Characteristics and Mechanical Properties of Acrylolpamidronate-Treated Strontium Containing Bioactive Bone Cement

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Abstract: The aim of the present study was to determine the influence of surface treatment on the mechanical properties of strontium-containing hydroxyapatite (Sr-HA) bioactive bone cement. Previously we developed an injectable bioactive cement (SrHAC) system composed of Sr-HA powders and bisphenol A diglycidylether dimethacrylate (Bis-GMA). In this study, the Sr-HA powder was subjected to surface treatment using acrylolpamidronate, a bisphosphonate derivative, which has a polymerizable group, to improve the interface between inorganic filler and organic matrix by binding Sr-HA and copolymerizing into the matrix. After surface treatment, the compression strength, bending strength, and stiffness of the resulting composites were defined by using a material testing machine (MTS) according to ISO 5833. The fracture surface of the bone cement specimen was observed with a scanning electron microscope. *In vitro* cytotoxicity of surface-treated SrHAC was also studied using a tetrazolium-based cell viability assay (MTS/pms) on human osteoblast-like cells, the SaOS-2 cell line. Cells were seeded at a density of 10^4/mL and allowed to grow in an incubator for 48 h at 37°C. Results indicated that after surface treatment, the compression strength and stiffness significantly improved by 22.68 and 14.51%, respectively. The bending strength and stiffness of the bioactive bone cement also showed 19.06 and 8.91% improvements via three-point bending test. The fracture surface micromorphology after compression and bending revealed that the bonding between the resin to surface-treated filler considerably improved. The cell viability indicated that the treated particles were nontoxic and did not inhibit cell growth. This study demonstrated a new surface chemistry route to enhance the covalent bonds between inorganic fillers and polymer matrix for improving the mechanical properties of bone cement. This method not only improves the overall mechanical performance but also increases osteoblastic activity. © 2007 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 83B: 464–471, 2007

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INTRODUCTION

Injectable acrylic bone cements are widely used in orthopaedic surgery to fix artificial prostheses. Conventional polymethylmethacrylate (PMMA) bone cement has been successfully used in arthroplastic procedures of the hip, knee, and other joints for the fixation of polymer or metallic prosthetic implants to living bone; however, it still has some potential problems and risks, such as poor adhesion of the bone cement to bone surface[1] and a high exothermic reaction during polymerization,[2] both which limit its application and may also lead to complications.[3,4] The drawbacks have promoted the search for alternative solutions.

The incorporation of bioactive fillers, such as hydroxyapatite (HA), inorganic bone particles, or bioactive glass into a methacrylate (MA) matrix as an alternative to PMMA bone cement was first reported by Hennig et al.[5] With the aim of replacing the traditional PMMA bone cement, some bioactive bone cements were developed. PMMA filled with inorganic fillers[6–9] or bisphenol A diglycidylether dimethacrylate (Bis-GMA) filled with inorganic fillers was commonly studied.[10–13] The incorporation of a bioactive filler into the organic-based bone cement can partially increase the biocompatibility, as well as osteoconductivity. Nevertheless, the addition of inorganic fillers embrittles the bone cement, because of the low ductility of the fillers and the weak interface bonding between the fillers and the matrix.

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Recently, the authors have described an injectable, bioactive bone-bonding cement especially for use in minimally invasive surgery.[14,15] The cement mainly comprises Bis-GMA and triethylene glycol dimethacrylate (TEGDMA) as the organic matrix, and Sr-HA as the inorganic filler. The use of strontium-containing hydroxyapatite (Sr-HA) has a number of key advantages, including the fact that Sr is a trace element in the human body, thus allowing for radiographic visualization without the need for adding radiopaque particles, which are known to decrease mechanical properties.[16] Some studies indicated that strontium could be localized in calcified bone, and it has been associated with improving osteoporosis.[17–19] Furthermore, this bioactive bone cement showed improved contact with living bone when compared with commercial PMMA cement.[20,21] Sr-HA bioactive bone cement was designed to have desirable properties for use in vertebroplasty and for the treatment of osteoporotic fracture.[15,22]

However, like other HA- or bioglass-containing bone cements, adding 40% Sr-HA filler into the organic matrix increases the brittleness of the cement. This is partially due to the weak interfacial interaction between the inorganic filler and organic matrix in a particle-filled composite.[23] Various studies have demonstrated that improved adhesion between filler and matrix resulted in enhanced mechanical properties of the cement.[9,24–27] One approach could be the surface modification of the inorganic fillers before mixing, to improve the interaction between the fillers and the matrix. Acrylic and MA esters have been used to strengthen ionic interaction between filler and matrix.[26] However, toxicity is still a concern for these monomers.

