In Vitro characterization of low modulus linoleic acid coated strontium-substituted hydroxyapatite containing PMMA bone cement

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Abstract: Poly (methyl methacrylate) (PMMA) bone cement is widely used in vertebral body augmentation procedures such as vertebroplasty and balloon kyphoplasty. Filling high modulus PMMA increases the modulus of filled verterbra, increasing the risk of fracture in the adjacent vertebra. On the other hand, in porous PMMA bone cements, wear particle generation and deterioration of mechanical performance are the major drawbacks. This study adopts a new approach by utilizing linoleic acid coated strontium substituted hydroxyapatite nanoparticle (Sr-5 HA) and linoleic acid as plasticizer reducing bone cement's modulus with minimal impact on its strength. We determined the compressive strength (UCS) and modulus (Ec), hydrophobicity, injectability, *in vitro* bioactivity and biocompatibility of this bone cement at different filler and linoleic acid loading. At 20 wt % Sr5-HA incorporation, UCS and Ec were reduced from 63 $\pm$ 2 MPa, 2142 $\pm$ 129 MPa to 58 $\pm$ 2 MPa, 1785 $\pm$ 64 MPa, respectively. UCS and Ec were further reduced to 49 $\pm$ 2 MPa and 774 $\pm$ 70 MPa respectively when 15 v/v of linoleic acid was incorporated. After 7 days of incubation, pre-osteoblast cells (MC3T3-E1) attached on 20 wt % Sr5-HA and 20 wt % Sr5-HA with 15 v/v of linoleic acid group were higher ($3.73 \pm 0.01 \times 10^4$, $2.27 \pm 0.02 \times 10^4$) than their PMMA counterpart ($1.83 \pm 0.04 \times 10^4$). Incorporation of Sr5-HA with linoleic acid in monomer phase is more effective in reducing the bone cement's stiffness than Sr5-HA alone. Combination of low stiffness and high mechanical strength gives the novel bone cement the potential for use in vertebroplasty cement applications. J Biomed Mater Res Part B: Appl Biomater 96B: 76–83, 2011.

Key Words: linoleic acid, strontium-substituted hydroxyapatite (Sr-HA), vertebroplasty, polymethylmethacrylate

INTRODUCTION

Polymethylmethacrylate (PMMA) with high radiopacifier content is widely used in vertebral augmentation procedures, such as vertebroplasty (VP) and balloon kyphoplasty (BKP).[1] Attractive cement properties such as appropriate viscosity upon injection into fractured vertebral body and capability to provide good mechanical augmentation make this cement type widely used in such applications. In general, vertebroplasty PMMA cement still has a few shortcomings such as high modulus, hydrophobicity, and lack of bioactivity.[2,3] PMMA bone cement modulus (2200 MPa)[4] is several times higher than that of the cancellous bone structure, whereas cancellous bones in osteoporosis patients have much lower compressive strength (UCS = 0.5–4.5 MPa) and compressive modulus (Ec = 100–450 MPa)[5] than healthy adults. Therefore, treatment with high stiffness PMMA cement usually leads to derangement of load transfer from stiffer, treated vertebra to adjacent weaker vertebral bodies, thereby increasing the risk of adjacent vertebral fracture.[6]

PMMA or Bis-GMA-based bioactive bone cement relies on incorporating strontium-substituted hydroxyapatite (Sr-HA),[7] hydroxyapatite or bioglass to increase the bone cement's bioactivity. There is growing literature on the preparation and characterization of porous, low stiffness bioactive vertebroplasty bone cement.[8] Therefore, porous PMMA cement was fabricated by incorporating various volume fractions of an aqueous sodium hyaluronate solution to reduce the cement modulus.[9,10] By this method, the compressive modulus significantly decreased from 930 to 50 MPa, however the compressive strength also reduced from 39 to 1.3 MPa. As the highly porous matrix of the cement injected into the vertebra is subjected to fatigue loading, deterioration of compressive strength is a concern. To provide necessary mechanical support, the cement strength should be higher than 2.3 MPa, the highest compressive stress at the nucleus pulpous of a nondegenerated intervetebral disc measured telemetrically.[11] Another approaches is to adopt poly(vinyl alcohol) (PVA) and amphiphilic reactive monomer diacetonacrylamide (DAA) to fabricate a hydrogel-based bone cement.[12] The elastic modulus and strength of such bone cements are in the range of 5–400 MPa and 5–75 MPa, respectively. Moreover, it can reach the final mechanical strength within 30 min postdelivery. However, it

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has been reported that the cement can be delivered by 18 and 20 gauge syringes, such a low viscosity of the cement may increase the risk of extravasation.

