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A novel sheep vertebral bone defect model for injectable bioactive vertebral augmentation materials

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Abstract New injectable bone substitutes have been developed that are, unlike polymethylmethacrylate, biologically active and have an osteogenic effect leading to osteogenesis and bone remodeling for vertebroplasty or kyphoplasty. In this study, we developed a sheep vertebral bone defect model to evaluate the new bioactive materials and assessed the feasibility of the model in vivo. Bone voids were experimentally created on lumbar vertebrae L2–L5 with L1 and L6 left intact as a normal control in mature sheep. The defect vertebrae L2–L5 in each sheep were randomized to receive augmentation with calcium phosphate cement (CPC) or sham. Vertebrae (L1–L6) were collected after 2 and 24 weeks of the cement augmentation and their strength and stiffness, as well as osseointegration activity and biodegradability, were evaluated. Finally, CPC significantly improved the strength and stiffness of vertebrae but did not yet restore it to the normal level at 24 weeks. Osteogenesis occurred at a substantially high level after 24 weeks of CPC augmentation or sham. Therefore, the sheep vertebral model with one void, 6.0 mm in diameter and 15.0 mm in depth, is replicable and can be used for evaluating the new injectable bioactive materials in vertebral augmentation or reconstruction.

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1 Introduction

Vertebral compression fractures (VCFs) are the most common injuries resulting from osteoporosis. Every year an estimated 1–4 million vertebral compression fractures that cause pain and disability and diminish quality of life [1, 2], come to clinical attention worldwide [3].

More recently, two percutaneous surgery procedures have been introduced for VCFs. Vertebroplasty is a percutaneous injection of viscous polymethylmethacrylate (PMMA) into the vertebral body and was first described by Galibert et al. [4]. Another procedure, termed kyphoplasty, consists of placing an inflatable bone tamp inside the compressed vertebral body via a cannula and inflating it in an attempt to elevate the vertebral endplates and create a void in the trabecular architecture. The void is then typically filled with PMMA cement. Good clinical results have been reported in several series of both vertebroplasty and kyphoplasty procedures [5–8].

Despite the success of vertebroplasty and kyphoplasty in alleviating pain, these two procedures have some potential drawbacks associated with the properties of PMMA, including thermal damage to adjacent tissues, excessive inherent stiffness which may increase fracture risk at adjacent levels, no biologic potential to remodel or integrate into the surrounding bone, no direct bone apposition and potential monomer toxicity.

More and more promising injectable bioactive cements have been developed to improve biocompatibility and absorbability [9]. The new cements have an osteogenic effect leading to osteogenesis. However, many of them were evaluated in animal proximal tibia or distal femur [10], outcome in extremities cannot give proof of that in vertebrae due to the different biomechanical environments in different parts. After the augmentation of the vertebrae

by a bioactive cement, the cement that degraded without new bone formation will cause the side-effects, as the induced absorption may weaken the vertebral body and promote further collapse. The biomechanical factor should be considered when assessing the osteoconductivity and osteoinductivity of the new bioactive bone substitute. To our knowledge, there was not proper animal model available to evaluate the bioactive cement for vertebral augmentation.

The present study was designed to develop an animal vertebral bone defect model and evaluate the feasibility of the model with bioactive injectable bone cement. Different size voids were created by different specification drills in the mature sheep lumbar vertebrae and the mechanical properties were determined by a material testing machine. Then, cement augmentation of bone defect vertebrae were performed in vivo to assess the feasibility of the current model.

2 Materials and methods

2.1 Development of the vertebral defect model in vitro

2.1.1 Specimens preparation

Forty vertebrae (L3–L6) were harvested from ten fresh mature female sheep cadavers (2–3 years, weight 40–50 kg). Each specimen was radiographically screened to rule out abnormalities which might impair the mechanical properties of lumbar spine. Rice bags were placed along the spine to serve as surrogate soft tissue [11], and dual-energy radiograph absorptiometry (Lunar DPX-IQ, Lunar Corp. Madison, WI) was used to determine the bone mineral density (BMD). The vertebrae were stripped of soft tissue, disarticulated. The discs were excised, and posterior elements were removed to facilitate mechanical testing. All vertebral bodies were randomly assigned to one of five groups to minimize the potential bias associated with bone mass.

