Effects of autogenous bone graft impaction and tri-calcium phosphate on anterior interbody fusion in the porcine lumbar spine

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Background **Impaction grafting can be achieved inside the spinal fusion cages, but its effect on bone graft incorporation and spinal fusion has not been studied.**

Animals and methods **We investigated the effect of impaction grafting on the bone graft healing and fusion potential of ß-tricalcium phosphate (ß-TCP) inside the carbon fiber reinforced spinal fusion device (Brantigan cage) in 10 Danish landrace pigs. Lumbar spine interbody fusion of L2/3, L4/5 and L6/7 using carbon fiber cages was performed on each pig. Cages filled with either loosely-packed autologous iliac bone graft, rod-impacted autologous bone graft or ß-TCP were randomly distributed to the three fusion levels. Half of the animals were followed for 8 weeks, and the other half for 16 weeks.**

Results **Radiographs and CT evaluations showed that autograft levels had significantly better results than ß-TCP levels ($p < 0.001$ Fisher's Exact Test). However, the difference between impacted and loosely-packed levels was not significant. Histomorphometric analysis showed no difference between the loosely-packed and impacted cages with regard to bone volume, bone marrow volume, cartilage and fibrous tissue volume, while both of the autograft levels differed from the ß-TCP levels in all of the aforementioned parameters. Fluorochrome studies demonstrated that bone mineral apposition rate was significantly higher in the impacted cages than in the loosely-packed cages at 16 weeks.**

Interpretation **Manual impaction of autologous bone graft into the carbon fiber cages resulted in a faster mineral apposition rate by 16 weeks. Bone ingrowth and spinal fusion were not influenced by impaction grafting. ß-TCP alone could not give fusion after 16 weeks in this model.**

Cage technology has become increasingly popular for spinal fusion. Regardless of their various designs, spinal interbody fusion cages are often filled with a certain amount of autogenous or allogenous bone graft. Bone graft impaction can be achieved inside the cage space using proper packing instruments. Theoretically, for an open box-shaped cage, impaction grafting may result in a load-sharing shift from the cage toward the graft and hence reduce stress-shielding of the graft. Mechanical stresses experienced by the graft material itself have been reported to affect fusion success rate (Evans 1985). However, the problems of delayed and/or incomplete bone incorporation may arise. Incompletely incorporated or entirely necrotic bone graft has been found to remain after 18–20 months in 4 intracorporal impaction grafted lumbar fractures (Tägil et al. 1999). A bone chamber study has shown that impaction of cancellous allograft bone can reduce the ingrowth of new bone after 6 weeks, but this reduction was not found when the period for ingrowth was extended to 12 weeks (Tägil and Aspenberg 1998).

We investigated the effect of impaction grafting on the graft incorporation and the fusion potential inside a carbon fiber interbody cage in a pig lumbar spine fusion model. In addition, the effect of ß-

tricalcium phosphate (ß-TCP) was investigated within the same context.

Animals and methods

Study design

10 female Danish landrace pigs, each 3 months old and weighing around 50 kg, were chosen as experimental animals. Lumbar spine interbody fusions of L2/3, L4/5 and L6/7 using carbon fiber reinforced spine fusion cages of 8 mm posterior height, 19 mm width and 15 mm depth (Brantigan I/F cage, AcroMed, Cleveland, OH, USA) were performed on each pig. Cages filled either with loosely-packed autologous iliac bone graft, rod-impacted autologous bone graft or ß-TCP (Vitoss, ß-tricalcium phosphate) were randomly distributed to the 3 levels. The pigs were housed separately and fed a standardized diet postoperatively. Half of the animals were observed for 8 weeks, and the other half for 16 weeks. The investigations complied with the Danish Law on Animal Experimentation and were approved by the Danish Ministry of Justice.

