

Histological Analysis of Bone Bonding and Ingrowth into Connected Porous Hydroxyapatite Spacers in Spinal Surgery

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Abstract. To evaluate the osteoconductive potential of connected porous hydroxyapatite (HAp), we histologically analyzed the newly formed bone inside unidirectional porous HAp (Regenos[®], Kuraray, Japan; 75% porosity, n=17) and interconnected porous HAp (Neobone[®], Covalent Materials, Japan; 75% porosity, n=10) 26 weeks after their implantation as bone spacers between the split lumbar laminae of goats. As a control, non-connected porous HAp spacers (Apaceram[®], Pentax, Japan; 50% porosity, n=5) were used. After staining non-decalcified samples with Villanueva Goldner, changes in pore shape were evaluated microscopically and new bone formation in HAp spacers was quantitatively analyzed. In addition, blood vessel distribution was evaluated by hematoxylin and eosin staining. Changes in pore shape were observed in 76% of the Regenos[®] spacers and 90% of the Neobone[®] spacers but were not detected in the Apaceram[®] spacers. Only limited new bone formation was observed in the Regenos[®] and Neobone[®] spacers, whereas vascular-like structures were detected in 82% of the Regenos[®], 70% of the Neobone[®], and 80% of the Apaceram[®] spacers. The changes in pore shape were thought to have resulted from the low initial compression strength of the connected porous HAp, which may have limited the inherent osteoconductive potential of connected HAp. Our findings suggest that the maintenance of pore shape is required for promoting new bone formation in connected porous HAp when used as lamina spacers in spinal surgery.

Introduction

Porous hydroxyapatite (HAp) has high osteoconductivity and is therefore widely used in orthopaedic applications. For example, the use of HAp for spine surgery, particularly as lamina spacers for double-door cervical laminoplasty, has become increasingly common. Recently, new types of HAp with connected pores have been developed and are clinically available. One such material is unidirectional porous HAp (Regenos[®]; Kuraray, Japan), which has a porosity of 75% and penetrating oval pores ranging in diameter from 100 to 300 μm [1]. Another promising material is interconnected porous calcium HAp (Neobone[®]; Covalent Materials, Japan), which has a fully interconnected porous structure with 75% porosity [2]. The porous microstructures of these HAp materials are advantageous for cell migration and angiogenesis [3]. However, new bone formation and bone remodeling in these HAp which are used in clinical situation remain unclear.

Here, to evaluate the osteoconductivity of connected porous HAp, we implanted Regenos[®] and Neobone[®] spacers between the split laminae of goats, and histologically analyzed the newly formed bone inside the HAp pores.

Materials and Methods

Regenos[®] and Neobone[®] spacers with a trapezoidal morphology (top, 7 mm; bottom, 9 mm; height, 7 mm; length, 8 mm) were used in this study. For suture fixation, a 1-mm diameter hole was made in the center of the side wall of each spacer. Seven goats (castrated one-year-old males, body weight >40 kg) were used. With animals in the prone position and under general anesthesia, we made a skin incision between L1 and L5 to expose the first 5 lumbar spinous processes and laminae. After the resection of spinous processes at the base, the middle of the laminae was split and side gutters were made using a 2-mm high-speed drill. Double-door laminoplasty was then performed by opening the split laminae, as previously described [4]. A 1-mm opening was made through each split lamina to allow for fixation of the HAp spacer between the split lamina (Fig. 1) using a 1-0 woven nylon suture (NESCOSUTURE[®]; Alfresa Pharma, Japan). The direction of the unidirectional pore was perpendicular to the axis of the spine. The laminoplasty operation and spacer implantation were similar to the clinical procedure.

