JOURNAL OF
biomaterials applications

Composites

Journal of Biomaterials Applications
2015, Vol. 30(1) 30–37

DOI: 10.1177/0885328215569092
jba.sagepub.com
SAGE

In vivo evaluation of bioactive PMMA-based bone cement with unchanged mechanical properties in a load-bearing model on rabbits

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Abstract

Polymethylmethacrylate-based bone cements are widely used for fixation of joint replacements. To improve the long-term outcome, bioactive bone cements are aspired to advance the bone–cement interface. This study evaluated the in vivo properties of a new polymethylmethacrylate-based bioactive bone cement with addition of amphiphilic phosphorylated 2-hydroxyethylmethacrylate. Previous in vitro studies confirmed bioactive properties in cell culture, as well as unchanged mechanical properties are tests according to ISO 5833:2002.

Three different variations of the cement (polymethylmethacrylate + phosphorylated 2-hydroxyethylmethacrylate, polymethylmethacrylate + phosphorylated 2-hydroxyethylmethacrylate + $CaCl_2$ and polymethylmethacrylate + phosphorylated 2-hydroxyethylmethacrylate + $CaCl_2$ + Na_2CO_3) were compared to conventional polymethylmethacrylate cement. To evaluate the properties under load-bearing conditions, a spacer prosthesis was implanted into the femoral diaphysis of 24 rabbits. Additionally, a cement plug was installed into the proximal tibia. After three months, polished sections with Giemsa surface staining were prepared. The bioactivity was determined using the bone affinity index.

The sections showed a good osseointegration of the bioactive bone cement without cement cracks under load-bearing conditions. Regarding the bone affinity index, the bioactive bone cement revealed a significantly higher value in the proximal tibia (25.9–37.7%) and around the spacer prosthesis (36.8–58.9%) compared to the conventional polymethylmethacrylate cement (12.8–17.0%).

The results confirm the in vivo bioactivity of this bone cement. The absence of cement cracks indicates a sufficient mechanical stability to fix prostheses with this bioactive cement, but for a final assessment long-term tests are necessary.

Keywords

Bioactive bone cement, polymethylmethacrylate, in vivo properties, bone affinity index, load bearing

Introduction

In the last five decades, bone cement based on polymethylmethacrylate (PMMA) has become the main material for fixation of prostheses in orthopedic surgery.[1] Especially for arthroplasties of the hip joint, clinical experiences have revealed that for the long-term results, the properties of PMMA and the cementation technique are more important than the design of the prosthesis.[2] For a good long time result, the main focus must be put on the fatigue properties of the cement and the cement–bone interface.[1,3] Therefore, many efforts have been made to improve bone cement

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Table 1. Overview and composition of the four different tested bone cements. For all groups, Palacos R (Heraeus Medical GmbH, Wehrheim, Germany) was used as PMMA resin.

Bone cement	Abbreviation	Composition
Bioactive cement A	MA3M	PMMA + HEMA-P
Bioactive cement B	MB3M	PMMA + HEMA-P + Na_2CO_3
Bioactive cement C	MC3M	PMMA + HEMA-P + Na_2CO_3 + $CaCl_2$
Reference cement	R3M	PMMA

Note: PMMA, polymethylmethacrylate; HEMA-P, phosphorylated 2-hydroxyethylmethacrylate.

by either enhancement of the mechanical properties of PMMA (i.e. fiber reinforcement, vacuum mixing) or improvement at the bone–cement interface through the development of bioactive bone cement.

Since PMMA does not adhere to bone,[3,4] a layer of intervening fibrous tissue is formed at the bone–cement interface.[5,6] Therefore, a sufficient penetration of the cement into cancellous bone is needed to provide adequate anchorage of the implant.[7] As a consequence, cemented femoral components using PMMA are not suitable for smooth bone surfaces often found in revision hip arthroplasty.[8] One way to solve this problem is seen in generating bone cement with bioactive properties.[9]

