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Acrylic Bone Cements: Effects of the Poly(methyl methacrylate) Powder Size and Chitosan Addition on Their Properties

Tugba Endogan,[1,2] Aysel Kiziltay,[2,3] Gamze Torun Kose,[4,8] Nil Comunoglu,[5] Tahsin Beyzadeoglu,[6] Nesrin Hasirci[1,3,7,8]

[1]Graduate Department of Polymer Science and Technology, Middle East Technical University, Ankara 06800, Turkey
[2]Central Laboratory, Middle East Technical University, Ankara 06800, Turkey
[3]Graduate Department of Biotechnology, Middle East Technical University, Ankara 06800, Turkey
[4]Department of Genetics and Bioengineering, Faculty of Engineering and Architecture, Yeditepe University, Istanbul 34755, Turkey
[5]Department of Pathology, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul 34098, Turkey
[6]Department of Orthopaedics and Traumatology, Faculty of Medicine, Yeditepe University, Istanbul 34755, Turkey
[7]Department of Chemistry, Middle East Technical University, Ankara 06800, Turkey
[8]BIOMATEN—Center of Excellence in Biomaterials and Tissue Engineering, Ankara 06800, Turkey
Correspondence to: N. Hasirci (E-mail: nhasirci@metu.edu.tr)

ABSTRACT: The effect of the particle size of poly(methyl methacrylate) (PMMA) and the incorporation of chitosan (CH) on the mechanical and thermal properties and the biocompatibility of acrylic bone cements were investigated. Three groups of bone cements were prepared with different PMMA particles. Groups 1 (BC1) and 2 (BC2) contained ground and sieved PMMA with particle sizes in the ranges 50–150 μm and 1–50 μm, and group 3 (BC3) contained synthesized PMMA microspheres with a size of about 1 μm. The mechanical properties of the three groups were similar, but their curing properties were significantly affected. The presence of CH improved the mechanical and thermal properties. For the BC1 group, the compressive strength increased more than 10 MPa, and the curing temperature decreased 12°. The cement having the optimum properties (BC1) was applied to rats, where it enhanced the bone bonding ability, and bioactivity was observed. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2013

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INTRODUCTION

Bone cements have been used as clinical grouting agents since the 1960s in orthopedic and dental applications for stabilizing implants with promising results. The main functions of acrylic bone cement are to serve as an interfacial phase between the implant and the bone and homogeneously transfer and distribute the body weight loads and cyclic loads to the whole skeleton.[1] There are various forms of bone supports, either as cements or scaffolds, which can be applied either as inorganic powders, polymers, or combinations of both.[2,3]

Acrylic cements are two-component, self-curing systems obtained from a mixture of a powder part, mainly containing poly(methyl methacrylate) (PMMA) polymer, and an initiator and liquid part, mainly containing methyl methacrylate (MMA) monomer and an activator. Bone cement pastes are easily molded and adapted to complex bone cavities or used in orthodontic applications to restore dental damage. In addition, PMMA can be modified by plasma to control the adsorption of human saliva proteins.[4] The main advantages of the use of cement are the excellent primary fixation between the bone and implant and, therefore, the faster recovery of the patient. Despite the good success rate of implant fixation with bone cements, they have some disadvantages. Local tissue damage due to exothermic polymerization reactions, a sudden drop of blood pressure as a result of the leaching of unreacted monomer, the high shrinkage of the cement after polymerization, and the stiffness mismatch between the bone and the cement are some drawbacks associated with PMMA-based bone cements.[5–7] Another problem is bone cement fracture, which is the main reason for its mechanical failure and the aseptic loosening of implants. It has been shown that PMMA bone cement cannot form covalent bonds with natural bone, and a lack of interactions may cause the loosening of the implant after a period of

Table I. Particle Sizes of the PMMA Used in the Formulations

Sample	PMMA	Particle size range (μm)	Average particle size (μm)
BC1	Commercial polymer, sieved powder	50–150	77
BC2	Commercial polymer, sieved powder	0–50	21
BC3	Synthesized polymer, microsphere	~1	1

time. A temperature increase in the surrounding tissues, in some cases of up to 90°C, also prevents the proper binding of cement to the tissue. These make the long-term stability of the cement questionable.[8,9] There is still ongoing research to improve the mechanical, thermal, and biological properties of bone cements to increase the performance and longevity of cemented prostheses. The preparation of cements with different mixing methods or the incorporation of additives, such as fibers,[10] mineral particles,[11–13] polymers,[14] or drugs,[15] to cements are reported. Among these modifications, the addition of bioactive fillers, such as hydroxyapatite (HAp)[16,17] or cuttlebone particles,[18,19] to enhance bioactivity has been extensively studied. It has also been reported that the application of plasma to PMMA films enhanced cell attachment to the surfaces and increased their biocompatibility.[20,21]