Seventeen Regenos[®] spacers and 10 Neobone[®] spacers were implanted into each goat, and the levels of implantation were randomized. As a control, 5 Apaceram[®] spacers (porosity 50%, unconnected pores; Pentax, Japan) were also implanted. The animals were sacrificed 26 weeks after implantation and the whole lumbar spines were excised and prepared for histological examination. Non-decalcified samples were stained with Villanueva Goldner, and changes in pore shape were evaluated microscopically. For the quantitative evaluation of newly formed bone, we measured the length of direct bonding (A) between the newly formed bone and the lamina, and the length of bone ingrowth (C) at the HAp spacer wall, and then calculated the ratio of A and C to the total length of the HAp spacer (B) (Fig.2). Bone bonding was defined as A/B , and bone ingrowth was defined as C/B . The mean bone bonding and bone ingrowth ratios were calculated by analyzing both the left and right walls of each HAp spacer. In addition, the distribution of blood vessels was evaluated by hematoxylin and eosin staining. The histological analyses were conducted by two orthopaedic surgeons who were blinded to the sample source.

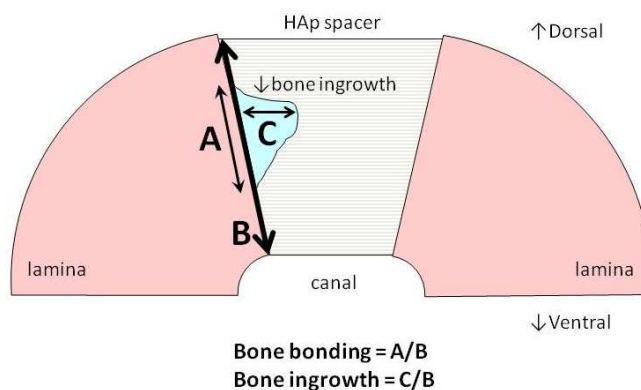
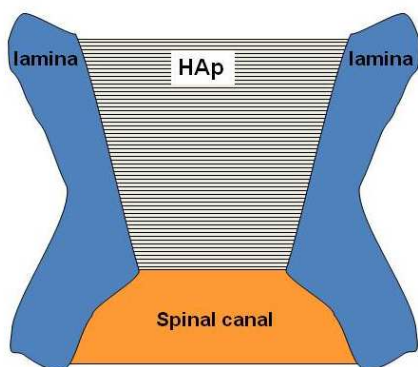


Fig. 1 Scheme of double-door laminoplasty

Fig. 2 Method used to determine bone bonding and bone ingrowth

Results

Changes in pore shape were observed in 13 (76%) Regenos[®] samples, 9 (90%) Neobone[®] samples, and 0 (0%) Apaceram[®] samples (Table 1; Fig. 3a-c). The Regenos[®] and Neobone[®] spacers had slightly lower mean bone bonding ratios (A/B) than the Apaceram[®] samples, but displayed markedly reduced mean bone ingrowth ratios (C/B) compared to the control spacers. Vascular-like structures were observed in the majority of samples for all HAp spacer types (Fig. 3d-f).

Table 1 Results of histological evaluation

| | Regenos | Neobone | Apaceram |
|--------------------------|----------|---------|----------|
| Number of spacers | 17 | 10 | 5 |
| Change in pore shape | 13 (76%) | 9 (90%) | 0 (0%) |
| Mean bone bonding (A/B) | 0.27 | 0.36 | 0.45 |
| Mean bone ingrowth (C/B) | 0.008 | 0.06 | 0.26 |
| Vascular-like structures | 14 (82%) | 7 (70%) | 4 (80%) |

