Preparation of epoxy–SiO_2 hybrid sol–gel material for bone cement

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Abstract: An organic–inorganic hybrid material, epoxy–SiO_2, was prepared by incorporating epoxy structure units covalently into a SiO_2 glass network via the sol–gel approach. The precursor was obtained by the reaction of diglycidyl ether of bisphenol A (DGEBA) with 3-aminopropyl trimethoxysilane (APTS). The precursor was then hydrolyzed and co-condensated with tetraethyl orthosilicate (TEOS) in tetrahydrofuran (THF) at room temperature to yield epoxy–SiO_2 hybrid sol–gel material having a 50 wt % SiO_2 content. Thermal properties of the hybrid material were characterized by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). The hybrid sol–gel material epoxy–SiO_2 was the solid, powder component of bone cement. The liquid component contains bis-phenol-A glycidyl methacrylate (Bis-GMA), triethyleneglycol dimethacrylate (TEGDMA), and methyl methacrylate (MMA) with 25, 55, and 20 vol %, respectively. We discuss the comparison between the new epoxy–SiO_2 bone cement and the commercial Simplex® P bone cement. Mechanical properties such as Young's modulus, compressive strength, hardness, and impact strength of the new epoxy–SiO_2 bone cement exceeded those of Simplex^R P bone cement. The tensile and bending strengths of the new epoxy–SiO_2 bone cement were approximately the same as those of Simplex® P bone cement. In order to evaluate the biocompatibility of the new bone cement, an MTT test and optical microscopy were conducted in cell culture. Results indicated that the new epoxy–SiO_2 bone cement exhibits very low cytotoxicity compared with Simplex® P bone cement. © 2002 Wiley Periodicals, Inc. J Biomed Mater Res 64A: 138–146, 2003

INTRODUCTION

Self-curing acrylic cements have been widely used in dentistry and orthopedic surgery as filling agents and for the fixation of joint prostheses.[1–3] Recent studies, with up to 20-year follow-up, have demonstrated the clinical success of prostheses implanted with cement.[4,5] However, there are still some disadvantages to the use of acrylic bone cement, including thermal necrosis of bone, chemical necrosis because of unreacted monomer release, shrinkage of the cement during polymerization, poor cement distribution around the implant, and property mismatch at the interfaces because the cement is orders of magnitude weaker than the bone or implant. To improve the performance of the existing surgical cement, many researchers have attempted to solve these problems by incorporating additional agents[6–14] into the conventional ingredients of the acrylic bone cement. Bisphenol-A glycidylmethacrylate (Bis-GMA) has been used as resin binder in dentistry since 1963.[15] In the study by Saito et al.,[16] a hydroxyapatite composite resin was developed with Bis-GMA as the base resin.

We have tried to improve the performance of bone cement in a series of studies[17–26] by using surgical-

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grade Radiopaque Surgical Simplex® P as the basic ingredient for the bone cement. Effects of the addition of tricalcium phosphate (TCP), hydroxyethyl meth acrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), and methyl methacrylate-grafted-ultrahighmolecular weight polyethylene fiber (MMA-g-UHMWPE) on tensile strength, tensile modulus, bending strength, bending modulus, compressive strength and compressive modulus, as well as dynamic mechanical analysis, were investigated. Effects of TCP, HEMA, and EGDMA on the polymerization of bone cement were also evaluated by differential scanning calorimetry (DSC).

In our previous study,[27] three organic–inorganic hybrid materials were prepared via the sol–gel approach. Because of the formation of covalent bonds and the elimination of macroscopic interfaces between polymers and inorganic components, physical properties of the organic–inorganic hybrid sol–gel materials could be designed and controlled by varying the nature and composition of both the polymer and the inorganic components. Therefore, the product of an organic–inorganic hybrid material poly(methyl methacrylate) (PMMA)–SiO_2 was applied to hard tissue applications such as fillers for dental composite resins and bone cement in our previous studies.[28,29]

In this study, an epoxy–SiO_2 hybrid sol–gel material was prepared by incorporating epoxy structure units covalently into an SiO_2 glass network via the sol–gel approach. The precursor was synthesized by the reaction of diglycidyl ether of bisphenol A (DGEBA) with 3-aminopropyl trimethoxysilane (APTS). The precursor was subsequently hydrolyzed and co-condensated with tetraethyl orthosilicate (TEOS) in tetrahydrofuran (THF) at room temperature to yield epoxy–SiO_2 hybrid sol–gel material.

The epoxy–SiO_2 hybrid sol–gel material was evaluated as the solid component of bone cement. Bis-GMA, EGDMA, and MMA were used as the liquid component of bone cement. The mechanical properties and biocompatibility of the new epoxy–SiO_2 bone cement were determined by comparison with commercial Simplex® P bone cement.