One bioactive filler is HAp, which forms the inorganic part of bone. Since HAp is biocompatible, bioactive, and osteoconductive, it strongly integrates with bone and encourages bone regeneration, forming bonds directly to the natural tissue. Therefore, the addition of HAp is preferred to enhance biocompatibility and mechanical strength of bone cements.[11,22] However, the mechanical and thermal properties of bone cement depend on the amount, size, and surface properties of the added HAp particles. An excess amount of HAp causes phase separation and worsens the quality and workability of the dough. Therefore, it is important to add an appropriate amount of HAp.

For any bone-supporting material, the presence of biodegradable substances may create pores as a result of hydrolytic and enzymatic degradation in biological media. As the biodegradable polymer disappears, new bone tissue fills the created pores. The migration of cells to these pores results in a stronger attachment between the bone and cement. This kind of partially biodegradable acrylic bone cement was developed by the modification of the formulations of the powder part with different biodegradable materials, such as poly(L-lactic acid), poly(β-hydroxybutyrate), starch, cellulose acetate, chitosan (CH), and gelatin.[14,23–26] CH is a natural polymer produced by the deacetylation of chitin, which is found in the exoskeleton of crustaceans such as crabs and shrimp and the cell walls of fungi. It has become an attractive polymer for biomedical applications because it is biocompatible, biodegradable, and antimicrobial. CH-derivative-coated methacrylate thermosets have been used for orthopedic and dental applications to enhance osseointegration, and it was shown that CH induced osteoblast proliferation and increased the alkaline phosphatase activity.[27] It was also reported that CH improved osseous healing and stimulated cell proliferation.[28]

In this study, we focused on the properties of acrylic bone cements prepared from PMMA particles having different sizes with the addition of inorganic HAp fillers and biodegradable CH polymer to enhance these thermal, mechanical, and biocompatibility properties. The compositions were optimized; those selected were used for *in vivo* applications on rat knees to examine their biological effects.

EXPERIMENTAL

Materials

In the synthesis of PMMA microspheres, MMA monomer (Acros Organics, New Jersey), poly(vinyl alcohol) (molecular weight = 88.000, Acros Organics), benzoyl peroxide (BPO; Sigma-Aldrich Chemie, Steinheim, Germany), and technical-grade ethanol (Tekel Sincan Organize Sanayi, Ankara, Turkey) were used. PMMA (Sigma-Aldrich Chemie), with an average molecular weight of 120.000, was used after grinding and sieving. Other chemicals used in cement preparations were HAp (Riedel-de Haën A.G., Seelze, Germany), barium sulfate ($BaSO_4$, Merck, Darmstadt, Germany), and *N,N*-dimethyl-*p*-toluidine (DMPT; Sigma-Aldrich Chemie). All chemicals, except MMA, were used as obtained without further purification. MMA was washed with a 10 wt % aqueous sodium hydroxide (J. T. Baker, Deventer, Holland) solution to remove the inhibitor before use.

Preparation of the PMMA Particles

The PMMA powder used in this study was prepared by two methods. In the first method, fine PMMA powder was obtained by the grinding of commercial PMMA particles with a water-cooled analytical mill (Tekmar, Janke and Kunkel GMBH Co. KG, Staufeb, Germany). The obtained powder was sieved first through 150-μm and then 50-μm sized sieves. Two groups of powders with different sizes were obtained. The cements prepared from PMMA powder with sizes of 50–150 and 0–50 μm were assigned as the BC1 group and BC2 group, respectively. In the second method, PMMA microspheres were synthesized according to a previously applied technique.[17] Briefly, a suspension polymerization was applied to MMA in ethanol/water (50/50 v/v) media with BPO as the initiator and poly(vinyl alcohol) as the stabilizer. The cements prepared with these microspheres were assigned as the BC3 group. Table I summarizes the types and particle sizes of all of the PMMA particles used in the bone cement formulations. The topographic shapes of the PMMA particles and microspheres were examined by scanning electron microscopy (SEM; FEI, Quanta 400F field emission scanning electron microscope, The Netherlands).