Another choice available in the market is calcium phosphate cement (CPC), which demonstrates osteoconductivity and bone biocompatibility.[13] However, surgical complications such as collapse of treated vertebra have been reported due to poor initial stability of CPC.[14]

In the dental field, plasticizer has been adopted to reduce the stiffness of temporary implants.[15] Plasticizer works by the principle of reducing the PMMA's molecular interaction inside the polymer matrix because of similar chemical composition modulus of vertebroplasty cement can be reduced by same mechanism. A previous study has reported that linoleic acid could react with MMA monomer to form a PMMA-linoleic acid copolymer with lower molecular weight and glass transition temperature (T_g).[16] On this basis, our group developed linoleic acid-functionalized strontium substituted hydroxyapatite particles (Sr5-HA) by using the liquid–solid solution method.[17] We hypothesize that the addition of Sr5-HA and linoleic acid in the liquid phase could reduce the modulus of the bone cement.

In this study, we aimed at investigating the cement properties: (1) + ultimate compressive strength (UCS), + compressive modulus (Ec), hydrophobicity, (2) bioactivity and biocompatibility, (3) radiopacity, and (4) injectability and curing properties.

MATERIALS AND METHODS

Materials

Linoleic acid functionalized strontium-substituted hydroxyapatite (Sr5-HA) powder was prepared using hydrothermal treatment. Polymethylmethacrylate (PMMA) powder was purchased from Depuy, SmartSet MV Endurance, United Kingdom (67.05 wt % PMMA, 21.10 wt % PMMA styrene co-polymer. 1.85% Benzoyl peroxide (BPO) and 10% barium sulfate ($BaSO_4$). The liquid monomer constituent were 98.2% methylmethacrylate (MMA) and 1.8% *N*,*N*-dimethyl *p*-toludiene (DMPT) and 75 ppm of hydroquinone. The commercially available vertebroplasty PMMA bone cement (Vertebroplastic™ Radiopaque Resinous Material, Depuy AcroMed, England) was used as control cement.

Preparation of bone cements

5 wt % Sr5-HA, 10 wt % Sr5-HA, and 20 wt % Sr5-HA bone cements were prepared by blending Sr5-HA (5, 10, and 20 wt %) with the PMMA powder (95, 90, and 80 wt %). On the basis of our preliminary trial, addition of 30 v/v linoleic acid leads to incomplete setting, 15 v/v linoleic acid Sr5-HA was prepared by replacing 15 v/v of MMA solution with linoleic acid. The blended powder mixture was mixed with the liquid monomer at a powder to monomer ratio of 2:1 in a bowl open to ambient air. Control cement was mixed according to the guidelines from Depuy.

Compressive properties

Sr-5-HA bone cement was injected into 6 mm diameter and 12 mm long Teflon molds to fabricate compressive test specimens. The specimens were cured in the mold for 24 h. Compressive strength (UCS) and modulus (Ec) were determined by material testing machine (MTS 858 Bionix; MTS System, Eden Prairie, Minneapolis, MN). Five specimens were evaluated in each group, operated at a cross-head displacement speed of 20 mm/min.

Bone cement injectability, curing properties

To measure the injectability of the Sr5-HA formulations, 3 cm^3 of cement was prepared and charged in a 2-cm^3 disposable syringe. A gauge 8 needle, 150 mm long was fixed to the syringe and the cement was injected to a recipient. The weight percent of cement injected to the recipient with respect to the total amount of cement charged in the syringe was considered as the injectability. Bone cement curing properties was determined according to ISO 5833 standards. 20 g of cement was mixed at (23°C ± 1°C) and place it in a shallow Teflon mold. The temperature was determined by a thermocouple (TM 914C, Lutron, Taiwan). The setting curve was recorded and used to determine the maximum temperature and setting time.

Bone cement radiopacity

Bone cement radiopacitiy was determined by Cabinet X-ray system (Faxitron X-ray corporation, IL) at 41 kV, 1.8 mA, and the film was developed by Okamoto X3. The specimens for radiopacity were 3.3 mm thick and 40 mm long.

Hydrophobicity and water absorption

The hydrophobicity of bone cement was determined by measuring contact angles using sessile drop technique (SL200B; Shanghai Solon Tech, China). Distilled water was used as the droplet. For each cement group, two specimens were used. Four points were measured at four widely dispersed points. For water absorption test, two specimens from each group were weighted in analytical balance (AUW320, Shimadzu, Japan) before and after immersion in normal saline solution (0.9% NaCl) for 1 day and 1 week.

In vitro bioactivity

Simulated body fluid (SBF) was prepared according to the Kokubo's method.[16] Reagent-grade NaCl, $NaHCO_3$, KCl, $K_2HPO_4{\cdot}3H_2O$, $MgCl_2{\cdot}6H_2O$, $CaCl_2$, and Na_2SO_4 were dissolved in distilled water, and pH was adjusted to 7.4 with Tris-HCl. *In vitro* bioactivity was determined by immersing the cement specimens in 80 mL of SBF for 1, 3, 8, and 14 days. SBF solution was kept at 37°C to stimulate body environment. The surface morphology and composition after 8 and 14 days were respectively characterized by SEM/EDX (LEO 1530 FESEM, Oxford, UK). In this experiment, only 20 wt % Sr5-HA and control cement were analyzed. The particle deposited on bone cement surface was scratched out and observed under TEM for morphological analysis. The phase property was evaluated by selected-area electron diffraction (SAED; Technai G2 20 STEM, FEI, Hillsboro, OR). At each time point, 7 mL SBF solution was collected for strontium ion concentration analysis with inductively coupled plasma atomic emission spectroscopy (ICP-AES, Varian Co., USA).