2.1.2 Vertebral bone defect creation

Four groups vertebrae were drilled perpendicularly to the center of the sagittal plane of vertebrae by different diameter drills: group B—Ø2.0 mm; group C—Ø4.0 mm; group D—Ø6.0 mm; group E—Ø8.0 mm. Drill stops were used to ensure that the defects did not extend more than 15 mm into the vertebral body. The left eight intact vertebrae served as control: group A (Fig. 1).

After the above procedures finished, each specimen was wrapped in saline-soaked gauze, sealed in plastic bags and stored at −20°C until the day before mechanical testing.

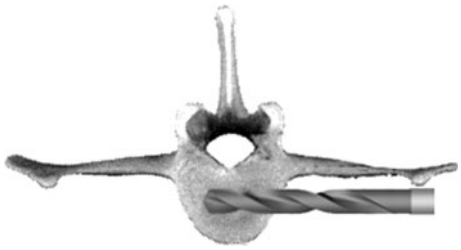

Fig. 1 The schematic diagram of sheep lumbar vertebral defect model. The drill went straight into the perpendicular direction to the center of the sagittal plane of vertebrae so as to create the vertebral bone defect

2.1.3 Biomechanical testing

All specimens were thawed at room temperature (20°C) for 24 h before testing. An impression of each endplate was made using a dental acrylic (Biomaterials Corp. Shanghai, China) to ensure uniform loading during compression testing. Each vertebral body was placed between its respective impressions and placed between loading platens on a materials testing machine (Instron 5500R; Canton, MA). A compressive preload of 200 N was applied for 1 min. Immediately thereafter, each vertebral body was compressed on the vertical axis through the center of the vertebral body [12] at a rate of 5 mm/min [13]. Compression was continued until the average height of the vertebrae decreased by 25% of the average initial vertebrae height. Force and deformation data were recorded at 10 Hz and the initial strength and stiffness of the vertebrae were measured. The strength was defined as the peak value at the inflection point of the load–displacement curve while the stiffness was calculated as the slope of the loading force over deformation.

2.2 Cement augmentation of vertebrae with bone voids in vivo

2.2.1 Surgical procedures

According to the results of biomechanical testing, we choose the specified bone void diameter as the model to be augmented in vivo by injectable bone substitute.

Eight spinal deformity-free adult female sheep with an average age of 2.5 ± 0.5 years and body weight 40–50 kg were provided by and housed in stalls with free access to food and water in the Experimental Animal Center of Soochow University (Suzhou, China). Under anesthesia with intravenous injection of 3% sodium pentobarbital, a bone void of a replicable size, which was determined by the results of bone defect vertebrae compressive testing, was created in each of the lumbar vertebrae L2–L5 by a drill in the center of the vertebrae while L1 and L6 was

kept intact as the control. Immediately after removing the broken bone debris, two vertebrae were randomly augmented with calcium phosphate cement (CPC, Shanghai Rebone Biomaterials Co. Ltd. Shanghai, China) and the other two served as sham. Once the CPC hardened, the wound was sutured. In the first 3 days after surgery, penicillin (800,000 U) was given intramuscularly per day to prevent infection.

Four animals were randomly chosen to be sacrificed with an overdose of sodium pentobarbital at the 2 and 24 weeks after surgery. Then, lumbar vertebrae L1–L6 were harvested. A total of 48 vertebral body specimens were prepared. Five vertebral body specimens from each group and time point combinations were subject to biomechanical evaluation with destructive uniaxial compression test. The vertebrae were prepared and biomechanical testing was performed as described above.

2.2.2 *Histomorphometric assessment of undecalcified vertebral bodies*

The remaining three vertebral specimens from CPC and sham groups were subject to histomorphometric analyses as previously described [14]. Briefly, vertebral bodies were fixed in 70% ethanol, dehydrated in graded series of alcohol without decalcification, embedded in polymethylmethacrylate, and sectioned to 150–200 μm in thickness. The sections were mounted onto Plexiglas slides, polished to 50 ± 5 μm under cooling water and stained with Van-Giemsa.

For each of the Giemsa-stained undecalcified sections, images partially overlapping different segments were captured and then stitched together, using Image-pro Plus Version 5.01 (Media Cybernetics Inc. Bethesda, MA) to generate a single large image that had a complete view of the whole void with the injected bone cement. The biodegradation of the bone cement, the formation of new bone and the cement-new bone interface were examined under a microscope for two time points. For the time points of 2 and 24 weeks, the area of the vertebra void (A_{void}), the area of new bone formation ($A_{new\ bone}$) and the area of the residual bone cement (A_{cement}) were determined. Accordingly, the new bone formation rate and the CPC degradation rate were calculated respectively as $A_{new\ bone}/A_{void}$ and $(1-A_{cement}/A_{void})$.