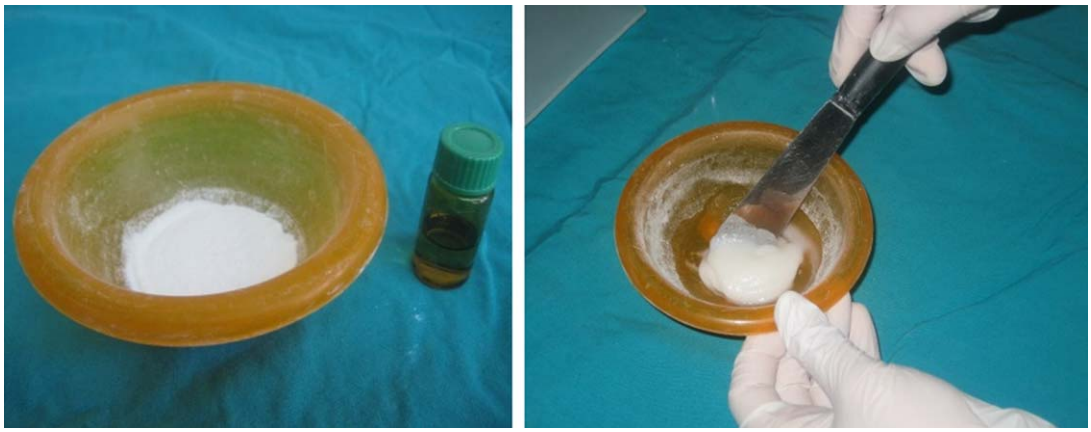

Figure 1. Representation of the bone cement preparation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Bone Cement Preparation

Acrylic bone cements are two-component systems and are formed upon the mixture of the liquid and powder parts for 2–3 min to obtain a workable dough for application to the patient. For bone cement preparation, either ground PMMA or microspheres of PMMA were mixed in a polypropylene cup with HAp, $BaSO_4$, and BPO to compose the powder part. Onto this mixture, the liquid part, consisting of MMA and DMPT, was added. All of the cement samples were prepared by hand mixing with a proper consistency, as shown in Figure 1.

The compositions of the prepared bone cement formulations are given in Table II. In all of the experiments, a constant amount of HAp (168 mg), $BaSO_4$ (604 mg), BPO initiator (45 mg), and DMPT accelerator (56 μL) were used. For the BC1 and BC2 groups, a half amount of MMA monomer was used for every gram of PMMA polymer. For group BC3, 1.5 times the amount of MMA monomer was used for every gram of PMMA polymer because more monomer was needed to wet the total microspheres because of the significantly higher surface-to-volume ratio of this group samples compared to the BC1 and BC2 groups. Modification with CH addition was achieved by the addition of two different amounts of CH (0.05 g of CH for the CH1 group and 0.1 g of CH for the CH2 group per gram of PMMA) into the powder part of the cement.

Table II. Formulations of the Prepared Bone Cements

Sample	Polymer/monomer (g/mL)	CH/PMMA (g/g)
BC1	2/1	—
BC1-CH1	2/1	0.05
BC1-CH2	2/1	0.1
BC2	2/1	—
BC2-CH1	2/1	0.05
BC2-CH2	2/1	0.1
BC3	2/3	—
BC3-CH1	2/3	0.05
BC3-CH2	2/3	0.1

Mechanical Tests

Tension and compression tests were performed to examine the mechanical properties of the prepared bone cement samples. Mechanical tests were performed with a Lloyd LRX 5K testing machine (Lloyd Instruments, Ltd., Fareham, Hampshire, United Kingdom) with a cell load of 5000 N at room temperature.

For the preparation of the tension test samples, the cement dough was rolled on a polyethylene surface, cut with dog-bone-shaped mold ($5 \times 0.5 \times 0.5$ cm^3) and allowed to cure for 1 h at room temperature. Then, the specimens were kept in physiological saline solution in a temperature-controlled water bath for 24 h at 37 ± 1°C before the mechanical tests. A tension force was applied with a crosshead speed of 1 mm/min at room temperature. For each sample, at least five specimens were tested, and the average values were obtained.

For compression tests, the samples were prepared by the pressing of the soft dough in a stainless steel mold, which had 56 holes with diameters of 6 mm, as described previously.[17] Tests were performed with a crosshead speed of 25 mm/min at room temperature. For each sample, at least eight specimens were tested, and their average values were obtained.