EXPERIMENTATION

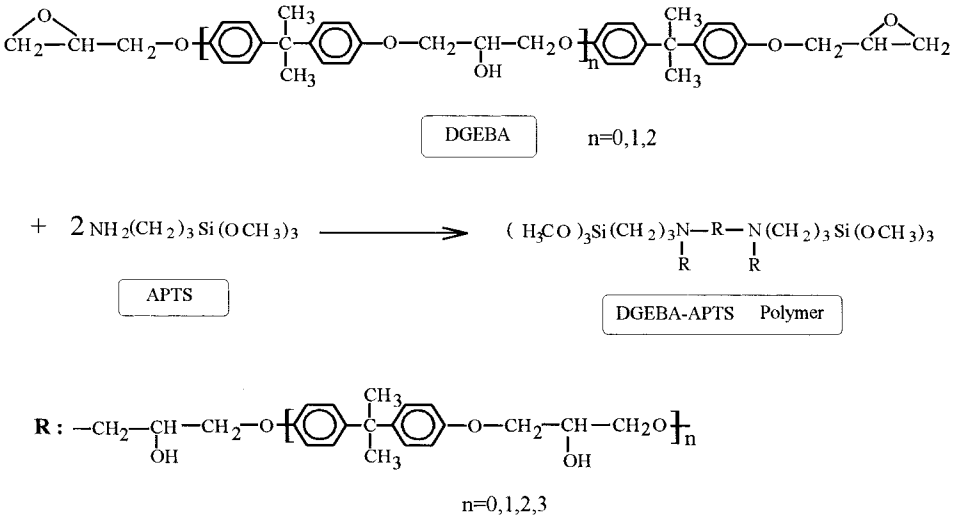
Synthesis of epoxy–SiO_2 hybrid sol–gel material

The precursor was synthesized under nitrogen in a thermostatted flask fitted with a constant speed stirrer, an inert gas inlet, a thermometer, and a condenser. The reaction flask contained a stoichiometric amount of DGEBA and APTS in toluene solution at 70°C under nitrogen to yield the precursor and then precipitated into *n*-hexane with stirring. The epoxy content of the product was titrated with HBr resulting from the reaction of perchloric acid with tetraethylammonium bromide with crystal violet as an indicator[30] to confirm completion of the reaction. The reaction is outlined in Scheme 1.

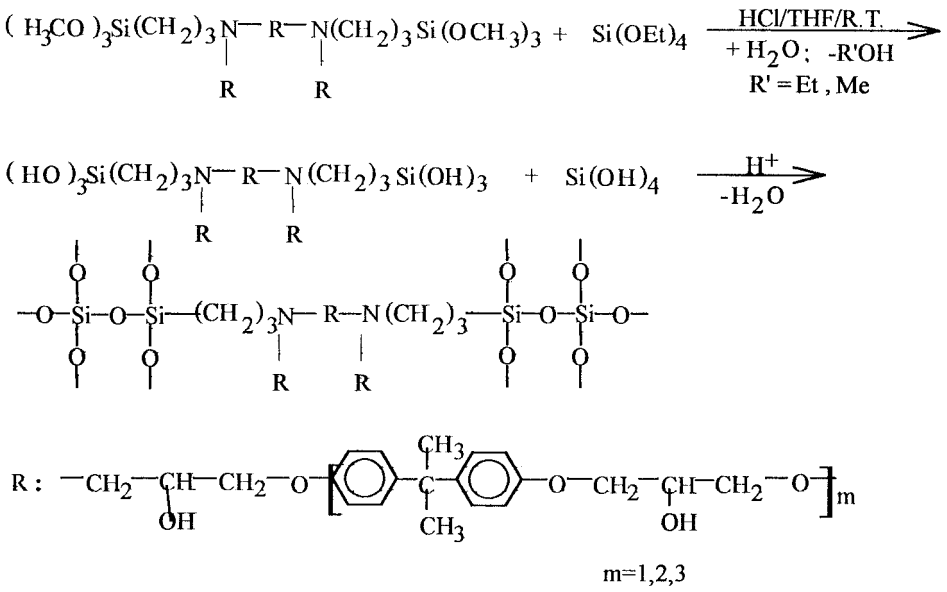
The precursor was hydrolyzed and co-condensated with TEOS with 0.4 *M* HCl as catalyst in THF at room temperature to yield epoxy–SiO_2 hybrid sol–gel material. The synthetic procedure was similar to that of the PMMA–SiO_2 hybrid sol–gel material described in our previous study.[27] The procedure is illustrated in Scheme 2, and the reaction is outlined in Scheme 3. The characteristics and thermal properties of the precursor and epoxy–SiO_2 hybrid sol–gel material were characterized by Fourier transform infrared spectroscopy (FTIR), DSC, and thermogravimetric analysis (TGA).

Preparation of new bone cement containing epoxy–SiO_2 hybrid sol–gel material

The chemical composition of the new bone cement consisted of a solid powder and a liquid component. The solid powder contained benzoyl peroxide (BPO) and epoxy–SiO_2



Scheme 1.



Scheme 2.