Degree of impaction

10 cages packed with pig cancellous bone graft were first subjected to micro-CT scanning (µCT40, Scanco Medical AG, Zürich, Switzerland) to determine the fractional change in bone volume after impaction grafting. The cancellous bone was collected from the iliac crest of an experimental 3-month-old pig. The bone graft was morselized to a particle size of about 10 mm^3 with scissors. 5 of the cages were loosely-packed with an amount that could be described as "just enough", while the other 5 were packed manually to maximum capacity using a special tamping rod. The amount of bone graft put into each cage was also weighed. Bone volume fractions (bone volume/total volume) inside the cages of the 2 packing methods were compared.

Surgery

Anesthesia and operation techniques have been reported in our preceding study (Li et al. 2002). Briefly, general anesthesia was maintained by inhalation of 1.5% isoflurane. Under sterile conditions, autologous bone graft was taken from the left iliac crest and morselized. For the impaction level, bone graft was tamped into the cage as much as possible by use of a rod. For the loosely-packed level, the bone graft was finger-packed to the degree of being "just enough". The amount of bone graft put into each cage was weighed and recorded. The pig was then placed in supine position. The anterior lumbar spine was exposed retroperitoneally by a paramedian abdominal incision. Before inserting the cage, the disc tissue was carefully removed by osteotomy together with the endplates and epiphysis to avoid rapid vertebral growth. Each cage was secured with two staples (22 × 16 mm, Howmedica GmbH, Schönklrchen, Germany) anteriorly. Prophylactic ampicillin was given immediately after surgery, and thereafter for 3 days. Postoperative analgesic buprenorphine (0.02 mg/kg) was routinely used for 3–5 days.

Follow-up

5 pigs were killed after 8 weeks, and the remaining 5 after 16 weeks. To provide fluorescent marking of bone mineralization, calcein (10 mg/kg) and tetracycline (25 mg/kg) were given at 3 and 12 days respectively before killing by intravenous infusion. Each pig was killed by an intravenous overdose injection of pentobarbital (0.4 mg/kg) under general anesthesia. The spinal column from L_1 to L_7 was removed en bloc, stripped of soft tissue, and kept frozen until examinations were done.

Radiography and CT

Plain radiographs of anterior-posterior and lateral views were taken. CT scanning of both sagittal and cross-sectional slices of 2 mm thickness was performed on each lumbar spine specimen. 2 independent observers evaluated the CT images. Fusion was defined as a continuous bony bridge across a cage with no obvious interruption, as indicated by a radiolucent line in even 1 of the serial sagittal images. Partial fusion distinguished the condition in which bone ingrowth was interrupted by either a radiolucent line in the middle or on the interface of a cage. Little or no bone ingrowth into the cage was graded as non-fusion.

Histomorphometry

The cages were harvested together with neighboring vertebral bone. Each was then sawed into left

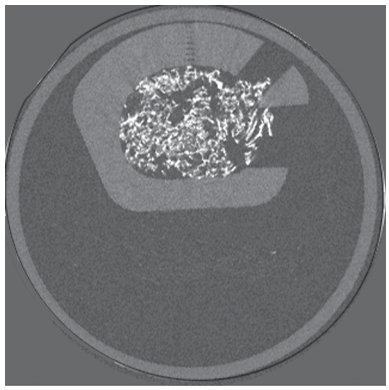
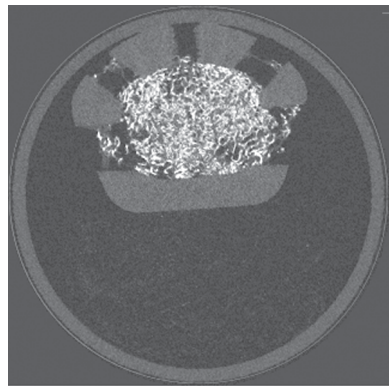


Figure 1. A. Loosely-packed cage.