Thermal Analysis

The maximum curing temperatures of the bone cements were measured with a thermocouple input module (SuperLogics, Natick). J-type thermocouple wires were cut into equal pieces of 5 cm, and one end was electrically welded to form a thermocouple junction. The cement dough was prepared and rounded to give a spherical shape with a radius of about 15 mm. Then, the welded end of the thermocouple, used as a temperature sensor, was placed in the center of the dough. The other end was connected to a data-acquisition device controlled by a computer. The temperature was recorded for 1200 s with a 1 data/s recording rate. Temperature versus time graphs showing the exothermic temperature changes were obtained for each sample. A typical curve is given in Figure 2. The peak temperature was the maximum temperature reached during the polymerization. The setting time of the bone cement was defined as the time when the temperature rise was at the halfway point between the maximum temperature (T_{max}) and the ambient temperature

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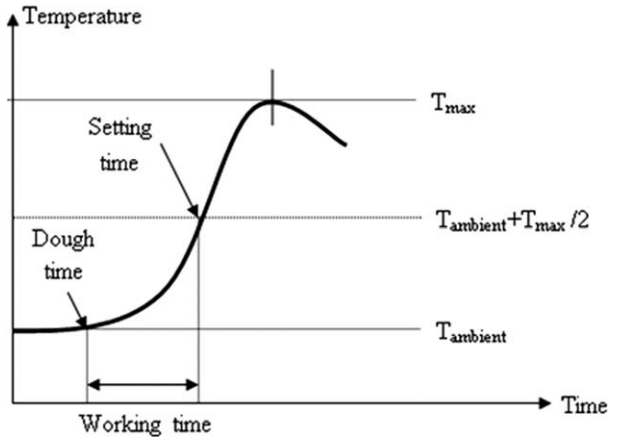

Figure 2. Typical temperature–time graph.

($T_{ambient}$), which was about 23°C. The setting temperature ($T_{setting}$) could be calculated with the following equation:

$$T_{setting} = (T_{ambient} + T_{max})/2$$

In Vivo Biological Activity Tests

After the measurements of the mechanical and thermal properties, BC1 and CH containing the BC1–CH1 (0.05 g of CH/g of PMMA) bone cements were chosen for *in vivo* tests with a rat model, and the biocompatibility results were evaluated and compared with those of the commercial CMW1 bone cement. Rats were anesthetized by intramuscular injection of Ketamine/xylazine. The skin of the animal was shaved and cleaned with Betadine. Defects were formed on both knees of the rats by anterior longitudinal incision. The powder and liquid parts of the bone cements were mixed, and the dough was immediately inserted with gentle pressing in a sufficient amount to fill the bone defect cavity on the right knees, whereas left knees were left unfilled as a control (Figure 3).

The cements were allowed to set *in vivo* after implantation, and then, the incisions were closed with a suture. A prophylactic antibiotic was applied to each animal to prevent postoperative infections. The animals were sacrificed 4 weeks after surgery. Histological examinations were performed on excised tissue, which was fixed in 10% buffered formalin, decalcified, and embedded in paraffin. Excised tissues having 5 μm thicknesses were stained with hematoxylin and eosin and examined under light microscopy (Olympus, Japan).

RESULTS AND DISCUSSION

PMMA Particles

Various acrylic bone cement formulations were prepared with PMMA powders having three different particle sizes; these were ground and sieved polymer particles with sizes of 50–150 μm (BC1 group) and 0–50 μm size (BC2 group) and synthesized microspheres with a size of 1 μm (BC3 group). SEM micrographs of the PMMA particles and microspheres are given in Figure 4. The particles prepared by grinding and sieving had irregular shapes and nonhomogeneous particle surfaces, and they were used in the preparation of the BC1 and BC2 group

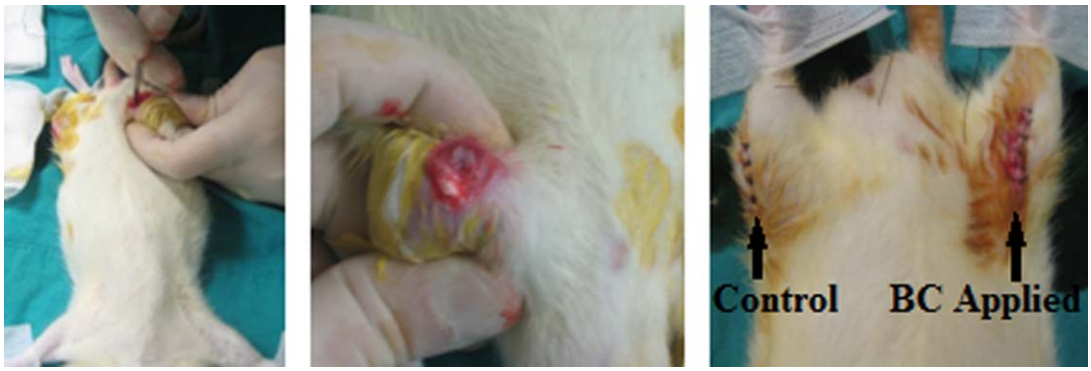

Figure 3. Application of bone cement to the knees of rats. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

