Micro-CT evaluation and histological analysis of screw-bone interface of expansive pedicle screw in osteoporotic sheep

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Objective: To investigate the properties of screw-bone interface of expansive pedicle screw (EPS) in osteoporotic sheep by micro-CT and histological observation.

Methods: Six female sheep with bilateral ovariectomy-induced osteoporosis were employed in this experiment. After EPS insertion in each femoral condyle, the sheep were randomly divided into two groups: 3 sheep were bred for 3 months (Group A), while the other 3 were bred for 6 months (Group B). After the animals being killed, the femoral condyles with EPS were obtained, which were three-dimensionally-imaged and reconstructed by micro-CT. Histological evaluation was made thereafter.

Results: The trabecular microstructure was denser at the screw-bone interface than in the distant parts in expansive section, especially within the spiral marking. In the non-expansive section, however, there was no significant difference between the interface and the distant parts. The regions of interest (ROI) adjacent to EPS were reconstructed and analyzed by micro-CT with the same thresholds. The three-dimensional (3-D) parameters, including tissue mineral density (TMD), bone volume fraction (BVF, BV/TV), bone surface/bone volume (BS/BV) ratio, trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp), were significantly better in expansive sections than non-expansive sections ($P < 0.05$). Histologically, newly-formed bony trabeculae crawled along the expansive fissures and into the center of EPS. The newly-formed bones, as well as the bones at the bone-screw interface, closely contacted with the EPS and constructed four compartments.

Conclusions: The findings of the current study, based on micro-CT and histological evaluation, suggest that EPS can significantly provide stabilization in osteoporotic cancellous bones.

Key words: Expansive pedicle screws; Osteoporosis; Screw-bone interface; Micro-CT; Three-dimensional parameters

Pedicle screws with plates or rods have been used to stabilize motional segments, correct spinal deformations, and provide segmental fixation so as to promote graft incorporation in patients with degenerative, traumatic or neoplastic disorders of the spine.1-5 But pedicle screw fixation is challenged in osteoporotic spine as the mechanical stability of the pedicle screw-bone contact is affected by bone mineral density (BMD).6-9 Poor rigidity of the bone-screw contact can lead to loosening of the implants in osteoporotic patients. Analysis of pull-out strength of pedicle screws in lumbar spine had been performed in the past with a view to optimize the screw size, the screw insertion depth or direction and the screw design.10-13 Besides, to improve the strength of the screw-bone interface in osteoporosis, mechanical tests have been made with augmentation using polymethylmethacrylate (PMMA), hydroxyapatite (HA) grout, calcium phosphate cement (CPC), and etc.3,14-18 These screw modifications and augmentation strategies are limited by possible complications and problems such as increased risks of pedicle fracture with resultant neural injury for larger screws, anterior body penetration with ensuing vascular or visceral injury for longer screws,19 and potential problems associated with a non-absorbable foreign body in the spinal canal 4,20,21 and with uncertain long-term rigidity on the course of substitution for those absorbable cements.

To address these issues, we designed expansive pedicle screws (EPS). EPS can improve bone fixation through increasing the screw tip diameter and
allowing for greater bone contact without increasing screw diameter or length.22 Biomechanical studies have demonstrated that an expansive screw design can significantly improve the fixation strength compared with conventional pedicle screws. However, no systemic evaluation has been reported concerning the properties of screw-bone interface and the stable mechanism of EPS, especially in a living osteoporosis body.

The principal purpose of this study was to investigate the screw–bone interface and expansive section of EPS in osteoporotic sheep using three-dimensional (3-D) image and reconstruction by micro-CT and histological observation of microtome sections under a microscope.