B. Impacted cage. Impaction of bone graft inside the cages increased the amount of graft by 51% in weight and by 100% in bone volume.

and right halves. One half was randomly chosen for histomorphometric processing and the other half was used for fluorochrome studies or alternative staining methods. Specimens for histomorphometric studies were dehydrated in graded ethanol (70–99%) containing 0.4% basic fuchsin, and embedded in methylmetacrylate. The surface was counterstained with 2% light green for 2 min before mounting the cover slip. Sections were cut with 500 µm in-between to obtain the maximal range of sampling. Each section was cut to a thickness of 50 µm using the sawing microtome KDG 95 (Meprotech, Heerhugowaard, the Netherlands). 4 sections were produced for histomorphometry, 1 for fluorochrome studies and 1 for optional Goldner's trichrome staining. Quantitative evaluations of slides were performed using the point counting technique (CAST-Grid Software, Olympus Denmark A/S, Glostrup, Denmark). Bone volume, bone marrow space, cartilage, remaining graft material and fibrous tissue were each calculated as percentage of the total volume. Distance between the double labels was also measured with the same software under ultraviolet light to calculate the mineral apposition rate (MAR). MAR measurements were corrected for obliquity of sections by multiplying by $\pi/4$, and were expressed in µm/day.

Statistics

Data were analyzed by means of SPSS and presented as mean (SD). A normality test (Q-Q plot) for approximation to normal distribution was used. Radiographs and CT results were analyzed using cross-tabulation (Fisher's Exact Test). Two-way ANOVA was used to analyze histomorphometric results with respect to the 3 treatment levels and different periods of time. MAR results were compared by either paired or non-paired t-test. $P < 0.05$ (2-tailed) was considered significant.

Results

Impaction efficacy

Micro-CT image analysis of loosely-packed and impacted cages showed that the bone volume/total volume (BV/TV) ratio rose from 24% (SD 0.6%) to 47% (SD 2.1%). The graft weight rose from 0.74 g (SD 0.04) to 1.23 g (SD 0.02) (Figure 1).

Surgery

All 10 pigs survived the surgery and underwent follow-up. No intraoperative or postoperative complications were observed. The average amount of bone graft placed into the loosely-packed cages was 0.83 g (SD 0.04), and into the impacted cages, 1.25 g (SD 0.04). The average amount of ß-TCP placed into the cages was 0.58 g (SD 0.04). The weight of the pigs increased by 15% after 8 weeks and by 22% after 16 weeks.

Radiography and CT

Radiographs of the specimens revealed neither obvious kyphosis nor any lateral or anterior implant migration (Figure 2). Both radiograph and CT results showed a significant difference between

