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Original Article

A novel injectable porous surface modified bioactive bone cement for vertebroplasty: an in vivo biomechanical and osteogenic study in a rabbit osteoporosis model

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Abstract: *Purpose:* The aim of this study is to determine the feasibility and effectiveness of a novel injectable Porous Surface Modified Bioactive Bone Cement (PSMBBC) for vertebroplasty of aiding osteoporotic vertebrae in an osteoporosis model. *Methods:* 72 osteoporosis rabbits were randomly divided into three groups: the Polymethyl Methacrylate (PMMA) group, the PSMBBC group and the control group. PMMA and PSMBBC were administrated to osteoporotic vertebrae in vertebroplasty, respectively. The animals were sacrificed at 1w, 4w, 12w after the procedure. Micro-CT analysis, biomechanical tests and histological analysis were performed at each time point. *Results:* From 4 to 12 weeks after the implantation of bone cements, the bone volume fraction (BV/TV) of the PSMBBC group increased from 28.27 ± 1.69% to 38.43 ± 1.34%. However, the BV/TV of the PMMA group showed no significant difference after the implantation. At 4 weeks, direct contact between the bone and the bone cement was observed in the PSMBBC group. At 12 weeks, it was discovered that new intact bone trabecular was formed in PSMBBC group. Furthermore, the maximum compressive strength values of the PSMBBC group were significantly higher than those of the control group at each time point after implantation. *Conclusions:* In summary, this study was the first investigation to evaluate the potential application of PSMBBC for vertebroplasty. Results demonstrated its beneficial effects on the trabecular ingrowth of new bone and bone mineral density increase. With further validation, PSMBBC can become a valuable biomaterial for aiding osteoporotic vertebrae and usable bone cement applied in vertebroplasty.

Keywords: Osteoporosis, vertebroplasty, bone cement, bioglass

Introduction

Osteoporosis is a common disease characterized by a systemic impairment of bone mass and micro-architecture that results in fragility fractures [1]. Vertebral compression fracture (VCF) is the most common complication in patients with osteoporosis [2]. Worldwide, osteoporosis causes 1.4 million clinical vertebral fractures annually [3]. In the United States, approximately 25% of women over the age of 50 suffer one or more vertebral compression fractures (VCFs) caused by osteoporosis [4]. In Asian countries, 50% of postmenopausal women have osteoporosis and 30% of elderly women have one or more VCFs [5].

Vertebroplasty and kyphoplasty are two percutaneous minimally invasive techniques in treating symptomatic VCFs patients who do not have neurological impairment [6, 7]. The most commonly used cement in vertebroplasty is polymethyl methacrylate (PMMA) [8]. However, the limitations of PMMA are noticeable, including low absorbability, poor biocompatibility, lack of osteoconductivity and excessive stiffness [2, 9-11]. These properties have prevented the implanted material from being

actively integrated into bone. Instead, it is encapsulated by a connective tissue layer. Furthermore, the excessive stiffness might lead to the likelihood of refracture [2]. Thus, to develop the modified bone cements become more and more necessary.

Our previous study [12] described the development and characteristics of a novel injectable Porous Surface Modified Bioactive Bone Cement (PSMBBC), which had osteogenic ability, enough biomechanical strength, partial degradability, and was composed of bioglass powder, chitosan powder and PMMA. However, the feasibility and effect of PSMBBC in strengthening osteoporotic the vertebrae still remains unknown. Thus, the purpose of this study is to determine how effective the proposed material is in terms of strengthening the osteoporotic vertebrae and increasing the trabecular ingrowth of the new bone following vertebroplasty *in vivo*.

Materials and methods

Preparation of materials

The raw materials were used for the preparation and composition of the solid component and the liquid component as our previous study [12]. Based on the ratio of PMMA in the bone powder (**Table 1**), we classified the samples into two types (PSMBBC and pure PMMA). The samples were prepared by mixing the powder with the liquid using a solid: liquid mass ratio of 3:2 under ambient conditions at the room temperature.

Establishment of rabbit osteoporosis model by ovariectomy

Animals: For the in vivo animal study, eighty 5-month-old female New Zealand white rabbits, weighing between 3.2 and 3.5 kg, were used. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal protocol was approved by the Animal Care and Use Committee at Fourth Military Medical University (Permit Number: 08-269). The rabbits were kept individually in cages and maintained with a cycle of 12 hours of light and 12 hours of darkness. The animals were fed standard rabbit chow (FMMU) and allowed to drink tap water freely.

