Evaluation of hydroxyapatite ceramic vertebral spacers with different porosities and their binding capability to the vertebral body: an experimental study in sheep

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Object. The aim of this study was to evaluate the degree of bone ingrowth and bonding stiffness at the surface of hydroxyapatite ceramic (HAC) spacers with different porosities in an animal model and to discuss the ideal porous characteristics of these spacers for anterior spinal reconstruction.

Methods. Twenty-one adult sheep (age 1–2 years, mean weight 70 kg) were used in this experiment. Surgery consisted of anterior lumbar interbody fusion at L2–3 and L4–5, insertion of an HAC spacer (10 × 13 × 24 mm) with three different porosities (0, 3, and 15%), and single-rod anterior instrumentation. At 4 and 6 months postoperatively, the lumbar spines were harvested. Bonding conditions at the bone–HAC spacer interface were evaluated using neuroimages and biomechanically. A histological evaluation was also conducted to examine the state of bone ingrowth at the surface of the HAC spacer.

Biomechanical testing showed that the bonding strength of HAC at 6 months postoperatively was 0.047 MPa in 0% porosity spacers, 0.39 MPa in 3%, and 0.49 MPa in 15% porosity spacers. The histological study showed that there was a soft-tissue layer at the surface of the HAC spacer with 0% porosity. Direct bonding was observed between bone and spacers with 3 or 15% porosity. Micro–computed tomography scans showed direct bonding between the bone and HAC with 3 or 15% porosity. No direct bonding was observed in HAC with 0% porosity.

Conclusions. Dense (0%) HAC anterior vertebral spacers did not achieve direct bonding to the bone in the sheep model. The HAC vertebral spacers with 3 or 15% porosity showed proof of direct bonding to the bone at 6 months postoperatively. The higher porosity HAC spacer showed better bonding stiffness to the bone.

Key Words • spinal fusion • anterior vertebral spacer • bone graft substitute • hydroxyapatite • sheep

Among various biomaterials, HACs have been widely used as bone graft substitutes in spinal surgery. There are two types of HAC that have been used for different locations and purposes. One type of HAC is solid and is commonly used for anterior spinal fusion after cervical discectomy or posterior lumbar interbody fusion.³,¹⁰,¹⁴,¹⁶,¹⁹,²³ The other type of HAC is a morcellized graft material with high porosity for posterior or posterolateral spinal fusion in patients with unstable lumbar spine and spinal deformity.³,¹¹,¹⁵,¹⁷ Up until now, there have been numerous animal and clinical studies in which the effectiveness of HAC spacers in spinal surgery have been evaluated, but the results have been conflicting. Authors of some studies have reported that HAC spacers were superior or equivalent to autogenous bone grafts.⁸,¹⁰,¹³,¹⁶ Pinter and colleagues¹³ showed that the fusion rate of dense HAC was similar to that of autogenous tricortical iliac bone graft in a goat model. Suetsuna et al.¹⁶ and Kim et al.¹⁰ reported good clinical results of anterior cervical fusion after using porous HAC spacers. On the other hand, there have been other reports regarding complications related to an HAC spacer, such as cracking, nonunion, and spinal cord compression due to its protrusion into the spinal canal.⁴,⁹,²³ Recently, some investigators have tried to use porous HAC as a carrier material for bone morphogenetic proteins and autogenous stem cells to supply osteoinductive and/or osteogenic components with osteoconductive HAC material.³,¹¹,¹⁵ As a synthetic interbody fusion material, there have been no clinical studies in which the authors have examined the use of porous HAC blocks or spacers for posterior lumbar interbody fusion and for anterior reconstruction surgery in the thoracolumbar spine. One of the main reasons for the scarcity of reports about the use of porous HAC spacers for load-sharing purposes may be due to the relative mechanical weakness and brittleness of porous HAC. To improve the mechanical strength of HAC for posterior lumbar inter-
body fusion or anterior reconstruction in the lumbar spine, authors of some clinical studies have tried using dense HAC spacers despite less bone ingrowth at the HAC–bone interface and the possibility of loosening. To date, the ideal porosity, pore size, and biomechanical strength of HAC for interbody fusion in load-bearing situations remain unclear. Because optimal porous characteristics and biomechanical strength of HAC may differ according to the areas in which it is applied in the spine, determination of pore sizes and orientations of the HAC spacer are indispensable for achievement of high-quality fusion at the bone–HAC interface as well as satisfactory long-term clinical results in anterior spinal reconstruction surgery.

In this study our goal was to evaluate the degree of bone ingrowth and bonding stiffness at the surface of different-porosity HAC spacers in an animal model and to discuss the ideal porosity characteristics of an HAC spacer for anterior spinal reconstruction.