There are different approaches to produce bioactive bone cement. To bioactivate PMMA-based bone cement different fillers based on calcium phosphates, hydroxyapatite, or bioactive glass–ceramics have been investigated.[10–13] Since the majority of the fillers are captured in the bone cement after polymerization, the large percentage weight necessary to generate bioactivity leads to a deterioration of the mechanical properties. Therefore, these cements are mechanically not strong enough for a use under weight-bearing conditions. To improve mechanical properties of bioactive bone cements, PMMA was replaced by bisphenol A-glycidyl-methacrylate (Bis-GMA) in several experiments.[14–16] These bioactive bone cements revealed high bioactivity along with sufficient stability under load-bearing conditions. But using Bis-GMA as resin brings up the disadvantages of altered handling properties, enlarged dissolution of monomers, and greater setting shrinkage.[9,10]

In an attempt to solve this problem, a new bioactive PMMA-based bone cement with comparable mechanical properties and handling characteristics as commercially produced PMMA bone cement was developed.[17,18] The purpose of this study was to evaluate the in vivo properties of this new PMMA-based bioactive bone cement under load-bearing conditions using a rabbit model.

Materials and methods

Bone cement

To achieve a bioactive PMMA-based bone cement with as few fillers as possible, the principle of biomineralization was applied.[19,20] As promoters, small amounts (0.5 wt%) of amphiphilic phosphorylated 2-hydroxyethylmethacrylate (HEMA-P) were added providing anionic phosphate groups. After the polymerization of the cement, these molecules served as nucleation sites for calcium phosphate phases leading to a bioactivation of the cement surface.

For this in vivo study, three different variations of the bioactive cement were used. Since admixture of $CaCl_2$ and Na_2CO_3 had positive effects on biomineralization in vitro, these additives were included in two groups. The composition of the three variations is shown in Table 1.

Implant preparation

A spacer prosthesis (Figure 1) replacing a part of the femur diaphysis was manufactured to evaluate the bone cement under load-bearing conditions. It was designed as a miniature version of standard spacer prostheses used for humans with tumor lesions in the diaphysis. The prosthesis consists of an interlocking section with a diameter of 9 mm and a total length of 20 mm. At this part, the prosthesis can be separated and reunited fixing the rotation with a locking system blocked in place with two small screws. Each part of the prosthesis has a 40 mm long stem for cemented fixation in the proximal and distal medullar canal of the femur. To ensure rotational stability, the 4 mm diameter stems are hexagonally shaped in the cross section.

Animal experiments

For this study, 24 New Zealand White rabbits were used. The rabbits were ex-breeders and their weight was between 4.0 and 5.5 kg. The experiments were

Figure 1. The custom made spacer prosthesis.

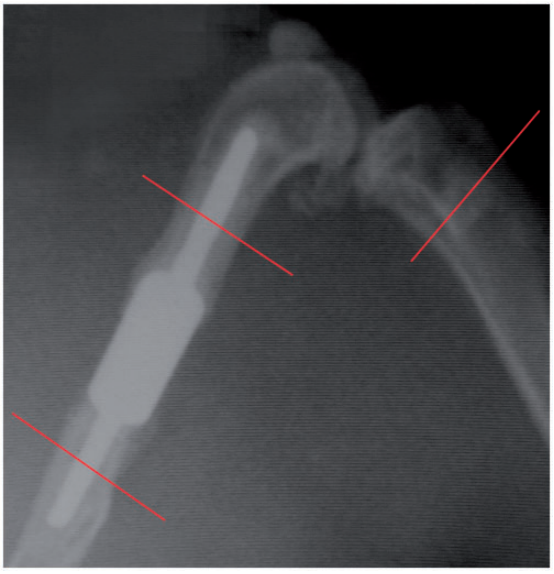

Figure 2. Radiograph of the right leg of a rabbit three months after implantation of a spacer prosthesis and a cement plug using the new bioactive bone cement (group MC3M). Red lines mark the level of the three cross sections.

conducted in the Walter Brendel Centre of Experimental Medicine, Ludwig-Maximillians-University Munich, Germany according to the guidelines for animal experiments and after approval of the Bavarian State Government. The 24 rabbits were divided randomly into four groups of the six animals.

All surgical procedures were performed under anesthesia induced with intramuscular injection of ketamine hydrochloride (Hameln pharmaceuticals, Hameln, Germany) and xylazine (Bayer AG, Leverkusen, Germany), and maintained intravenously over the ear vein. As a prophylactic antibiotic, 250 mg cefuroxime (Fresenius, Bad Homburg, Germany) was administered intravenously.