Osteoporotic rabbit model: After the rabbits were acclimatized to the new situation for two weeks, they were randomly divided into two groups. The ovariectomy group (OVX) (n = 72) received bilateral ovariectomy. The sham group (n = 8) only received the sham operation. The OVX operation was performed as previously described [13]. Postoperatively, antibiotics (ampicillin, 0.1 g/kg/day, China) were administered subcutaneously for three continuous days. A combination of ketamine hydrochloride (50 mg/kg, IM) and fentanile (0.17 mg/kg, IM) were used as the general anesthesia. Meanwhile 2% lidocaine with 1:100000 epinephrine was injected as the local anesthesia.

Bone mineral density (BMD): Dual-energy X-ray absorptiometry (DXA) analysis was performed with a Hologic Discovery Wi using linear fan beam technology (Hologic Inc, Bedford MA, USA) and switching between two X-ray potentials (100 and 140 kVp) from an X-ray source mounted beneath the subject [14]. During the process, the Hologic Discovery analysis software version 12.7 was used, whereas the small animal-scanning mode was applied for all scanning. For all animals, the baseline BMD was measured for lumbar vertebrae before the surgical procedure. Six months after the OVX, the animals underwent the BMD scan again. The mean of L3-L5 vertebral BMD values was calculated to represent the lumbar vertebrae.

Vertebroplasty procedure

After six months, the rabbits of the OVX group were randomly assigned to three groups. The PMMA group, the PSMBBC group and control group. Among those, the PMMA and PSMBBC group received vertebroplasty.

Between 0.1 and 0.2 ml of cement per level was injected into the L4, L5 lumbar vertebrae in each animal. The exact amount of cement was recorded in each case.

Animals were monitored during their recovery from general anesthesia, at which time they were placed back into their respective cages. Analgesics were administered by the veterinary staff. The animals were observed for any evidence of abnormality of function or behavior. For the next following three days, we administered antibiotic therapy with ampicillin to animals.

Table 1. Compositions of the bone cements prepared[a]

	PSMBBC	PMMA
Solid component		
PMMA	48.5	98.5
Bioglass	40.0	0
CS	10.0	0
BPO	1.5	1.5
Liquid component		
MMA	99.0	99.0
DMPT	1.0	1.0

[a]by weight ratio (wt%) of solid component and liquid component, respectively.

Micro-CT analysis

At 1, 4 and 12 weeks after the vertebroplasty procedure, the rabbits were sacrificed with an overdose of sodium pentobarbital. During necropsy, the lumbar spine (L4, L5) was removed, labeled and subsequently stripped of all soft tissue.

Bones harvested were frozen at −80°C until the time of scanning by micro-CT (eXplore Locus SP, GE Healthcare, USA). The micro-CT system was used at a spatial resolution of 14.435 m, while the CT images were reconstructed in 1024ere placed represent the lumbar vertebrae and switchi. The region of interest (ROI) of the cancellous bone was then chosen for analysis. The ROI of vertebrae (1.5 mm of 14.435 m), and the CT images were reconstructed in 1024ere placed represent the lumbar vertebrae and switchi. Trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), bone surface/bone volume (BS/BV) and bone volume/total volume (BV/TV) were examined [15]. The mean value of the two sides was calculated for each specimen.

Biomechanical tests

As aforementioned, bones harvested were frozen at -80°C until the time of testing. Bones were thawed and kept fully moist before the mechanical testing. Prior to testing on vertebrae (L4, L5), the endplates, spinous, transverse and articulate processes were cut with a motor wafersaw to obtain a sample with parallel surfaces [16]. The vertebral samples were placed centrally between two steel parallel plates attached to the materials-testing machine (AGS-10kNG, SHIMADZU, JAPAN) and compressed at a nominal deformation rate of 5 mm/min [17]. A constant displacement rate of 0.1 mm/s was applied until failure [18]. Displacement (mm) and force (N) were measured at 10 Hz until failure. Load-deformation curves were recorded during the test. The ultimate load was taken as the maximum force on the curve, and the compressive strength was calculated from the compressive load and geometric area of the samples.