Materials and Methods

Animal Model and Surgical Technique

Twenty-one adult male Suffolk sheep (age 1–2 years; weight 65–80 kg, mean 70 kg) were used in this experimental protocol approved by the institutional animal review board. Anesthesia was induced by intravenous administration of ketamine (10 mg/kg) and diazepam (0.15 mg/kg) and was maintained with endotracheal inhalation of 2% isoflurane throughout the operation. The animals were placed in the right lateral decubitus position; after sterile preparation the left sides of the lumbar vertebrae were exposed via a retroperitoneal approach. After total removal of the L2–3 and L4–5 intervertebral discs as well as the upper and lower cartilage endplates at both levels, an HAC spacer (PENTAX Co.) was inserted in these spaces under mild distraction. The HAC used in this study was chemically synthesized by sintering HA powder at 1200˚C and shaping it into a 10 × 13 × 24–mm block after fabrication (Fig. 1). During synthesis, foaming liquid was mixed with HA powder to produce different pore sizes in an HAC block. The animals were randomly divided into the following three groups (seven in each) according to the porosity of HAC: Group 1, 0% porosity HAC spacer (dense HAC); Group 2, 3% porosity HAC spacer; and Group 3, 15% porosity HAC spacer. The surface image of each HAC observed using a scanning electron microscope is shown in Fig. 2. The compressive stiffness of 0, 3, and 15% porosity spacers was 735, 710, and 245 MPa, respectively. Two HAC spacers of the same porosity were implanted at L2–3 and L4–5 in each animal. After complete discectomy and placement of an HAC spacer at L2–3 and L4–5, a single screw/rod system (Kaneda-SK, Depuy Acromed) was applied across L2–3 and L4–5 to afford immediate stability over the surgical sites.

Three animals were killed at 4 months postoperatively and the other four animals at 6 months postoperatively. At that time, the entire lumbar spine was harvested.

Imaging Analysis

Bonding conditions at the bone–HAC interface were evaluated using CT scanning. Two HAC–bone interfaces were evaluated in each animal. Bonding conditions between HAC and adjacent VBs on CT scans were classified into four grades: protrusion, suspicious fusion, probable fusion, and absolute fusion (Fig. 3). After obtaining the CT scans, all soft tissues and spinal implants were removed from the lumbar spine.

Biomechanical Testing

Six and four motion segments containing the HAC spacer in each animal group killed at 6 and 4 months postoperatively, respectively, were examined biomechanically. The tensile strength at the interface between the HAC spacer and the VB was evaluated by a detachment test under displacement control by using the servohydraulic MTS 858 Mini Bionix 2 System (MTS Systems). The VBs around the HAC spacer were removed by an automated diamond bar so as to preserve the HAC–VB interface. Then, the upper and lower VBs were anchored with stainless-steel screws and secured in metal fixtures with polyester resin (Fig. 4). The tensile load was applied to the top of the upper VB at a constant speed of 0.5 mm/second. Load-displacement curves were recorded by a data sampling program (MultiPurpose TestWare, MTS Systems Corp.) on a personal computer (Compaq Deskpro EN). The curves were analyzed to yield peak loads at tensile failure. Tensile failure strength (in MPa) was calculated as the failure load (in N) divided by the cross-sectional area of the HAC–bone interface. The detached surface of the VB was recorded using a digital camera (Nikon D1, Nikon Co.) immediately after the detachment test, and the cross-sectional area of the HAC on digital images was measured by using Image J Software (National Institutes of Health).

Histological Analysis

Four motion segments from each group were examined histologically. Among the four segments in each group, two segments were taken from the animals killed at 4 months postoperatively and the other two were from the animals killed at 6 months. The histological analysis was also conducted to examine the state of bone ingrowth at the surface of the HAC spacer. The specimens were subjected to undecalcified tissue processing, and frontal sections of the spinal unit containing the spacer were examined by light microscopy. The specimens were sectioned by a diamond saw into an appropriate thickness and were then ground 1 mm thick. Staining with H & E and toluidine blue O was performed. Direct bonding of the HAC spacer to the bone was measured on a histological slide by obtaining the ratio of the direct bonding surface to the total surface of the HAC spacer. Three slices of one HAC spacer were randomly selected for measurement, and the average was chosen for the representative value (direct bonding ratio).

Microimaging Analysis

Microimaging evaluation was conducted using micro-CT scanning (Hitachi MTC-225CR, Medico Corp.) to examine the HAC–VB interface obtained from representative animals in each group.