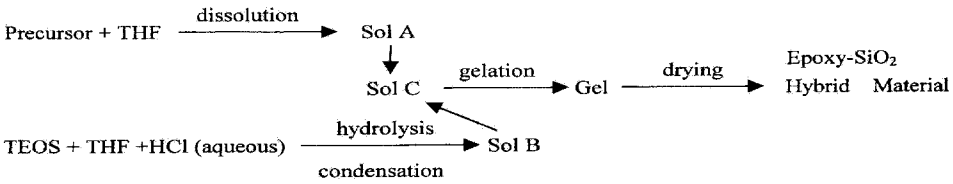
hybrid sol–gel material that was prepared following the above description. The median particle size was about 26.6 μm. The liquid component contained Bis-GMA, triethyleneglycol dimethacrylate (TEGDMA), and MMA with 25, 55, and 20 vol %, respectively. *N,N*-dimethyl-*p*-toluidine was used as accelerator. All tests were performed keeping the constant ratio of powder to liquid components at 1.2:1.0. To reduce the porosity during the mixing of the liquid component and the solid powder, the process was performed in a Simplex® Enhancement Mixer (Howmedical Int., Ltd.). Each specimen was cast in a mold; a uniform stress (10 kPa) was exerted by placement of a known weight.

In order to study the effect of particle size on the compressive strength of bone cement, SiO_2 hybrid sol–gel materials with different median particle sizes of 20.5, 26.6, 32.7, and 39.2 μm were also prepared.

Measurements

The tensile strength, Young's modulus, bending strength, compressive strength, and impact strength of the new epoxy–SiO_2 hybrid sol–gel bone cement were compared with those of commercial Simplex® P bone cement obtained from Howmedical Int. Ltd. Each packet contained 40 g of prepolymerized powder and 20 mL of liquid monomer. The liquid monomer component is composed of MMA monomer with *N,N*-dimethyl-*p*-toluidine as accelerator. The solid component is composed of 75 wt % of MMA-styrene copolymer, 15 wt % of PMMA, 10 wt % of $BaSO_4$, and a tiny amount of BPO as initiator.

Four kinds of specimen were prepared for these measurements. Specimens of each sample were cut to a dumbbell of 6.25 × 2 mm for the tensile strength measurements. The tensile strength was measured with a Material Test System (MTS Model 810 from MTS Systems Corp.) at 25°C with a crosshead speed of 0.5 mm/min. Rectangular beams (5 × 5 × 40mm) were prepared for the measurement of bending strength with an MTS at 25°C, with a crosshead speed of 0.5 mm/min. Rectangular beams (10cm × 2mm × 10mm) were prepared for the measurement of impact strength in accordance with the procedure outlined in American Society for Testing and Materials (ASTM) E23. Cylindrical specimens of 10-mm diameter and 10-mm length were prepared for the compressive measurement with a MTS at 25°C, with a crosshead speed of 2 mm/min. The hardness of the samples was measured with a microhardness tester (Matsuzawa, MXT 50) with a load of 50 g and contact time of 10 s. The tests for the mechanical properties of each specimen were conducted for 10 samples to ensure good reproducibility of the measurements. The time and temperature of polymerization of the new bone cement were the same as in our previous study.[29] The solid component was poured into the liquid component and spatulated. A thermocouple was used to monitor the time–temperature profile of the bone cement. The maximum temperature during curing was determined directly, and the time was also recorded.



Scheme 3.

Cell-culturing method

Osteoblasts were isolated according to the method of Boonekamp, with some modifications.[31–33] Briefly, calvaria from 20-day-old rat embryos were excised aseptically and incubated for 2 × 10 min at 37°C with 4 m*M* ethylenediaminetetraacetic acid (EDTA) in phosphate-buffered saline (PBS). After rinsing the calvaria for 3 × 5 min with PBS, they were incubated for 10 min with collagenase (1 mg/mL PBS) at 37°C. After removal of the periostea, the cell suspension containing periosteal fibroblasts was discarded. Then osteoblasts were isolated by further collagenase treatment for 2 × 30 min. The supernatant was centrifuged for 5 min at 1500 rpm. The pellet obtained was resuspended in culture medium: minimum essential medium (α-MEM) containing 5% inactivated fetal calf serum, 1mg/mL glucose, and 90 μg/mL gentamycin. The sterilized substructures were placed in 6-well culture dishes. Samples for cell population and rate of cell growth were seeded with a higher cell density of approximately 5×10^4 cells/mL; 1mL of cells was added in each well and incubated at 37°C with 5% CO_2 for 1, 3, and 7 days, respectively. Half of the medium was replaced every 72 h in this culturing period.

MTT measurement

The end point of a microtitration assay is usually an estimate of cell number. Although this can be directly tested by cell counts or by indirect methods such as isotope incorporation, MTT reduction as a cell viability measurement is now widely chosen as the optimal end point. MTT is a yellow, water-soluble tetrazolium dye that is reduced by live, but not dead, cells to a purple formazan product that is insoluble in aqueous solution but can be dissolved in dimethylsulfoxide (DMSO). We briefly describe here the procedure of MTT measurement. The medium was carefully removed from the cultured well after 1-, 3-, and 7-day culture. The well was washed with PBS three times, then 400 μL of medium was added to each well; 100 μL of MTT solution was added to each well in an environment without light. The culture dish was packed with aluminum foil paper and incubated 4 h. The cultured medium was then removed by pipette, and 200 μL of DMSO was added to each well and then mixed for few minutes. Then, 100 μL mixed solution was removed from each well and put into a 96-well culture dish. The solution of the well was examined with an enzyme-linked immunosorbent assay (ELISA) with 570 nm of absorbency wavelength.