The right femur was selected for all implantations. The skin on the lateral side of the leg was shaved and sterilized according to standard techniques. The skin incision was performed over a length of 5 cm along the palpable lateral side of the femoral bone. After longitudinal splitting of the fascia, approximately 4 cm of the bone was exposed by blunt dissection of the overlying muscles. A 20 mm long part in the middle of the femur diaphysis was resected using an oscillating saw and the medullary canal of the remaining segments was rasped to a diameter of 5 mm and irrigated. The bioactive bone cement or the reference PMMA cement was hand mixed and injected into each medullary canal through a syringe. Both parts of the spacer prosthesis were inserted and held in position until the cement was set while the rest of the cement streaming out was removed. The two parts were united at the locking system and fixed with two screws after adjusting the correct rotation. Afterward the operation field was closed in layers.

For the installation of the cement plug, a 1 cm skin incision was applied on the medial flank of the proximal tibia. A 3.5 mm bore hole was drilled and another portion of the cement was hand mixed. A total of 0.5 ml of the cement was injected using a syringe. After the operation, no immobilization or support was used.

Histological examination

Three months after implantation, the rabbits were killed by an overdose of pentobarbital (Narcoren, Streuli Pharma AG, Uznach, Switzerland) and X-rays of the right femur were taken in two planes (Figure 2). The femur and the proximal tibia were removed and the spacer prosthesis was disconnected. The resulting three parts were dehydrated with rising alcohol concentrations (70% to absolute). In the next 11 days, the sections were embedded in PMMA using alcohol–PMMA mixtures with rising concentrations of the embedding media Technovit 7200 VLC (Heraeus Kulzer GmbH, Wehrheim, Germany).

After hardening, the sections were cut 20 mm from the interlocking section of the spacer prosthesis on the distal and proximal femur as well as in the middle of the cement plug on the tibia. Then, they were grinded to a thickness of 70 µm using a grinding machine (EXACT 400 CP Micro Grinding System, Norderstedt,

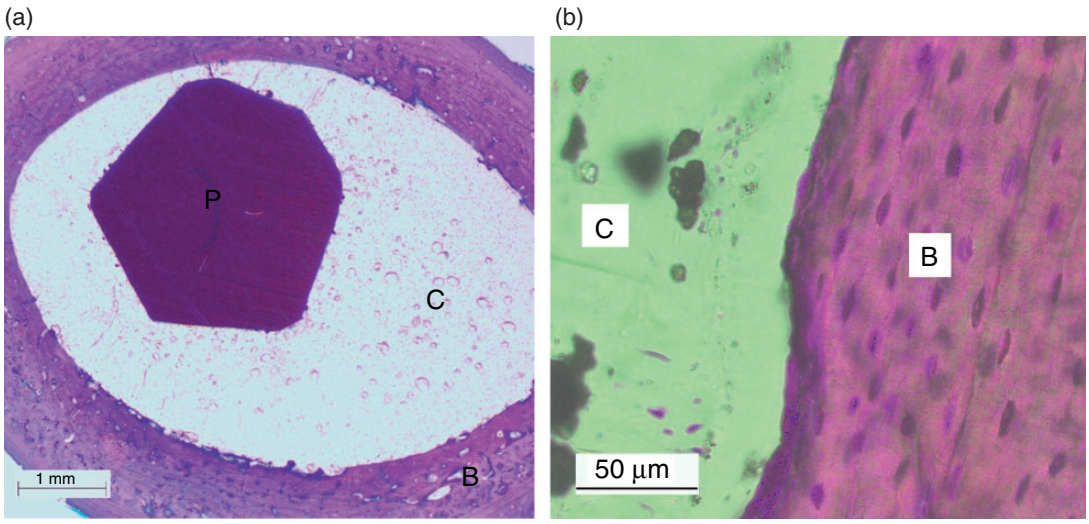

Figure 3. (a,b) Giemsa surface staining of a rabbit femur three months after implantation of a spacer prosthesis using the new bioactive bone cement (group MA3M). (a) Overview (original magnification × 125) showing a good bonding of the cement (C) to the surface of the prosthesis (P) and the bone (B). (b) Intimate contact between cement and bone without any fibrous layer intervening was also confirmed on a closer view (original magnification × 40).