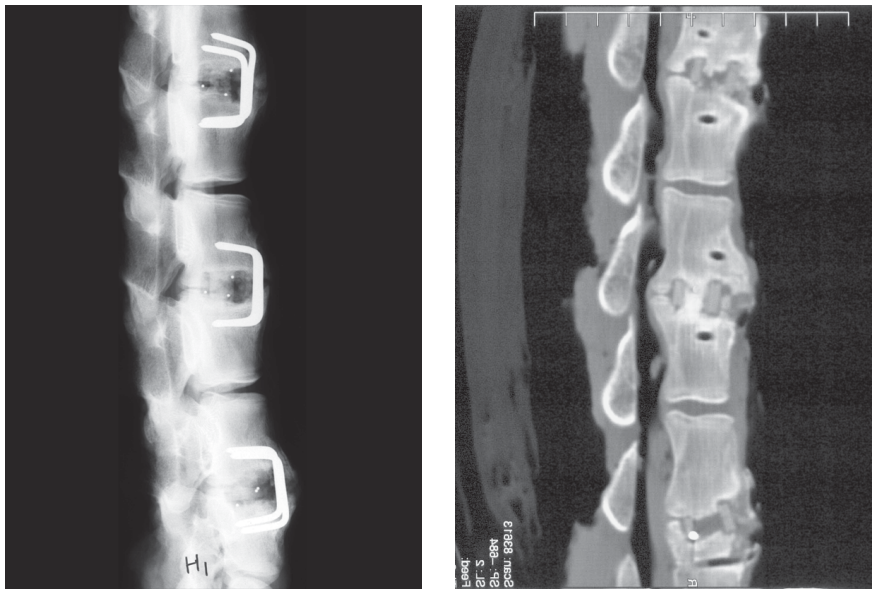


Figure 2. Lateral radiograph (left) and sagittal CT image (right) from pig H1 at 16 weeks. Radiograph shows our experimental setup. The evaluation criteria can be demonstrated from these two images. Partial fusion = bone ingrowth with radiolucent line (upper level, L2/3); fusion = continuous bone bridge (middle level, L4/5); nonfusion = nearly no bone ingrowth (lower level, L5/6).

Table 1. Fusion status in loosely-packed, impacted and ß-TCP levels by radiography and CT at 8 and 16 weeks

Fusion status	Loosely-packed levels [a]		Impacted levels [a]		ß-TCP levels [a]	
	Radiography 8 (16)	CT [b] 8 (16)	Radiography 8 (16)	CT [b] 8 (16)	Radiography 8 (16)	CT [b] 8 (16) weeks
Fusion	1 (3)	2 (2)	0 (4)	1 (4)	0 (0)	0 (0)
Partial fusion	3 (2)	3 (3)	5 (1)	4 (1)	2 (3)	0 (2)
Nonfusion	1 (0)	0 (0)	0 (0)	0 (0)	3 (2)	5 (3)

[a] Both of the autograft levels differed from the ß-TCP level by CT evaluation ($p = 0.001$; Fisher's Exact Test)
[b] CT evaluation showed significant differences between the three levels ($p = 0.001$ at 8 weeks, $p = 0.05$ at 16 weeks; Fisher's Exact Test).

levels of autograft and ß-TCP ($p < 0.001$), while impaction and loosely impacted grafts showed no significant difference (Table 1).

Histomorphometry

The staining methods produced good contrasts of bone and soft tissue with the implant in situ (Figure 3a,b). Bone graft in both loosely-packed and impacted cages showed good incorporation as early as 8 weeks. The TCP-filled cages were still largely occupied by fibrous tissue by 16 weeks. Implant subsidence was observed in 1 specimen of the loosely-packed cage group. Its insufficiently removed epiphyseal plate can be seen to curve a full 90 degrees into the cage (Figure 3b). Fluorochrome labeling appeared throughout the cage and defined areas of new bone formation. The 2 separate labels could be observed even in stained sections under fluorescent light (Figure 3d). Due to some diffusely labeled areas and the irregularity of bone formation, mineralized surface and total bone surface (MS/TS) were technically difficult to differentiate quantitatively. Well-separated double labels appeared in reason-