Statistical Analysis

The chi-square test was used to analyze the data obtained on CT images for neuroimaging assessment of fusion. The unpaired t-test was used to assess the interfacial tensile strength and direct bonding.
Hydroxyapatite spacers for anterior reconstruction

Table 1. Protrusion of the 0 and 3% porosity HAC spacers from the disc space was noted in two and four animals, respectively. No protrusion was seen on CT scans in animals with 15% porosity HAC spacers. Dense HAC spacers (0% porosity) demonstrated the least amount of fusion at both 4 and 6 months after surgery. Fusion rates with the 0% porosity HAC spacers were 0 and 25% at 4 and 6 months, respectively. The 3% porosity HAC spacers had a 50% fusion rate at 6 months, whereas the 15% porosity HAC spacers had the highest fusion rate (75%) at 6 months postoperatively. There was no significant statistical difference among the groups.

Biomechanical Evaluation

At 4 months postoperatively, the mean bonding strength was $0.071 \pm 0.018$, $0.061 \pm 0.028$, and $0.058 \pm 0.058$ MPa, respectively, for the 15, 0, and 3% porosity HAC spacers. There was no statistical difference among the groups. Bonding stiffness in all groups was extremely low at 4 months. At 6 months postoperatively, the mean bonding strength was $0.047 \pm 0.026$, $0.39 \pm 0.32$, and $0.49 \pm 0.29$ MPa, respectively, for 0, 3, and 15% HAC spacers. Statistical significance was observed in bonding strength between 4 and 6 months postoperatively in the 3 and 15% porosity HAC spacer groups. There were significant statistical differences in bonding stiffness between 0 and 3% porosity HAC spacers and between 0 and 15% porosity HAC spacers at 6 months after surgery.

After the detachment test, remnants of 3 and 15% porosity HAC spacers were left at the detachment surface of the VBs, which showed that bonding stiffness between HAC and the bone was greater than the mechanical stiffness of HAC itself (Fig. 5).

Histological Evaluation of the HAC–Bone Interface

A soft-tissue layer was observed at the interface between the 0% porosity HAC spacers and the VBs. There was also cartilage tissue as well as fibrous tissue that surrounded the 0% porosity HAC spacer. There was no direct bonding between the 0% porosity HAC spacer and the bone (Fig. 6 left). On the other hand, there were areas indicating direct bonding between 3 and 15% porosity HAC spacers and the VBs (Fig. 6 right), although there were some areas showing soft tissue between HAC spacers and the bone. There was also new bone formation in the pores adjacent to the VBs in the groups with 3 and 15% porosity HAC spacers.

The direct bonding ratio of 0% porosity HAC spacers was 0% at both 4 and 6 months postoperatively. The direct bonding ratio of 3% porosity HAC spacers at 4 and 6 months was $11.3 \pm 13.5$ and $17.7 \pm 20.5\%$, respectively, and that of 15% porosity HAC spacers was $9.1 \pm 8.0$ and $20.8 \pm 27.1\%$, respectively. Although very porous HA tended to be associated with a greater direct bonding ratio, there was no statistically significant difference among the groups.

Micro–Computed Tomography Analysis

Micro–computed tomography scans of 15% HAC porosity spacers at postoperative 6 months are shown in Fig. 7. There was no gap between trabecular bones of the VBs and the surface of the HAC spacer.

Results

All the animals tolerated the surgery and remained alive throughout the observation periods with no evidence of severe pain or neurological impairment. One animal had a postoperative superficial wound infection that healed within 2 weeks after surgery.

Neuroimaging Analysis

The results of the neuroimaging evaluation are shown in

![Fig. 2. Scanning electron microscope images showing the differences in surface of the three types of HAC spacers. A: The 0% porosity HAC spacer. This surface is smooth and nonporous. B: The 3% porosity HAC spacer. Numerous small pores (diameter ~ 1 µm) are seen on its surface. C: The 15% porosity HAC spacer. Small (1-µm) and large (20-µm) pores can be seen. Original magnification × 3000.](image-url)
Discussion

There have been many experimental and clinical studies in which authors have shown the clinical benefits and drawbacks of using HAC constructs in spinal surgery.\textsuperscript{4,6,9,13,21} The main use of these constructs in spinal surgery has been as bone graft expanders for posterolateral spinal fusion, interspinous blocks after cervical laminoplasty, and anterior strut grafts after cervical discectomy.\textsuperscript{7,15} Very por-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Computed tomography scans demonstrating the HAC spacer protruding (A), and with suspicious fusion (B), probable fusion (C), and definite fusion (D).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Photograph showing the biomechanical testing setup used in this study. The pure detachment strength at the HAC–bone interface was measured. The VBs excluding the area of pure HAC–bone interface were removed using an automated diamond bur.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Photograph showing the surface of a VB after the detachment test. The remaining HAC was left on the VB. Because the bonding strength of HAC to the bone was stronger than the mechanical stiffness of HAC, the cracks occurred inside the HAC spacers with 3 and 15\% porosity.}
\end{figure}
ous HAC has been reported to be more beneficial compared with less porous HAC in attaining better bone ingrowth. Therefore, industrial companies have been striving to produce higher-porous HACs for posterolateral spinal fusion or interspinous blocks after cervical laminoplasty. However, given that higher-porosity HACs showed biomechanical weakness and brittleness, which often led to cracks or collapse, authors of several reports have not recommended porous HACs for anterior column support after resection of intervertebral discs. Therefore, there have been some reports in which dense HAC spacers have been used for anterior cervical fusion after discectomy. Our literature search revealed that the porosity of HAC spacers used clinically for anterior spinal fusion ranged widely (from 0 to 70%).

