the cortex around the margin of them while the confronting bone remained attached to the cement at 16 weeks. The values of the interfacial tensile strengths, that is bone-bonding strength, of G2B1 and CMW1, and the statistical comparisons are summarized in Table 1, and the graph was

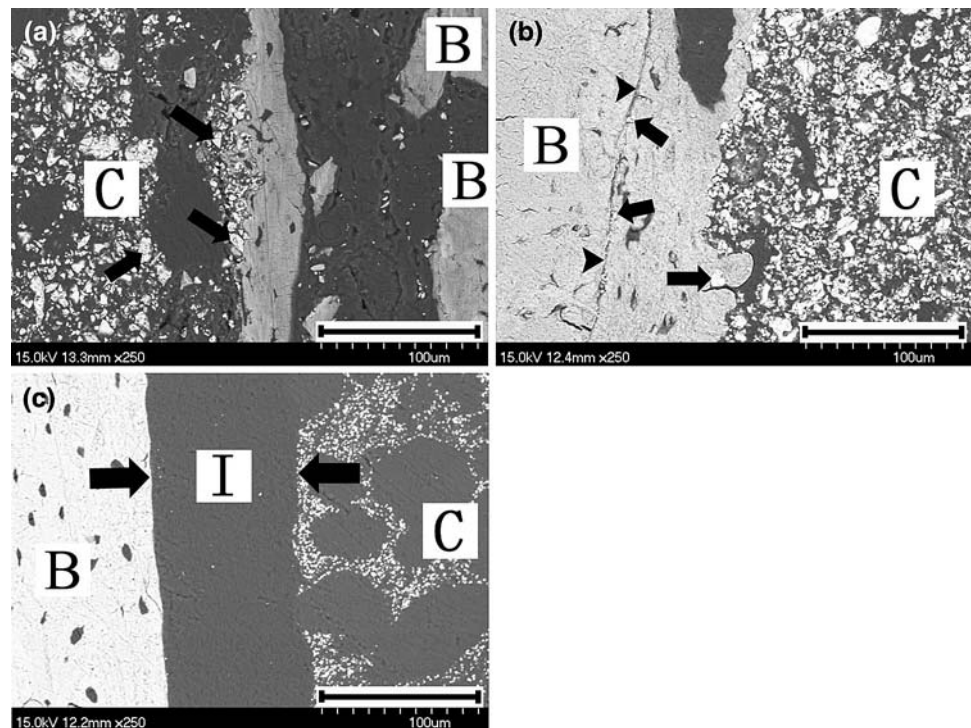
shown in Fig. 7. The bone-bonding strength of G2B1 was significantly higher than that of CMW1 at each time interval, and significantly increased from 4 weeks to 8 and 12 weeks, while it decreased significantly from 12 weeks to 16 weeks. On the other hand, bone-bonding strength of CMW1 gradually increased up to 16 weeks.

4 Discussion

Histological findings of G2B1 revealed that new bone formation started on the surfaces of the cement specimens as well as those of the cortex surfaces by 4 weeks after implantation, and the space of the fibrous tissue or body fluid between the bone on the cement surface and cortex was partially filled up with newly formed bony tissue at 8 weeks after implantation. These findings were not observed with CMW1 and indicated excellent biocompatibility and osteoconductivity of G2B1. On the other hand, the border line between the original cortex and the newly formed bony tissue as was observed in Fig. 5b indicated a possibility that bony tissue did not extend from the outer surface of the cortex in the process of osteoconduction of G2B1. As for the morphological change of the cortex in the implanted site, CMR revealed that the thinning of the cortex and the cortical porosis [10, 11] occurred in the rabbit's tibiae. In this study, frames were fixed subperiosteally on the cortex so that the blood supply to the bone beneath the frame might be impaired. As Unthoff et al. mentioned, cortical porosis under plates may occur in response to either altered cortical perfusion or stress-shielding [11]. In this study cortical porosis occurred with G2B1 and CMW1 respectively, and the difference of the morphological change of bone beneath the frame was apparent between G2B1 and CMW1. Because the alteration of cortical perfusion was presumably equivalent in both the case with G2B1 and CMW1, this result indicated that stress shielding might significantly affect the morphological change of the cortex that is bone remodeling, in the case of bioactive materials like G2B1. G2B1 connected to the bone directly and this presumably resulted in the stiffening effect of the cement in the implant area of the tibia. On the other hand, CMW1 did not attach to the bone, and its stiffening effect was supposed to be lower than G2B1. As a result, stress shielding of the cortex presumably occurred more in the case with G2B1. Yoshii et al. fixed bioactive AW-glass ceramics and bioinert alumina ceramics respectively to the surface of bone cortex in their experiments and mentioned that the thinning of the bone cortex at the base of the implants seen in the case of AW-glass ceramics was not observed with alumina ceramics [9]. Their results was consistent with ours.