**METHODS**

**EPS**

The newly-designed EPS (being produced by Weigao Orthopedic Material Co. Ltd, Shandong Province, China) was barrel-shaped, with an outer diameter of 6.5 mm, a 2.5-mm bore and a 3-mm pitch. The anterior half of the screw was split lengthwisely by two perpendicular grooves to form four anterior fins. A smaller gauge screw was inserted into the threaded interior of EPS and the fins were opened concentrically as it was advanced. This system increased the diameter of the expanding screw tip by approximately 2.5 mm (Fig. 1). The diameter of the posterior portion of the screw remained constant in order to prevent the fracture of the pedicle during the expansion of the screws.22

**Animals**

Osteoporosis was made on 6 female Chinese white sheep (aged 3.5 years ± 0.8 years and weighing 45.1 kg ± 5.2 kg) with a combined protocol of transperitoneal bilateral ovariectomy, calcium-reduced diet and movement restriction.23,24 During operation, the cancellous BMD at the lumbar vertebrae was reduced by 30% on average compared with the initial values and did not recover until the end of the experiment. The bilateral femoral condyles of 6 sheep (12 femoral condyles) were employed in this experiment.

**Surgical preparation**

All sheep were anesthetized with sumianxin (0.2 ml/kg body weight), a complex prescription composed of xyldinothiazoline, edathamil, etorphine and aloperidin. Each femoral condyle was exposed by the lateral approach, and an inclined and retroverted hole was drilled from the proximal end of the ectocondyles to the center of the entocondyles with a drill of 5.5 mm in diameter. It should be assured that the drill did not break through the medial cortical substance of bone or penetrate into the fossa intercondyloidea femoris (Fig. 2). EPS was inserted into each bone hole. After operation, the sheep were randomly selected and divided into two groups: 3 sheep were bred for 3 months (Group A), while the other 3 were bred for 6 months (Group B).

**Micro-CT scanning and 3-D reconstruction**

After the sheep being killed, the bilateral whole femurs were obtained and the soft tissues were removed carefully. Then the 12 specimens were incised into cylinders of 20 mm in diameter, centered by EPS. The specimens were examined with a micro-CT system (Explore Locus SP, GE Healthcare Company, USA). Then they were mounted on a stage and scanned. The images consisted of 886 slices with a voxel size of 21 µm in all three axes. Cancellated bone and EPS in the specimens were three-dimensionally imaged and reconstructed. Regions of interest (ROI) adjacent to EPS with the same size were reconstructed and analyzed by micro-CT with the same thresholds. The database was analyzed, which led to 3-D parameters of each ROI. Each pair of these parameters in the same specimen was compared for each group.

**Histological examination**

After CT scanning, the specimens were removed and prepared for histological examination. The specimens were fixed in 10% phosphate-buffered formalin (pH=7.25) for 7 days and dehydrated through a series of increasing concentrations of ethanol (70%, 80%, 90%, 99%, 100%, and 100% v/v) for 18 hours in each. The specimens were then embedded in polyester resin for 3 weeks. Thick sections (30-100 µm) were obtained with a band saw (Leica-LA 2500, Germany) strictly perpendicular to the axis of the implants. The sections were then stained with bone meal (a complex prescription composed of fast green, orange yellow, azure and aniline red) and ponceau. A thorough microscopic analysis was performed on the sections using transmitted light microscopy (Leica-LA, Germany) combined with a digital camera (Pixera Pro600cl, USA).
Statistical analysis

Statistical comparisons were carried out with software SPSS13.0. The parameters of the two groups between the expansive and non-expansive sections were compared by the Mann-Whitney U-test. The values were expressed as mean ± SD and considered significant at a probability (P) of <0.05 (Table 1).