2.3 Statistical analysis

All data were expressed as mean ± SD. The differences in BMD among groups were checked for significance by Student–Newman–Keuls test. We also evaluated the effect of vertebral bone defect size and cement augmentation on vertebral body strength and stiffness by the Dunnett-*t* test. Statistical analyses were performed by SPSS (release 13.0; SPSS Inc. Chicago, IL) software package. Significance was set at $P < 0.05$, unless otherwise specified.

3 Results

3.1 Bone mineral density

The BMD maximum and minimum values of all vertebrae were 1.124 and 0.709 g/cm^2, respectively. Thereafter, there were no significant differences among these five groups ($F = 0.12$; $P > 0.05$) (Fig. 2).

3.2 Biomechanical properties of the vertebral bone defect model

The changes of the specimen strength with the bone defect diameter increasing are showed in Fig. 3. The strength of vertebrae decreased slightly when the bone defect was minor. There is no significant difference when the mean strength of group B with Ø2.0 mm void and group C with Ø4.0 mm compared to group A, respectively. While the defect diameter of group D increased to Ø6.0 mm, the strength decreased obviously. The strength of group D (3663.64 ± 439.41 N) was equivalent to 55.60% of group A, So the difference was significant between group A and D ($P < 0.05$). When the void diameter was up to Ø8.0 mm, the mean strength decreased to the 2289.29 ± 377.72 N, 34.74% of group A. The stiffness of the vertebrae changed very similarly to the strength as the diameter of the defect changed.

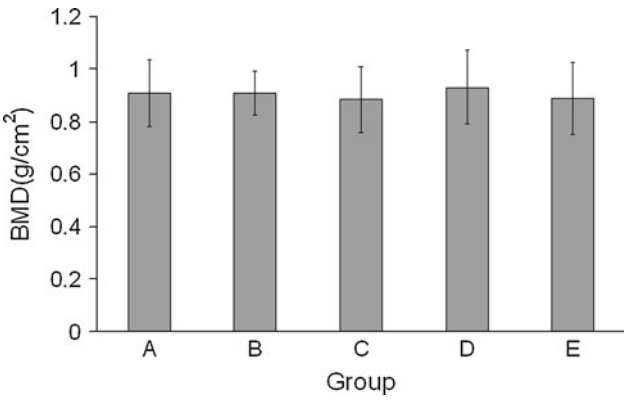

Fig. 2 BMD of vertebral bodies in study groups. The BMD maximum value (1.124 g/cm^2) and minimum value (0.709 g/cm^2) were both in group E. Thereafter, there is no significant difference among these groups, values are showed as mean and SD for each group ($P > 0.05$)

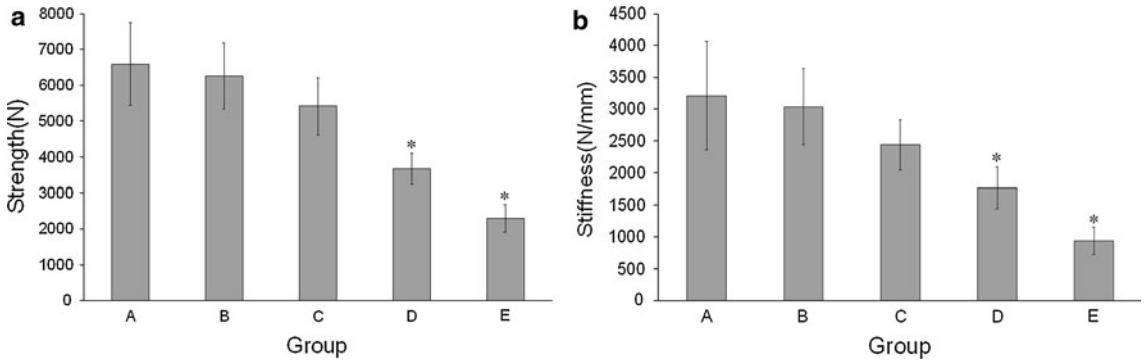

Fig. 3 Diagrams of strength (**a**) and stiffness (**b**) for each group. The strength and stiffness of group D and E decreased significantly respectively, compared to those of group A. For each group, the data are expressed as means and standard errors. *$P < 0.05$, by Dunnett-*t* test