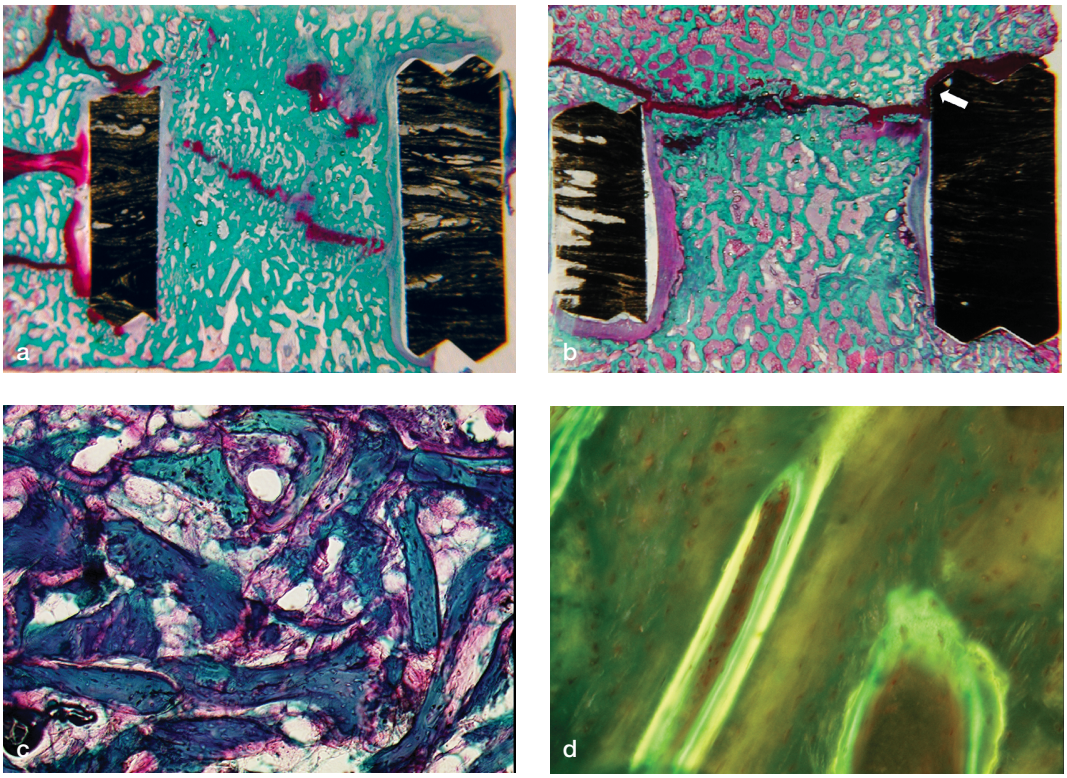


Figure 3.
a. Histological section of an impacted cage (pig number 2) at 16 weeks, with good bone ingrowth. Fibro-cartilaginous line remains in the middle. Staining method: basic fuchsin and light green.
b. A section from a loosely-packed cage (pig number 10) at 8 weeks. Bone ingrowth travels through the cage, but the epiphyseal plate failed to be removed. Note the epiphyseal plate (white arrow) curved at right-angle into the cage, caused by implant subsidence.
c. Unincorporated bone graft surrounded by fibrous stroma at 8 weeks (pig number 9). (Magnification: ×20).
d. Double labels of tetracycline (yellow) and calcein (green) can be visualized under UV light. (Magnification: ×100).

able quantity for the autograft cages, but not in the TCP-filled cages. Mineral apposition rate in the impaction cages was significantly higher than that in loosely-packed cages at 16 weeks; the value was also higher at 8 weeks than at 16 weeks (Table 2). Small islands of graft bone surrounded by viable bone could be seen in some specimens, but these were less than 1% of the total volume. In 1 specimen, pig number 9, a large area of graft bone (10%, V/V) was found in the impacted level (L6/7) at 8 weeks (Figure 3c). However, the histomorphometric results showed no statistical difference between the loosely-packed and impacted cages with regard to total bone volume, bone marrow volume, cartilage and fibrous tissue volume at either 8 weeks or 16 weeks, while both of the autologous graft levels differed from the ß-TCP level in all of the above parameters (Table 3).

Table 2. Mineral apposition rates (µm/day) in the autograft packed cages. Values are mean (SD)

	8 weeks	16 weeks	P-value [b]	95% CI
Nonimpaction	2.51 (0.33)	2.20 (0.21)	0.1	–0.11–0.74
Impaction	2.98 (0.13)	2.40 (0.25)	0.004	0.26–0.91
P-value [a]	0.07	0.04		
95% CI	–0.08–1.03	0.01– 0.40		

[a] paired t-test.
[b] independent sample t-test.