<table>
<thead>
<tr>
<th>Items</th>
<th>Group A (n=3)</th>
<th>u</th>
<th>P</th>
<th>Group B (n=3)</th>
<th>u</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expansive ROI</td>
<td>Non-expansive ROI</td>
<td></td>
<td>Expansive ROI</td>
<td>Non-expansive ROI</td>
<td></td>
</tr>
<tr>
<td>TMD (mg/cc)</td>
<td>503.64±91.79</td>
<td>383.64±46.14*</td>
<td>4.00</td>
<td>0.025</td>
<td>466.62±51.83</td>
<td>402.16±31.53*</td>
</tr>
<tr>
<td>SMI (%)</td>
<td>1.68±0.47</td>
<td>2.11±0.64</td>
<td>3.00</td>
<td>0.510</td>
<td>5.19±1.71</td>
<td>8.05±1.65*</td>
</tr>
<tr>
<td>BV/TV (%)</td>
<td>0.53±0.10*</td>
<td>0.35±0.07*</td>
<td>2.00</td>
<td>0.010</td>
<td>58.52±7.37</td>
<td>66.37±5.91</td>
</tr>
<tr>
<td>BS/BV (mm)</td>
<td>6.84±1.88</td>
<td>9.89±1.27*</td>
<td>3.00</td>
<td>0.015</td>
<td>2.48±0.14</td>
<td>2.50±0.06*</td>
</tr>
<tr>
<td>Tb.Th (mm)</td>
<td>0.31±0.09</td>
<td>0.21±0.03*</td>
<td>3.00</td>
<td>0.015</td>
<td>1.41±0.20</td>
<td>1.75±0.46*</td>
</tr>
<tr>
<td>Tb.N (mm)</td>
<td>1.74±0.29</td>
<td>1.72±0.47</td>
<td>39.00</td>
<td>0.015</td>
<td>0.27±0.04</td>
<td>0.47±0.10*</td>
</tr>
<tr>
<td>Tb.Sp (mm)</td>
<td>0.27±0.04</td>
<td>0.47±0.10*</td>
<td>0.00</td>
<td>1.000</td>
<td>0.23±0.09</td>
<td>0.40±0.12*</td>
</tr>
</tbody>
</table>

*P<0.05, compared with Group A.

RESULTS

Trabecular microstructure around EPS by micro-CT and 3-D reconstruction

The specimens of the two groups were three-dimensionally imaged and reconstructed (Fig. 3). It showed that the trabecular microstructure of the screw-bone interface was denser significantly than that of the distant parts in the expansive sections, especially within the spiral marking. While in the non-expansive sections, there was no significant difference between the interface and the distant part.

Same size of ROI adjacent to the EPS was reconstructed and analyzed with the same thresholds (1 000). In Group A, the tissue mineral density (TMD, 503.64 mg/cc ± 91.79 mg/cc), the bone volume fraction (BVF, 53.25% ± 10.16%), and the trabecular thickness (Tb.Th, 0.31 mm ± 0.09 mm) of ROIs in the expansive sections were significantly greater than those of the non-expansive sections (TMD, 383.64 mg/cc ± 46.14 mg/cc; BVF, 35.18% ± 7.69% and Tb.Th, 0.21 mm ± 0.03 mm. P < 0.05). While the bone surface/bone volume ratio (BS/BV, 6.84 /mm ± 1.88 /mm) and the trabecular separation (Tb.Sp, 0.27 mm ± 0.04 mm) were significantly lower than those of the non-expansive sections (BS/BV, 9.89 /mm ± 1.27 /mm; Tb.Sp, 0.47 mm ± 0.10 mm. P < 0.05). Whereas there was no significant difference (P > 0.05) in the trabecular number (Tb.N) and structure model index (SMI) between the expansive sections (Tb.N, 1.74 /mm ± 0.29 /mm; SMI, 1.68 ± 0.47) and the non-expansive sections (Tb.N, 1.72 /mm ± 0.47 /mm; SMI, 2.11 ± 0.64 ).

In Group B, the TMD (466.62 mg/cc; 51.83 mg/cc), BVF (58.52% ± 7.37%), and Tb.Th (0.42 mm ± 0.14 mm) of ROIs in the expansive sections were significantly greater than those of the non-expansive sections (TMD, 402.16 mg/cc ± 31.53 mg/cc; BVF, 38.37% ± 6.91%; and Tb.Th, 0.25 mm ± 0.06 mm. P=0.05). While BS/BV (5.19 /mm ± 1.71 /mm) and Tb.Sp (0.23 mm ± 0.09 mm) were significantly lower than those of the non-expansive sections (BS/BV, 8.05 /mm ± 1.65 /mm; Tb.Sp, 0.40 mm ± 0.12 mm. P=0.05). Whereas there was no significant difference (P=0.05) in Tb.N and SMI between the expansive sections (Tb.N, 1.41 /mm ± 0.20 /mm; SMI, 2.48 ± 0.84) and the non-expansive sections (Tb.N, 1.75 /mm ± 0.46 /mm; SMI, 2.81 ± 0.74 ).