Histological analysis

The specimens were fixed in 10% phosphate-buffered formalin pH 7.25 for seven continuous days. After stepwise ethanol dehydration, the specimens were embedded in Spurr's plastic (Polysciences, Inc., Warrington, PA) without decalcification. Thick sections (100 μm) were cut with a diamond saw (Leica-LA 2500, Germany) strictly perpendicular to the long axis of the implant material. The sections were stained with Van Gieson's Stain and examined with a light microscope (Nikon Microphot FXA).

Statistical analysis

All statistical processing was completed using SPSS 16.0 (SPSS, Chicago, IL). A student's t-test was used to analyze data between two groups. Differences between three groups were tested by the oneway analysis of variance (ANOVA). *P* values less than 0.05 were considered statistically significant. All errors are given as standard deviations.

Results

Synthesis of the novel bone cement

To observe the surface morphologies of the novel PSMBBC, SEM analysis was performed. As shown in **Figure 1A**, the glass and CS particles were uniformly distributed in the polymeric matrix for dry cement samples. The space around the particles was existent and the particles were not firmly integrated into PMMA matrix.

Mechanical and degradation properties

In order to measure the mechanical properties of the novel bone cement, the compressive strength values were examined. **Figure 1B** showed the compressive strength of the PSMBBC and the PMMA bone cement before and after the degradation. Apparently, the PSMBBC had a lower initial compressive strength than