Germany). Afterward, the surface was polished with #4000 garnet paper. At the end, the sections were stained with Giemsa solution for histological observation under a light microscope (Axioskop 40, Carl Zeiss, Jena, Germany) connected to a digital camera (AxioCam MRc5, Leica, Carl Zeiss, Jena, Germany).

Affinity index

To compare the bioactivity of the different PMMA-based bone cements, the affinity index (AI) was measured on the Giemsa surface staining sections. The percentage values of the AI were calculated by dividing the length of regions with direct contact of the bone to the cement without any intervening connective tissue (contact length) with the total length of the bone–cement interface (total length) multiplied by 100[21] (AI = contact length/total length × 100%). All measurements were performed using image processing and analysis software (Leica Qwin, Leica Microsystems Imaging Solutions Ltd., Cambridge, UK).

The values were expressed as mean ± standard deviation. Statistical analysis was performed using one-way analysis of variance (ANOVA). Additionally, a post hoc test using the Student–Newman–Keuls method was applied. P values less than 0.05 were considered to be statistically significant.

Results

After recovery from the operation, all rabbits were able to bear their own weight and were moving freely within one week. Until the end of the experiments, no failures such as fractures, loosening, or deep infections occurred. In all groups, the fixation of the bone cement to the prosthesis and the bone was stable under load-bearing conditions. This was also confirmed by the X-rays taken after three months (Figure 2).

Histological evaluation

In the groups of the bioactive bone cement and in the control group as well, the cement bonded firmly around the hexagonally shaped surface of the prosthesis in the proximal and distal femur. In wide areas, no intervening soft tissue was observed at the implant–cement interface of all 24 specimens (Figures 3(a) and 4(a)).

Regarding the femur, all Giemsa surface staining sections showed no signs of inflammatory reactions around the cement. In the groups of the bioactive bone cement, the surface of the cement contacted the bone in the most parts without intervening soft tissue (Figure 3(a,b)). In contrast, there was an intervening layer of soft tissue measuring 10–100 µm in most areas of the bone–cement interface in the control group (Figure 4(a,b)).

Similar results were seen in the sections of the proximal tibia. There were more areas of intimate contact between the surface of the cement and the bone around the bioactive cement (Figure 5(a)) compared to the reference PMMA cement (Figure 5(b)).

Affinity index

An overview of the affinity indices of all groups is displayed in Figure 6(a–c). In all three examined regions (proximal femur, distal femur, and proximal tibia), the one-way ANOVA reached significant levels ($p = 0.002$;

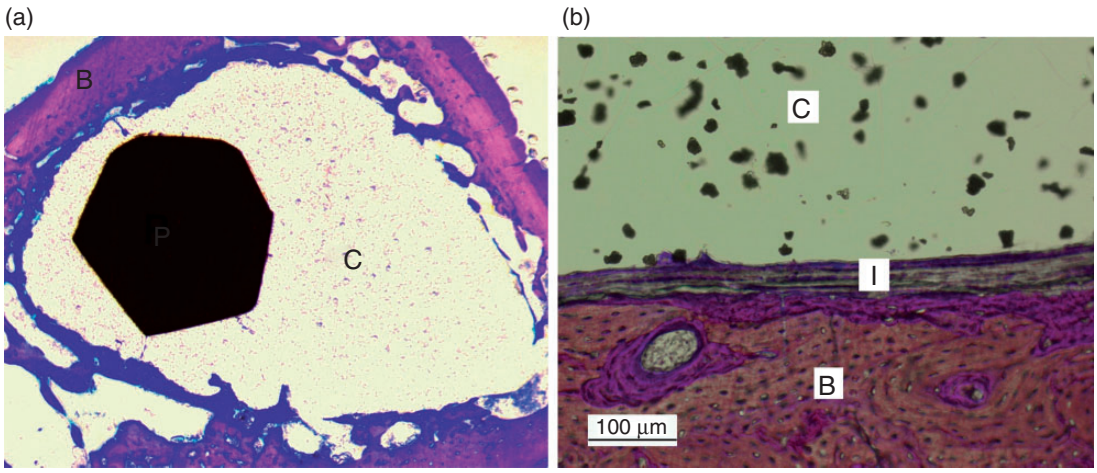

Figure 4. (a,b) Giemsa surface staining of a rabbit femur three months after implantation of a spacer prosthesis using the reference PMMA bone cement. (a) Overview (original magnification × 125) showing a good bonding of the cement (C) to the surface of the prosthesis (P). (b) An intervening layer of soft tissue (I) of at least 10 µm was apparent at the cement–bone interface.