TABLE I. Compressive Strength, Modulus, and Cell Number at Day 3, 7 Determined by WST-1 Assay of 5, 10, 20 wt % Sr5-HA and Control Bone Cement

	UCs	Ec	Cell Number at Day 3	Cell Number at Day 7
Control cement	63 ± 2	2142 ± 129	$1.01 \pm 0.19 \times 10^4$	$1.83 \pm 0.04 \times 10^4$
5 wt % + Sr5-HA	96 ± 3[a]	2793 ± 163[b]	$8.69 \pm 0.16 \times 10^3$	$1.68 \times 10^4 \pm 0.17 \times 10^4$
10 wt % + Sr5-HA	63 ± 2	1875 ± 131	$8.67 \pm 0.00 \times 10^3$	$1.37 \pm 0.01 \times 10^4$[c]
20 wt % Sr5-HA	58 ± 2[a]	1785 ± 64	$2.80 \pm 0.01 \times 10^3$[d]	$3.73 \pm 0.02 \times 10^4$[c]
20 wt % Sr5-HA with 15 v/v linoleic acid	49 ± 2[a]	774 ± 70[b]	$1.42 \pm 0.01 \times 10^3$[d]	$2.27 \pm 0.02 \times 10^4$[c]

[a] Based on one-way ANOVA, 5, 20, and 20 wt % Sr5-HA with 15 v/v linoleic acid compressive strength is significant different from control cement.

[b] 5 and 20 wt % with 15 v/v linoleic acid compressive modulus is significant different from control cement. ($p < 0.05$)

[c] Based on one-way ANOVA, cell number on 10, 20, and 20 wt % Sr5-HA with 15 v/v linoleic acid cement is significant different from control cement ($p < 0.05$)

[d] Based on one-way ANOVA, cell number on 20 and 20 wt % Sr5-HA with 15 v/v linoleic acid is significant different from control cement ($p < 0.05$).

Cell proliferation rate

Mouse preosteoblast cells (MC3T3-E1, RIKEN Cell Bank (Tsukuba, Japan) were thawed and suspended in α-Modified Eagle Medium (DMEM) (Invitrogen, TX) containing 10% (v/v) fetal bovine serum (FBS Biowest, Miami, USA) into culture flasks and cultured at 37°C in an incubator (5% CO_2). MC3T3-E1 were trypsinized, and then seeded on the bone cement surface (12 mm diameter) at 1×10^4 cells per well (24 well plate), and then incubated for 3 and 7 days. After incubation, WST-1 assay was applied according to Dojindo guideline.[18] One hundred microliter of WST-1 solution was added to each well and further incubated for 3 h. The absorbance was measured by plate reader (SpectraMax 340, Molecular Devices, USA) at 440 nm, 630 nm as reference. For each kind of cement, three specimens were evaluated.

Cell morphology

MC3T3-E1 cells were seeded on the bone cement surface and incubated for 3 days. After incubation bone cement surface was rinsed with phosphate-buffered saline (PBS) to remove nonadherent cells. The cement specimens were fixed by 2.5% glutaraldehyde with cacodylate buffer and then washed in cacodylate buffer with sucrose. After dehydration with gradient alcohol, the specimens were sputter coated with gold palladium. A scanning electron microscopy (3400N, Hitachi, Japan) was used to characterize the cells' morphology and density.

Statistical analysis

For all parameters determined, the results are presented as mean ± standard deviation. Test of significance between results from study pairs was conducted by using Origin 8 (OriginLab Corporation, One Roundhouse Plaza, Suite 303, Northampton, MA) one-way ANOVA with significance $p < 0.05$.

RESULTS

Compressive properties

Incorporation of 20 wt % Sr-5HA lowered the modulus of the bone cement by 17% (Table I). With an increase in the Sr5-HA particle loading from 5 to 20 wt % the cement's compressive strength reduced from 63 ± 2 MPa to 58 ± 2 MPa.

Incorporation of linoleic acid in the monomer reduced the compressive strength and modulus of the cement to 49 ± 2 MPa and 774 ± 70 MPa, respectively. The modulus of the linoleic acid containing Sr5-HA cement was much lower than that of the 20 wt % Sr5-HA group.

Bone cement injectability and curing properties

It can be noticed that the injectability of the bone cement improved to 100% as 15 v/v MMA was replaced by linoleic acid (Table II). Without the addition of linoleic acid, the injectability barely exceeded 10% as the high BPO and DMPT content lead to a rapid increase in the viscosity.

The exotherms curve of the experimental formulation was measured at 24°C ± 1°C, and the values of the curing parameters are as summarized in Figure 1. All Sr5-HA bone cement groups fulfilled the setting time and temperature requirements (>3 min and <90°C). Addition of linoleic acid to the monomer phase brought down the temperature from 74 to 42°C and prolonged the setting time to 27 min. The setting time is longer than for the control cement (14–20 min).