Preparation for optical microscopy

After being cultured for a period of time, the substrata were carefully removed from the cultured well dish. Both substrata and well dishes were fixed in 2.5% glutaraldehyde in 0.1 *M* sodium cacodylate buffer for 2 h, rinsed with PBS (3 × 5 min), and dehydrated in a graded ethanol series. The well dishes were stained (Stute method) with hematoxylin and eosin for population observation (Nickon FX-150, Tokyo, Japan). The alkaline phosphate stain was used to prove the osteoblasts did not transfer into fibroblasts or other tumor cells.[34]

Statistical analysis

Mean, standard deviation (SD), and graphs were used to describe the data. An unpaired *t* test was conducted to compare the difference in tensile strength, Young's modulus, bending strength, compressive strength, and hardness and impact strength between Simplex® P and epoxy–SiO_2. The difference in the maximum temperature of cement during curing and the time it took specimens to reach the maximum temperature were also compared by unpaired *t* test. Compressive strength was compared among four particle sizes of epoxy–SiO_2 using one-way analysis of variance (ANOVA). Duncan multiple comparison was further performed in a significant ANOVA. Two-way ANOVA with repeated measure was performed on cell growth in Simplex® P, epoxy–SiO_2, and control. When interaction between time (24, 72, and 168 h) and specimens (Simplex® P, epoxy–SiO_2, and control) was significant, one-way ANOVA with repeated measure was made to determine whether the OD_{570} grew significantly in each specimen. One-way ANOVA was made to assess the difference in OD_{570} at different hours among three specimens. A 95% confidence interval (CI) was computed for OD_{570} in each specimen. All *p* values were two-sided and significant level was 0.05. SAS/win 8.1 was used to conduct all statistical analysis.

RESULTS AND DISCUSSION

Characteristics of precursor and epoxide–SiO_2 hybrid sol–gel material

Figure 1 shows the absorption spectra of pure APTS, DGEBA, and precursor prepared by reaction in Scheme 1. The characteristic absorption bands for the N–H and Si–O bonds of APTS appear at 1580 and 1088 cm^{-1}, respectively, and that for epoxide group of DGEBA appears at 915 cm^{-1}, whereas the absorption peak of epoxide group at 915 cm^{-1} was not found in the precursor.

The FTIR spectra of precursor are essentially the sum of APTS and DGEBA except for the difference in the relative intensities of the absorption bands, and without the epoxide group. Because the APTS and DGEBA have been extracted with *n*-hexane and the epoxy content was not apparent in the titration as described in experiment, the presence of this absorption band confirmed that the precursor was the product of the reaction between APTS and DGEBA.

The thermal property of the hybrid sol–gel material was characterized using TGA and DSC (results are shown in Figs. 2 and 3). In the TGA illustrated in Figure 2, the thermal stability of the epoxide–SiO_2 sol–gel material increased significantly in comparison with the precursor. The onset decomposition temperature increased from about 280 to 320°C. The major weight loss of the epoxy–SiO_2 hybrid sol–gel material

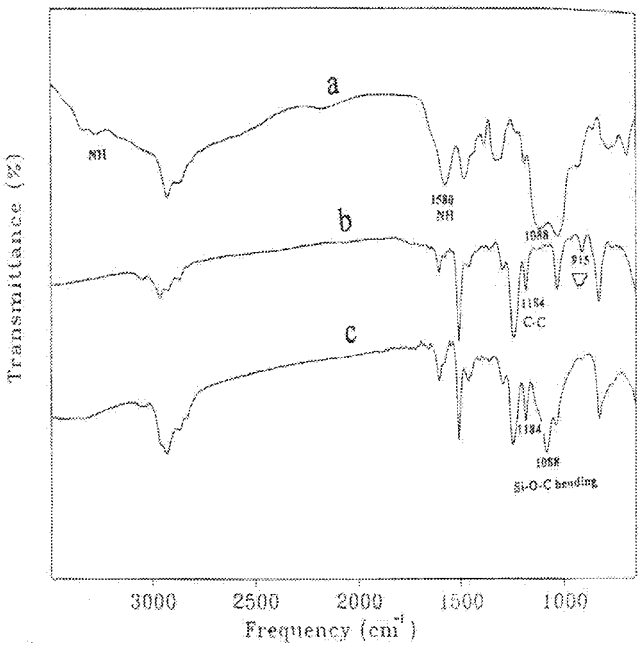


Figure 1. Fourier transfer infrared spectrum of (a) 3-aminopropyl trimethosysilane, (b) diglycidyl ether of bisphenol A, and (c) precursor. The characteristic absorption bands for the N–H and Si–O bonds of APTS appear at 1580 and 1088 cm^{-1}, respectively, and that for epoxide group of DGEBA appears at 915 cm^{-1}, whereas the absorption peak of epoxide group at 915 cm^{-1} was not found in the precursor.

