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Original Article

Characterization of a novel caudal vertebral interbody fusion in a rat tail model: An implication for future material and mechanical testing

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ABSTRACT

Background: Of the proposed animal interbody fusion models, rat caudal discs have gained popularity in disc research due to their strong resemblance to human discs with respect to geometry, composition and mechanical properties. The purpose of this study is to demonstrate an efficient, repeatable and easily accessible animal model of interbody fusion for future research into mechanical testing and graft materials.

Methods: Twelve 12-week-old female Sprague–Dawley (SD) rats underwent caudal interbody fusion of the third and fourth coccygeal vertebrae of the tail. Serial radiological evaluation, and histological evaluation and manual palpation after sacrifice were performed to assess the fusion quality. Mechanical testing of functional units (FUs) of non-operated and operated segments was compared using a three-point bending test.

Results: At postoperative 12 weeks, callus formation was observed at the fusion sites in all rats, with the mean radiological evaluations of 2.75/3 according to the Bransford classification. Newly formed bone tissue was also observed in all rats with the mean histological score of 5.85/7, according to the Emery grading system. No palpable gaps and obvious change of bending stiffness was observed in the operated segments. The mean bending stiffness of the FUs was statistically higher than that of the control FUs (26.57 ± 6.71 N/mm vs. 12.45 ± 3.21 N/mm, $p < 0.01$).

Conclusion: The rat caudal disc interbody fusion model proved to be an efficient, repeatable and easily accessible model. Future research into adjuvant treatments like growth factor injection and alternative fusion materials under conditions of osteoporosis using this model would be worthwhile.

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At a glance commentary

Scientific background on the subject

Rat caudal discs have gained popularity in disc research due to their strong resemblance to human discs.

What this study adds to the field

This study has demonstrated a rat caudal disc interbody fusion model, which is efficient, repeatable and easily accessible.

Interbody spinal fusion is currently the most commonly performed surgical procedure for a number of spinal conditions, including degenerative disc disease (DDD), spondylolisthesis, and spinal deformity. All can potentially cause compression, stretching or angulation of the nerve roots, and lead to radiculopathy or myelopathy [1–3].

Spinal stability is restored by fusing two or more vertebrae together with interbody spacers, consisting of bone autografts, allografts or synthetic materials. Then, pedicle screw fixation provides supplemental stabilization, and dynamically restores lumbar lordosis in which interbody spacers serve as a cantilever [4]. Also, interbody fusion with the use of pedicle screw fixation has been reported to significantly improve fusion rates [5,6].

Previous studies have proposed several animal models to study interbody fusion with adjuvant treatment; for example, anterior lumbar interbody fusion with platelet-rich plasma (PRP) in a porcine model, lumbar interspinous process fusion with beta-tricalcium phosphate and recombinant human bone morphogenetic protein-2 (rhBMP-2) in a rabbit model, and posterior lumbar fusion with hyperbaric oxygen (HBO) therapy in a rabbit model [7–9]. In addition, the effects of anti-resorptive agents, including alendronate and zoledronic acid, in interbody fusion have also been investigated [9,10].

In spinal fusion animal models, lumbar discs have been the most commonly studied; however, rat caudal discs have recently become an attractive model in disc research due to their strong resemblance to human discs, with regard to geometry, composition and mechanical properties [11,12]. The purpose of this study is to demonstrate an efficient, repeatable and easily accessible animal model of interbody fusion for future research into mechanical testing and graft materials.

Methods

Study design

This study was approved by the Animal Care and Ethics Committee of our institute. 12-week-old female Sprague–Dawley (SD) rats were obtained from the Laboratory Animal Center of our institute, and were housed in environmentally controlled cages. The study protocol was designed in accordance with the guidelines of the National Research Council for the Care and Use of Laboratory Animals.

All the rats underwent caudal interbody fusion of the third and fourth coccygeal vertebrae of the tail, and an X-ray assessment of each rat tail was conducted. Radiological evaluation, manual manipulation and histological evaluation were performed to assess fusion quality.