Histological findings

Histologically, newly-formed bony trabeculae crawled along the expansive fissures (Fig.4). The bony trabeculae in the fissures of the fins were thickened (Fig. 5) and tended to the center of the EPS 6 months after surgery (Fig.6). The newly-formed bones, as well as the bones at the bone-screw interface, closely contacted the EPS and constructed four compartments, though there existed significant osteoporosis around the EPS.
DISCUSSION

In the early study, we evaluated the newly-designed EPS biomechanically with fresh pedicles from calf lumbar vertebrae in comparison with conventional pedicle screws, CD Horizon (CDH), Universal Spine System pedicle screw (USS) and Tenor (Sofamor Danek). The results showed that the turning-back torque and pulling-out force of EPS were significantly greater than those of USS, Tenor and CDH screws. The use of an expansive screw design significantly improved the biomechanical fixation strength of the screw instantly.

The EPS, with an outer diameter of 6.5 mm, could not be inserted into sheep lumbar vertebrae because its width of pedicle was small (4.2 mm ± 0.8 mm). In the present study, femoral condyles, which were regarded as a reasonable substitute for the body of ver-
tebra in the experiment because of their geometric properties and BMD of cancellous bones, were employed for in vivo evaluation of the EPS. We also used the method of scanning and 3-D reconstruction by micro-CT in this study. The findings from the current study demonstrated that those methods were an advance over the conventional radiography and medical CT because they can accurately resolve micro-sized structures, which made up the cancellous bones around the EPS. And from these images a wide array of parameters, which have been demonstrated to be related to the mechanical properties of cancellous bones, can be measured. It was demonstrated that the parameters in the expansive sections, including TMD, BVF, BS/BV, Tb.Th and Tb.Sp, were significantly better than those of the non-expansive sections.

Many biomechanical studies have demonstrated that pedicle screw fixation is highly correlated to BMD. In the present experiment, the fins of EPS were expanded and detached, which consequently made the bone trabeculae around the expansive sections micro-fractured and compressed to some extent and became denser than before. Therefore, the fixation strength and stiffness were augmented for the increased BMD of the anterior screw-bone interface. Furthermore, according to Wolff’s law, it is believed that the bone tissue structure adapts to the corresponding mechanical load. It is also believed that the change of bone mass during the bone remodeling can be achieved through changing the density or geometry. The mechanical loads provided by the expanded fins of EPS can be taken as regulators of bone remodeling, which increased bone mass of anterior screw-bone interface. Therefore, we could find that the trabecular microstructure of screw-bone interface were significantly better in the expansive sections than that of the non-expansive sections. The continuous pressure of the EPS fins to the bony trabeculae of the expansive sections ensured the EPS’s mechanical stabilization at the earlier stage, which could prevent EPS from loosening effectively.

Histologically, with time went by, more and more newly-formed bones occurred continuously from the screw-bone interface and crawled into the fissure of the anterior fins, even at the center of anterior fins. Both the newly-formed bones and the bones in the interface directly contacted with the EPS without connective tissue layers (Fig. 5), which showed that EPS had excellent biocompatibility, and they constructed four compartments and wrapped up the pins tightly. Theoretically, this special 3-D structure, in which bones contained pins as well as pins contained bones, kept fixation strength and stiffness of EPS enduringly.

In conclusion, because of the continuous pressure of EPS fins on the trabecular interface, as well as the special 3-D structure, EPS can significantly provide stabilization in cancellous bones after osteoporosis.

REFERENCES


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