A Novel Composite PMMA-based Bone Cement with Reduced Potential for Thermal Necrosis

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ABSTRACT: Percutaneous vertebroplasty (VP) and balloon kyphoplasty (BKP) are now widely used to treat patients who suffer painful vertebral compression fractures. In each of these treatments, a bone cement paste is injected into the fractured vertebral body/bodies, and the cement of choice is a poly(methyl methacrylate) (PMMA) bone cement. One drawback of this cement is the very high exothermic temperature, which, it has been suggested, causes thermal necrosis of surrounding tissue. In the present work, we prepared novel composite PMMA bone cement where microcapsules containing a phase change material (paraffin) (PCMc) were mixed with the powder of the cement. A PCM absorbs generated heat and, as such, its presence in the cement may lead to reduction in thermal necrosis. We determined a number of properties of the composite cement. Compared to the values for a control cement (a commercially available PMMA cement used in VP and BKP), each composite cement was found to have significantly lower maximum exothermic temperature, increased setting time, significantly lower compressive strength, significantly lower compressive modulus, comparable biocompatibility, and significantly smaller thermal necrosis zone. Composite cement containing 20% PCMc may be suitable for use in VP and BKP and thus deserves further evaluation.

KEYWORDS: PMMA bone cement, phase-change microcapsule, thermal necrosis, setting time, compressive strength, modulus
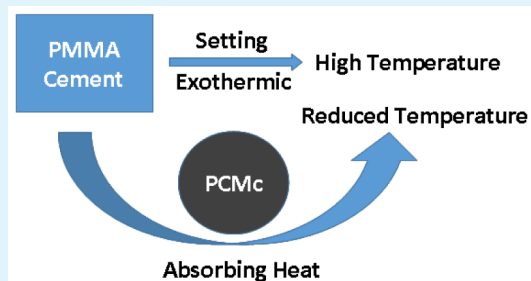
1. INTRODUCTION

Osteoporosis, literally "porous bone", is a disease characterized by weak bones and has been a major public health issue. Hundreds of millions of people, predominantly postmenopausal women, suffer from it.[1] The most common consequence of osteoporosis is bone fractures. It is estimated that, worldwide, ~33% women and ~20% of men over the age of 50 suffer osteoporotic fracture(s).[1] In the European Union, there are ~3.5 million new onset osteoporotic fractures every year, incurring a huge medical cost of over €37 billion.[2] Vertebroplasty (VP) and balloon kyphoplasty (BKP) are now widely used in the treatment of patients who experience severe pain arising from osteoporotic vertebral compression fractures. Each of these treatments involves percutaneous injection of a paste of a poly(methyl methacrylate) (PMMA) bone cement into the fractured vertebral body/bodies.[1]

PMMA bone cement has a number of attractions, notably, ease of preparation and manipulation and high compressive strength. However, the cement has its share of shortcomings, one being that the maximum exothermic temperature experienced during its polymerization (T_{max}) is very high, which may cause thermal necrosis.[3−6] In bone cement augmentation of fractures and anchoring of implants, there have been reports of thermal damage of cartilage and periosteum, leading to nonunion of fractures and loosening of implants.[5,6] In VP and BKP, there have been reports of (1) serious injury to neuromuscular structures by the exothermic reaction, which may cause catastrophic complications such as paralysis and bleeding or even death[7,8] and (2) collapse of augmented vertebrae because of the poor mechanical strength caused by thermal necrosis.[7] However, in VP, it has been postulated that thermal damage to intraosseus neural tissue, as a result of high T_{max}, is what accounts for the pain relief provided by this procedure.[8]