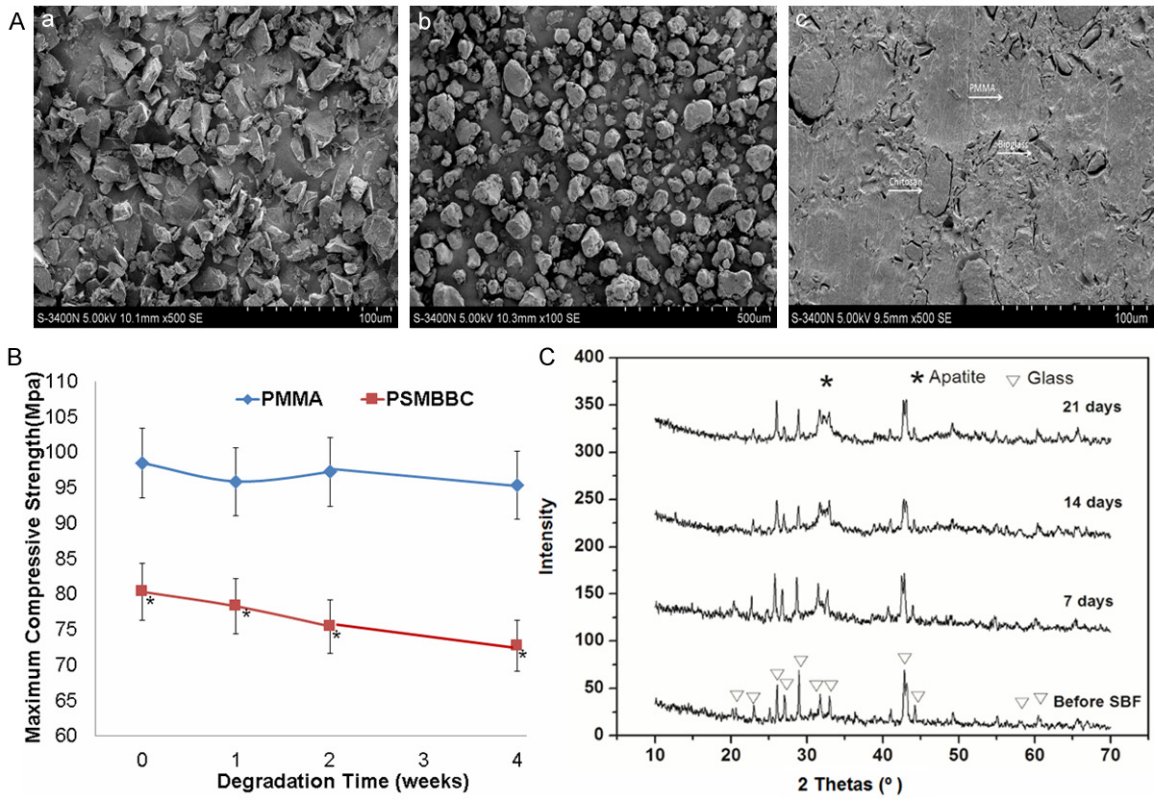


Figure 1. A. SEM images of the bioactive bone cement freshly prepared. a. The glass particles consisted of numerous fine grains. The average diameter of the particles was about 40 μm; b. The CS particles displayed irregular shapes with the average diameter of about 200 μm; c. The glass and CS particles were uniformly distributed in the polymeric matrix for dry cement samples. B. The degradation of mechanical properties of the PMMA and the porous surface modified bioactive bone cement. A significantly lower compressive strength ($P < 0.05$) was observed for the PSMBBC compared to the PMMA bone cement at each degradation time. C. DRX patterns of bioactive bone cement. *The newly formed apatites were confirmed after 14 days.

Table 2. BMD in lumbar vertebral ($\bar{x} \pm s$) (mg/cm^2)

Group	Pre-ovx	Post-ovx
Sham (n = 8)	266.35 ± 11.71	255.58 ± 24.78
OVX (n = 72)	268.47 ± 26.77	194.96 ± 12.43[a]

[a]Significantly different from sham group, $p < 0.01$.

the PMMA bone cement, 80.31 ± 9.46 VS 98.48 ± 9.08 ($p < 0.05$). After 28 days of degradation, the strength of the PSMBBC decreased to 72.71 ± 3.65Mpa, which still meets the criterion (>70 MPa) listed in ISO 5833.

XRD analysis of the novel bone cement

In order to assess the *in vitro* bioactivity of the PSMBBC, the samples were soaked in SBF. As depicted in **Figure 1C**, result showed that the newly formed apatites were confirmed by XRD at day 14- a new peak (2θ 32°) assigned to diffractions of apatite (according to the standard JCPDS cards 09-0432).

BMD measurements

In order to evaluate whether the osteoporosis rabbit models were established, BMD values were measured. The mean BMD (mg/cm^2) values of the lumbar vertebral are shown in **Table 2**. In the sham group, there is no significant difference in BMD (post-surgery) as compared to the baseline values. However, in the OVX group, BMD (post-OVX) decreased by 27.4 ± 2.1% ($p < 0.01$), as compared to the same baseline value.

Events of the study

As a result, all 80 rabbits tolerated the procedure well. The amount of cement that could be injected into the vertebral bodies ranged from 0.1 to 0.2 ml (**Figure 2**). No complications, such as paralysis from cement extravasations, were observed. After surgery, the rabbits did not