Bone cement radiopacity

The radiopacity of 20 wt % of Sr5-HA and 20 wt % Sr5-HA with 15 v/v linoleic acid bone cement were lower than that of the control cement. Sr5-HA specimens showed good homogenecity with only a few bright spots observed on X-ray photography (Figure 2).

Hydrophobicity

Below 10 wt %, the Sr5-HA bone cement's water contact angle was not different from that of the control cement (Table III). The addition of 15 v/v linoleic acid decreased the water contact angle to 60° ± 3°. From the water

TABLE II. Injectability of 5, 10, and 20 wt % Sr5-HA, 20 wt % Sr5-HA with 15 v/v Linoleic Acid and Control Bone Cement

	Injectability (%)
5 wt %	0
10 wt %	4
20 wt % Sr5-HA	10.2
20 wt % Sr5-HA with 15 v/v linoleic acid	100

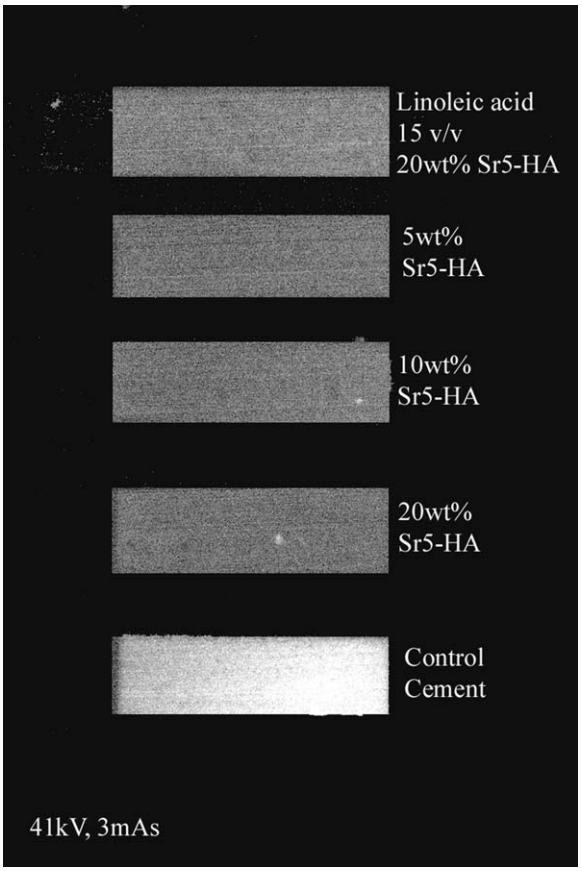

FIGURE 1. Radiopacity of (a) 5 wt % Sr5-HA, (b) 10 wt % Sr5-HA, (c) 20 wt % Sr5-HA, (d) 20 wt % Sr5-HA with 15 v/v linoleic acid, and (e) control bone cement taken at 41 kV and 1.8 mAs.

absorption data, it can be noticed that the linoleic acid group had a higher water absorption rate than the all pure Sr5-HA group.

Bioactivity

Apatite-like particle formed on the 20 wt % Sr5-HA bone cement surface. (Figure 3) No material deposition was found on the control cement surface despite immersion in SBF solution for 14 days. TEM images showed plate-like particle deposition in the Sr5-HA group's cement surface (Figure 4). From the SAED pattern's d-spacing measurement, these plate-like particles were confirmed as apatite.

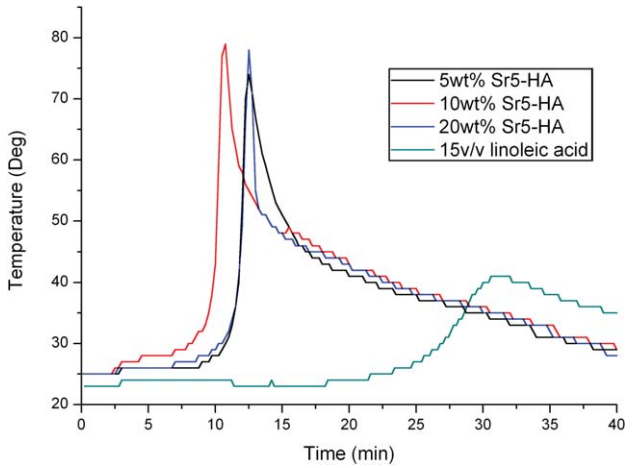

FIGURE 2. Setting curve of (a) 5 wt % Sr5-HA, (b) 10 wt % Sr5-HA, (c) 20 wt % Sr5-HA, and (d) 20 wt % Sr5-HA with 15 v/v linoleic acid bone cement. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

From Figure 5, Sr5-HA group's strontium ion concentration was significantly higher than that of the control cement. At day 3 and 14, a decrease in strontium ion concentration was observed in the Sr5-HA group.