A number of approaches have been taken to reduce T_{max} in the case PMMA bone cements, for example, (1) reducing the ambient temperature in the operating theater or cooling the cement powder and/or cement liquid prior to preparation of the cement paste,[9,10] but the improvement is modest; (2) reducing the molecular weight (M_w) of the cement powder, but it is difficult to control the paste viscosity of a low-M_w cement, making it hard to inject the paste;[11,12] and (3) adding filler
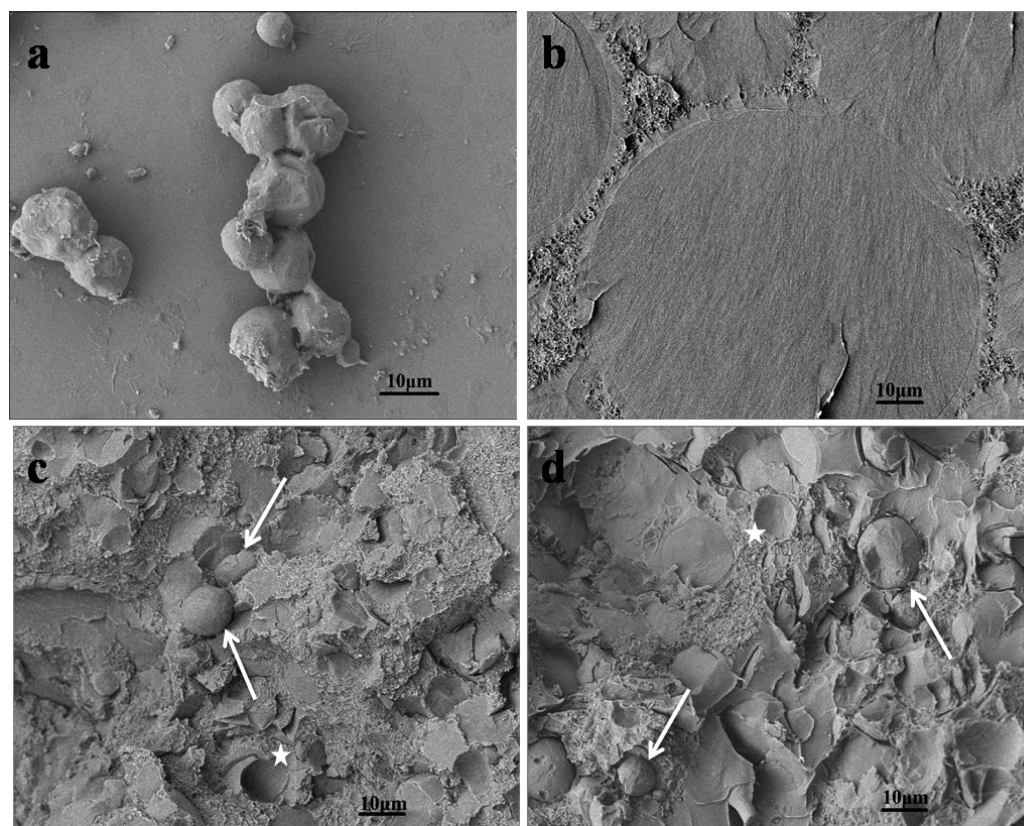
Figure 1. Surface morphologies of the (a) PCMc, (b) PMMA cement, (c) PMMA10 cement, and (d) PMMA20 cement. For the cement specimens, the arrows indicate the PCMcs.

(strontium titanate or ZrO_2 particles) to PMMA, but here again, the improvement is modest.[13,14]

A phase change material (PCM) absorbs a large amount of heat within a narrow temperature window, in the process transforming to a liquid.[15,16] To avoid leaking of the liquid, the PCM may be enclosed in a solid shell, thereby obtaining a PCM microcapsule (PCMc). We hypothesize that, compared to commercially available PMMA bone cement used in VP and BKP, PMMA bone cement with the same composition but with PCMcs mixed in with the cement powder, has significantly lower T_{max} and, hence, significantly lower potential for causing thermal necrosis. In the present work, we determined T_{max}, extent of thermal necrosis, and a number of other properties of these two types of cements.

2. MATERIALS AND METHODS

2.1. Materials. The conventional PMMA bone cement used was a brand that is in clinical use (Mendec Spine; Tecres SpA, Verona, Italy). Its powder (20 g) comprised 67.5 wt % PMMA, 30.0 wt % barium sulfate, 2.5 wt % benzoyl peroxide; and its liquid (9.4 g) comprised 99.1 wt % methyl methacrylate and N,N-dimethyl-p-toluidine, and 75 ppm hydroquinone. The PCM used was paraffin (Shanghai Huayong Paraffin, Ltd., Shanghai, China). Amidopropyltrimethoxysilane (APTMS), and phenyltriethoxysilane (PTES) were purchased from Acros (Brussel, Belgium). Tetraethyl orthosilicate (TEOS) was purchased from Sinopharm Chemical Reagent (Beijing, China). All commercial products were used as received. Hydrolyzed styrene-maleic anhydride (HSMA) copolymer was synthesized and purified, as described in a previous report.[15]