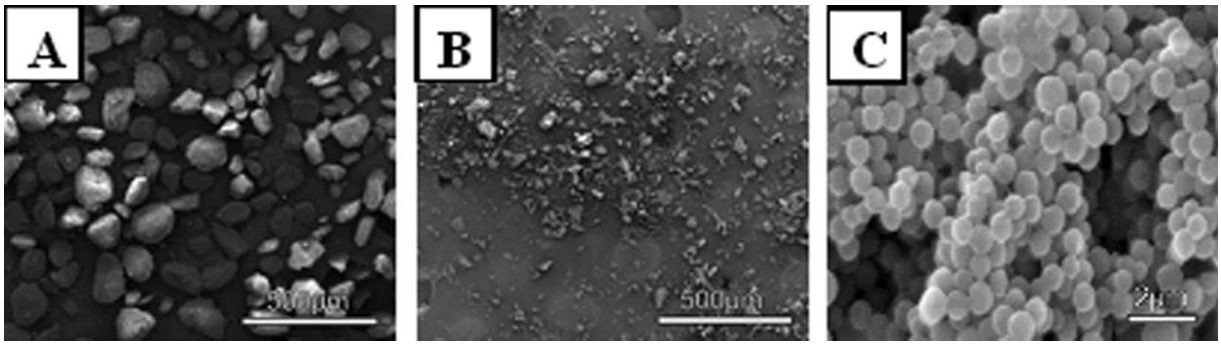

Figure 4. SEM micrographs of the PMMA particles: (A) 50–150, (B) 0–50, and (C) 1 μm.

Table III. Mechanical Properties of the Prepared Bone Cements

		Tensile properties		Compressive properties	
Sample	CH/PMMA (g/g)	UTS (MPa)	E_T (GPa)	UCS (MPa)	E_C (GPa)
BC1	—	19.65 ± 2.70	0.39 ± 0.05	81.51 ± 3.43	0.57 ± 0.04
BC1—CH1	0.05	20.16 ± 2.58	0.46 ± 0.02	94.04 ± 3.77	0.57 ± 0.03
BC1—CH2	0.1	18.72 ± 4.17	0.46 ± 0.07	96.62 ± 4.70	0.58 ± 0.04
BC2	—	19.77 ± 1.78	0.39 ± 0.04	75.37 ± 7.47	0.48 ± 0.05
BC2—CH1	0.05	17.53 ± 0.81	0.41 ± 0.04	98.40 ± 6.02	0.55 ± 0.06
BC2—CH2	0.1	19.66 ± 3.11	0.42 ± 0.07	89.29 ± 7.89	0.57 ± 0.02
BC3	—	18.27 ± 5.14	0.38 ± 0.04	75.96 ± 2.21	0.53 ± 0.03
BC3—CH1	0.05	23.53 ± 3.25	0.43 ± 0.06	80.26 ± 6.07	0.59 ± 0.02
BC3—CH2	0.1	22.72 ± 4.20	0.42 ± 0.05	81.64 ± 7.14	0.63 ± 0.02
CMW1	—	20.05 ± 2.98	0.51 ± 0.06	100.20 ± 5.45	0.56 ± 0.04

bone cement formulations. The use of blades during grinding caused microparticles with random edges. The particles synthesized by suspension polymerization were very homogeneous and monodisperse with perfect spherical shapes and with sizes of approximately 1 μm. These small-sized particles were used in the preparation of the BC3 group bone cement formulations.

In the bone cement preparation, the particle size of the PMMA powder greatly affected the quality, handling, and setting properties of the resulting dough. In case of formulations prepared with particles larger than 150 μm, large particles did not dissolve homogeneously in the monomer component and caused phase separation. Therefore, a uniform and workable cement dough could not be obtained. In the literature, it was reported that commercial bone cement formulations contained PMMA particles with average particle sizes in the range 30–150 μm [e.g., Simplex P (34 μm), CMW1 (44 μm), and Palacos R-40 (55 μm)].[29,30]

Mechanical Properties

The powder part, composed of PMMA polymer, BPO initiator, barium sulfate radiopaque agent, and HAp, was mixed with the liquid part, consisting of the MMA monomer and DMPT accelerator. The powder part of acrylic bone cement was modified with CH incorporation. Then, the effects of modification on the mechanical and thermal properties were investigated. To examine the mechanical properties, tension and compression tests were applied, whereas to examine the thermal properties, the maximum curing temperatures and setting times were studied.

The obtained tensile and compressive test results are given in Table III. It was observed that CH addition did not have a significant effect on the tensile strength. The ultimate tensile strength (UTS) values of the BC1 and BC2 group cements remained almost the same, whereas the UTS value increased from 18.27 to 22.72 MPa for the BC3 group with the addition of CH. The tensile elastic modulus (E_T) values increased about 18, 8, and 11% for the BC1, BC2 and BC3 formulations, respectively, with CH incorporation. When the bone cement formulations prepared with different PMMA particles were considered, we observed that the particle size of the polymer did not have a significant effect on the mechanical properties.