Several attempts have been made to compensate the biomechanical weakness of high-porous HAC spacers in clinical situations by using a titanium cage as an outside shell; the titanium cage sustains the load so that the inner HAC spacer only fuses to the adjacent VBs. Considering the biomechanical characteristics of HAC with different porosities, hybrid types of HAC vertebral spacers would be the best so that the dense HAC would be on the outside of the spacer for load bearing and porous HAC on the inside to provide direct bonding. At present, however, these composite materials of HAC are not commercially available for clinical practice.

To pursue the biomechanical stiffness of HAC, some industrial companies are producing dense HAC spacers for anterior spinal reconstruction surgery. There have been no clinical and experimental studies, however, in which researchers have determined the lowest porosity of HAC for providing direct bonding between an HAC spacer and bone when used for anterior column support in spinal surgery. In the present animal study we have shown that an HAC spacer with 0% porosity had no possibility of direct bonding to the bone at 6 months after implantation. It is still unknown whether a 0% HAC spacer could obtain direct bonding to the bone beyond 6 months postoperatively. In this study we found that the HAC spacer should be at least 3% porous for an anterior strut graft to obtain direct bonding between the spacer and bone. Although there was no statistical difference in direct bonding ratio between the spacers with 3 and 15% porosity during the histological evaluation, the biomechanical bonding strength of the latter spacer tended to be superior to that of the former. This result was compatible with those in previous studies indicating that higher porosity offered greater possibility for bone ingrowth into the surface of the HAC spacer.

Taking into account the fact that compressive strength of

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**TABLE 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0% Porosity</th>
<th>3% Porosity</th>
<th>15% Porosity</th>
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<td></td>
<td>4 Mos</td>
<td>6 Mos</td>
<td>4 Mos</td>
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<tr>
<td>fusion (no. of levels)*</td>
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<td>suspicious</td>
<td>5</td>
<td>5</td>
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<td>fusion rate (%)</td>
<td>0/6 (0)</td>
<td>2/8 (25)</td>
<td>0/6 (0)</td>
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* Absolute or probable fusion was defined as CT-documented fusion at the HAC–bone interface.

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**FIG. 6.** Photomicrographs. **Left:** The interface between a 0% porosity HAC spacer (H) and bone (B). A soft-tissue or cartilage (C) layer is seen at the interface between the 0% porosity HAC spacer and the bone. There was no evidence of direct bonding of 0% porosity HAC to the bone. **Right:** The interface between a 15% porosity HAC spacer and bone. Direct bonding is evident without any soft-tissue layer between the HAC and the bone. Original magnification × 100.
the 15% porosity HAC spacer is equal to human cortical bone of the femur, from a biomechanical standpoint porosity less than 15% can be used as an alternative graft material for anterior spinal reconstruction surgery. The biomechanical strength of HAC decreases as its porosity increases. When HAC spacers with high porosities are used alone for anterior strut grafts, the grafts may be associated with higher rates of collapse because of their biomechanical weakness. Authors of some studies have recommended the combined use of metal instrumentation with HAC spacers to afford more biomechanical stiffness to the surgical site for better fusion.\textsuperscript{18,23} The ideal porosity and biomechanical strength of an HAC spacer used as an anterior strut graft need to be identified in future studies.

Another factor to obtain better direct bonding between the HAC spacer and the bone was the spacer’s surface characteristics. In the present study we used smooth-surfaced HAC spacers; thus there were some animals whose 0 or 3% HAC spacer was protruding at the final follow up. The lower rate of protrusion in animals with 15% HAC spacers may be because of their rougher surface. Optimal surface design is another important factor for preventing HAC spacers from protruding and obtaining better contact between the HAC spacer and bone. Expanding on the current manufacturing process of HAC spacers may help to advance the surface design of HAC spacer in the future.

Conclusions

Dense HAC anterior vertebral spacers did not achieve direct bonding to the bone at 6 months after surgery in a sheep model. The HAC vertebral spacers with 3 or 15% porosity showed the capability of direct bonding to the bone at 6 months postoperatively. Although there was no statistical significance, there was a tendency for the 15% porosity HAC spacer to achieve greater bonding with the bone than the 3% porosity spacer.

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