Bisphosphonates are simple chemical compounds based on a phosphorous-carbon-phosphorous (P-C-P) motif. This template forms a three-dimensional structure capable of binding to divalent metal ions, such as Ca^{2+}, Mg^{2+}, and Fe^{2+}, in a bidentate manner through the coordination of one oxygen from each phosphate group with the divalent cation.[28,29] Moreover, bisphosphonates are now the most widely used drugs for diseases associated with loss of bone mass, such as osteoporosis.[30] Earlier studies showed that bisphosphonates could effectively bind to HA.[31] However, no study has been reported on whether surface treatment of HA by bisphosphonates can increase the mechanical properties of bioactive bone cement. The specific objective of this study was to investigate the mechanical properties, fracture surface and *in vitro* cytotoxicity of the Sr-HA bioactive bone cement with acrylolpamidronate treatment.

MATERIALS AND METHODS

Synthesis of Sr-HA Powder

The Sr-HA powder was developed in our laboratory with the method previously reported.[14,15,20–22,26] Briefly, Sr-HA powder was made through the wet method, suitable for mass production of both small crystalline and noncrystalline HA powders. After filtration of the slurry, the product was dried at 110°C. An alumina ball mill was used to pulverize the granular product into fine powder, and the product under 200 mesh (<75 μm) was calcined in a high-temperature Muffle furnace (F46240CM; Thermolyne, Dubuque, IA) at 800°C for 3 h. The particle size distribution of HA powder was measured by using a MAM500-5 powder analyzer (Ivern, UK). The average particle diameter was 8.99 μm.

Surface Treatment of Sr-HA Powder

In pilot studies, Sr-HA powder was treated by acrylolpamidronate with different concentrations. Based on the pilot trials, the best group of mechanical and handling properties was used in this study and the ratio (w/w) of acrylolpamidronate to Sr-HA was 0.25%. Fifty milligrams of acrylolpamidronate was dissolved in 50 mL distilled water. Twenty grams of Sr-HA powder, dried at 110°C for 24 h, was then added into the acrylolpamidronate solution. A homogeneous paste was achieved by gently stirring under nitrogen flow for 3 h. This paste was frozen at −70°C for 1 h and lyophilized to achieve a dry powder. This treated powder was stored in a dark dry box with 28% humidity at 23°C until use.

Preparation of the Resin

The resin was prepared from 50 wt % Bis-GMA (Aldrich, UK), 40 wt % TEGDMA (Aldrich), and 9.75 wt % polyethylene glycol methacrylate (PEGMA) (Aldrich). *N,N*-dimethyl-*p*-toluidine was dissolved into the mixture at 0.25% per unit weight of resin. To increase the homogeneity of the components, the resin was mixed with a mechanical stirrer for 72 h. Mixing was performed at 23°C under a dark hood.

Preparation of Cement Samples

SrHAC specimens were prepared by mixing 40 wt % original Sr-HA or surface-treated Sr-HA powder into 60 wt % resin. Briefly, the liquid resin was poured directly into the MixEvac® Bone Cement Mixer (Stryker Instruments, MI) and then the powder was poured into the bowl on top of the liquid resin. The mixture was stirred for 2–3 min in MixEvac unit when the pressure level reached 20–22 in.Hg, then it was injected into a cylindrical mold, and held until fully cured. The specimens were pushed out from the mold after setting.

Setting Time and Maximum Temperature Determination

The setting time and maximum temperature were recorded in the method previously reported.[14] In brief, the Sr-HA bone cement was reconstituted in standardized conditions (room temperature of 23°C ± 1°C, humidity of 50% ± 10%), and poured into a mold of 60 mm diameter and 6 mm