Operative technique

Inhalational general anesthesia with 2% isoflurane was administered before the operation. The rat was placed in a lateral recumbent position, and an approximately 2.5 cm dorsal skin incision was made. In order to obtain full exposure of the caudal vertebrae, the underlying tendons were partially removed. The caudal disc between the third and fourth coccygeal vertebrae was completely removed with a rongeur. Grafton DMB®, a commercial bone allograft containing demineralized bone matrix, was placed in the disc space as fusion material. Finally, the wound was closed in layers with sutures. In addition, to stabilize the surgical site, a sterile silicon drainage tube was attached to the rat tail. The tube was cut in half horizontally, glued to the rat tail by super glue, and further fixed with surgical suture. An intramuscular injection of 80-mg cefazolin and a local neomycin application on the surgical site were used to prevent postoperative infection. Each rat was housed individually in a cage to prevent other rats from inadvertently contacting the wound [Fig. 1].

Radiological evaluation

Anteroposterior and lateral plain films of the fused caudal vertebrae were taken at 0, 4, 8, and 12 weeks postoperatively. All the radiographs were taken under the same radiographic exposure factors (penetration power: 42 kV, output current: 320 mA, distance: 120 cm, and exposure time: 8 mA).

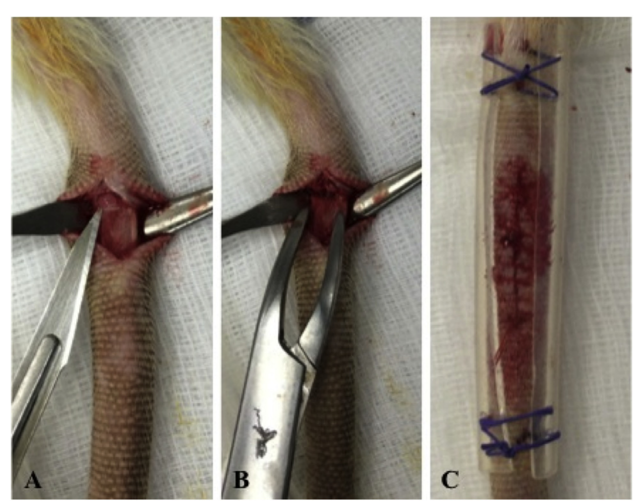

Fig. 1 Photographs showing the surgical procedure for interbody fusion at the rat tail. (A) An approximately 2.5 cm dorsal skin incision was made, and underlying tendons were partially removed. (B) The caudal disc between the 3rd and 4th coccygeal vertebrae was removed using a rongeur. (C) The wound was closed in layers with an additional sterile silicon drainage tube as protection.

Radiological evaluations were made by two different research fellows in a blinded fashion in accordance with the classification proposed by Bransford et al. [13]. Degree 1 was defined as many bone-graft particles clearly in the fusion area. Degree 2 was defined as some bone-graft particles in the fusion area as well as new-bone generation. Degree 3 was defined as a few bone-graft particles in the fusion area and a large amount of new-bone generation in many areas.

Manual palpation

All the rats were sacrificed at postoperative 12 weeks. After sacrifice, the fusion segments of the rat tails were harvested to manually evaluate the structural integrity of the fused caudal vertebrae. Manual palpation was tested and graded according to Abe et al. [14]: solid union when no motion was observed between the segments; partial union when slight motion was observed; nonunion when wide motions equivalent to adjacent segments were detected.

Histological evaluation

After tail harvest, the specimens of caudal vertebrae and discs were fixed with 10% buffered neutral formalin, then decalcified in Surgipath Decalcifier II solution, followed by embedding in paraffin. Slides with the dried paraffin-embedded sections were deparaffinized in xylene, and rehydrated with a series of ethanol washes. The sections were stained with hematoxylin and eosin and Masson's trichrome before they were examined under light microscopy. The histological assessment was evaluated by two different research fellows under the guidance of an experienced pathologist, in accordance with the grading system of Emery et al. [15]. The fusion status was graded using a histological score of 0–7: score 0, empty islets; score 1, fibrosis tissue only; score 2, fibrosis tissue more than fibrocartilage tissue; score 3, fibrocartilage tissue more than fibrosis tissue; score 4, fibrocartilage tissue only; score 5, fibrocartilage tissue more than bony tissue; score 6, bone tissue more than fibrocartilage tissue and fibrosis tissue; score 7, bone tissue only.