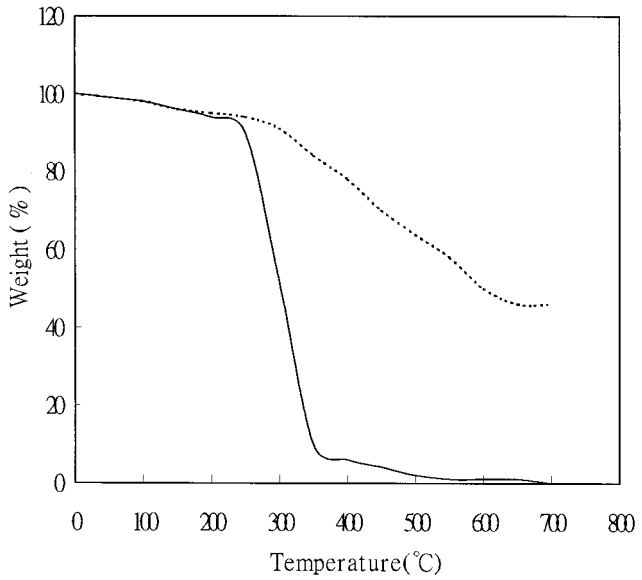


Figure 2. The weight percentage versus temperature of precursor (solid line) and epoxy–SiO_2 hybrid sol gel (dotted line) material. The onset decomposition temperature is about 280°C for the precursor, whereas the onset decomposition temperature is about 320°C for the epoxide–SiO_2 hybrid sol–gel material. At temperatures higher than 650°C, the TGA curve for the epoxy–SiO_2 hybrid sol–gel material levels off.

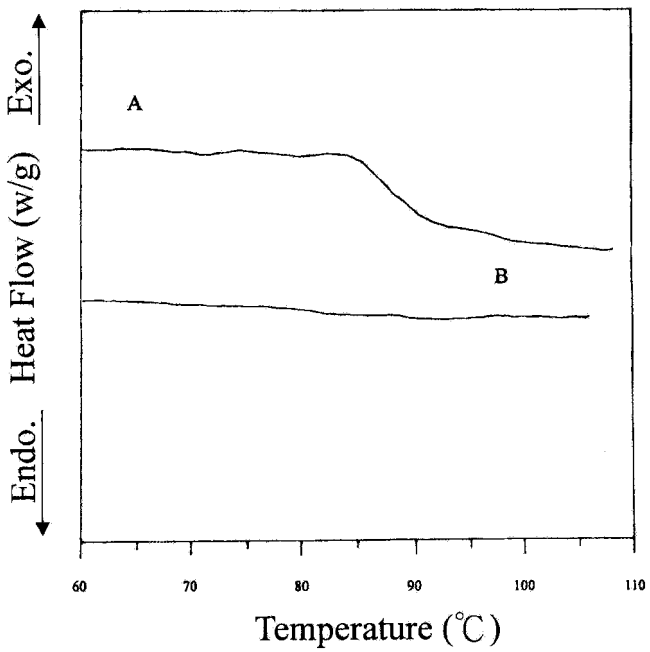


Figure 3. DSC curves of (A) precursor and (B) epoxy–SiO_2 hybrid sol–gel material. The glass transition temperature was about 85°C for the precursor. The DSC curve of the epoxy–SiO_2 hybrid sol–gel material does not evidence any distinct thermal transition in the test region.

at the onset temperature of about 320°C was attributed to the decomposition of the precursor because of its organic component, which was completely removed at temperatures higher than 650°C, and there was no residue at temperatures higher than 650°C. But the TGA curve leveled off for the epoxy–SiO_2 hybrid sol–gel material at temperatures higher than 650°C, because of the existence of the inorganic SiO_2 component.

In the DSC curves in Figure 3, the glass transition temperature was about 85°C for the precursor, whereas there was no distinct thermal transition in the test region for the epoxy–SiO_2 hybrid sol–gel material. This lack of glass transition suggested that the precursor chains might be uniformly distributed in the inorganic SiO_2 glass network.

As described in our previous study,[27] the thermal stability of the hybrid sol–gel materials increased significantly with the SiO_2 content. These results are consistent with the theory that the polymer chains are uniformly distributed in and covalently bonded to the amorphous SiO_2 matrices.[35]

Properties of new epoxide–SiO_2 hybrid sol–gel bone cement

In Table I, the mechanical properties of new epoxide–SiO_2 hybrid sol–gel bone cement are compared

TABLE I
Mechanical Properties of Bone Cement

Properties	Simplex® P	Epoxy–SiO_2	P Value[a]
Tensile strength (MPa)	35.9 ± 2.1[b]	37.6 ± 2.9	0.1510
Young's modulus (GPa)	2.8 ± 0.2	5.9 ± 0.5	<0.0001
Bending strength (MPa)	69.3 ± 2.9	62.6 ± 5.7	0.0120
Compressive strength (MPa)	90.1 ± 2.4	126.0 ± 2.9	<0.0001
Hardness (HAD)	70.5 ± 1.1	81.7 ± 1.2	<0.0001
Impact strength (Kg-m/cm^2)	80.0 ± 0.7	85.3 ± 0.8	<0.0001

[a]Unpaired *t* test
[b]Mean standard deviation

with those of commercial Simplex® P bone cement. We found the new epoxide–SiO_2 hybrid sol–gel bone cement significantly superior to commercial Simplex® P bone cement. The Young's modulus of new epoxide–SiO_2 hybrid sol–gel bone cement was 5.9 GPa, about twice that of commercial Simplex® P bone cement ($p < 0.0001$). The compressive strength, impact strength, and hardness were 126 MPa, 85.3 Kg-m/cm^2, and 81.7, respectively. They were higher than those of commercial Simplex® P bone cement ($p < 0.0001$ for compressive strength, impact strength, and hardness, respectively). Tensile strength was about the same for both bone cements ($p = 0.1510$). The bending strength of commercial Simplex® P bone cement was higher than that of new epoxide–SiO_2 hybrid sol–gel bone cement ($p = 0.0120$).