3.3 Biomechanical properties of the augmented vertebrae with injectable cement

Time-dependent changes of the biomechanical strength and stiffness in normal control L1 and L6 vertebrae and in the vertebrae augmented, respectively with CPC and sham cement are presented in Fig. 4. At the two time points, the defect vertebrae without cement augmentation had a significantly lower biomechanical strength than the control vertebrae ($P < 0.01$). The CPC-augmented defect vertebrae significantly restored strength ($P < 0.01$) but not yet to the level of normal lumbar vertebrae. Along the time course studied, the biomechanical strength increased from 2 to 24 weeks in the CPC-augmented defect vertebrae as the defect vertebrae without cement augmentation. The stiffness showed the change pattern very similar to that of the strength.

3.4 Biodegradability and osteogenesis

There was no indication of inflammation of 2 weeks after surgery. Cement degradation and new bone formation were not observed at 2 weeks in all the vertebrae CPC-augmented but became evident at 24 weeks (Fig. 5). The trabecular bone surrounding the cavities had become organized into concentric rings, the residual material was seen in the newly formed bone ring and contact closely to it. No obvious boundary was seen between the material and the new bone. The trabecular bone surrounding the cavities had become organized into concentric rings by 24 weeks post-surgery in sham group, and a regeneration of most adipose marrow and hematopoiesis was observed. The new bone formation rate and the cement degradation rate are shown in the Table 1.

4 Discussion

In our study, we chose the sheep as the experimental model. Sheep spines were demonstrated as a valid biomechanical model for the similarities of sheep and human spine [15, 16]. The sheep spine might not only be useful as a model for disc surgery or bone healing processes, but also serve as an alternative for the evaluation of spinal implants. Sheep also was considered as the idea in vivo model for study the human spine [17]. The quadruped spine was essentially loaded in the same way as that of human and the axial compression stress in quadrupeds was higher, which leads to higher bone densities in the vertebrae.

The sheep vertebrae bone defect model was firstly used to assess the osteogenic effects of nacre, a promising bone substitute for vertebroplasty, and the bone cavity diameter was 3.0 mm [18]. Although 3.0 mm cavity in vertebrae would not be cured itself [19], the biomechanical factor was neglected in 3.0 mm cavity model. In our study, as the void with 3.0 mm in diameter did not impair the

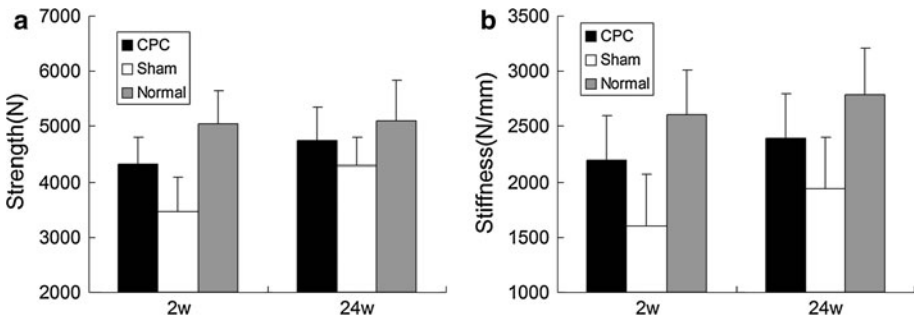

Fig. 4 Diagrams of the strength (**a**) and stiffness (**b**) of the vertebrae with 6 mm bone defect at 2 and 24 weeks after augmentation by injectable calcium phosphate cement