The ultimate compression strength (UCS) values increased from 81.51 to 96.62 MPa, from 75.37 to 89.29 MPa, and from 75.96 to 81.64 MPa for BC1, BC2, and BC3, respectively, upon CH addition. The compressive elastic modulus (E_C) values also increased 2, 19, and 19% for the BC1, BC2, and BC3 bone cement formulations, respectively, with CH incorporation. We concluded that CH acted as a load carrier and improved compressive properties in all of the bone cement groups. CH has a rigid D-glucosamine structure, a high crystallinity, and the ability to form hydrogen bonds, and these properties lead to a high mechanical stability. CH also has a high resistance to heat because of its strong intramolecular hydrogen bonds. CH is expected to degrade *in vivo* over time as new bone tissue forms; this leads to a stronger bond between the bone and bone cement and extends the survival of implants. Several studies in the literature reported that CH increased the mechanical properties of composites.[31,32] Hansen and Jensen[33] reported a typical range of values for the compressive strength of bone cement as 80–105 MPa, and the compressive strength values of CH-containing bone cements are within this range. It was observed that all of the prepared bone cements fulfilled the minimum compressive strength (70 MPa) requirement specified by the ASTM standard specification for acrylic bone cement.[34] The results show that the prepared cement formulations had enough mechanical strength for clinical applications. The mechanical properties of commercially available bone cement CMW1 were also measured, and the tensile strength and compressive strength were found to be 20.05 and 100.20 MPa, respectively. The tensile strength values were similar to those of the prepared cement formulations, whereas the compressive strength value of CMW1 was about 6.6% higher than the UCS value of the BC1–CH1 group. On the other hand, the compressive strength values of all of the prepared cement formulations were in an acceptable range according to the ASTM standard.

Thermal Properties

The polymerization of the MMA–PMMA system is highly exothermic and leads to an increase in the local temperature. This

Table IV. Thermal Properties of the Prepared Bone Cements

Bone cement	CH/PMMA (g/g)	Thermal properties	
		T_{max} (°C)	$T_{setting}$ (s)
BC1	—	71.60 ± 9.31	312 ± 17
BC1-CH1	0.05	68.58 ± 8.92	253 ± 28
BC1-CH2	0.1	59.04 ± 9.59	274 ± 19
BC2	—	83.48 ± 7.35	190 ± 20
BC2-CH1	0.05	85.40 ± 4.73	174 ± 7
BC2-CH2	0.1	86.78 ± 3.73	180 ± 11
BC3	—	116.24 ± 4.94	406 ± 8
BC3-CH1	0.05	123.12 ± 4.16	456 ± 34
BC3-CH2	0.1	116.32 ± 5.14	395 ± 32
CMW1	—	96.03 ± 3.78	405 ± 10

increase in temperature is dependent on the MMA to PMMA ratio, the composition of the liquid and solid components, the concentrations of the initiator and accelerator, the presence of a chain-transfer agent, and the particle size of the PMMA. When the maximum curing temperature is high, significant damage in the surrounding bone tissue occurs after the application of the bone cements. Therefore, scientists are trying to make new formulations to lower the curing temperature without destroying the mechanical properties of the cements. In this study, the maximum curing temperatures and setting times of the bone cements varied with the particle size of the PMMA particles and CH addition (Table IV). The maximum curing temperature was lower in group BC1 than in group BC2 and BC3 with or without CH addition, and in the BC1 group, the presence of CH caused a decrease from 71.60 to 59.04°C. This could be attributed to the presence of CH and the size of the PMMA particles, which both acted as a heat sink and absorbed the released heat. It was stated in literature that the maximum curing temperature decreased when the polymer particle size became larger. In one study,[10] the T_{max} value of a bioactive titania–PMMA composite bone cement was lowered to 74°C with large PMMA particles with a diameter of 34 μm. PMMA particles larger than 50–60 μm could absorb the produced heat during the setting process, and those smaller than 20 μm underwent complete dissolution in the polymerizing MMA medium; therefore, it may have caused increases in the viscosity and curing temperature of the cement.[35] However, on the other hand, for the BC2 and BC3 groups, CH caused an increase in the curing temperature. When the size got smaller, the surface-to-volume ratio of the particles and their interaction with the MMA monomer increased; this caused a rise in the heat generation (as in BC3). On the other hand, CH acted as a heat sink and decreased the curing temperature. In the BC1 group, the effect of CH and, in the BC3 group, the effect of particle size were dominant. These two factors compensated each other for the BC2 samples. It was clear that less damage in the surrounding bone tissue occurred during *in situ* applications when the maximum curing temperature was lower. Moreover, the maximum curing temperature values were quite high between 116 and 123°C in the BC3 group. It should be noted that for the BC3 group, there were two factors affecting the results. The monomer-to-polymer ratio was higher, and the particle size was smaller (1 μm) compared to the other groups; this may have led to the complete dissolution of the particles in its monomer. Both factors can cause higher curing temperatures and increase the polymerization rate and decrease the absorption of released heat by particles.