On bone-bonding strength of G2B1 there was a significant increase from 4 weeks to 12 weeks without significant

Fig. 5 SEM images of G2B1 in rabbit tibiae **a** 4 and **b** 8 weeks after implantation; **c** CMW1 in rabbit tibiae 8 weeks after implantation. *C* cement; *B* bone; *I* (between *arrows*) intervening fibrous tissue. Arrowheads indicate the border line between the original cortex and the newly formed bony tissue. Arrows indicate β -TCP fillers. Bar = 100 μ m



difference between the values of 8 and 12 weeks. However there was a significant decrease from 12 weeks to 16 weeks. It was presumably because mechanical stress through the bone was distributed via G2B1, and the cortex under it was absorbed in the process of bone remodeling. As a result, bone-bonding strength of G2B1 decreased as the bone–cement contact area became small. On the other hand, bone-bonding strength of CMW1 gradually increased up to 16 weeks, which indicated that bone remodeling of the cortex below the cement had no dominant effect on bone bonding strength of CMW1. This result is consistent with the result of the histological evaluation in which there was always intervening fibrous tissue between CMW1 and bone.

As was revealed by SEM observation at the bone–cement interface, fragmented β -TCP particles in the margin of the newly formed bony tissue and bony tissue intrusion into the margin of G2B1 demonstrated degradable property of G2B1. However degradation of G2B1 appeared not to progress as the time interval became longer until 16 weeks. The extent of G2B1's degradation in the present study seemed to be less than that in the previous study [8] where G2B1 was implanted in the medullary canals of rat tibiae. It was not surprising because the cement was encapsulated by the frame and plate in this study, and there is only contact to bone on one side, where the blood supply was reduced due to the removal of periosteum.

There have been many studies on bone-bonding strength of bone cements. Each experimental method has some merits and demerits respectively. Detachment tests with

pre-hardened cement specimens, could be performed easily as compared with the other experimental models, but did not reflect the real bone–bonding ability of bone cement in clinical use [5, 12, 13]. Push-out tests using diaphyses with their medullary canals replaced by prosthesis with cements or cements only, could mimic clinical use of bone cements and represent load bearing model of the bonding-strength tests [14, 15]; however they could not indicate the actual bonding property of the interface, because the loading geometry, surface roughness of bone, and the mismatch of the modulus of cement and bone could influence significantly the experimental results [16]. Repair of segmental bone defects of rabbit tibiae with bone cements, reported by Okada et al. [6], could indeed reproduce load bearing condition of bone cements confirmly, but the failure loads by the tension tests could not correspond to the bonding strength of the cement–bone interface because of the ambient callus formation and the cement intrusion into the medullary canal. As compared with the other experimental methods, the experimental method used in this study may evaluate quantitatively bone bonding strength of bone cements because the bone–cement contact area is always uniform and the influence of the initial surface roughness of the cortex on bone-bonding strength is negligible. However this method is under non-load bearing condition so that the result of this experiment could not always promise the stability of bone–cement interface during vertebroplasty with G2B1 in which the cement within vertebral bodies must bear the body weight. Furthermore

Fig. 6 SEM images of the surfaces of G2B1 specimens after detaching tests **a** 4, **b** 8, and **e** 16 weeks after implantation; **c** and **d** are SEM-EDX mapping images of calcium and phosphorus respectively in the same area of **(b)**. *C* cement; *B* bone. Bar = 100 μm