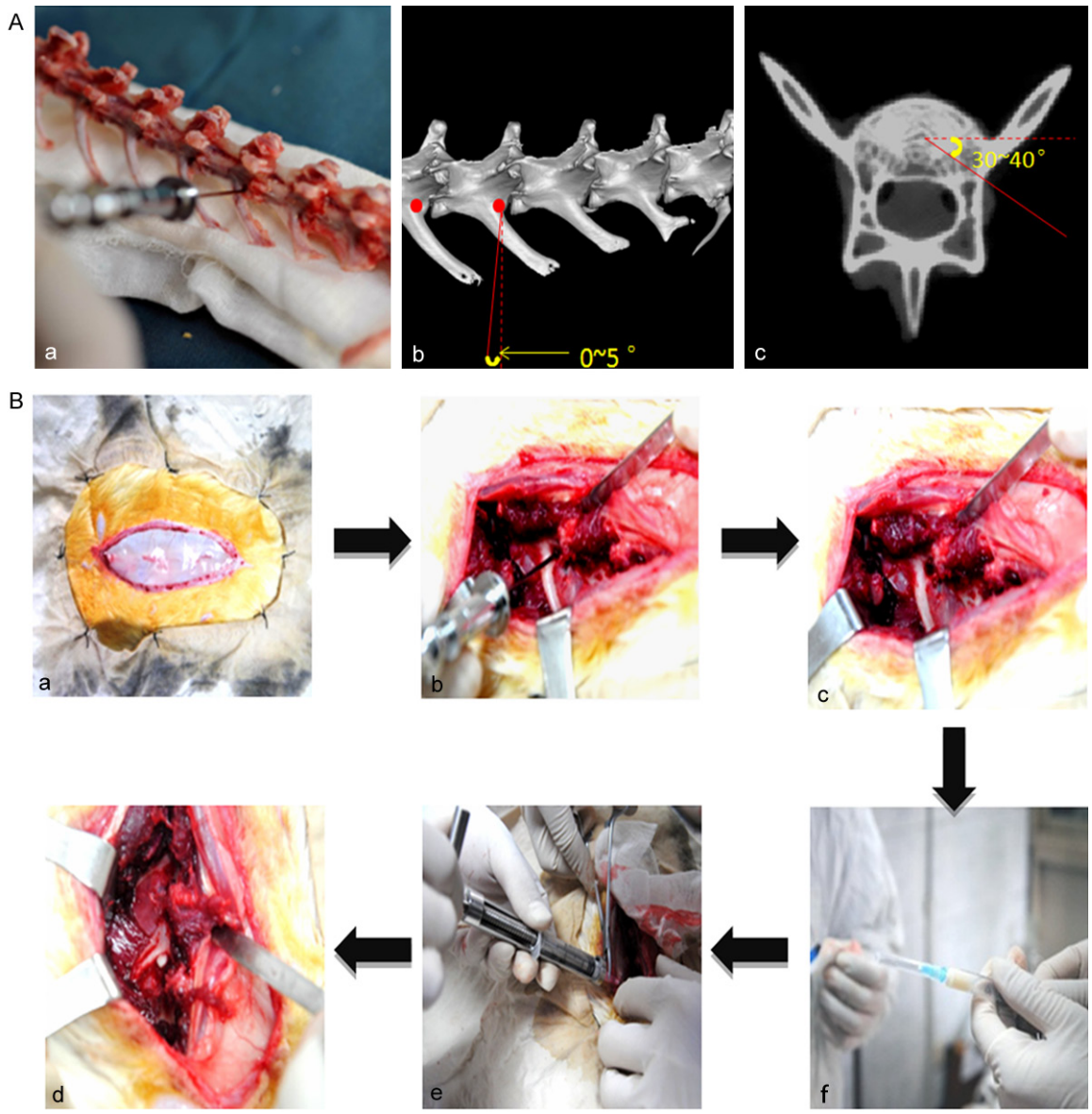
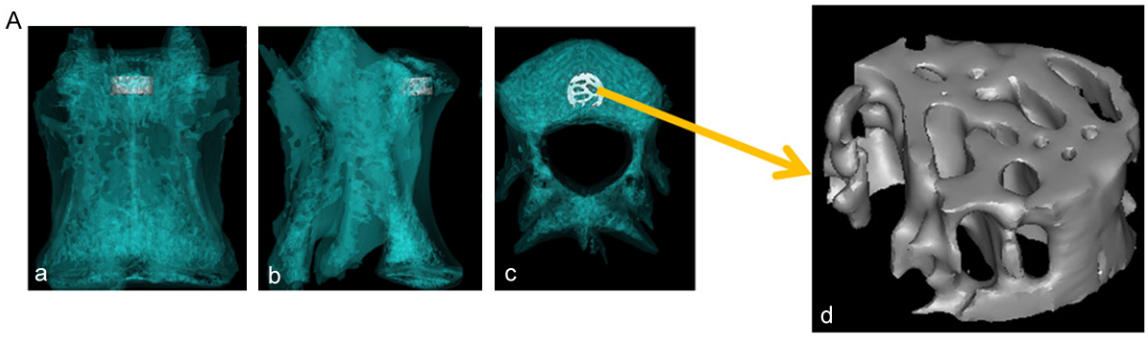


Figure 2. A. Vertebroplasty in rabbit vertebra. A procedure Insertion of trochar in rabbit vertebra. a. A 12 French myelogram trochar was inserted into the bone at a point that was 1 to 2 mm caudal to the junction of the base of transverse apophysis and the vertebral body; b, c. The trochar should be angled 60° towards the midline, 5° caudally, and penetrate a depth of 5 to 7 mm. B. Vertebroplasty procedure. a. The skin over the dorsolumbar region was then shaved, prepared with an iodine-impregnated solution, and draped in the usual sterile fashion; b, c. A 12 French myelogram trochar was inserted into the bone; d. The cement mixture was drawn into a 2 ml syringe that was then Luer-locked to the trochar; e, f. The cement mixture was injected into the 2 adjacent lumbar vertebrae.

show any abnormal changes in their physical condition or daily activities.