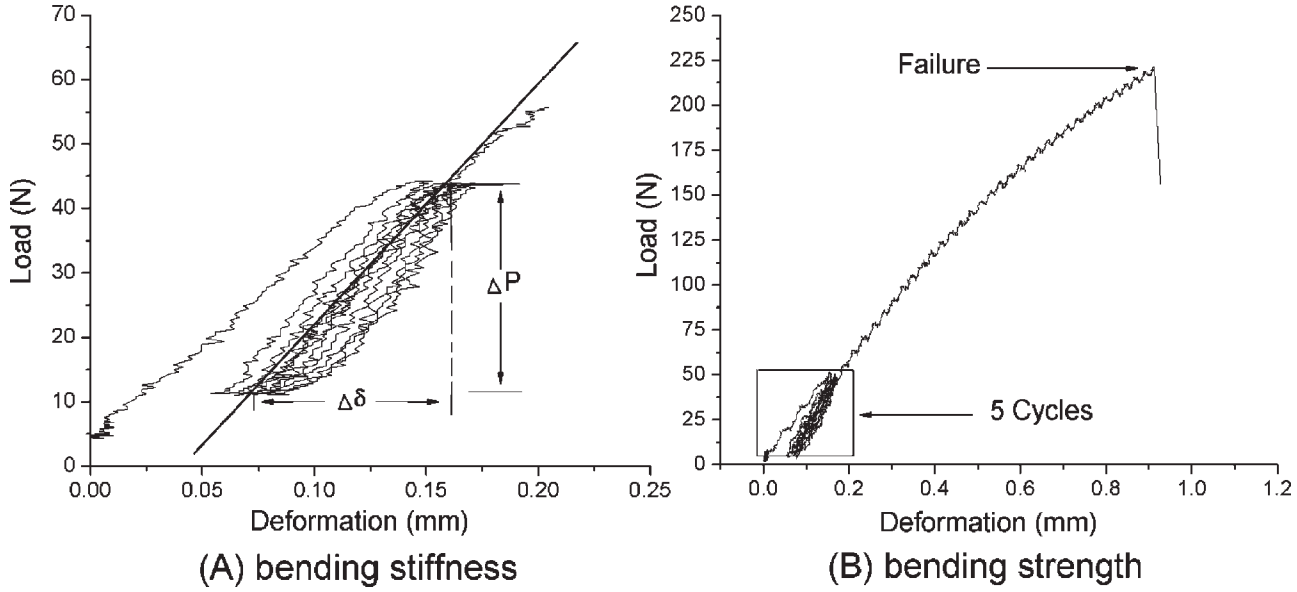

Figure 1. Bending test curves of original Sr-HA bone cement. A: Five cycles of loading were applied, and linear regression was used to calculate stiffness; ΔP is the force applied, $\Delta\delta$ is the deformation; B: Bending curve for strength determination.

height. A thermocouple was inserted into the center of the mold, and the temperature measured at 1-min intervals.

Mechanical Testing

SrHAC specimen were cut into rods with a diamond saw (Exakt 300CP, Germany) for bending testing (6 mm diameter × 25 mm length) and for compression testing (6 mm diameter × 12 mm length). The specimens were soaked in distilled water at 37°C for 24 h before being tested at 23°C.

All mechanical tests were conducted on a servo-hydraulic material testing machine (MTS 858 bionix machine; MTS System, Minneapolis, MN). The mean value and standard deviation were calculated from each group of 12 specimens.

The compression strength was tested according to the standard ISO 5833. Five cycles of compressive loading were applied to each specimen at the range of 100–400 N and the load deformation data was collected. The stiffness, evaluated by the slope of the load-deformation curve, was determined. After five loading cycles, the specimens were loaded to failure. In all loading regimes, a speed of 1 mm/min was used.

In this study, a three-point bending test was performed as previously described.[11,26] The dimension of the span was defined as that between the supports for the three-point bending test, and it equaled 17.65 mm in this study. The cross-head speed use was 0.5 mm/min. As with the compressive test described earlier, we recorded the load deformation data and evaluated it by the slope of the load-deformation curves. Five loading cycles were applied for calculating the bending stiffness with the loading range of 5–50 N [Figure 1(A)]. The specimens were subsequently loaded to failure after cyclic testing to define the bending strength [Figure 1(B)].

Characterization

Scanning electron microscopy (SEM) was used to observe the fracture surface of the samples after compression and bending test. The fracture sections of the Sr-HA specimens were gold coated, and SEM observation was carried out with the use of a Leica S440 (Cambridge, England) SEM.

In Vitro Cytotoxicity Assessment

To assess the short-term cytotoxicity of the original and treated powders, the following ISO/EN 10993 Part 5 guidelines[32] were used. The original and treated particles were extracted for 72 h at 37°C, using Dulbecco's modified Eagle's medium (DMEM; Sigma) as the extraction fluid.[33] The culture medium was also used as a negative control. In the test groups, the ratio of particle weight to extract fluid was constant at 0.05 g/mL.

Cell Culture

The present experiment used a cell line of human osteoblast-like cells (SaOS-2). Cells were grown as monolayers in DMEM supplemented with 10% fetal calf serum (Biowest), 100 U/mL penicillin–streptomycin (GIBCO), 4 μg/mL fungizone (GIBCO), and 2 m*M* L-glutamine at 37°C in a humid atmosphere containing 5% CO_2. Cells were trypsinized before the experiments. For the viability test, cells were seeded in 96-well plates ($n = 12$), at a density of 2×10^4 cells per well, and incubated for 48 h at 37°C, in a humidified atmosphere containing 5% CO_2.

TABLE I. Mechanical Properties of Original and Treated Sr-HA Bone Cement

	Compression Strength (MPa)	Compression Stiffness (GPa)	Bending Strength (MPa)	Bending Stiffness (MPa)
SrHAC with original Sr-HA	115.68 ± 16.39	1.93 ± 0.21	42.96 ± 4.34	818.10 ± 65.67
SrHAC with 25 mg/10 g treated	141.92 ± 17.72	2.21 ± 0.26	51.15 ± 8.04	890.99 ± 65.36
Improvement (%)	22.68	14.51	19.06	8.91
p value	0.01	0.01	0.01	0.02

Cell Viability Test (MTS/pms)

3-(4,5-Dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2(4-sulfofenyl)-2*H*-tetrazolium (MTS) (CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay Promega) is commonly used for cell viability evaluation. Before the MTS/pms test, culture medium was removed from the wells and an identical volume, 200 μL, of serum-free culture medium replaced. After 24 h, the serum-free culture medium was replaced by extraction fluid. Cell response was evaluated after 48 h of