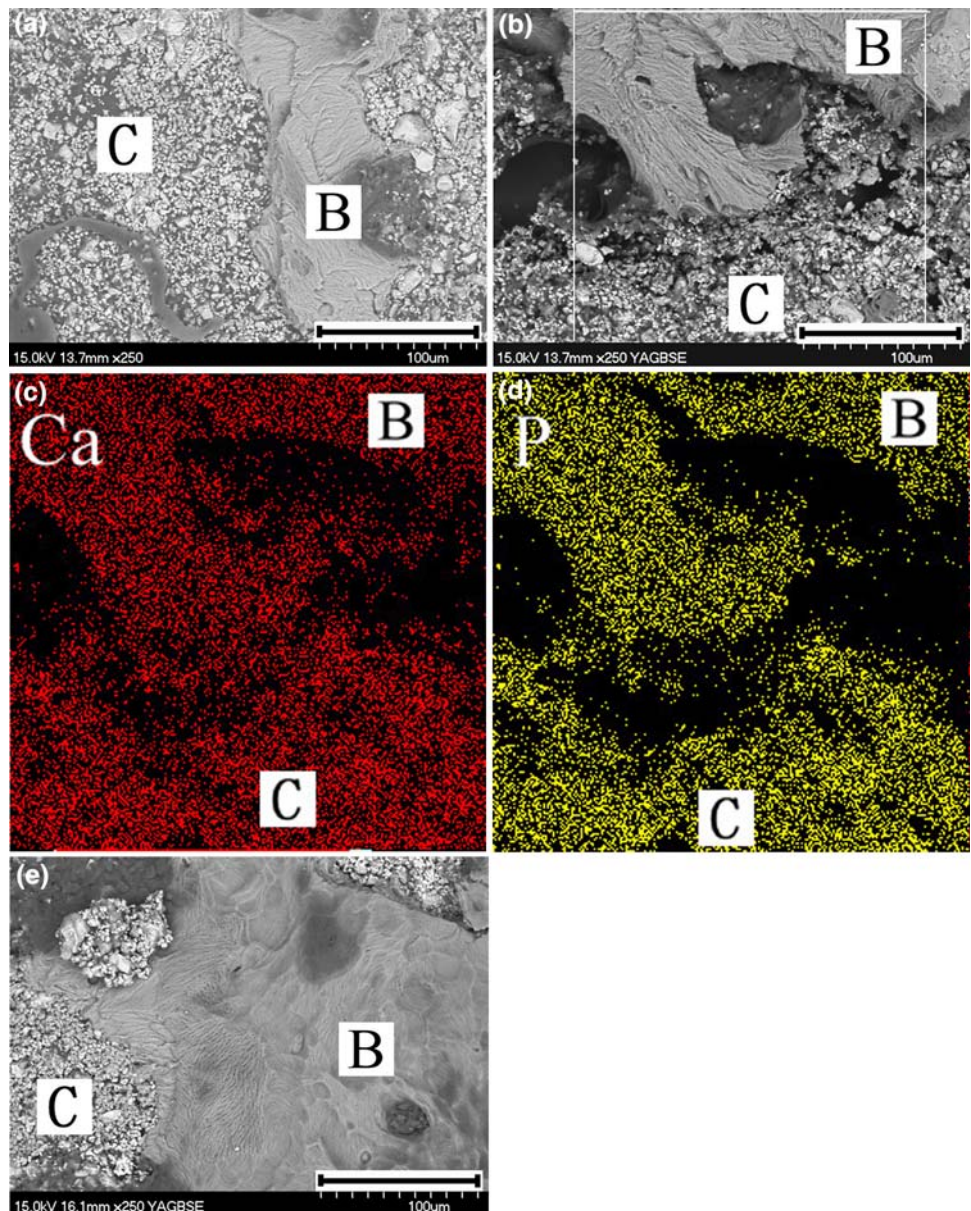


Table 1 The bonding strength between the cements and the surface of bone cortex (kgf/cm²)

Time after implantation (weeks)	G2B1 ^a	CMW1
4	3.92 ± 2.03	0.80 ± 0.97
8	6.72 ± 2.61 ^b	1.51 ± 1.44
12	8.74 ± 2.85 ^b	1.89 ± 1.86
16	4.99 ± 2.59 ^c	2.59 ± 1.12

Values were expressed as Means ± SD; *N* = 8

^a Significantly different from CMW1 at each time interval

^b Significantly different from 4 weeks for G2B1

^c Significantly different from 12 weeks for G2B1

this method tends to be much influenced by bone remodeling so that evaluation of bone-bonding strength in the long term is difficult. Nevertheless this method can still be beneficial to evaluate real bioactivity of bone cements quantitatively in the short term and to predict the morphological change of bone–cement interface of bioactive bone cements in clinical use.

Finally the present study demonstrated that the bone bonding ability of G2B1 was significantly higher than that of PMMA. G2B1 which contains β-TCP particles has the degradable property as well as excellent osteoconductivity as revealed in the previous study. G2B1 was developed as a bone substitute for vertebroplasty and has enough

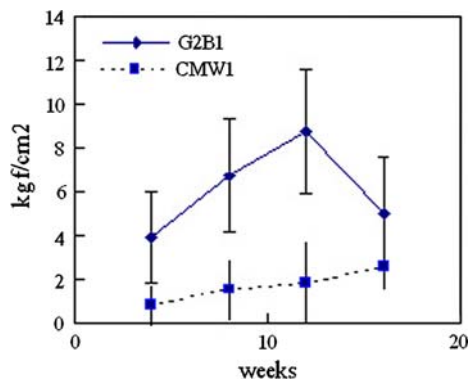


Fig. 7 Graph showing the change of the bone-bonding strength of G2B1 and CMW1 from 4 to 16 weeks after implantation

mechanical properties. For vertebroplasty, the injected bone cement must bear the compressive load in the long term. Therefore the bone bonding strength in the load bearing condition and the aging mechanical properties of G2B1 must be examined before it is applied to clinical use.