2.2. Preparation of the PCMc. First, 15 mL of 10 wt % HSMA solution was dissolved in 75 mL of water. The solution was kept at 70 °C after adjusting the pH to 2.5 with 2 M hydrochloric acid. At 70 °C, 25.0 g

of paraffin was mixed with 5.2 g of TEOS, 0.92 g of APTMS, and 1.2 g of PTES under stirring. The oil mixture was dispersed into the aqueous solution with a homogenizer at a speed of 13 000 rpm for 5 min, forming an oil-in-water emulsion. The emulsion was kept at 70 °C for 12 h for a self-organized sol−gel process at the emulsion interface. After the resultant emulsion was cooled to room temperature, the core/shell particles (PCMc) with paraffin core and silica shell were obtained by a sequential filtration and wash with water. A white powder was obtained after freeze-drying.

2.3. Preparation of PMMA/PCMc Composite Bone Cement. For the commercially available cement ("PMMA cement"), the powder and liquid were manually mixed (powder-to-liquid ratio (PLC) = 2 g/mL) to form a paste. For a composite cement, the powder of the commercially available cement and the desired amount of the PCMc were thoroughly mixed and then ground, yielding a homogeneous mixture. Three compositions of the composite cement were prepared, with the powder mixture containing 10% PCMcs ("PMMA10 cement"), 20% PCMcs ("PMMA20 cement"), and 30% PCMcs ("PMMA30 cement"). Each powder mixture was manually mixed with the liquid of the commercially available cement (PLC = 2 g/mL) to form a paste.

2.4. Characterization. *2.4.1. Surface Morphologies of PCMc and Cements.* The cement powder mixture and liquid were mixed in a plastic cylindrical mold with a diameter of 5 mm and a height of 10 mm and then the paste was injected into the Teflon mold. After setting, the specimen was removed from the mold, cut into small pieces (4 × 3 × 2 mm), coated with a thin layer of gold (Au) by sputtering (SCD 500), and observed using a scanning electron microscope (SEM) (JEOL-6700, JEOL, Japan), operated at an acceleration voltage of 15 kV. For each of the cement composites prepared, the SEM examination was done in triplicate.

2.4.2. Exothermic Temperature and Setting Time of Cement. The powder mixture and the cement liquid were manually mixed for about 2 min at room temperature. Then, a contact thermocouple was inserted into the paste to record the temperature of the paste as it polymerized.
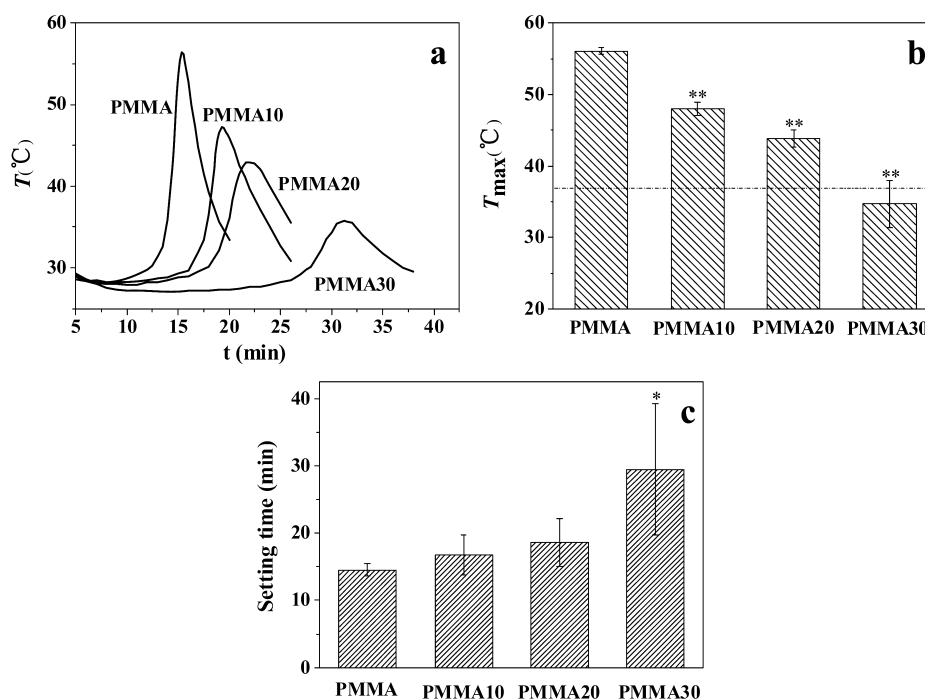
Figure 2. Temperature profile of the cements: (a) temperature of setting cement paste versus mixing time (t), (b) summary of the maximum exothermic temperature (T_{max}) results, (c) and summary of the setting time results. In panel b, the dashed line indicates mean normal body temperature (37 °C). Statistical significance: **$p < 0.01$; *$p < 0.05$.