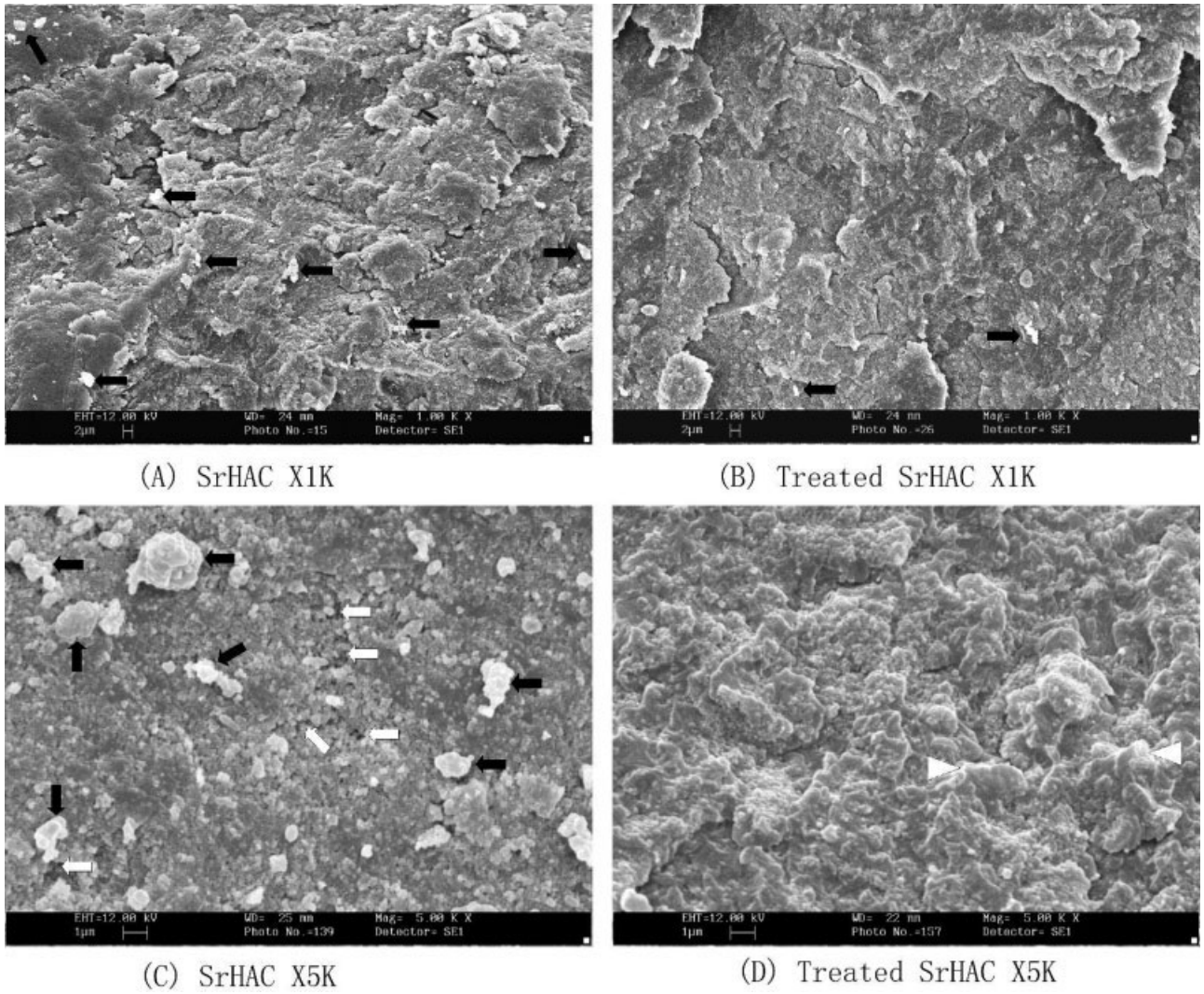

Figure 2. SEM micrographs of the Sr-HA bone cement fracture surface with original Sr-HA powder (A, C) and treated Sr-HA powder (B, D). The compressive fracture surface of the treated Sr-HA cement was relatively flat (B) compared with the control (A). There were many Sr-HA particles (black arrows, A) on the fracture surface of the control sample. However, fewer filler particles (black arrows, B) can be seen on the fracture surface of treated bone cement. Sr-HA particles could be identified on the bending fracture surface (black arrows, C). It showed that gaps and pores (white arrows, C) existed between the particles and resin. After treating with acrylolpamidronate, the cement exhibited a rough and uneven surface, and no distinguishable particles could be seen on the fracture surface. The treated bone cement showed a "wavy" fracture surface (white triangle, D).