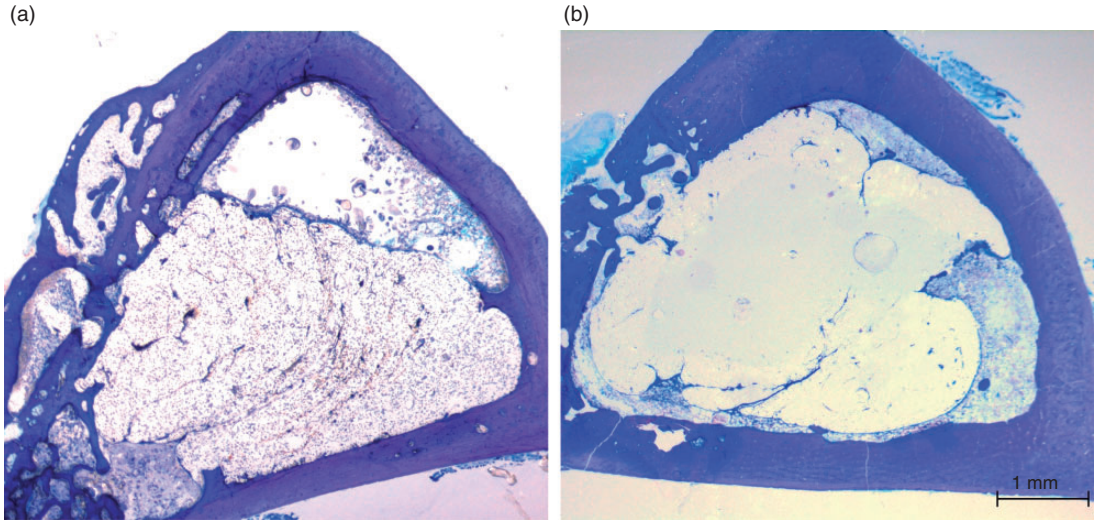

Figure 5. (a,b) Overview of Giemsa surface staining of a rabbit tibia three months after implantation of a cement plug. (a) In many areas around the bioactive cement (group MA3M), a good bonding to the bone could be achieved. (b) Around the reference PMMA cement, a layer of soft tissue was given in most parts of the bone–cement interface.

$p = 0.001$; $p = 0.044$). The Student–Newman–Keuls test revealed a significant difference between each group of the bioactive bone cement and the reference PMMA cement under load-bearing conditions on the proximal and distal femur. On the proximal tibia, a significant level was only achieved between the bioactive cement with solitary HEMA-P added (group MA3M) and the reference PMMA cement.

Discussion

These animal experiments confirmed that the application of biomineralization on a PMMA-based bone cement leads to an in vivo bioactivity of the cement.

In all the three tested regions (with and without load bearing), the AI revealed a significant difference using one-way ANOVA. But the three different variations of the new bioactive bone cement reached different extents of bioactivity. The best result was achieved by the variation of the cement with only HEMA-P added (group MA3M). Only this variation revealed a significant difference compared to the referent PMMA cement in all three regions using the Student–Newman–Keuls method. This result is contrary to previous in vitro studies with incubation of this new bioactive cement in cell culture medium.[18] The addition of $CaCl_2$ and Na_2CO_3 improved the signs of biomineralization in vitro but showed a decreased AI in vivo. A possible

