Introduction: The widespread use of fusion procedures in the management of spinal disorders has led investigators to explore the use of growth and differentiation factors in such procedures. Recombinant human BMP-2 (rhBMP-2), as one of the first factors identified in the process of endochondral bone formation, appears to improve fusion rates after spinal arthrodesis in both animal models (Sandhu HS, et al. 2002; Ohyama T, et al. 2004) and humans (Barkus JK, et al. 2002 and 2003; Villavicencio AT, et al. 2005). As an adjuvant to allograft bone or as a replacement for harvested autograft, rhBMP-2 could reduce the donor-site morbidity previously associated with such procedures; however, it is only effective in high physiological concentrations (Vehof JW, et al. 2001, 2002, and 2003). Recombinant BMPs, therefore, limits their clinical use because it is very expensive.

Colloss, a lyophilized complex of the extracellular matrix proteins extracted from diaphyseal equine bone, containing native BMPs, have been proven to be cost-effective alternatives to recombinant BMPs. Osteoinductive potential for this equine-derived material has been proven in the ectopic rat model (Nienhuijs ME et al. 2006; Li H et al. 2006). Equine osseogenetic collagen complex has been shown as an effective bone graft alternative in experimental posterolateral lumbar spine arthrodesis in rabbits (Boden SD et al. 2005) and experimental anterior lumbar spine arthrodesis in pigs (Li H et al. 2006).

The assessment of bone regeneration, from the equine collagen lyophilisate or rhBMP-2, is important in better understanding of the mechanism of action of these materials currently being developed as bone graft alternatives. Noninvasive functional imaging techniques such as bone imaging and mapping of living intact humans and non-human subjects by positron emission tomography (PET) have become a powerful approach to the understanding of bone metabolism in bone regeneration. The aim of the present study is quantitatively to assess the time course of spinal fusion healing with an osteoinductive equine bone collagen extract compared to rhBMP-2 and autograft by use of positron emission tomography (PET) in an anterior lumbar interbody fusion (ALIF) model in pigs.

Materials and Methods: ALIF was performed on 18 Danish female landrace pigs, weighing around 50kg. After harvesting of autograft from the iliac crest and pedicle screw instrumentation in L3 to L6, ALIF procedures were done with retroperitoneal approach. After carefully removal of the intervertebral disc and adjacent endplates, each level was randomly inserted with a PEEK cage containing different treatment of 1.27 g (SD 0.06) autograft, 3.0 mg InFuse (rhBMP-2) dissolved on one quarter of the enclosed collagen sponge or 40 mg of Colloss E. The animals were divided into three groups of 6 animals, with postoperative observation periods of 2, 4 and 8 weeks. Before sacrifice, the pigs were scanned by means of PET/CT with 18-fluoride (18F) tracer. The activity of the fluoride uptake in bone corresponding to the metabolic activity within the cage, represented by K-values, was evaluated using a Gjedde-Patlak plot (Gjedde 1981, 1982; Patlak et al. 1983). This relies on the hypothesis of irreversible incorporation of fluoride into the bone. Data was analyzed by two-way ANOVA (time * treatment).

Results: 18 pigs went through the observation. One pig from the 8 week observation group was excluded from the analysis due to severe infection at a late stage of the observation period. Implant breaking, loosening or spinal deformity was not observed after 2, 4 and 8 weeks on radiographic examination.

K-values, represented the activity of the incorporated 18F with different treatments in an observation period of 2, 4 or 8 weeks. This is summarized in table 1 and depicted on figure 1.

Table 1: K-values (Mean±SEM)

<table>
<thead>
<tr>
<th></th>
<th>2 weeks</th>
<th>5 weeks</th>
<th>8 weeks</th>
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<tbody>
<tr>
<td>InFuse</td>
<td>0.13±0.0318</td>
<td>0.27±0.04</td>
<td>0.03±0.005</td>
</tr>
<tr>
<td>Colloss E</td>
<td>0.13±0.0322</td>
<td>0.23±0.036</td>
<td>0.05±0.006</td>
</tr>
<tr>
<td>Autograft</td>
<td>0.16±0.0163</td>
<td>0.15±0.031</td>
<td>0.04±0.006</td>
</tr>
</tbody>
</table>

Two-way ANOVA analyses showed significant difference in main effects (P<0.001). Two weeks postoperatively, difference in the activity was found in both the InFuse and Colloss E level compared to autograft (P<0.05), but no difference between the InFuse and Colloss E. At 4 weeks, InFuse had a significantly higher activity than both autograft and Colloss E (P<0.05), but no difference between autograft and Colloss E. Eight weeks postoperatively, InFuse showed a significantly lower activity than both Colloss E and autograft (P<0.05), with no difference between the two.

Discussion: With kinetic of 18F PET/CT scanning, quantitative images were produced for interpretation as new bone formation in the volume of interest within the interbody cage localized from CT findings. After surgery, rapid new bone formation had increased greatly in the cage with rhBMP-2 and Colloss E at 2 weeks. At 4 weeks bone formation was highest in the cage with rhBMP-2, but activity had declined to the lowest level in the cage with Colloss E. At 8 weeks bone formation were about the same, but activity was lowest in the cage with rhBMP-2. In the present ALIF study, these findings using the PET technique showed that new bone formation occurred early after use of rhBMP-2 and Colloss E at 2 weeks, rapidly increased with rhBMP-2 at 4 weeks, and reached the consolidation and remodeling stage at 8 weeks, which corresponded to a significant higher bone metabolism than autograft.

We found that PET/CT is a sensitive method for evaluating bone formation in the ALIF device. It indicates the regional bone hypermetabolism with the growth factors compared to autograft for spinal fusion.


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