Mechanical testing

Twenty-four functional units (FUs) of fresh rat tail were studied and separated into two groups (12 per group), based on the different segments from the rat tail: (1) intact (control) and (2) fusion (T). Functional unit was defined as two bodies of adjacent vertebrae connecting by an intervertebral disc for the intact group and fused materials for the fusion group. After sacrifice, the operated third and fourth coccygeal segments were harvested as the fusion functional units, and the non-operated fifth and sixth coccygeal segments as the intact functional units. Three-point bending tests in a forward direction normal to the longitudinal axis of the rat tail were performed using an Instron testing machine (model 5544, Instron Inc., Canton, MA, USA) to compare bending stiffness between the groups. The FU in each group was positioned on the supporting pins with a span of 18 mm, and clamped on the lower side of the Instron frame. A plunger at the mid-point of the span was clamped on the upper side of the Instron grip and connected to the load cell. After positioning the construct, an axial compressive force was applied at a constant crosshead rate of 2 mm/min. The relationship between force and displacement (deflection) was continuously recorded in 0.05-mm increments (sampling rate: 0.67 Hz) using Instron Merline software. The deflection of the specimen was measured to evaluate bending stiffness between the two groups. The experimental set-up and testing configuration are shown in [Fig. 2].

Results

At postoperative 12 weeks, callus formation was observed at the fusion sites in all rats [Fig. 3]. Moderate to high amount of new-bone formation was observed at the fusion sites with the mean radiological evaluations scored 2.75/3 based on the classification of Bransford et al.

The transverse section of the rat tail demonstrated a bilateral symmetrical arrangement of coccygeal muscles and blood vessels adjacent to the caudal vertebrae, including one dorsal vein, two lateral veins and one ventral artery [Fig. 4]. The mean histological score was 5.85. Newly formed bone tissue was observed at the fusion site in all 12 rats using H&E stain [Fig. 5].

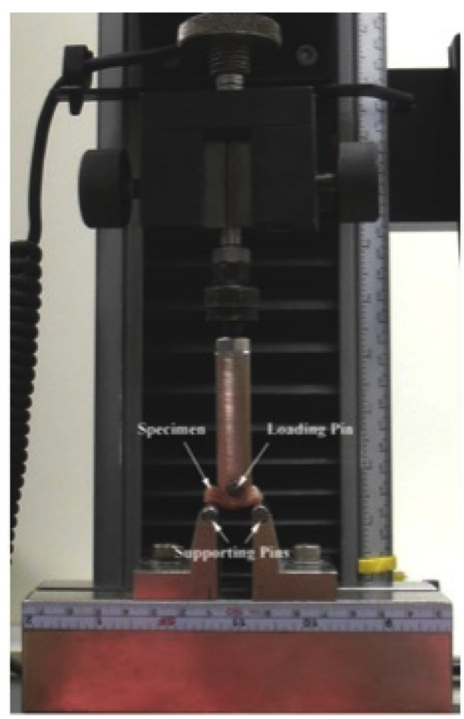

Fig. 2 Photograph showing a test fixture on the Instron testing machine for a three-point bending test. The prepared FU specimen was positioned on the supporting pins with a span of 18 mm, and a loading pin at the mid-point of the span was clamped onto the upper side of the Instron grip. After positioning the construct, an axial compressive force was applied at a constant crosshead rate of 2 mm/min. The deflection of each FU specimen was measured to evaluate the bending stiffness between two FU groups.

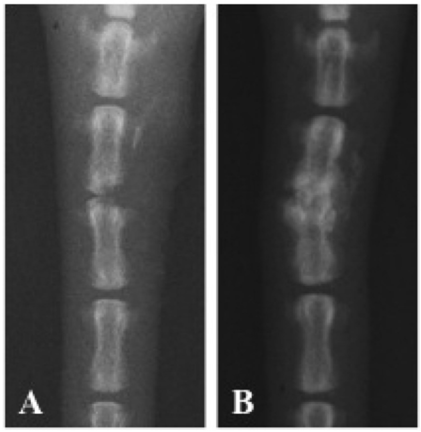

Fig. 3 Radiographic results showing callus formation at the fusion site (A) immediately after fusion, and (B) post-fusion 12 weeks.