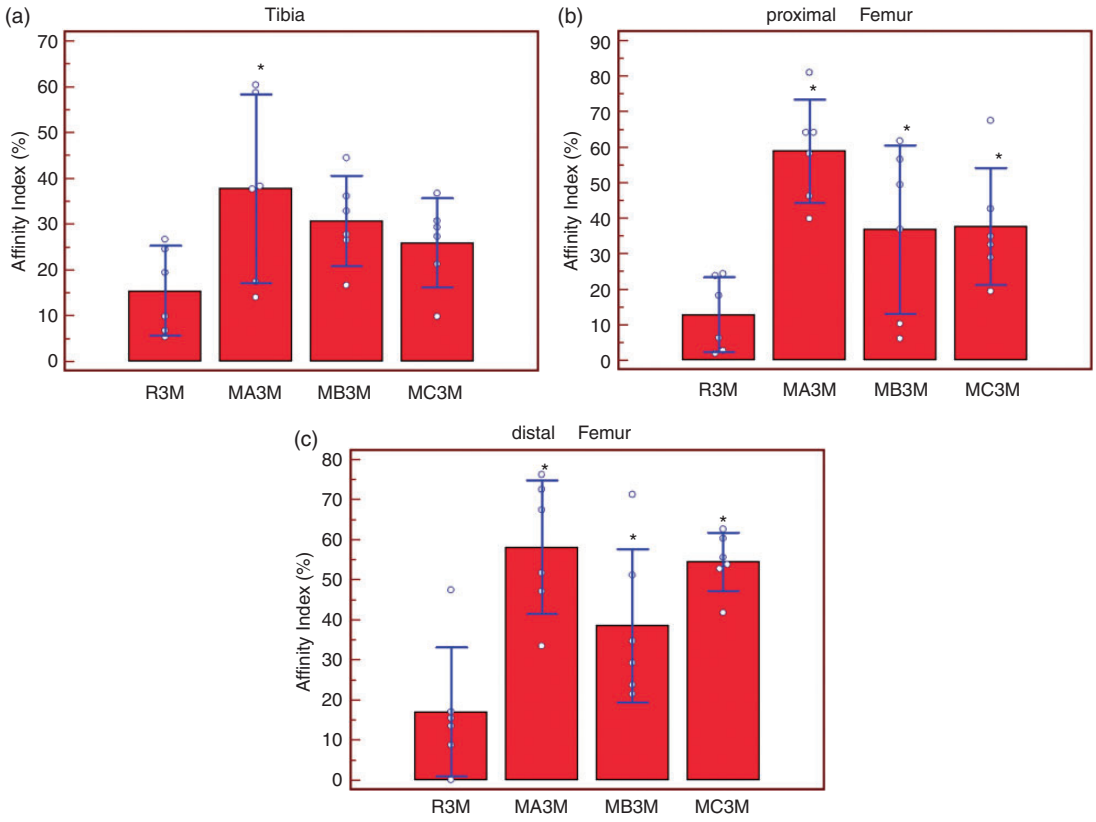

Figure 6. (a–c) Statistical figures of affinity indices of the four different tested bone cements. (a) Tibia, (b) proximal femur, and (c) distal femur. ○ marks each measured affinity index. *Significant difference compared to control group (R3M).

explanation for these findings could be seen in the potential cell toxicity of the admixture of $CaCl_2$. In the previous cell culture experiments, this additive caused a dying off of SaOs-2 cells.[18]

Comparing the achieved extent of bioactivity of this new bioactive bone cement with other bioactive cements is difficult. In many cases a comparison is not possible, because the bone AI was not determined.[14,16,22–24] Regarding the remaining comparable publications,[13,15,25–29] the comparability of the AI is limited by the differences in the experimental setup (different animals, tested regions, load conditions) and different histological examinations (section thickness, scanning electron microscopy vs. light microscopy).

Overall, the extent of bioactivity of this new bone cement is comparable to most of the other tested bioactive bone cements in the literature. In this study, the AI of the bioactive bone cement was 25.9–58.9% compared to 12.8–17.0% for the reference PMMA cement.

Compared to our results, Matsuda et al.[27] achieved an AI of 26.3–38.1% using bis-GMA cement with bioactive glass powder for fixation of hip prostheses in dogs. Fujita et al.[25] also used bioactive bis-GMA cement (with apatite-wollastonite (AW) glass–ceramic) for hip prostheses in dogs reaching affinity indices of 19.2–40.1%. Under unloaded conditions, Mousa et al.[28] revealed an AI of 32.8–68.4% using PMMA cement with different admixtures of AW glass–ceramic in the tibia of rats. Under similar conditions, Shinzato et al.[13] tested PMMA cement with different bioactive powders achieving an AI of 29.9–64.8%. Goto et al.[26] reached an AI of 66.0% after 12 weeks using modified acrylate cement (G2B1) with beta tricalcium phosphate under unloaded conditions in the proximal tibia of rats.