The temperature was recorded at intervals of 1 min. For each of the cement composites prepared, the test was run in triplicate. As specified by ISO 5833, the maximum temperature (T_{max}) attained by the bulk was recorded, and the setting time (t_{set}) was determined as the time taken to reach a temperature midway from room temperature (T_{amb}) and T_{max}.

2.4.3. Compressive Properties of Cement. The cement paste was prepared as was done for the t_{set} tests and then poured into a rectangular Teflon mold, yielding test specimens measuring $4 \times 8 \times 12$ mm. In a test, the specimen was positioned in a general-purpose materials testing machine (Instron 3365; Intron Corp., St. Paul, MN) and compressed, using a 5 kN load cell, at a crosshead displacement speed of 0.5 mm/min. Three compressive properties of the cement were determined; namely, failure strain (e_f), compressive strength (UCS), and compressive modulus (E_c). For each of the cement composites prepared, the test was run five times.

2.4.4. Cell Compatibility Evaluation. L929 cells were used to evaluate the cell compatibility of resultant cement materials. L929 cells were incubated with 4 mL of 0.25% trypsin (Sigma, St.Louis, MO) for 20 min with gentle shaking at room temperature. The trypsin was removed, and fetal bovine serum (FBS, HyClone, Logan, UT) was added to terminate digestion. After digestion, the fragments were uniformly attached to each culture flask bottom and were cultured in a humidified atmosphere of 5% CO_2 at 37 °C. After 24 h, Dulbecco's modified Eagle's medium (DMEM, Sigma) supplemented with 1% penicillin/streptomycin antibiotics (Sigma) and 10% FBS, was added to the flasks. The medium was changed every 2 days. Cell subcultures at passage 2 were used in the following studies.

The composite cements were previously sterilized under UV light for 12 h, and then soaked in α-MEM, preheated to 37 °C (extracting vehicle ratio = 0.2 g/mL) for 3 days as specified by ISO 10993−12:2007.[17] Under sterile conditions, α-MEM was filtered to eliminate cements. The extracts were further diluted and used as culture medium after adding 10% fetal bovine serum (FBS).

A Cell Count Kit-8 (CCK-8, Beyotime, China) was employed to quantitatively evaluate cell viability. L929 cells were cultured in the presence of different extracts of the composite cements on 96-well culture plates at a density of 5×10^5 cells/ml. The cells cultured in 96-well without cement extracts were used as a control. The cells of different groups were cultured for 24 h. The CCK-8 solution (20 μL per well) was added to each well, followed by cultured at 5% CO_2 and 37 °C for 4 h, and 100 μL of the reacted reagent from each well was transferred to 96-well plates. The absorbance was measured at 450 nm using a microplate spectrophotometer (Multiskan MK3, Thermo, Waltham, MA). Six parallel replicates of each condition were tested.