When examining manual bending of the operated segments, obvious change in bending stiffness was observed compared to the adjacent non-operated segments. The manual palpation results indicated that the interbody fusion in the rat caudal disc model was successful and achieved good quality of fusion.

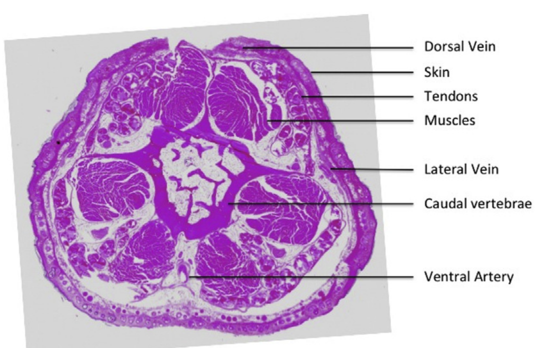

Fig. 4 Transverse sectional view of the rat tail showing anatomic structures.

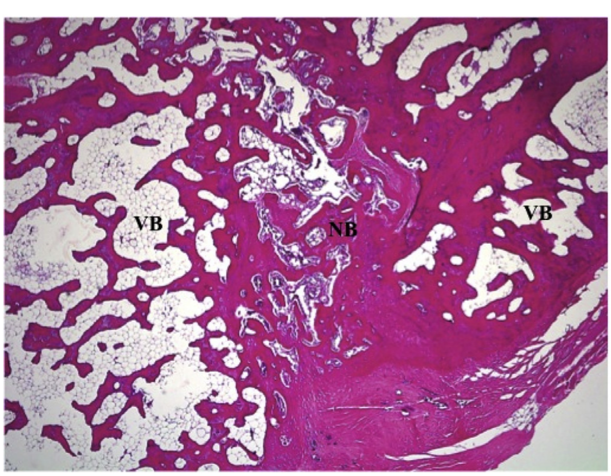

Fig. 5 Histological results showing newly formed bone tissues (NB) at the fusion site between two vertebral bodies (VB) using H&E stain.

[Fig. 6A] depicts a typical force vs. displacement curve for the three-point bending test. For the two FU groups, a lower increasing rate of force was observed in the initial phase. This might have been due to the presence of soft tissue in the FU specimens. In order to exclude the effects of this kind of soft tissue, bending stiffness was defined as the slope of the straight line connecting the two force values required to cause, respectively, 2.0 mm and 3.0 mm of displacement. Based on the definition, the mean bending stiffness values for the control FUs, and fusion FUs were 12.45 ± 3.21 N/mm and 26.57 ± 6.71 N/mm, respectively [Fig. 6B]. Compared to the control FU group, the fusion FU group exhibited a statistically higher bending stiffness ($p < 0.01$). The results indicate that interbody fusion surgery may improve the bending stiffness of FUs.

Discussion

Interbody fusion is the preferred method among all spinal fusion techniques owing to its low risks of both non-fusion (pseudarthrosis) and postoperative complications [16]. In addition, substantial evidence suggests that interbody fusion is associated with a significantly greater fusion rate than conventional posterolateral fusion (PLF) in patients undergoing lumbar fusion [5,17–19]. Furthermore, placing bone grafts within the load-bearing spinal column is beneficial in terms of biomechanical properties. Since interbody bone grafts occupy 90% of the intervertebral surface area and support 80% of spinal loads, theoretically they can better restore coronal and sagittal balance and yield better stability [16].

The interbody fusion procedure has been performed for more than fifty years, since it was first introduced by Cloward [20]. Its surgical approaches have undergone several modifications since its original description. There are currently four main approaches that are primarily utilized for lumbar interbody fusion. These include anterior lumbar interbody fusion (ALIF), posterior lumbar interbody fusion (PLIF), transforaminal lumbar interbody fusion (TLIF) and lateral lumbar interbody fusion (LLIF), all of which can be performed in a mini-open or minimally invasive (MIS) fashion. However, there is no conclusive evidence that one surgical approach is clinically superior to another, and each approach is characterized by its unique set of advantages and complications [19].