Cell proliferation rate

At day 3 and 7, the WST-1 assay showed that the cell proliferation rate was on the order of 20 wt % Sr5-HA > 20 wt % Sr5-HA with 15 v/v linoleic acid group > 10 wt % Sr5-HA > 5 wt % Sr5-HA > control cement specimen (Table I). On the basis of Table I, 20 wt % Sr5-HA and 20 wt % Sr5-HA with 15 v/v linoleic acid had significantly higher number of cells attached on the cement surface than on the control cement ($p < 0.05$).

Cell morphology

Regarding *in vitro* biocompatibility, the results obtained from the direct seeding of cells on the cement surface indicate that there was a slight cell length difference between the culture on the control cement and the 20 wt % Sr5-HA cement. After incubation for 3 days, the 20 wt % Sr5-HA bone cement surface showed more cells adhered on the cement surface than in the control 5 and 10 wt % groups (Figure 6).

TABLE III. Water Contact Angle and Water Absorption at Day 1 and 7

	Water Contact Angle	Water Adsorption (wt %) Day 1	Water Adsorption (wt %) Day 7
Control cement	74 ± 5	0.67 ± 0.07	0.60 ± 0.07
5 wt %	93 ± 4[a]	0.83 ± 0.07	1.21 ± 0.62
10 wt %	84 ± 6[a]	0.78 ± 0.07	0.88 ± 0.22
20 wt % Sr5-HA	69 ± 3	0.45 ± 0.10	1.17 ± 0.27
20 wt % Sr5-HA with 15 v/v linoleic acid	60 ± 3[a]	2.23 ± 1.41	1.87 ± 0.68

[a] Based on one-way ANOVA, 5, 10, and 20 wt % Sr5-HA with 15 v/v linoleic acid contact angle value is significant different from control cement ($p < 0.05$).

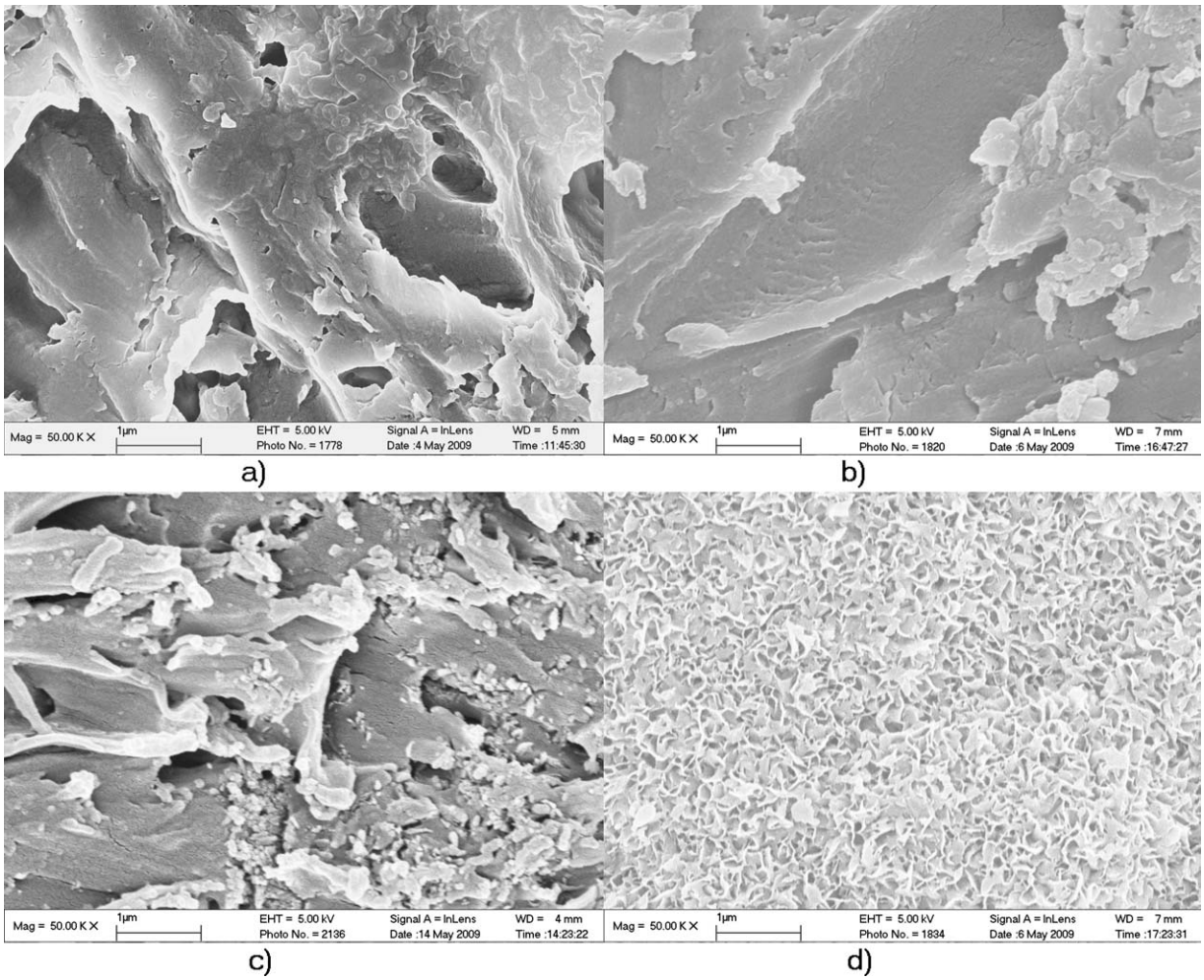

FIGURE 3. Surface morphology of bone cements before and after immersed in SBF at 37°C for 14 days: (a) control cement and (b) 5 wt % Sr5-HA, (c) 10 wt % Sr5-HA, (d) 20 wt % Sr5-HA, and (e) 20 wt % Sr5-HA with 15 v/v linoleic acid.