2.4.5. Extent of Thermal Necrosis of Cement. Nine fresh bovine lumbar vertebrae (L1) obtained from three 6-year-old healthy oxen, were assigned randomly into one of three groups of three vertebrae for each type of implant. The cement paste of either the PMMA cement or the PMMA20 cement (1.5 mL) was injected into the pedicles of each of the vertebral bodies using the standard bilateral transpedicular approach. Twenty-four hours after the injection, a three-dimensional computer tomography (CT) scan of the vertebral body was taken (Siemens, Munich, Germany). The size of the thermal necrosis zone was measured using picture archiving and communication systems (PACS, GE, New York, NY). After that, a power saw was used to slice the vertebra for macroscopic examination.

2.5. Statistical Analysis. All quantitative results were given as mean ± standard deviation. The Kruskal−Wallis one-way analysis of variance was used for intergroup comparison, with significant difference being denoted when $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Surface Morphologies. The PCMc is spherical, with diameter of 10 ± 3 μm (Figure 1a). Cross section of the pure PMMA cement has a plain texture (Figure 1b), but that of a composite cement is coarse, and the PCMcs are clearly seen (Figure 1c,d). At higher PCMc content, no coalescence among the PCMc is found. No gap between the capsules shell and the PMMA matrix is observed. This implies that the PCMc particles are well compatible with the PMMA cement matrix. It is noted that the PCMc particles preserve their original spherical shape after the polymerization of the bone cement. This indicates that the PCMc shell is very tough. The tough shell is beneficial to avoid the capsules breakage and paraffin leakage under stress.

3.2. Setting Properties. The temperature-versus-mixing time profile is the same for both the control and the composite
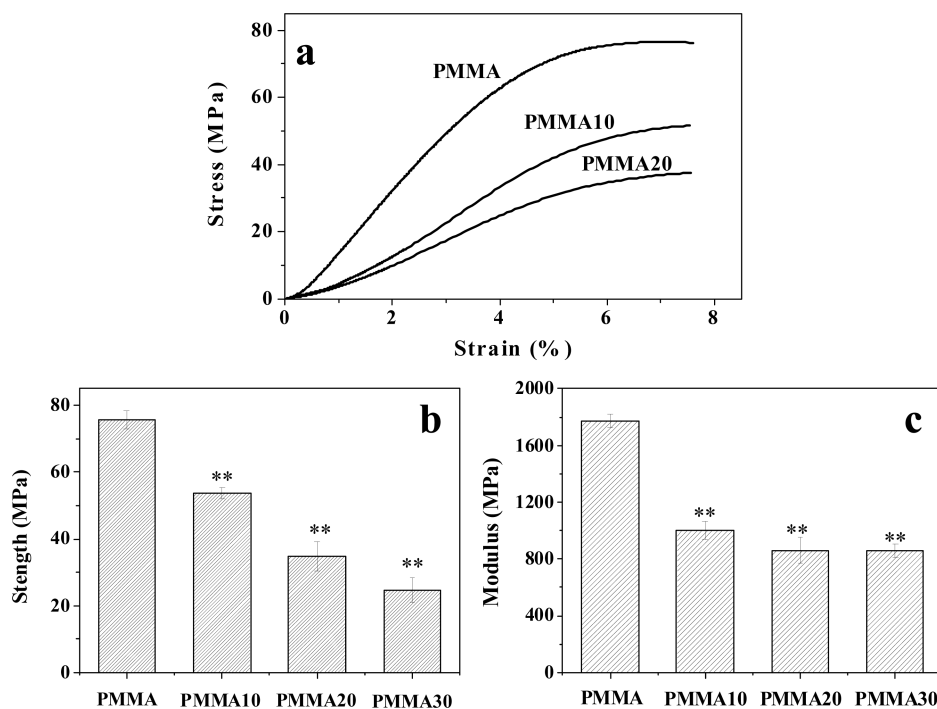
Figure 3. (a) Typical compressive stress-versus-compressive strain resultscancellous bones; (b) summary of the compressive strength results; and (c) summary of the compressive modulus results (statistical significance: **$p < 0.01$).

bone cements (Figure 2a). However, there is a monotonic decrease in T_{max} with increase in the PCMc content of the composite cement, with the value for each of the composite cements being significantly lower than that of the control cement (Figures 2b). The possible reason for this trend is that the PCMcs have high latent heat and suitable phase transition temperatures for thermal energy storage and retrieval, which make them have capacity to store large amounts of thermal energy in narrow temperature ranges.[15] In the case of t_{set}, while there is a monotonic increase with increase in PCMc content of the composite cement, the increase of this parameter, relative to its value for the control cement, is significant only when PCMc content is >20 wt % (Figure 2c). Setting time is 15, 18, 20, and 32 min for PMMA, PMMA10, PMMA20, and PMMA30, respectively. According to clinical practice, the setting time is recommended within 25 min so that the surgeries can be finished in time.[8] Therefore, from a practical point of view, the PCMc content should be controlled below 20 wt % to ensure a setting time shorter than ~20 min. A maximum temperature of around 44 °C for PMMA20 is also acceptable.