Spinal fusion in rats is most commonly performed at the lumbar spine. However, rat caudal discs are also biomechanically similar to rat lumbar discs, and performing interbody fusion at rat tail is attractive because it is easily manipulated, cost effective and rapid recovery. Gehhard et al. [21] developed an in vivo model of total disc replacement in the rat caudal spine with a tissue-engineered composite disc implant, and the functional results were assessed by X-rays and magnetic resonance imaging. Other than radiographic assessments, our study has further examined fusion status with manual palpation, histological evaluation, and mechanical testing.

Larger animals such as cattle, sheep, dogs and pigs have been considered to be good research models in terms of studying human discs, because they can be prepared directly and gripped to test the motion segments [22–25]. However,

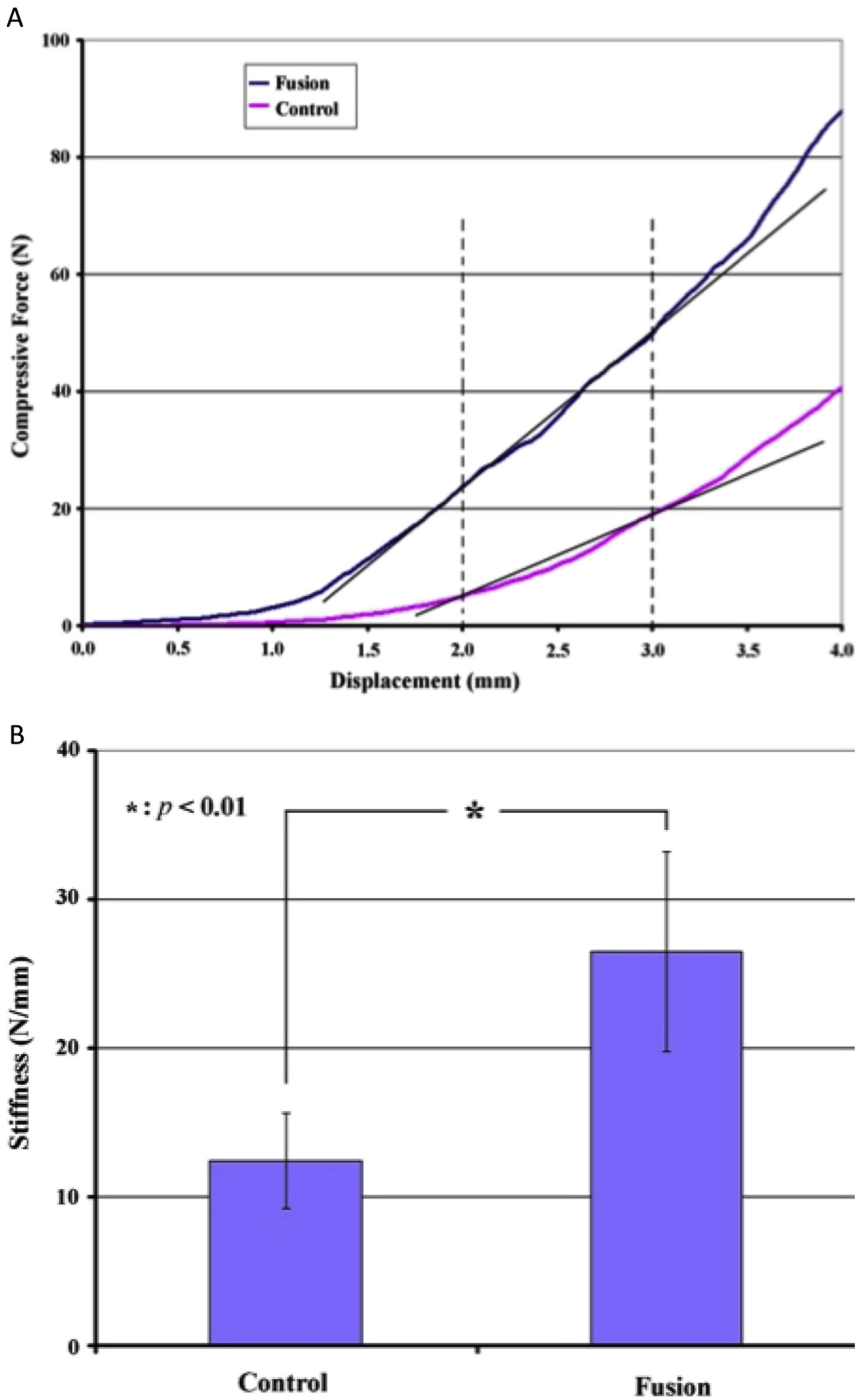

Fig. 6 (A) The typical force vs. displacement curves for the two FU groups in the three-point bending test. In both FU groups, a lower increasing rate of force was observed in the initial phase. Bending stiffness was defined as the slope of the straight line connecting the two force values required to cause 2.0 mm and 3.0 mm of displacement. (B) A graphical comparison of the mean bending stiffness values of the two FU groups. Compared to the control FU group, the fusion FU group exhibited a statistically higher bending stiffness ($p < 0.01$). The results indicate that interbody fusion surgery may improve the bending stiffness of FUs.

smaller animals such as rabbits and rats have emerged as desirable candidates due to the advantages of reduced cost, easy handling and faster healing [26]. In addition, rat lumbar and caudal discs have been shown to be good mechanical models for studying human discs in terms of their comparable tension, compressive and torsional mechanics and geometric nature [11,12]. Moreover, large animal discs for interbody fusion have strong resemblance to human bony structures which allow the insertion of actual clinical instruments into the pedicles [27,28], and are capable of investigating the feasibility of robotic assisted surgery [29]. Also, large animal models provide better evaluation for fusion status when using same setting for CT and MRI assessments [30]. On the other hand, small animal discs are often used to investigate the effect of drug treatment and growth factor supplementation even in ovariectomized model due to short generation time, high metabolic rate, and easy to manipulation for small animals [8,31,32]. Rat caudal discs are more easily accessible in terms of manipulation and surgical approaches. Faster recovery and less blood loss were also important reasons to choose rat caudal discs over rat lumbar discs in this study.