As given in Table IV, the setting times of the BC1–CH1 and BC2–CH1 samples were shorter than those of the BC1 and BC2 samples. When the monomer-to-powder ratio was considered, the amount of MMA monomer in CH containing bone cements was lower because the addition of CH increased the amount of powder part. In the literature, it was stated that the polymerization time got longer when the amount of MMA monomer increased.[36] As a result, the decrease in the monomer-to-powder ratio might have been the reason for the shorter setting time of the BC1–CH1 and BC2–CH1 samples compared with those of the BC1 and BC2 samples. When the amount of CH increased, the setting time of samples elongated. This might have been because of the inhibition effect of the increased CH amount on the polymerization reaction of the MMA monomer. In the literature, a similar trend was reported for different kinds of additives. It was reported that the addition of magnetite particles shortened the setting time but then increased with increasing magnetite particle concentration. This behavior was explained by the fact that as the inhibition effect of particles on the polymerization reaction of MMA became significant with increased amount of magnetite particles.[36]

When the BC1, BC2, and BC3 groups were compared, the setting time of BC3 was found to be longer than the others. This was most probably because of the longer time requirement of higher amounts of MMA monomer for complete polymerization. On the other hand, the thermal properties of the CMW1 commercial bone cement were also examined, and the maximum curing temperature was found to be 96.03°C; this was higher than that of the BC1 group bone cement samples.

In Vivo Tests

In bone cement preparation, the amount of additives and the particle size of the powder highly affected the quality and handling properties of the resulting dough. We observed that the bone cement composed of PMMA, with particle sizes between 50 and 150 μm (BC1), seemed to be more favorable for the mechanical, thermal, and handling properties compared to the BC2 and BC3 group samples. Therefore, the CH-containing BC1 group bone cement formulation was chosen, and further, *in vivo* tests were carried out with these samples.

For *in vivo* tests, the bone cements with and without CH incorporation (the BC1 and BC1–CH1 groups) were applied to the defects formed on the rat knee. After 4 weeks, histological examination was done on the excised tissue sections, and the interface region between the implant and the cancellous bone was examined for fibrous tissue and new bone formation between the osseous tissue and the bone cement.

Microscopic images of neighborhood tissue 4 weeks after the implantation of the bone cements are given in Figure 5. In