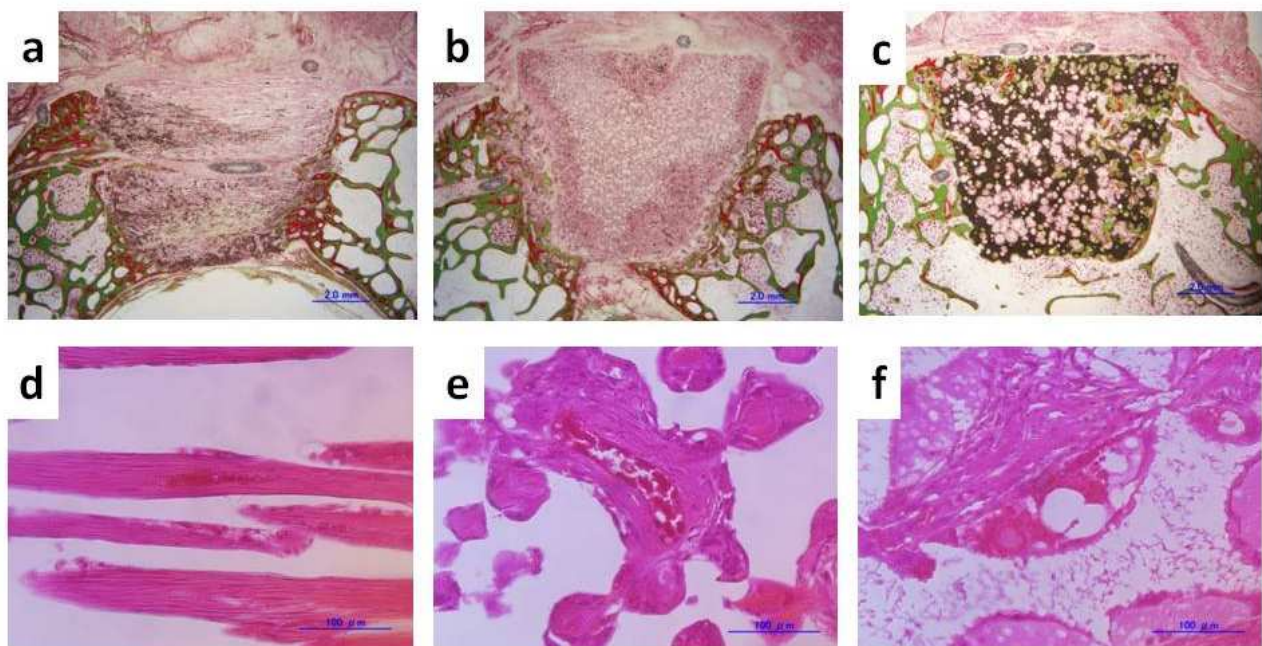


Fig. 3 Histological examination of implanted HAp spacers at 26 weeks. *Upper*, Villanueva Goldner staining (original magnification: 100X); *Lower*, hematoxylin and eosin staining (original magnification: 400X). The shape of pores had changed and only limited new bone formation was seen in the Regenos[®] (a) and Neobone[®] spacers (b). In contrast, pore shape was maintained and partial new bone formation was detected in the Apaceram[®] spacers (c). Vascular-like structures were observed in the majority of the Regenos[®] (d), Neobone[®] (e), and Apaceram[®] spacers (f).

Discussion

Previously, we demonstrated that unidirectional porous HAp (Regenos[®]) promotes the migration of osteogenic and angiogenic cells as early as two weeks after implantation in the femoral marrow of rabbits [1]. This implantation model was suitable for studying osteogenesis with respect to mesenchymal stem cells. Unidirectional porous HAp (Regenos[®]) was also advantageous for treating cortical bone defects in the proximal tibia of rabbits [5]. This bone defect model was suitable for studying osteogenesis with respect to not only blood flow, but also the compression force. In the present study, only limited new bone formation was observed in the connected porous HAp spacers. The observed changes in pore shape for the majority of Regenos[®] and Neobone[®] spacers were thought to have occurred due to the low initial compression strength of connected porous HAp resulting from its higher porosity [2, 6]. Notably, the blood flow from the split laminae and compression force on the spacers, which were located between the split laminae, were considered to be minimal in this implantation model. These factors may have limited the inherent osteoconductive potential of connected porous HAp, although vascular-like structures were observed in most of the connected porous HAp spacers.

Conclusions

The maintenance of pore shape is required for promoting new bone formation in connected porous HAp when used as lamina spacers in spinal surgery.

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