The effect of epoxide–SiO_2 hybrid sol–gel particle size on the compressive strength is shown in Table II. We found that compressive strength increased with decreasing the particle size of the solid epoxide–SiO_2 hybrid sol–gel ($p < 0.0001$; Duncan's multiple comparison revealed that compressive strength significantly differed in 4 particle sizes).

The maximum polymerization temperature of new epoxide–SiO_2 hybrid sol–gel bone cement was about 74°C, and the corresponding time was about 155 s. The values obtained for commercial Simplex® P bone cement under the same conditions were 97°C and 323 s, respectively (Table III).

As we know, the properties of acrylic bone cements are affected by intrinsic and extrinsic factors.[36] The

TABLE II
The Effect of Particle Size of Epoxy–SiO_2 on Compressive Strength

Particle Size of Epoxy–SiO_2 (μm)	Compressive Strength (MPa)
20.5	147.91 ± 13.70
26.6	125.99 ± 2.93
32.7	81.33 ± 3.71
39.2	70.00 ± 4.70
P value[a]	<0.0001

[a]One-way ANOVA

TABLE III
Maximum Temperature of Bone Cement During Curing and Time to Reach Maximum Temperature

Specimen	Maximum Temperature (°C)	Time (s)
Simplex® P	97 ± 6	323 ± 10
Epoxy–SiO_2	74 ± 5	155 ± 12
p value[a]	<0.0001	<0.0001

[a]Unpaired *t* test

intrinsic factors include the composition of the liquid and the powder phases, the powder particle size, the shape and distribution, and the liquid–powder ratio. As indicated in the discussion of the characteristics of the epoxy–SiO_2 hybrid sol–gel material in this article and in the study by Wei et al.,[37] the bulk properties of the SiO_2-containing hybrid sol–gel materials differ significantly from their precursors. The densities of organic polymers are usually lower than those of inorganic glasses (e.g. 2.06 g/cm^3 for the SiO_2 sol–gel glass). Higher bulk density and hardness can be achieved by increasing the content of SiO_2 in the hybrid materials. Because the epoxy–SiO_2 is a rigid, organic–inorganic hybrid sol–gel material, both the Young's modulus and compressive strength (5.9 GPa and 126 MPa, respectively) of the new epoxy–SiO_2 bone cement are higher than those of Simplex® P bone cement (2.8 GPa and 90 MPa, respectively).

The ASTM standard specifies that the minimum compressive strength of bone cement is 70 MPa. The compressive strength of our new epoxy–SiO_2 bone cement is significantly higher. Because the commercial cement is orders of magnitude weaker than the bone or implant, the result is property mismatch at the interfaces that contributes to the loosening phenomenon. Given that the modulus of our new epoxy–SiO_2 bone cement is about twice that of commercial Simplex® P bone cement, the disadvantage of clinical loosening may be alleviated with our new epoxy–SiO_2 bone cement.

The exothermic character of the polymerization reaction may play a role in the thermal necrosis of the bone, which could induce early loosening of an implant. At standard ASTM conditions, the maximum temperature during the exothermic polymerization is 90°C. When we compared the results of new epoxy–SiO_2 bone cement and commercial Simplex® P bone cement, the maximum polymerization temperature of new epoxy–SiO_2 bone cement was lower than that of Simplex® P bone cement. Because of the difference during the testing and extrinsic factors associated with the preparation method of the samples, the maximum polymerization temperature of Simplex® P bone cement was higher than that of the ASTM condition. Given that the maximum polymerization temperature of our new epoxy–SiO_2 bone cement is lower than that of Simplex® P bone cement and the ASTM standard

condition, the thermal necrosis may be reduced by our new epoxy–SiO_2 bone cement.

Cell culture

The biocompatibility of new epoxide–SiO_2 hybrid sol–gel bone cement and commercial Simplex® P bone cement was evaluated by MTT testing. The results of osteoblast cells grown on these surfaces are shown in Table IV. Because interaction between time and cement was significant, one-way ANOVA with repeated measure revealed that the OD_{570} grew significantly in each sample (p = 0.0213 for Simplex® P, 0.0110 for epoxy–SiO_2, and 0.0016 for control). OD_{570} was not significantly different at 24 h among three samples (p = 0.1056), but it was significantly different at 72 and 168 h among three samples (p < 0.0001 and 0.0002, respectively).