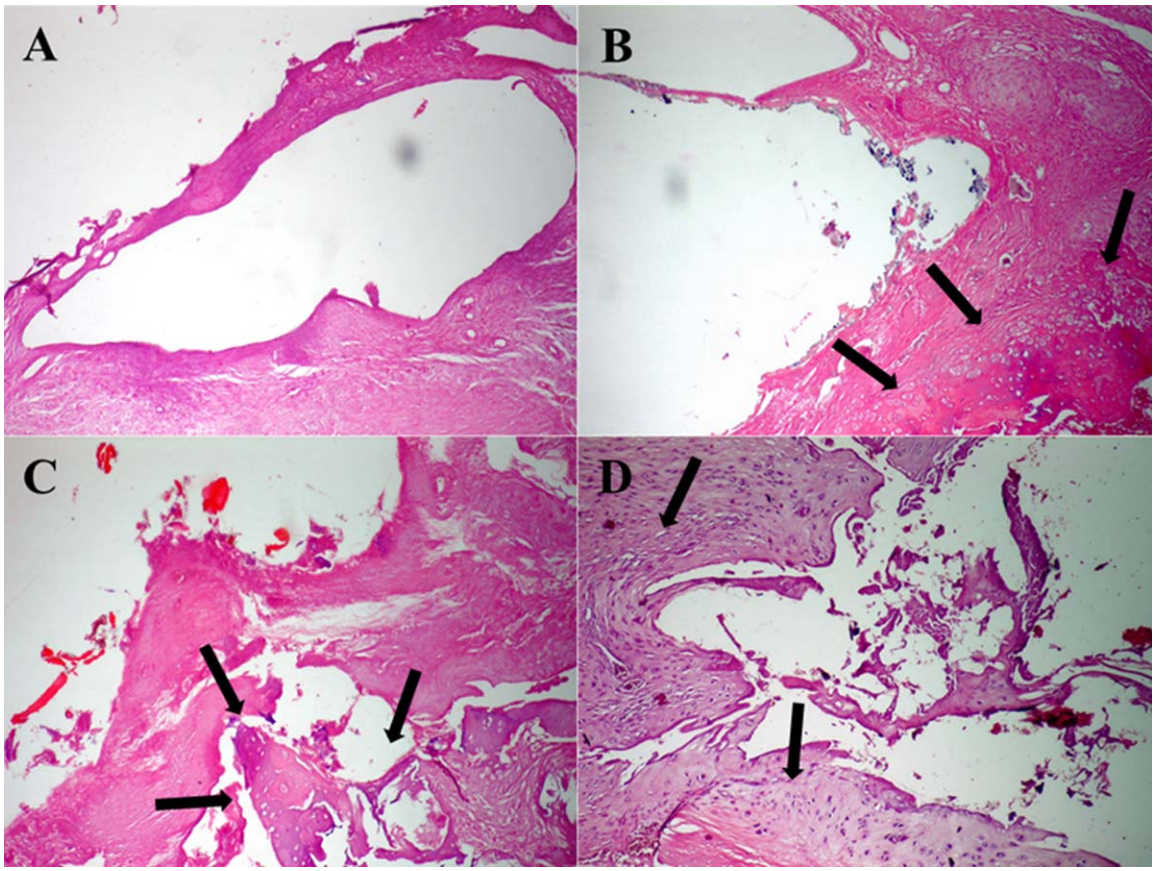

Figure 5. Microscopic images of neighborhood tissue 4 weeks after the implantation of the bone cements: (A) control, (B) BC1, (C) BC1–CH1, and (D) CMW1 (arrows show new osteoid formation). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

histology, osteoid is the unmineralized, organic portion of the bone matrix that forms before the maturation of bone tissue. Osteoblasts begin the process of forming bone tissue by secreting the osteoid. When the osteoid becomes mineralized, it and the adjacent bone cells develop into new bone tissue. In the control group (with no cement), a normal cavity in the fibrous tissue was observed, whereas there was no osteoid formation. CH-modified bone cement samples (BC1–CH1) were surrounded by newly formed osteoid bone [Figure 5(C)], whereas the BC1 cement samples were surrounded with fibrous tissue with a few focal osteoid formations [Figure 5(B)]. New osteoid formation is shown with black arrows. The lack of fibrous tissue interface between the host bone and the implant material is an indication of good integration of the CH-modified cement sample with the bone tissue. In the case of the CMW1 cement sample, similar to BC1–CH1 samples, new bone formation was observed in many areas around the implant. New osteoid formation was observed only in 40% of the samples treated with BC1 bone cements, whereas all of the samples treated with CH-containing (BC1–CH1) and commercial CMW1 bone cements showed new osteoid formation after 4 weeks of application in the defected area.

CH and various calcium phosphates have been extensively used together in composite materials to combine the advantages of the two. The favorable effects of CH, when used in composites, have been proven in many investigations.[37,38] Its positive contributions were also shown as coating materials for titanium implants. Park et al.[39] investigated the effect of the surface chemistry on the response of human MG63 osteoblast-like cells and found that CH-coated microstructured titanium surfaces induced a greater osteoblast-specific protein production compared to the uncoated ones. CH with a positive-charge (NH_3^+) surface induced a greater osteocalcin and osteoprotegerin production than other coating components; this suggested that the surface chemical composition played a role in controlling the osteoblast differentiation.

CONCLUSIONS

It is important to adjust the mechanical, thermal, and handling properties of acrylic bone cement by modifying the composition so that the bone cement will be bioactive, biocompatible, and suitable for orthopedic and orthodontic applications. In this study, PMMA particles with various sizes were used in cement preparation, and CH was incorporated into the formulations to examine the effects on the mechanical, thermal, and biological properties. The use of differently sized PMMA particles did not cause a significant change in the mechanical properties, although the curing parameters were altered. CH did not have a significant effect on the tensile properties but had a positive effect on the compressive strength. Moreover, it reduced the

maximum curing temperature from 71.60 to 59.04°C when it was added to the BC1 group. In addition, CH is expected to degrade *in vivo* over time as the new bone tissue forms; this will lead to a stronger bond between the host bone and bone cement and extend the survival of the implant. Therefore, we concluded that use of a CH-containing construct in bone cement formulations appears to be an advantageous method for enhancing the physical properties and *in vivo* osteogenic activity of cement. In any case, before any clinical applications, further analyses should be carried out.

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