Micro-CT analysis

To evaluate the *in vivo* resorption of the implanted cements, the 3D reconstruction images of the residual materials were used at 4 and 12 weeks after implantation (**Figure 3**). The 3D reconstruction image of the control subject revealed the thinning and loss of connectivity in cortical and trabecular. By contrast, the images of the PSMBBC and PMMA group demonstrated the promotion of the bone as well as the thickening of the cortical and trabecular. Furthermore, at 12w, in the PSMBBC group, the density and thickness of the trabecular both increased while compared with those measured at 4w. Conversely, the PMMA bone cement group showed no observable changes through 12 weeks after vertebroplasty. More specifically, from 4 to 12 weeks, the BV/TV (%)



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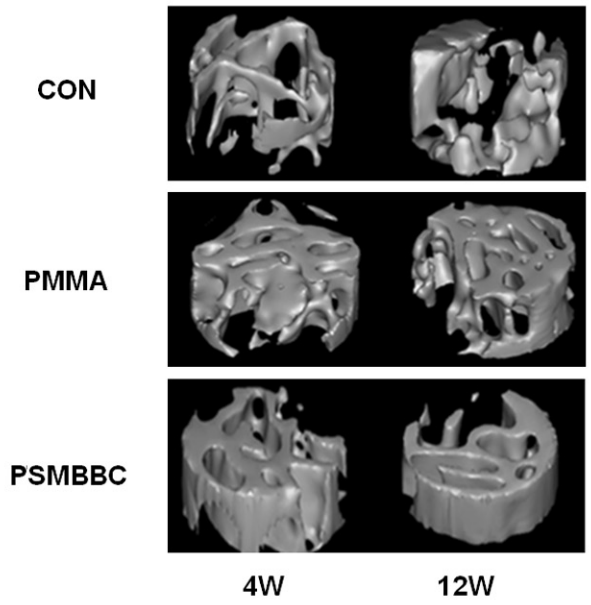

C

Time point	BV/TV(%)		
	CON	PMMA	PSM BBC
4w	24.83± 2.22	41.35± 2.17	28.27± 1.69
12w	18.81± 1.79	41.83± 1.98	38.43± 1.34

Time point	TB.N(mm^{-1})		
	CON	PMMA	PSM BBC
4w	1.40± 0.17	2.03± 0.15	1.40± 0.19
12w	1.34± 0.14	1.85± 0.17	1.93± 0.16

Figure 3. Micro-CT analysis of the porous surface modified bioactive bone cement. A. The method of analyzing cortical bone with micro-CT. a. Anteroposterior; b. Lateral; c. Cross-section; d. 3D reconstruction images of the ROI. B. Three-dimensional trabecular micro-architectural images of ROI at different time point after implantation. 3D reconstruction images of the ROI in the untreated control subject shows cortical and trabecular thinning and a loss of connectivity; in the PSMBBC group and PMMA group, promotes the deposition of bone and cortical and trabecular thickening. C. Values of BV/TV and TB.N at each time point after implantation.

of the PSMBBC group increased from 28.27 ± 1.69% to 38.43 ± 1.34%, while the BV/TV (%) of the PMMA group showed no significant difference. The results suggested that as compared to the PMMA bone cement, the PSMBBC induced more bone ingrowth and increased more bone volume at both 4 and 12 weeks ($p < 0.05$).

Biomechanical test

In order to better determine the potential clinical application in vertebroplasty and to evaluate the mechanical properties of PSMBBC *in vivo*, compressive strength values were also analyzed. The mechanical properties of each specimen at different time point are shown in **Figure 4**. From 4w to 12w, the maximum compressive strength values of the PSMBBC group and the PMMA group both increased and were both significantly different from the control group ($P < 0.01$). At 4w after implantation, the maximum compressive strength of the PSMBBC group reached 69.74 ± 7.83 Mpa, and at 12w, it reached 83.88 ± 8.56 Mpa.