As shown in Figure 4, the results revealed that the cells not only remained viable but also proliferated on the surface of the new epoxide–SiO_2 hybrid sol–gel bone cement, as indicated by the positive slopes of the cell growth curves. The results of the regression are listed in Table V. The relative rate of cell growth was calculated by dividing the slope of the cell growth curves of both bone cements by the slope of the cell growth curve of the control material (polystyrene). The growth rate of commercial Simplex® P bone cement (about 0.17) was significantly lower than the relative rate of cell growth in the new epoxide–SiO_2 hybrid sol–gel bone cement (about 0.89).

The optical microscopy in Figure 5(a and b) reveals that our new epoxide–SiO_2 hybrid sol–gel bone cement and Simplex® P bone cement would not harm the growth of the osteoblast. The morphology of the osteoblast still retains its phenotype. After staining with alkinephosphatase [Fig. 5(c,d)], the cytosol turned dark green in color, indicating that the osteoblast cocultured with our new epoxide–SiO_2 hybrid sol–gel bone cement and Simplex® P bone cement retained its original function in osteolinage without any transformation. From the structure, the cytoskeleton can be clearly observed in order from structure. It reflects that the osteoblasts are favored to stay with our new epoxide–SiO_2 hybrid sol–gel bone cement.

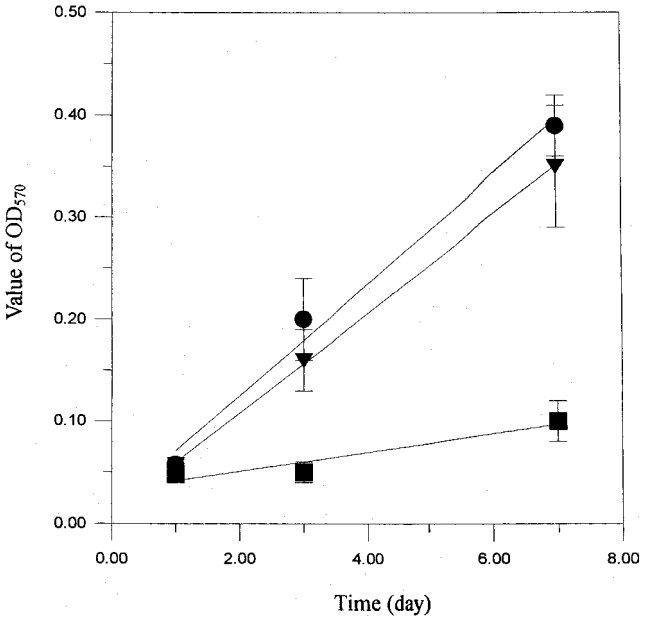


Figure 4. Typical cell growth on various samples: (●) control, (▼) epoxy–SiO_2 bone cement, and (■) Simplex® P bone cement. The relative rate of cell growth was calculated by dividing the slope of the cell growth curves of both bone cements by the slope of the cell growth curve of the control material (polystyrene). The growth rate of commercial Simplex® P bone cement (about 0.17) was significantly lower than the relative rate of cell growth in the new epoxide–SiO_2 hybrid sol–gel bone cement (about 0.89).

It has been clearly demonstrated that the MMA monomer shows cytotoxic levels in all fractions of the tissue medium in the study by Kusy.[38] When the liquid and solid components of commercial Simplex® P bone cement are mixed, the polymerization of the MMA monomer is initiated. Because the polymerization is not complete, residual unreacted MMA monomer in the bone cement *in vivo* is implicated in chemical necrosis of the bone.

The results illustrated in Table IV and Figure 4 reveal that the cell population and rate of cell growth are significantly inhibited by commercial Simplex® P bone cement. Figure 5 indicates that the new epoxy–SiO_2 hybrid sol–gel bone cement exhibits almost no or low toxicity with respect to osteoblast cells *in vitro*.

TABLE IV
OD_{570} Values of Cell Grown in Various Samples

Hours	Simplex® P	Epoxy–SiO_2	Control	p Value[a]
24	0.048 ± 0.005	0.057 ± 0.005	0.049 ± 0.002	0.1056
72	0.050 ± 0.008	0.153 ± 0.021	0.205 ± 0.004	<0.0001
168	0.100 ± 0.020	0.349 ± 0.061	0.394 ± 0.029	0.0002
p value[b]	0.0213	0.0110	0.0016	

[a]One-way ANOVA
[b]One-way ANOVA with repeated measure

TABLE V
Regression Results of the Cell Growth Curves for Various Samples

	Linear Regression	
Sample	Formula	R^2
Simplex® P	Y = 0.00921429X + 0.0322143	0.913
Epoxy–SiO_2	Y = 0.0486429X + 0.0106429	0.999
Control	Y = 0.0543571X + 0.0163571	0.988