3.3. Compressive Properties. For each of the cements tested, e_f was ~7% (Figure 3a), indicating their mechanical compatibility with both cancellous and cortical bones.[18,19] Compared to values for the control cement, both UCS and E_c of a composite cement were significantly lower (Figure 3b). In the absence of PCMc, the PMMA cement has the highest compressive stress and modulus (~76 and ~1770 MPa, respectively, Figure 3b). They decreased to ~54 and ~1000 MPa, respectively, for PMMA10. With even more PCMc incorporated, those for PMMA20 further dropped to ~35 and ~860 MPa, respectively (Figure 3b). As known in daily orthopedic practice, the mechanical property of bone substitutes should ideally be close to cancellous bones (20 and 800 MPa for compressive strength and modulus, respectively.[19-21]). Indeed, PMMA cement was found to be too rigid for bone substitutes,

which could easily cause adjacent fracture after implanted into osteoporotic bones, mainly due to the stress screen effect.[8,22,23] When the strength and modulus of bone cement are reduced to the level of surrounding bone, the stress screen effect can be avoided. Therefore, with PCMc incorporated, the PMMA/PCMc cements might be a better bone substitute, even in terms of mechanical properties, although they actually become weaker than PMMA cement itself.

3.4. Cell Compatibility. The cell viability values were normalized to that of the control cement at the same cell culture interval; thus, 100% and above denotes excellent biocompatibility. On the whole, composite cement was as biocompatible as the control cement or better (Figure 4). These results showed that PMMA/PCMc cements up to 20 wt % PCMc did not have any negative effect on the growth of cells, indicating they had good biocompatibility.
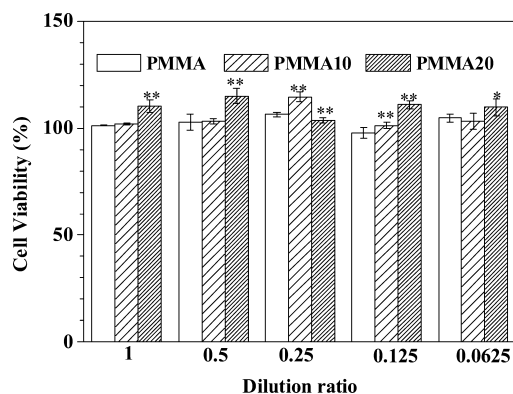
Figure 4. Summary of the cell viability results: CCK-8 assay for proliferation of L929 cells cultured with extracts of PMMA and PMMA/PCMc cements. Statistical significance: **$p < 0.01$, *$p < 0.05$.
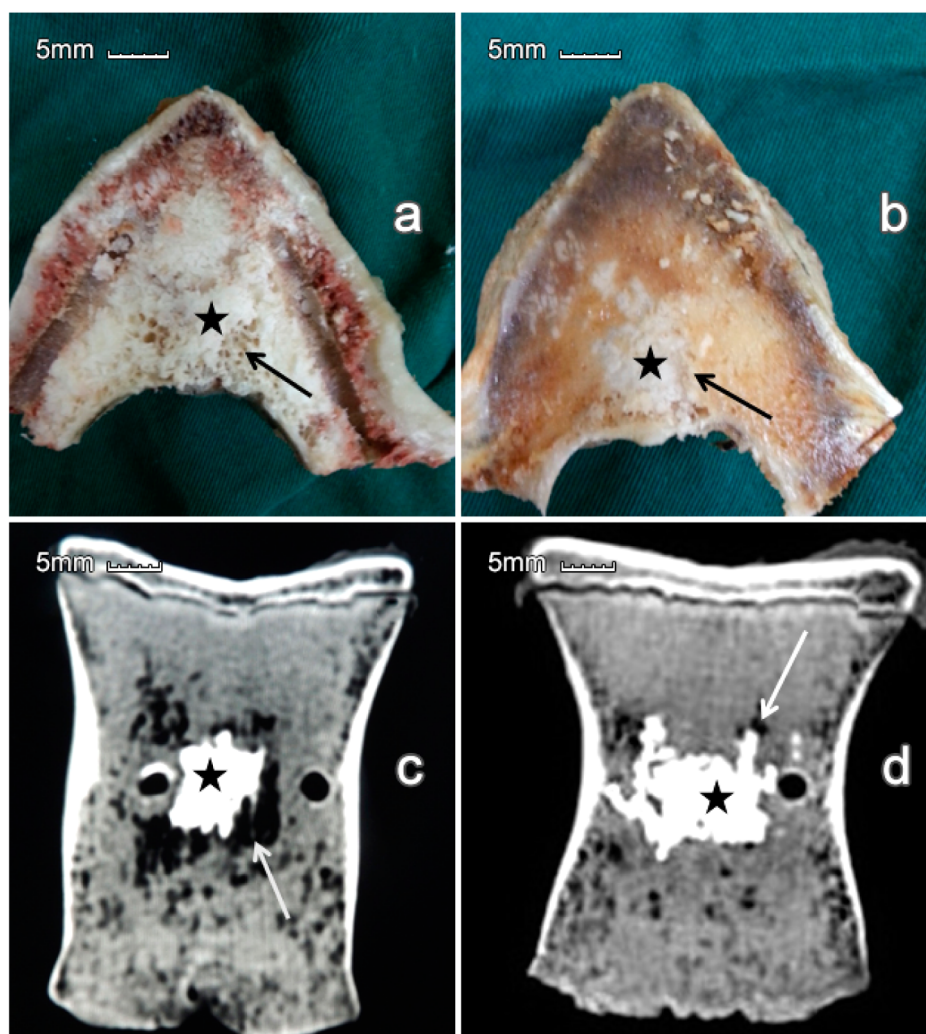