Histological analysis

In order to further investigate the effects of different bone cement on osteoporotic vertebrae,

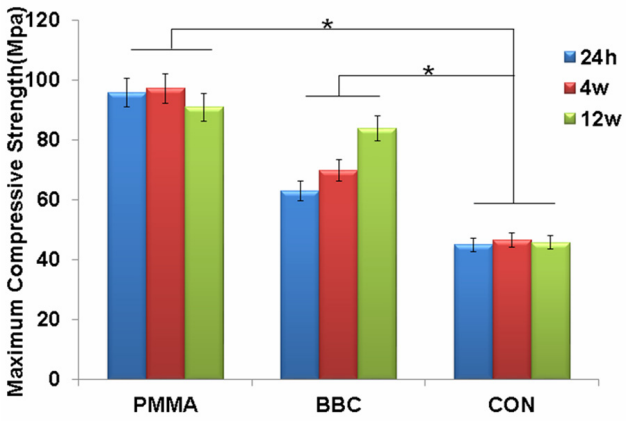

Figure 4. The mechanical properties of each specimen at different time point. *Significantly different from control group, $p < 0.01$.

the specimens were stained with Van Gieson's Stain and observed with a light microscope at 1, 4 and 12 weeks, respectively (**Figure 5**). At 4 weeks after implantation, the connectivity of the new bone was observed at the interface of the PSMBBC. In addition, the bone connectivity became thicker and the intact bone trabecular was observed at 12 weeks after implantation. By contrast, neither new bone trabecular nor bone ingrowth was observed in the PMMA group.

Discussion

In this study, for the first time, we have showed the feasibility and effectiveness of the novel PSMBBC, -for vertebroplasty in terms of strengthening osteoporotic vertebrae and increasing new bone trabecular ingrowth *in vivo*. PSMBBC composed of 40% bioglass bone powder, 10% chitosan powder, 48.5% commercially available PMMA bone cement and 1.5% BPO.

Osteoporosis is one of the global threatens to human health. It can affect people of all ethnic backgrounds and can result in challenging complications, ranging from compression fractures of vertebral bodies to femoral neck fractures. 35%-50% of all women over 50 had at least one vertebral fracture. In the United States, 700,000 vertebral fractures occur annually, but only about a third are recognized. In a 15-year study that included a series of 9704 women aged 68.8 on average, 324 had already suffered a vertebral fracture at entry into the study and 18.2% developed a vertebral fracture, but that risk rose to 41.4% in women who had a previous vertebral fracture [19].

Vertebroplasty and kyphoplasty are by far the most commonly used techniques in treating symptomatic VCFs patients who do not have neurological impairment. The filling materials used in vertebroplasty require proper biocompatibility, biomechanical strength and stiffness, and certain radiopacity for fluoroscopy guided procedures. At present, PMMA is still the most popular filling material in vertebroplasty considering the following advantages: low viscosity, easy to perfuse, capacity to strengthen and stiffen vertebral body quickly, and relatively cheaper price [20]. However, it also has some limitations, including insufficient adhesion ability to bone surface (no bioactivity) [21], monomer toxicity, high exothermic reaction temperature and excessive stiffness [11]. Recently, it's reported that some updated PMMA formulations are now available for clinical application. Unfortunately, they are still lack of enough osteogenic ability [21].

In recent years, considerable attention has been given to chitosan composite materials and their applications in the field of bone tissue engineering, because of their minimal foreign body reactions, intrinsic antibacterial nature, biocompatibility, biodegradability, and the ability to be molded into various geometries and forms, such as porous structures, which are suitable for cell ingrowth and osteoconduction [22]. In 2010, Hautamäki M [23] reported that surface porous fiber reinforced PMMA was biocompatible and had osteogenicability. In 2012, the study of Honglue Tan [24] showed that PMMA loaded with chitosan or chitosan derivatives could improve osteogenic ability. More recently, it has been demonstrated that bioactive glass possesses better osteoconductive properties and can increase the structural strength when administrated with PMMA [25].

Compared with pure PMMA, PSMBBC demonstrated better handling characteristics and adequate stiffness. In our study, at 4 and 12 weeks after implantation, biomechanics results suggested that the PSMBBC could provide enough strength for the lumbar vertebrae and the mechanical properties could be increased along with the bone ingrowth. More important-