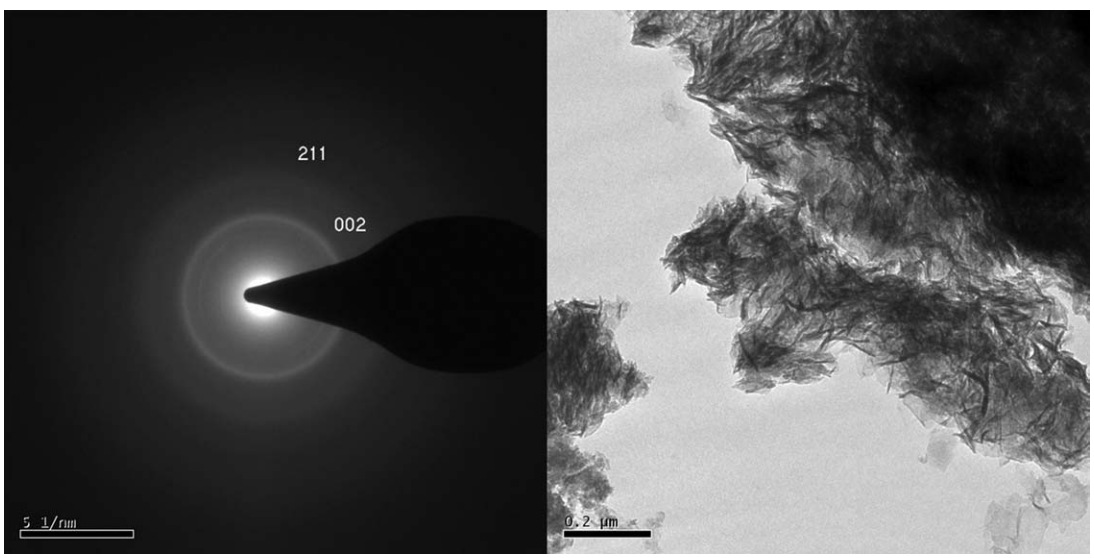

FIGURE 4. TEM and SAED pattern of precipitated particle deposited on 20 wt % Sr5-HA cement surface after 14 days; the coating was easily scratched from the surface after cleaning and drying.

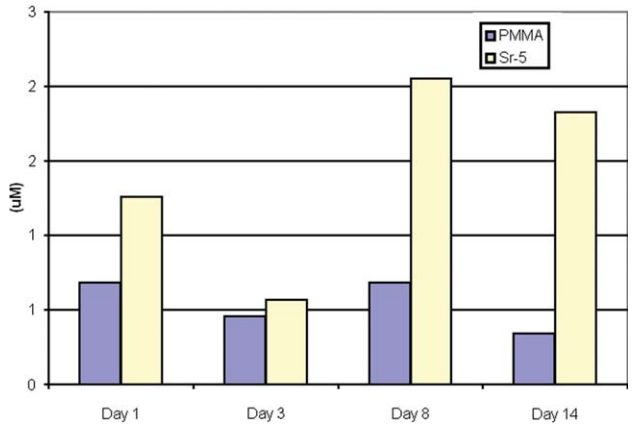

FIGURE 5. Strontium concentration of control cement and 20 wt % Sr5-HA bone cements after soaking in SBF at 37°C for 1, 3, 8, and 14 days. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

DISCUSSION

While the current porous PMMA cement can reduce the cement modulus to a level close to the osteoporotic bony tissue, generation of wear particle and low mechanical strength remain the major limitations. Therefore, in this study we aimed to develop a low modulus cement based on an alternative mechanism that has minimal impact on the mechanical strength. Since the Sr5-HA cement's stiffness is lowered by the linoleic acid's plasticizer effect and formation of linoleic acid copolymer on Sr5-HA–PMMA interface, the mechanical strength of the bone cement does not deteriorate to the level of its porous PMMA and hydrogel counterparts.[9,12]

Compressive properties

At high filler loading rates, increased Sr5-HA filler interaction leads to aggregate formation. Aggregate acts as a stress concentrator and reduces the mechanical properties of the bone cement.[18] From Table I, it can be noticed that the modulus drops when Sr5-HA loading increases from 5 to 10 wt %. As the Sr5-HA filler loading increases to 20 wt %, the aggregate size growth and mechanical deterioration level off.