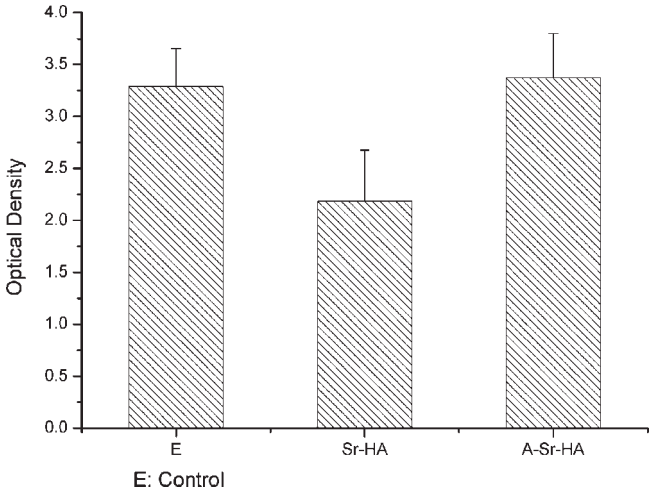

Figure 3. SaOS-2 cell viability after incubation with the test and culture medium over a period of 48 h. Results are based on optical density measurements. (E: culture medium for control; Sr-HA: Sr-HA extraction; A-Sr-HA: treated Sr-HA extraction.) Acrylolpamidronate-treated Sr-HA particles (A-Sr-HA) did not cause growth inhibition after 48 h, whereas it increased the SaOS-2 cell activity slightly when comparing with the results that were obtained in control (E). Acrylolpamidronate treatment significantly ($p < 0.05$) increased the cell activity over the original Sr-HA powder (Sr-HA).

incubation time, by adding 20 μL of MTS reagent to each well. Cells were then incubated for 3 h at 37°C in a humidified atmosphere containing 5% CO_2. At this time optical density was measured with a microplate reader (Molecular Devices model no. 300, Sunnyvale, CA) at 490 nm.

Statistical Analysis

Results are expressed as mean ± SD. A Student's t test was used to compare the statistical significance between the treated and untreated cements. Significant results were accepted when $p \leq 0.05$.

RESULTS

Setting and Handling Properties

The setting time of Sr-HA and modified bone cement was 8–12 min. The peak curing temperature of original and modified Sr-HA bone cement was 58°C. During the process of handling, it was observed that Sr-HA bone cement had equivalent properties to PMMA in terms of ease of injection. After surface treatment of Sr-HA powders, the fluidity of the mixture decreased, while its viscosity increased slightly.

Mechanical Properties

The mechanical properties of the original and treated Sr-HA bone cement are listed in Table I. The compression strength and compression stiffness of the bone cement after surface treatment were significantly improved by 22.68 and 14.51%, respectively. The bending strength and bending stiffness of the treated bone cement were significantly improved by 19.06 and 8.91%, respectively.

Micrographic Observation

Figure 2 shows the compressive fracture surfaces of the Sr-HA bone cement with original (A) and acrylolpamidronate-treated (B) Sr-HA particles, respectively. The acrylolpamidronate-treated Sr-HA bone cement had a different appearance from the original bone cement. At high resolution, the surface of the acrylolpamidronate-treated Sr-HA bone cement (B) showed a higher integrity than the control sample (A). There were many Sr-HA particles [black arrows, Figure 2(A)] on the fracture surface of the control sample. However, fewer filler particles [black arrows, Figure 2(B)] can be seen on the fracture surface of treated bone cement. The surface of the control sample also exhibited a considerable lamellar fracture morphology compared with the acrylolpamidronate-treated Sr-HA bone cement.

The bending fracture surface of the control sample [Figure 2(C)] appeared to be smooth. The Sr-HA particles were embedded in the resin matrix, but could still be identified on the fracture surface [black arrows, Figure 2(C)]. After treatment with acrylolpamidronate [Figure 2(D)], the Sr-HA bone cement exhibited a rough and uneven surface, and no distinguishable particles could be seen on the fracture surface. The fracture surface of the control sample showed that gaps and pores [white arrows, Figure 2(C)] existed between the particles and resin. But after acrylolpamidronate treatment, the gap was not apparent. The treated bone cement showed a "wavy" [white triangle, Figure 2(D)] fracture surface. The acrylolpamidronate-treated Sr-HA particles appeared well bonded to the matrix in the cement in comparison to the original Sr-HA cements.

Cell Viability

Regarding short-term MTS/pms extraction tests, the results (Figure 3) demonstrated that leachables from the acrylolpamidronate-treated Sr-HA particles did not cause growth inhibition after 48 h, while it increased the SaOS-2 cell activity slightly when comparing with the results that were obtained in control culture medium. Acrylolpamidronate treatment significantly ($p < 0.05$) increased the cell activity over the original Sr-HA powder.

DISCUSSION

The modified Sr-HA bioactive bone cement has equivalent properties to PMMA in terms of ease of injection and setting times. From safety aspects, Sr-HA bone cement was superior to PMMA cement (90°C) in its lower setting temperature. The slightly increased viscosity reduced the risk of leak during the surgical process.