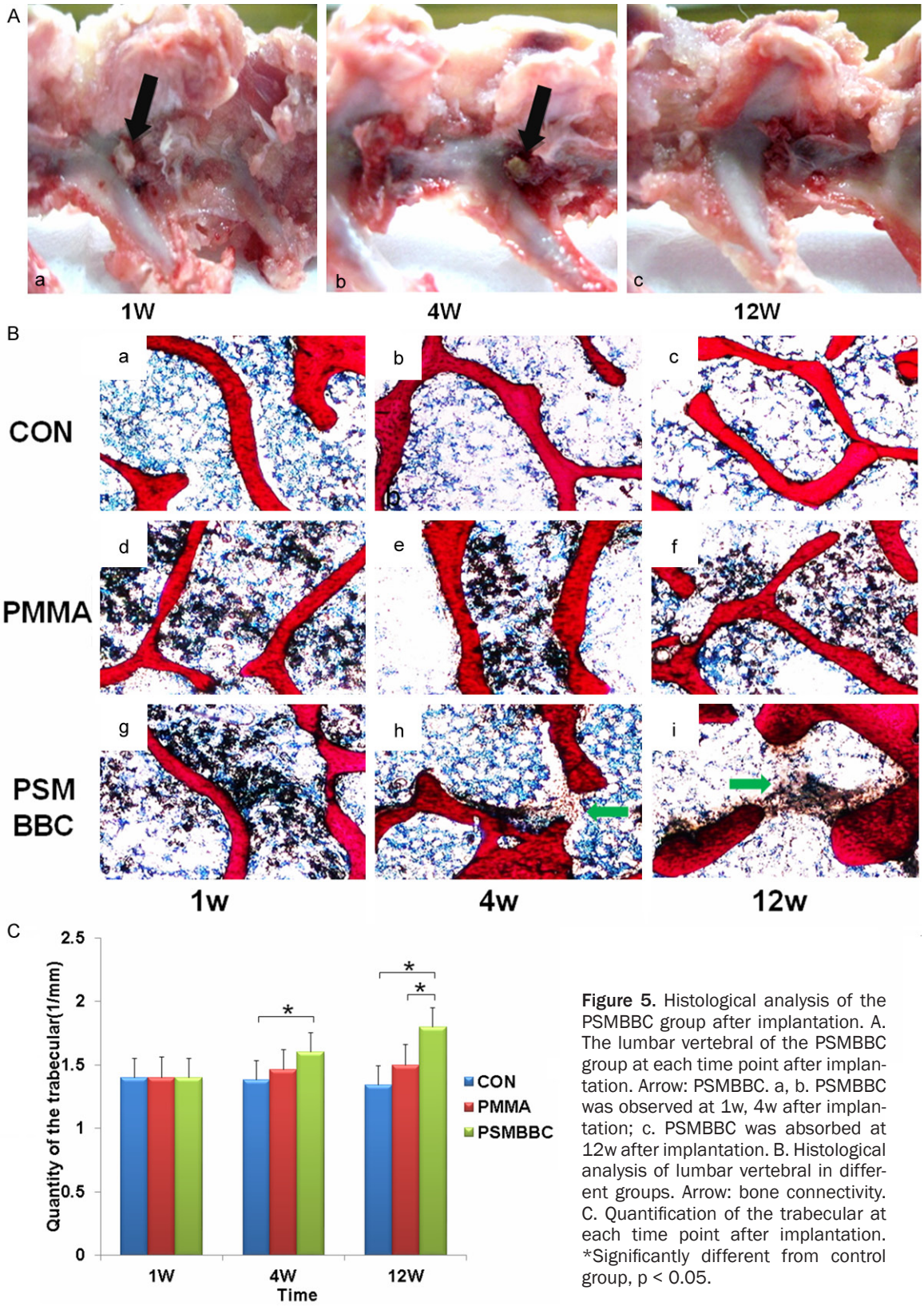

Figure 5. Histological analysis of the PSMBBC group after implantation. A. The lumbar vertebral of the PSMBBC group at each time point after implantation. Arrow: PSMBBC. a, b. PSMBBC was observed at 1w, 4w after implantation; c. PSMBBC was absorbed at 12w after implantation. B. Histological analysis of lumbar vertebral in different groups. Arrow: bone connectivity. C. Quantification of the trabecular at each time point after implantation. *Significantly different from control group, $p < 0.05$.

ly, Micro-CT and histological analysis suggested that the PSMBBC had the ability of osteogenesis and was able to connect with bone surface. In summary, these findings indicated

that the novel injectable PSMBBC contained good osteogenic ability, enough biomechanical strength and bio-degradability. With further validation, PSMBBC has the potential to become a valuable filling biomaterial for inducing osteogenesis, strengthening osteoporotic vertebrae and usable bone cement applied in vertebroplasty.

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Disclosure of conflict of interest

None.

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