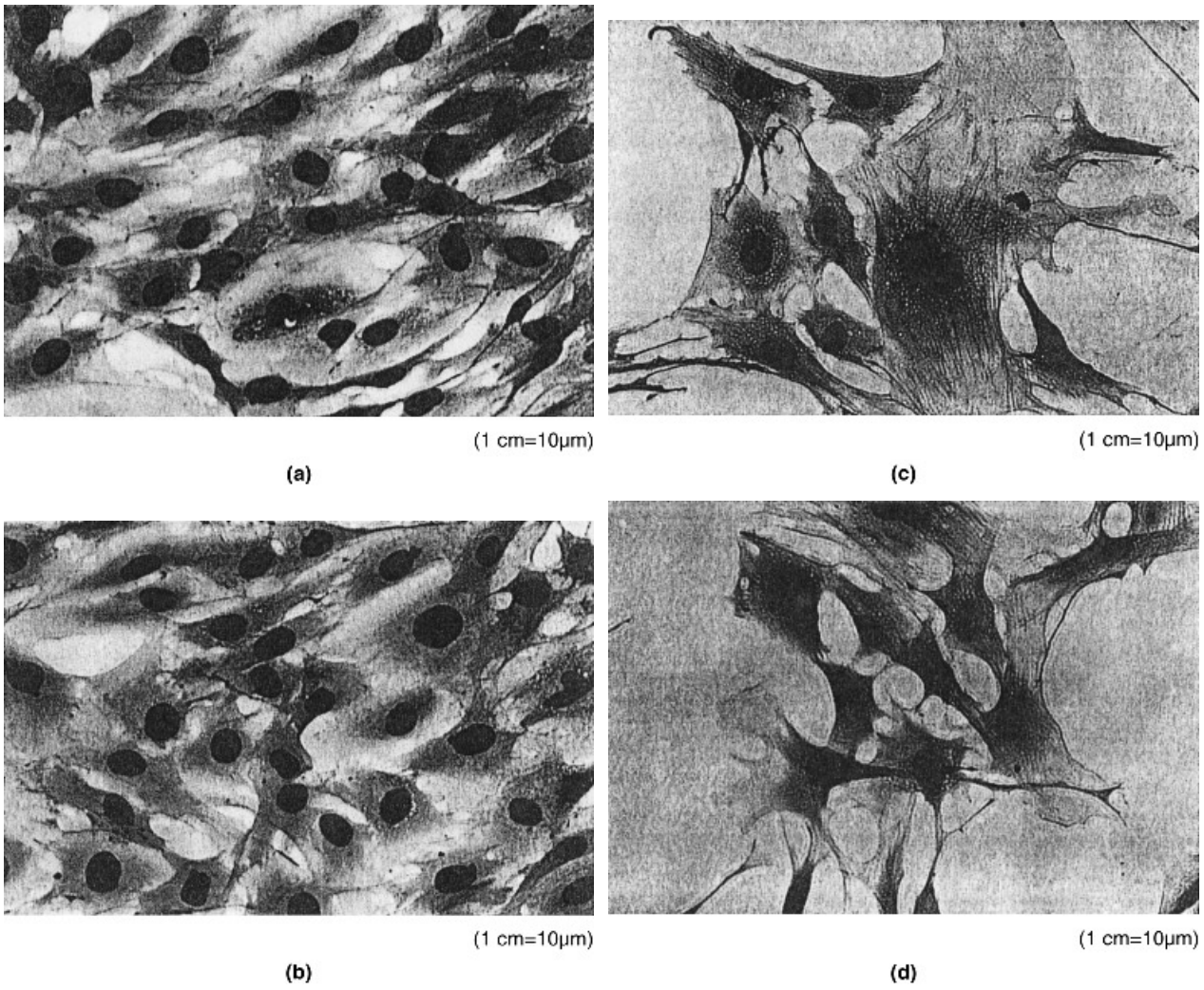


Figure 5. Observation of the morphology of osteoblast by optical microscopy reveals that (a) the epoxide–SiO_2 hybrid sol–gel bone cement does not harm the growth of the osteoblast. The morphology of the osteoblast still remains in its phenotype (1 cm = 10 μm). The commercial Simplex® P bone cement (b) does not affect the proliferation of the osteoblast. The morphology of the osteoblast still remains in its phenotype (1 cm = 10 μm). After staining with alkinephosphatase, the cytosol (c) turned dark green in color, indicating that the osteoblast cocultured with the epoxide–SiO_2 hybrid sol–gel bone cement retains its original function in osteolinage without any transformation. The cytoskeleton can be clearly observed in an order from structure, which means that the osteoblasts are favored to remain with the epoxide–SiO_2 hybrid sol–gel bone cement (1 cm = 10 μm). After staining with alkinephosphatase, the cytosol (d) turned dark green in color, indicating that the osteoblast cocultured with the commercial Simplex® P bone cement keeps its original function in osteolinage without any transformation (1 cm = 10 μm).

CONCLUSIONS

In this study, the organic–inorganic hybrid sol–gel material, epoxy– SiO_2, was prepared by a sol–gel approach. The epoxy– SiO_2 hybrid sol–gel material with about 50 wt % SiO_2 inorganic components was used as the solid component of new bone cement. The liquid component of new bone cement included Bis-GMA, TEGDMA, and MMA of 25, 55, and 20 vol %, respectively. Because the mechanical properties of the new epoxy–SiO_2 hybrid sol–gel bone cement are superior to those of commercial Simplex® P bone cement, with low polymerization temperature and very low cytotoxicity in comparison to Simplex® P bone cement, we will conduct a detailed study of the new epoxy–SiO_2 hybrid sol–gel bone cement in the future.

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