Figure 5. Optical images of the bovine lumber vertebrae implanted with (a) PMMA and (b) PMMA20 and (c and d, respectively) their corresponding coronal CT images of the necrosis zone 24 h after injection of the cement samples. The arrows point to the thermal necrosis zone and stars (★) mark the positions of implanted cements.

3.5. Macroscopic Aspects, CT Images, and Extent of Thermal Necrosis. Twenty-four hours after implantation, direct macroscopic observations of bovine lumbar vertebrae implanted with both PMMA and PMMA20 showed clear differences (Figure 5a,b). The former had many pores, while the latter had no pores, as judged using the unaided eye. This difference was probably caused by the different temperature the samples had endured, that is, different necrosis effect. Coronal CT reconstruction images further confirmed this difference (Figure 5c,d).

The extent of the thermal necrosis zone in vertebrae implanted with PMMA20 cement (mean diameter, 0.6 mm; Figure 5c) was significantly smaller than that in vertebrae implanted with the control cement (mean diameter, 4.5 mm; Figure 5d; $p < 0.001$)

4. CONCLUSIONS

A novel composite PMMA bone cement was formulated in which the powder comprised microcapsules containing a phase change material (paraffin) (PCMc) that were thoroughly mixed with the powder of a commercial PMMA cement used in vertebroplasty (VP) and balloon kyphoplasty (BKP), and the liquid was that of the commercially available cement. In preparing the composite cement paste, a powder/liquid ratio

of 2 g/mL was used. Three variants of this composite cement were prepared, containing 10, 20, and 30% PCMcs

Compared to the value for the control cement, the composite cement had significantly lower maximum exotherm temperature, higher setting time, significantly lower compressive strength, significantly lower compressive modulus, comparable biocompatibility, and significantly smaller thermal necrosis zone.

When all the present results and desirable property values for a cement for use in VP and BKP were considered, a composite cement containing 20% PCMc may be a promising cement to use in these treatments. Thus, this composite cement should be studied further.

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Notes

The authors declare no competing financial interest.

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