5 Conclusions

Bone-bonding strength of G2B1 was significantly higher than that of CMW1, and histological examination demonstrated that G2B1 contacted to bone directly within 4 weeks, while CMW1 did not up to 16 weeks. Though the experimental method used in this study is influenced by bone remodeling and the evaluation of bone bonding strength in the long term is difficult, this method can be beneficial to evaluate real bioactivity of bone cements quantitatively and to predict the morphological change of bone–cement interface of bioactive bone cements in clinical use.

References

1. Charnley J. Anchorage of the femoral head prosthesis to the shaft of the femur. *J Bone Joint Surg Br.* 1960;42-B:28–30.
2. Freeman MA, Bradley GW, Revel PA. Observations upon the interface between bone, polymethylmethacrylate cement. *J Bone Joint Surg Br.* 1982;64:489–93.
3. Goldring SR, Schiller AL, Roelke M, Rourke CM, O'neil DA, Harris WH. The synovial-like membrane at the bone-cement interface in loose total hip replacements, its proposed role in bone lysis. *J Bone Joint Surg Am.* 1983;65:575–84.
4. Harper EJ. Bioactive bone cements. *Proc Instn Mech Engrs.* 1998;212:113–20.
5. Tamura J, Kitsugi T, Iida H, Fujita H, Nakamura T, Kokubo T, et al. Bone bonding ability of bioactive bone cements. *Clin Orthop Relat Res.* 1997;343:183–91.
6. Okada Y, Kawanabe K, Fujita H, Nishio K, Nakamura T. Repair of segmental bone defects using bioactive bone cement: comparison with PMMA bone cement. *J Biomed Mater Res.* 1999; 47(3):353–9.
7. Kamimura M, Tamura J, Shinzato S, Kawanabe K, Neo M, Kokubo T, et al. Interfacial tensile strength between polymethylmethacrylate-based bioactive bone cements and bone. *J Biomed Mater Res.* 2002;61(4):564–71.
8. Goto K, Shinzato S, Fujibayashi S, Tamura J, Kawanabe K, Hasegawa S, et al. The biocompatibility and osteoconductivity of a cement containing beta-TCP for use in vertebroplasty. *J Biomed Mater Res A.* 2006;78(3):629–37.
9. Yoshii S, Kakutani Y, Yamamuro T, Nakamura T, Kitsugi T, Oka M, et al. Strength of bonding between A-W glass-ceramic and the surface of bone cortex. *J Biomed Mater Res.* 1988;22(3 Suppl): 327–38.
10. Klaue K, Fengels I, Perren SM. Long-term effects of plate osteosynthesis: comparison of four different plates. *Injury.* 2000; 31(Suppl 2):S-B51–62.
11. Uhthoff HK, Boisvert D, Finnegan M. Cortical porosis under plates. Reaction to unloading or to necrosis? *J Bone Joint Surg Am.* 1994;76(10):1507–12.
12. Fujita H, Nakamura T, Tamura J, Kobayashi M, Katsura Y, Kokubo T, et al. Bioactive bone cement: effect of the amount of glass-ceramic powder on bone-bonding strength. *J Biomed Mater Res.* 1998;40(1):145–52.
13. Kobayashi M, Nakamura T, Tamura J, Kikutani T, Nishiguchi S, Mousa WF, et al. Osteoconductivity and bone-bonding strength of high- and low-viscous bioactive bone cements. *J Biomed Mater Res.* 1999;48(3):265–76.
14. Senaha Y, Nakamura T, Tamura J, Kawanabe K, Iida H, Yamamuro T. Intercalary replacement of canine femora using a new bioactive bone cement. *J Bone Joint Surg Br.* 1996;78(1): 26–31.
15. Takahashi A, Koshino T. Compressive and bone-bonding strength of hydroxyapatite thermal decomposition product implanted in the femur of rabbit as a bioactive ceramic bone cement. *Biomaterials.* 1995;16(12):937–43.
16. Lucksanasombool P, Higgs WA, Higgs RJ, Swain MV. Interfacial fracture toughness between bovine cortical bone and cements. *Biomaterials.* 2003;24(7):1159–66.