Linoleic acid acts as a plasticizer[19] by increasing the free volume between the polymer chains and the Sr5-HA filler, allowing more chain movement.[20] Addition of linoleic acid could reduce the modulus by two mechanisms. Formation of low molecular weight linoleic acid-PMMA copolymer can reduces the bone cement modulus in a load dependent manner. Since linoleic acid is miscible with PMMA, it can act as an internal lubricant by reducing the frictional force between the polymer chains. On the basis of report by Boger et al., reduction of endplate fracture can be achieved with low modulus cement. The refracture risk increases in low modulus bone cement reinforced vertebra at high ultimate loads. Compared with the porous PMMA cement, the 20 wt % Sr5-HA with 15 v/v linoleic acid group has a higher mechanical strength that may reduce the refracture risk while slightly increasing the endplate fracture risk due to higher stiffness. The refracture risk efficiency of new low modulus cement cannot be confirmed further animal studies.

Bone cement injectability and curing properties

Higher DMPT or BPO content increases the free radical reaction rate and reduces the working time of Sr5-HA bone cement.[21] The setting time is also affected by Sr5-HA filler loading, longer setting time is obtained as Sr5-HA filler loading increases. These findings match with other group's PMMA Sr-HA research data.[7]

The powder-to-liquid ratio (PLR) of bone cement plays an important role in controlling the setting time and temperature, higher PLR prolongs setting time and lowers setting temperature. The PLR of control cement (Vertebroplasty Radiopaque Resinous Material) is 2.47 g/mL while it is 2.0 g/mL for the Sr5-HA cement. Lower PLR in Sr5-HA was adopted to compensate the increase in viscosity of less density Sr5-HA filler incorporation. Lower PLR also leads to higher setting temperature and shorter working time.

Bone cement radiopacity

Radiopacity of Sr5-HA bone cement is lower than control cement as the radiopacifer content is lower. Because of low strontium content incorporated in hydroxyapatite, Sr5-HA has lower radiopacity than $BaSO_4$ or ZrO_2. Vertebroplasty cement generally contains 30–36 wt % $BaSO_4$ or ZrO_2 that is much higher than Sr5-HA cement ($\sim$10 wt % of $BaSO_4$). Therefore, an addition of 5–10 wt % radiopacifier such as $BaSO_4$ or ZrO_2 is needed to improve the cement's X-ray contrast.

Hydrophobicity

Hydrophobicity of the bone cement surface plays an important role in optimizing its performance in cell growth and proliferation.[22] PMMA bone cements are highly hydrophobic, a limited hydration capacity of about 2–3 wt % and higher water contact angle have been reported for joint replacement bone cements.[23] Radiopacifier content of verterbroplasty bone cement can approach 36 wt % to improve X-ray contrast about 3–3.6 times higher than their joint replacement counterparts. Radiopacifier content ($BaSO_4$ or ZrO_2) increases the porosity of bone cements and water adsorption.

Sr5-HA filler has a lower water contact angle while its water absorption is similar to its control cement counterpart.[24] Other researchers have shown that 2–15 wt % HA incorporation could increase the water absorption of the PMMA composite from 0.04 and 0.64 wt % after 24 h of water immersion. On the other hand, incorporation of linoleic acid leads to formation of less hydrophobic linoleic acid–PMMA copolymer surface, increasing water absorption to 2.14 wt % after 1 day of water immersion.

Bioactivity, cell adhesion, and proliferation rate

In vivo bioactivity of bone cement is manifested through a deposition of apatite which converts to new bone formation providing direct contact between bone cement and bony