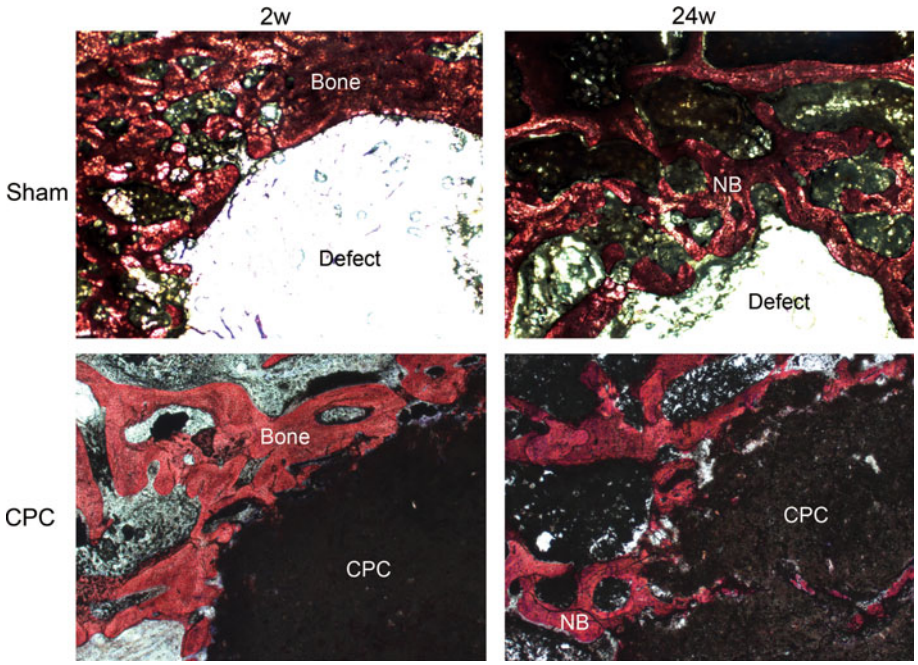

Fig. 5 Representative digital images of undecalcified sections of indicated vertebrae. It showed the time-dependent changes in the histological properties of the vertebrae. The CPC was gradually replaced by new bone (NB) without fibrous tissue or inflammations in CPC group at 24 weeks

Table 1 New bone formation rate (mean ± SD) and CPC degradation rate in the vertebrae at the 24 weeks after augmentation

	New bone formation rate (%)	Material degradation rate (%)
Sham	20.14 ± 0.67	–
CPC	15.47 ± 0.98	16.48 ± 1.84
P	<0.05	–

mechanical properties of the vertebral bodies significantly. The largish vertebral bone defect models were carried to assess several bone substitutes for vertebral augmentation [20–23]. The diameter of the voids ranged from 3.0 to 8.0 mm, the depth from 10 to 15 mm. However, these studies did not recommend the relatively reasonable size void in sheep vertebrae as the vertebral defect model for vertebral reconstruction. Wilke has studied the anatomy of the sheep spine, and compared it to human spine [16], which shows the width of the sheep L3–L6 was approximately 28 mm, from 26.5 ± 0.9 to 36.4 ± 1.6 mm. The mechanical testing results showed that the strength and stiffness of the vertebrae with 6.0 mm bone void decreased significantly when compared to the intact. So we choose the vertebrae containing 6.0 mm void as the model for cement augmentation. Since size limitation of the sheep lumbar vertebrae, the larger of the voids, the more risk of the leakage of the injectable materials to the spinal canal and paravertebral tissues.

From the results of the sheep vertebrae augmentation in vivo, it is obvious that the model with 6.0 mm voids can be effectively augmented by injectable calcium phosphate cement, and at 24 weeks after surgery, apparent osteogenesis can be seen in the histological images.

Although the model was demonstrated as effective to assess the injectable bioactive bone substitute for spine reconstruction, it can be used as a model to evaluate other spinal bioactive materials. It still has some disadvantages when it was used for vertebroplasty or kyphoplasty. (1) It is a bone defect model, not the vertebral compressive fracture model. Although, they have such common mechanical features as they both undermined the mechanical properties of the vertebrae, they also have their individual features. (2) The cement was injected directly into the bone voids in this model, while in vertebroplasty or kyphoplasty procedures, it would be injected via the pedicle canal.

5 Conclusions

In this article, we developed a sheep vertebral bone defect model for injectable vertebral augmentation materials and evaluated the feasibility of this model as well. The biomechanical test results showed that the strength and stiffness of the vertebrae with 6.0 mm bone defect in diameter were significantly impaired. Therefore, they could be augmented effectively by bone substitute materials in vivo. As the time went on, due to the osteogenesis and bone remodeling, the augmentation effect showed as more and more apparent.

The sheep vertebral model with bone defect (6.0 mm in diameter and 15 mm in depth) presented here is replicable and effective for assessing injectable bioactive bone

substitutes in vertebral reconstruction. It would be an alternative animal model for assessing future new bioactive bone substitutes, especially injectable vertebral augmentation materials. However, further researches would be necessary to develop better animal models, as well as to assess the applications of the new bioactive bone substitutes.

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