Higher affinity indices were only measured in the studies of Shinzato et al.[29] 2001 and Ni et al.[15] 2006. By adding phosphoric ester to PMMA cement with bioactive glass beads Shinzato et al.[29] could increase the AI up to 79.9% under unloaded conditions. Under load-bearing conditions, only Ni et al.[15] achieved a high AI of 85.1% using bis-GMA cement with strontium-containing hydroxyapatite for the fixation of hip prostheses in rabbits.

None of these mentioned bioactive bone cements could get ready for the market to fix prostheses due to poor mechanical stability or unfavorable ''sticky'' handling properties of bis-GMA cements.[9,10]

Within the scope of these experiments under load-bearing conditions no failure of the specimens,

debonding at the implant–cement interface or cracks in the histological examination of the cement was observed. Hence, the unchanged mechanical properties of this new PMMA-based bioactive bone cement could be confirmed. In previous in vitro studies by Vorndran et al.[17] and Wolf-Brandstetter et al.[18] mechanical tests according to ISO 5833:2002 could confirm unchanged mechanical properties after addition of HEMA-P to commercial PMMA-based cement compared to the plain bone cement. The further admixture of $CaCl_2$ and Na_2CO_3 neither had an influence on the mechanical stability. For example, Wolf-Brandstetter et al.[18] measured a compressive strength of 75.8 MPa for PMMA, 74.5 MPa for PMMA + HEMA-P, and 78.2 MPa for PMMA + HEMA-P + 5% of $CaCl_2$ and Na_2CO_3.

Since only a limited admixture of HEMA-P is necessary to induce in vivo bioactive properties through biomineralization, this cement seems to be suitable for fixation of prostheses. However, further long-term in vivo experiments are needed to evaluate the impact of sustained loading on bone remodeling and fatigue behavior of this new bioactive bone cement.

Conclusion

Using the principle of biomineralization is a new promising way to achieve bioactive bone cements without significant reduction of mechanical properties. According to the results of this in vivo study, a clinical use for stabilization of vertebral body fractures and fixation of cemented knee and hip prostheses might be possible. In order to give a final recommendation, further long-term experiments are needed.

Authors' note

This study was part of the dissertation of Mr. Denis Kitanovic.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

This study was supported by the research Grant (01 EZ 0611) of the German Federal Ministry of Education and Research (BMBF).