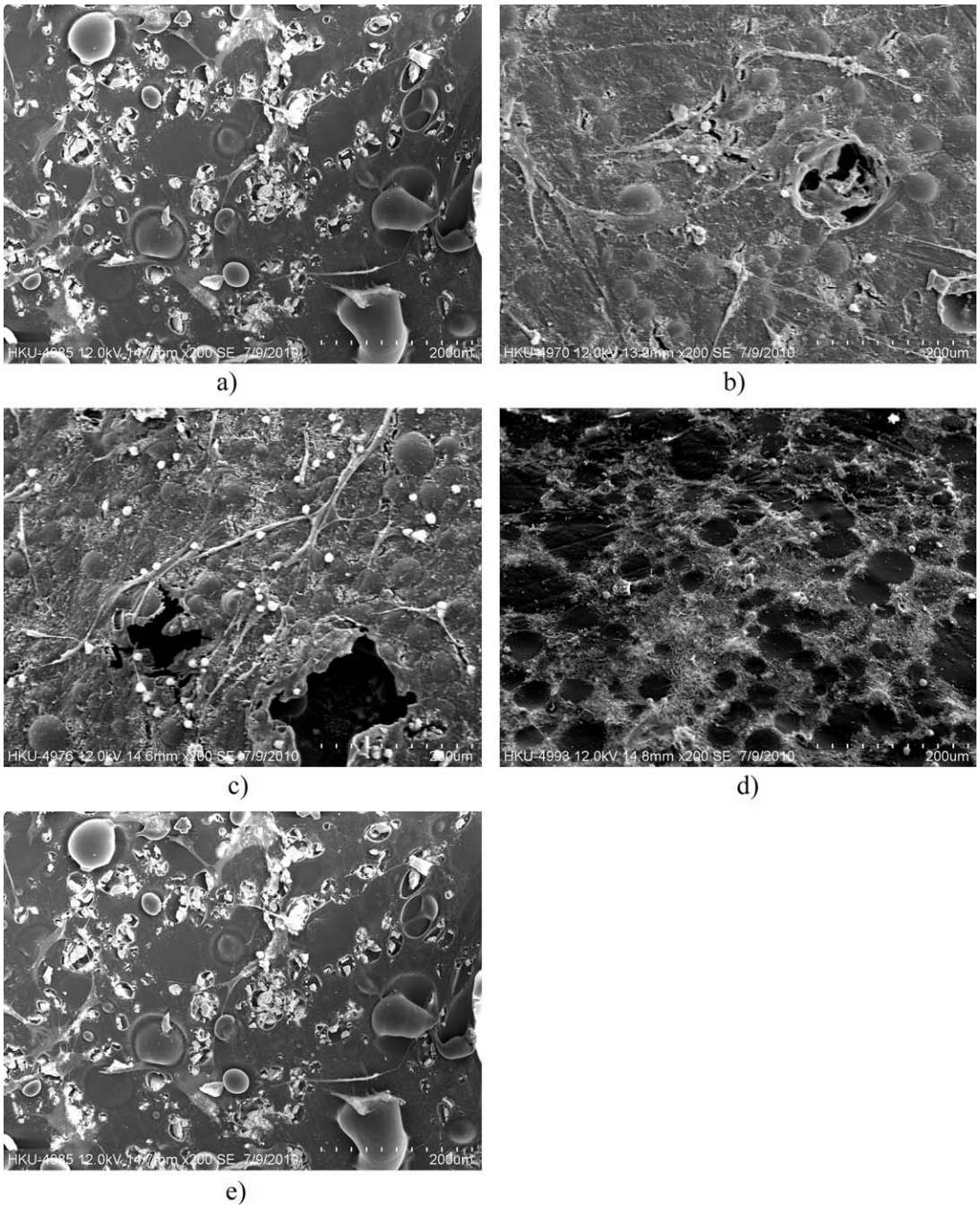

FIGURE 6. SEM image of cell adhesion on control bone cements and (a) 5 wt %, (b) 10 wt %, (c) 20 wt % Sr5-HA, (d) 20 wt % Sr5-HA with 15 v/v linoleic acid bone cement, and (e) control cement surface seeded with MC3T3-E1 respectively for 3 days.

tissue. From Kokubo study, formation of apatite is a good indicator of bone-bonding behavior.[25] From various studies, strontium substituted hydroxyapatite is known to improve the bioactivity of PMMA and Bis-GMA bone cements.[9,24] From the studies performed on cured cement, 20 wt % Sr5-HA cement is seen to produce the deposited layer on their cement surface. On the other hand, no deposited layer was observed in the control group. Strontium ions released from Sr5-HA increase apatite nucleation rate by over saturation.[26] Traces of strontium ions observed in the control cement extract may be related to impurity in $CaCl_2$ and $MgCl_2$ component in the SBF solution.

From the WST-1 assay, on day 7, the cell number increased from 1.68×10^4 to 3.73×10^4/well as the Sr5-HA content increased from 5 to 20 wt %. Higher Sr5-HA content increases strontium release via larger area of exposed filler surface and higher cement porosity. Linoleic acid release and strontium ions increase the MC3T3 proliferation rate in Sr5-HA bone cement.[7,9,27] Despite linoleic acid–PMMA provides more hydrophilic environment that is

favorable to cell adhesion and proliferation, 15 v/v linoleic acid group has a lower proliferation rate than 20 wt % Sr5-HA group. Linoleic acid lowered down the monomer conversion and increased the DMPT release that may inhibit cell growth. WST-1 results matched with apatite layer formation in SEM images. Formation of thick layer deposit reduces cell proliferation on the linoleic acid surface. From the SEM images, more spherical cells were found on the linoleic acid bone cement surface.

Limitation of this study

Reduction of DMPT content is suggested to improve cement injectability in the linoleic acid-free group. Additional experiments are needed to determine their mechanical properties based on construct of cadaveric vertebral bodies. Quasi-static and fatigue compressive properties of bone cement should be evaluated by new fractured vertebra model to determine long-term stability.

CONCLUSION

The addition of 10–20 wt % Sr5-HA effectively decreased the modulus of PMMA bone cement while retaining its compressive strength. Formation of linoleic acid—PMMA polymer on the filler—cement interface leads to stiffness reduction in Sr5-HA bone cement. The compressive modulus can be significantly reduced by the addition of 15 v/v linoleic acid into the MMA monomer phase to 49 $\pm$ 2 MPa and to 774 $\pm$ 70 MPa when 15 v/v of linoleic acid is added to the liquid phase. Because of the strontium ion release, the 20 wt % Sr5-HA group and the linoleic acid Sr5-HA bone cement group have higher cell proliferation rate than the control cement.

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