Blood vessels of the rat tail consist of one ventral artery, two lateral veins, and one dorsal vein. Adjacent to the vertebrae, there are three groups of bilaterally symmetrical coccygeal muscles (dorsal, lateral and ventral). Therefore, a dorsal skin incision was made first, while avoiding major vasculatures with ligation of the dorsal vein. The bilateral symmetric coccygeal muscles were then retracted to expose the vertebrae. At first, we performed the interbody fusion alone, without any additional procedure. However, skin necrosis with poor wound healing developed afterward. Therefore, we modified the procedure by applying a sterile silicon drainage tube to the surgical site after closing the wound to provide support and protect the wound. In addition, each rat was isolated in an individual cage to minimize contact with the wound by other rats. As a result, wound healing was significantly improved with this additional protection.

The main limitation of this study is the differences in the mechanics and geometry of the disc between human lumbar discs and rat caudal discs. Rat caudal discs do not experience large compression loads resulting from muscle action during ambulation, similar to human lumbar discs, but they have a more prominent large flexion displacement. Besides, pedicle screws, cages and other instrumentations which often serve as supplementation of interbody fusion in human subjects may not be applicable in this rat caudal disc model due to a mismatch in size. However, the potential advantages of easy accessibility, lower cost and direct manipulation may outweigh the mechanical differences and the inability to insert extra instruments. The rat caudal vertebral fusion model is still an easily accessible, repeatable, measurable and low cost way to directly assess fusion status.

Conclusions

In conclusion, our study proposed an efficient, repeatable and easily accessible animal model to evaluate interbody fusion using radiological assessment, manual palpation, histological evaluation and mechanical testing to measure and monitor fusion status. Future research into adjuvant treatments like growth factor injection and alternative fusion materials in an osteoporotic condition using this model would be worthwhile.

Conflicts of interest

There is no conflicts of interest.

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