Subsequent mechanical tests with MTS after surface treatment showed that the mechanical properties of the

SrHAC were significantly improved ($p < 0.05$). Previous studies have indicated that surface treatment of inorganic fillers, such as bioglass, HA, and silica is an important approach to achieve stronger bonds between the inorganic filler and the organic matrix, forming mechanically stronger composites. A significant fraction of organic reactive hydroxyl groups can be made to graft organic molecules onto the surface of HA particles.[34] The hydroxyl groups have been proven to have the ability to react with organic functional groups.[35,36]

The results presented in this study are the first to illustrate the effects of modifying Sr-HA with acrylolpamidronate and the particular chemical structure of acrylolpamidronate[37] may contribute to the observed improvement in mechanical properties of the bioactive bone cement. The acrylolpamidronate belongs to the family of bisphosphonates, which is structurally similar to endogenous pyrophosphates, but with a carbon molecule replacing the central oxygen molecule enabling the accommodation of two additional substituents, R1 and R2.[28,29,38] Acylolpamidronate produced by grafting of an acrylic group, which contains an unsaturated double bond, onto the pamidronate[39] enabled its binding to the Sr-HA effectively. The imine in the R2 side chain of the acrylolpamidronate increased its affinity to Sr-HA and an acrylic group in the side chain facilitated its crosslinking with Bis-GMA by free-radical polymerization. These reactions, therefore, promoted the adhesion between the Sr-HA particles to the organic matrix. Apart from Sr-HA, acrylolpamidronate can also bind to HA, divalent cation-substituted HA, and other calcium-containing ceramics effectively, and it can also crosslink with Bis-GMA and other Bis-GMA-based or MA-based resins. Consequently, the application of this interesting surface treatment method could be extended to other polymer-based bioactive bone cements.

Results of the morphology after compression suggest that there was better integration between acrylolpamidronate-treated SrHAC [Figure 2(B)] than pristine SrHAC [Figure 2(A)]. The two components, powder and liquid, were mixed to form a dough or paste. Setting of the cement occurs by way of polymerization of the mixture, which is initiated by free radicals produced by the reaction of benzoyl peroxide present in the powder with *N,N*-dimethyl-*p*-toluidine present in the liquid. The acrylolpamidronate covered and bound to the Sr-HA particles effectively after surface treatment. The unsaturated double bond on the R2 side chain of acrylolpamidronate may crosslink with the Bis-GMA by free-radical polymerization. In the present case, the increase in the compressive strength and stiffness can be explained by the Sr-HA treatment with acrylolpamidronate, which promoted a better adhesion between the Sr-HA filler and the Bis-GMA matrix.

If the Sr-HA particles are present and distributed nonhomogeneously in acrylic bone cement, it will result in aggregation of particles and cause poor adhesion to the matrix. The agglomerates are weak points, and break when stress is applied.[40] For untreated Sr-HA bone cement, a typical smooth type I brittle fracture surface was observed [Figure 2(C)], which meant that there was no or little plastic deformation when bending, whereas a rougher type II/III brittle fracture surface was observed in treated Sr-HA bone cement [Figure 2(D)]. Acrylolpamidronate covering the surface of the Sr-HA improved the compatibility of the filler. Thus, the surface tension as well as the viscosity of the mixture decreased, whereas its fluidity increased and consequently, less voids were formed by the trapped air. This property change benefits the mixing of the inorganic fillers in the organic liquid, decreasing the aggregation of Sr-HA particles and leading to a more homogeneous dispersion of the Sr-HA particles in the organic matrix. The morphology of treated SrHAC after bending testing showed that the fracture surface was much more jagged, irregular, and rough [Figure 2(D)]. As a result, the bending strength and stiffness of the bioactive bone cement were improved.

The cell viability assay in this study is particularly aimed at establishing the possible toxic effects of leachables released from the particles during extraction. Bisphosphonates are well-known potent inhibitors of osteoclast activity. The mechanisms by which bisphosphonates reduce bone resorption directly acting on osteoclasts are now largely clarified even at a molecular level. But research concerning the bisphosphonate's effects on osteoblasts has instead shown variable results. Many *in vitro* studies have reported positive effects on osteoblasts proliferation and mineralization for several bisphosphonates.[41–43] The MTS/pms test indicated that the developed surface treatment method may be nontoxic. In addition, it may also promote osteoblastic bone formation.

CONCLUSION

In the present work, composites based on Bis-GMA and Sr-HA were prepared as a potential bioactive bone cement for orthopaedics or other clinical applications. The surface modification of Sr-HA particles using acrylolpamidronate was demonstrated to be an effective way to improve the interface bonding between the polymer matrix and Sr-HA filler. The compressive strength, compressive stiffness, bending strength, and stiffness of the injectable bioactive bone cement were significantly improved with surface treatment. Further, acrylolpamidronate-treated Sr-HA particles did not show cytotoxic effects *in vitro*.