References

1. Webb JC and Spencer RF. The role of polymethylmethacrylate bone cement in modern orthopaedic surgery. *J Bone Joint Surg Br* 2007; 89: 851–857.
2. Malchau H, Herberts P, Eisler T, et al. The Swedish total hip replacement register. *J Bone Joint Surg Am* 2002; 84-A: 2–20.
3. Breusch SJ and Malchau H. What is modern cementing technique? In: Breusch SJ, Malchau H (eds) *The well-cemented total hip arthroplasty—theory and practice*. Heidelberg, NY: Springer–Berlin, 2005, pp.146–149.
4. Lewis G. Properties of acrylic bone cement: state of the art review. *J Biomed Mater Res (Appl Biomater)* 1997; 38: 155–188.
5. Jasty M, Maloney WJ, Bragdon CR, et al. Histomorphological studies of the long-term skeletal responses to well-fixed cemented femoral component. *J Bone Joint Surg* 1990; 72A: 1220–1225.
6. Freeman MAR, Bradley GW and Revell PA. Observation upon the interface between bone and polymethylmethacrylate cement. *J Bone Joint Surg* 1982; 64B: 489–493.
7. Noble PC, Collier MB, Maltry JA, et al. Pressurization and centralization enhance the quality and reproducibility of cement mantles. *Clin Orthop Relat Res* 1998; 355: 77–89.
8. Dohmae Y, Bechtold JE and Sherman RE. Reduction in cement–bone interface shear strength between primary and revision arthroplasty. *Clin Orthop* 1988; 236: 214–220.
9. Kenny SM and Buggy M. Bone cements and fillers: a review. *J Mater Sci Mater Med* 2003; 14: 923–938.
10. Harper EJ. Bioactive bone cements. *Proc Inst Mech Eng Part H* 1998; 212: 113–120.
11. Heikkila JT, Aho AJ, Kangasniemi I, et al. Polymethylmethacrylate composites: disturbed bone formation at the surface of bioactive glass and hydroxyapatite. *Biomaterials* 1996; 17: 1755–1760.
12. Hennig W, Blencke BA, Brömer H, et al. Investigations with bioactivated polymethylmethacrylate. *J Biomed Mater Res* 1979; 13: 89–99.
13. Shinzato S, Kobayashi M, Mousa WF, et al. Bioactive polymethyl methacrylate-based bone cement: comparison of glass beads, apatite- and wollastonite-containing glass-ceramic, and hydroxyapatite fillers on mechanical and biological properties. *J Biomed Mater Res* 2000; 51: 258–272.
14. Okada Y, Kobayashi M, Neo M, et al. Ultrastructure of the interface between bioactive composite and bone: comparison of apatite and wollastonite containing glass–cer amic filler with hydroxyapatite and beta-tricalcium phosphate fillers. *J Biomed Mater Res* 2001; 57: 101–107.
15. Ni GX, Lu WW, Chiu KY, et al. Strontium-containing hydroxyapatite (Sr-HA) bioactive cement for primary hip replacement: an in vivo study. *J Biomed Mater Res* 2006; 77B: 409–415.
16. Senaha Y, Nakamura T, Tamura J, et al. Intercalary replacement of canine femora using a new bioactive bone cement. *J Bone Joint Surg* 1996; 78B: 26–31.
17. Vorndran E, Spohn N, Nies B, et al. Mechanical properties and drug release behavior of bioactivated PMMA cements. *J Biomater Appl* 2012; 26: 581–594.
18. Wolf-Brandstetter C, Roessler S, Storch S, et al. Physicochemical and cell biological characterization of PMMA bone cements modified with additives to increase bioactivity. *J Biomed Mater Res B Appl Biomater* 2013; 101: 599–609.

19. Kawai T, Ohtsuki C, Kamitakahara M, et al. Coating of an apatite layer on polyamide films containing sulfonic groups by a biomimetic process. *Biomaterials* 2004; 25: 4529–4534.
20. Kokubo T, Hanakawa M, Kawashita M, et al. Apatite formation on non-woven fabric of carboxymethylated chitin in SBF. *Biomaterials* 2004; 25: 4485–4488.
21. Hayashi K, Uenoyama K, Matsuguchi M, et al. The affinity of bone to hydroxyapatite and alumina in experimentally induced osteoporosis. *J Arthroplasty* 1989; 4: 257–262.
22. Fukuda C, Goto K, Imamura M, et al. Bone bonding ability and handling properties of a titania-polymethylmethacrylate (PMMA) composite bioactive bone cement modified with a unique PMMA powder. *Acta Biomater* 2011; 7: 3595–3600.
23. Ni GX, Choy YS, Lu WW, et al. Nano-mechanics of bone and bioactive bone cement interfaces in a load-bearing model. *Biomaterials* 2006; 27: 1963–1970.
24. Sugino A, Miyazaki T, Kawachi G, et al. Relationship between apatite-forming ability and mechanical properties of bioactive PMMA-based bone cement modified with calcium salts and alkoxysilane. *J Mater Sci Mater Med* 2008; 19: 1399–1405.
25. Fujita H, Ido K, Matsuda Y, et al. Evaluation of bioactive bone cement in canine total hip arthroplasty. *J Biomed Mater Res* 2000; 49: 273–288.
26. Goto K, Shinzato S, Fujibayashi S, et al. The biocompatibility and osteoconductivity of a cement containing beta-TCP for use in vertebroplasty. *J Biomed Mater Res A* 2006; 78: 629–637.
27. Matsuda Y, Ido K, Nakamura T, et al. Prosthetic replacement of the hip in dogs using bioactive bone cement. *Clin Orthop Relat Res* 1997; 336: 263–277.
28. Mousa WF, Kobayashi M, Shinzato S, et al. Biological and mechanical properties of PMMA-based bioactive bone cements. *Biomaterials* 2000; 21: 2137–2146.
29. Shinzato S, Nakamura T, Tamura J, et al. Bioactive bone cement: effects of phosphoric ester monomer on mechanical properties and osteoconductivity. *J Biomed Mater Res* 2001; 56: 571–577.