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REFERENCES

1. Harrigan TP, Kareh JA, O'Connor DO, Burke DW, Harris WH. A finite element study of the initiation of failure of fixation in cemented femoral total hip components. J Orthop Res 1992;10:134–144.

2. Wang JS, Franzen H, Toksvig-Larsen S, Lidgren L. Does vacuum mixing of bone cement affect heat generation? Analysis of four cement brands. J Appl Biomater 1995;6:105–108.
3. Jasty M, Maloney WJ, Bragdon CR, O'Connor DO, Haire T, Harris WH. The initiation of failure in cemented femoral components of hip arthroplasties. J Bone Joint Surg Br 1991; 73:551–558.
4. Leeson MC, Lippitt SB. Thermal aspects of the use of polymethylmethacrylate in large metaphyseal defects in bone. A clinical review and laboratory study. Clin Orthop Relat Res 1993;295:239–245.
5. Hennig W, Blencke BA, Bromer H, Deutscher KK, Gross A, Ege W. Investigations with bioactivated polymethylmethacrylates. J Biomed Mater Res 1979;13:89–99.
6. Shinzato S, Nakamura T, Kokubo T, Kitamura Y. PMMA-based bioactive cement: Effect of glass bead filler content and histological change with time. J Biomed Mater Res 2002; 59:225–232.
7. Kamimura M, Tamura J, Shinzato S, Kawanabe K, Neo M, Kokubo T, Nakamura T. Interfacial tensile strength between polymethylmethacrylate-based bioactive bone cements and bone. J Biomed Mater Res 2002;61:564–571.
8. Shinzato S, Nakamura T, Ando K, Kokubo T, Kitamura Y. Mechanical properties and osteoconductivity of new bioactive composites consisting of partially crystallized glass beads and poly(methyl methacrylate). J Biomed Mater Res 2002;60: 556–563.
9. Morejon L, Mendizabal AE, Garcia-Menocal JA, Ginebra MP, Aparicio C, Mur FJ, Marsal M, Davidenko N, Ballesteros ME, Planell JA. Static mechanical properties of hydroxyapatite (HA) powder-filled acrylic bone cements: Effect of type of HA powder. J Biomed Mater Res B Appl Biomater 2005; 72:345–352.
10. Erbe EM, Clineff TD, Gualtieri G. Comparison of a new bisphenol-a-glycidyl dimethacrylate-based cortical bone void filler with polymethyl methacrylate. Eur Spine J 2001;10 (Suppl. 2):S147–S152.
11. DiCicco M, Compton R, Jansen-Varnum SA. *In vitro* evaluation of orthopedic composite cytotoxicity: Assessing the potential for postsurgical production of hydroxyl radicals. J Biomed Mater Res B Appl Biomater 2005;72:146–155.
12. Lee JH, Um CM, Lee IB. Rheological properties of resin composites according to variations in monomer and filler composition. Dent Mater 2006;22:515–526.
13. Zandinejad AA, Atai M, Pahlevan A. The effect of ceramic and porous fillers on the mechanical properties of experimental dental composites. Dent Mater 2006;22:382–387.
14. Li YW, Leong JC, Lu WW, Luk KD, Cheung KM, Chiu KY, Chow SP. A novel injectable bioactive bone cement for spinal surgery: A developmental and preclinical study. J Biomed Mater Res 2000;52:164–170.
15. Lu WW, Cheung KM, Li YW, Luk KD, Holmes AD, Zhu QA, Leong JC. Bioactive bone cement as a principal fixture for spinal burst fracture: An *in vitro* biomechanical and morphologic study. Spine 2001;26:2684–2690.
16. Christoffersen J, Christoffersen MR, Kolthoff N, Barenholdt O. Effects of strontium ions on growth and dissolution of hydroxyapatite and on bone mineral detection. Bone 1997;20: 47–54.
17. Dahl SG, Allain P, Marie PJ, Mauras Y, Boivin G, Ammann P, Tsouderos Y, Delmas PD, Christiansen C. Incorporation and distribution of strontium in bone. Bone 2001;28:446–453.
18. Meunier PJ, Roux C, Seeman E, Ortolani S, Badurski JE, Spector TD, Cannata J, Balogh A, Lemmel EM, Pors-Nielsen S, Rizzoli R, Genant HK, Reginster JY. The effects of strontium ranelate on the risk of vertebral fracture in women with postmenopausal osteoporosis. N Engl J Med 2004;350:459–468.
19. Farlay D, Boivin G, Panczer G, Lalande A, Meunier PJ. Long-term strontium ranelate administration in monkeys preserves characteristics of bone mineral crystals and degree of mineralization of bone. J Bone Miner Res 2005;20:1569–1578.
20. Ni GX, Lu WW, Chiu KY, Li ZY, Fong DY, Luk KD. Strontium-containing hydroxyapatite (Sr-HA) bioactive cement for primary hip replacement: An *in vivo* study. J Biomed Mater Res B Appl Biomater 2006;77:409–415.
21. Ni GX, Choy YS, Lu WW, Ngan AH, Chiu KY, Li ZY, Tang B, Luk KD. Nano-mechanics of bone and bioactive bone cement interfaces in a load-bearing model. Biomaterials 2006;27: 1963–1970.
22. Cheung KM, Lu WW, Luk KD, Wong CT, Chan D, Shen JX, Qiu GX, Zheng ZM, Li CH, Liu SL, Chan WK, Leong JC. Vertebroplasty by use of a strontium-containing bioactive bone cement. Spine 2005;30 (Suppl. 17):S84–S91.
23. FL M, RD R. Composite Materials: Engineering and Science. Cambridge: Woodhead Publishing; 1999.
24. Mousa WF, Kobayashi M, Kitamura Y, Zeineldin IA, Nakamura T. Effect of silane treatment and different resin compositions on biological properties of bioactive bone cement containing apatite-wollastonite glass ceramic powder. J Biomed Mater Res 1999;47:336–344.
25. Roether JA, Deb S. The effect of surface treatment of hydroxyapatite on the properties of a bioactive bone cement. J Mater Sci Mater Med 2004;15:413–418.
26. Zhao F, Lu WW, Luk KD, Cheung KM, Wong CT, Leong JC, Yao KD. Surface treatment of injectable strontium-containing bioactive bone cement for vertebroplasty. J Biomed Mater Res B Appl Biomater 2004;69:79–86.
27. Dupraz AM, de Wijn JR, v d Meer SA, de Groot K. Characterization of silane-treated hydroxyapatite powders for use as filler in biodegradable composites. J Biomed Mater Res 1996; 30:231–238.
28. Fleisch H. Bisphosphonate pharmacology. Semin Arthrit Rheum 1994;23:261–262.
29. Nancollas GH, Tang R, Phipps RJ, Henneman Z, Gulde S, Wu W, Mangood A, Russell RG, Ebetino FH. Novel insights into actions of bisphosphonates on bone: Differences in interactions with hydroxyapatite. Bone 2006;38:617–627.
30. Watts NB. Bisphosphonate treatment of osteoporosis. Clin Geriatr Med 2003;19:395–414.
31. Zaheer A, Lenkinski RE, Mahmood A, Jones AG, Cantley LC, Frangioni JV. *In vivo* near-infrared fluorescence imaging of osteoblastic activity. Nat Biotechnol 2001;19:1148–1154.
32. ISO document 10993. Biological Compatibility of Medical Devices. 5: Test for cytotoxicity: *In vitro* methods; 1992.
33. Bondemark L. Orthodontic magnets. A study of force and field pattern, biocompatibility and clinical effects. Swed Dent J Suppl 1994;99 (Suppl):1–148.
34. Yesinowski JP, Eckert H. Hydrogen environments in calcium phosphates: 1H MAS NMR at high spinning speeds. J Am Chem Soc 1987;109:6274–6282.
35. Liu Q, de Wijn JR, de Groot K, van Blitterswijk CA. Surface modification of nano-apatite by grafting organic polymer. Biomaterials 1998;19:1067–1072.
36. Furuzono T, Sonoda K, Tanaka J. A hydroxyapatite coating covalently linked onto a silicone implant material. J Biomed Mater Res 2001;56:9–16.
37. Wang L, Zhang M, Xu B. The first pamidronate containing polymer and copolymer. Chem Commun 2006;2006:2795–2797.

38. Heymann D, Ory B, Gouin F, Green JR, Redini F. Bisphosphonates: New therapeutic agents for the treatment of bone tumors. Trends Mol Med 2004;10:337–343.
39. Mekraldi S, Toromanoff A, Rizzoli R, Ammann P. Pamidronate prevents bone loss and decreased bone strength in adult female and male rats fed an isocaloric low-protein diet. J Bone Miner Res 2005;20:1365–1371.
40. Islas-Blancas ME, Cervantes JM, Vargas-Coronado R, Cauich-Rodriguez JV, Vera-Graziano R, Martinez-Richa A. Characterization of bone cements prepared with functionalized methacrylates and hydroxyapatite. J Biomater Sci Polym Ed 2001;12:893–910.
41. Von Knoch F, Jaquiery C, Kowalsky M, Schaeren S, Alabre C, Martin I, Rubash HE, Shanbhag AS. Effects of bisphosphonates on proliferation and osteoblast differentiation of human bone marrow stromal cells. Biomaterials 2005;26: 6941–6949.
42. Frediani B, Spreafico A, Capperucci C, Chellini F, Gambera D, Ferrata P, Baldi F, Falsetti P, Santucci A, Bocchi L, Marcolongo R. Long-term effects of neridronate on human osteoblastic cell cultures. Bone 2004;35:859–869.
43. Im GI, Qureshi SA, Kenney J, Rubash HE, Shanbhag AS. Osteoblast proliferation and maturation by bisphosphonates. Biomaterials 2004;25:4105–4115.