Table 3. Histomorphometric evaluation of the different grafts inside the cages (expressed in percentage of the total volume). Values are mean (SD)

	ß-TCP level	Loosely-packed level	P-value [a]	Impacted level	P-value [b]	95% CI [c]
Lamella bone	4.1 (4.0)	9.7 (5.1)	0.03	11 (4.3)	0.01	-4.1–5.8
Woven bone	13 (10.9)	28 (11)	0.006	31 (13)	0.001	-7.9–14
Bone marrow	8.7 (7.5)	24 (11)	0.006	24 (12)	0.008	-12–11
Cartilage	1.6 (1.8)	11 (7.3)	0.007	9.2 (6.8)	0.031	-8.6–5.1
Fibrous tissue	59 (19)	27 (22)	0.002	24 (18)	0.001	-23–17

[a] two-way ANOVA. Post hoc multiple comparison between ß-TCP and loosely-packed levels.
[b] two-way ANOVA. Post hoc multiple comparison between ß-TCP and impacted levels.
[c] 95% confidence intervals of the difference between the loosely-packed and impacted levels. None of these are significant.

ß-TCP particles were only slightly visible at both 8 weeks and 16 weeks.

Discussion

Depending on the cage type used in spinal fusion, the bone graft housed inside the cage normally bears very little or even no weight. For vascular infiltration of the graft and the formation of new bone, the cages should provide the most spacious interface possible between the grafts and the fusion beds. For this reason, cages are usually fenestrated. Even so, the cage is a relatively closed environment with limited openings. It has, for example, been reported that the BAK cage allows a maximum graft-bed interface of only 10% of the surface area of the endplate (Weiner and Fraser 1998). It is to be expected that packing more bone graft into the cage means more osteogenic cells and growth factors. We know from experiments with allograft, however, that impaction grafting can reduce bone ingrowth (Tägil and Aspenberg 1998), which in turn has implications for the success of the spinal fusion. We found no difference between the impacted and loosely-packed cages in relation to new bone volume, bone marrow volume, cartilage tissue and fibrous tissue volume. However, bone mineral apposition rate (MAR) was higher in the impacted cages than it was in the loosely-packed cages at both 16 weeks and, to a lesser degree, at 8 weeks. The reason for this is probably related to different loading effects on the graft. Load has been shown to increase the active incorporation area of impacted allograft in a goat model (van der Donk et al. 2002). MAR increase was also noted in the tibiae of mice, where increased load was induced by performance of jumping exercises (Kodama et al. 2000). However, how the signals from load variation are transduced to the osteoblast or osteoclast to accelerate bone apposition is not yet clear.

Due to the difficulty in using a standardized loading force under sterile conditions in the operation room, we chose to weigh the grafts first, thereby ensuring packing of the same amount of bone into both impaction or loosely-packed cages. Standardizing graft weight thus controlled the initial bone impaction density. The impacted cages developed a higher percentage of new bone formation, but there was no statistically significant difference between the loosely-packed and impacted levels in any of the four parameters as evaluated from histomorphometry. This means that given this combination of factors, putting more than 50% extra bone graft into the impacted cage does not change the static histological results. However, the dynamic data retrieved from fluorochrome labeling indicated higher mineral apposition rate in impacted cages. When bone graft is impacted in carbon reinforced cage, which has a modulus of elasticity close to that of human cortical bone, the graft shares a greater load than it would in a loosely-packed cage—and is therefore remodeled at a quicker pace (Tägil 2000). Furthermore, the Brantigan cages we used have a relatively large contact area with the host bone. The fibrous tissue and vessels could therefore penetrate the graft more easily, even though we had packed them with high manual pressure.

Bone graft behavior in a cage is complex and can be influenced by the degree or density of bone graft impaction. Factors such as donor bone quality, local environment, systemic conditions and individual patient variations can all affect graft incorporation. In addition to its natural porosity and open architecture which facilitates penetration by ingrowth vessels, autologous cancellous bone graft has great osteogenic potential because of its large number of surviving bone marrow cells. Osteoid can be deposited directly onto the surface of trabecular graft in the presence or absence of preceding resorption. In the present study, residual bone graft could not be seen after 16 weeks, but at 8 weeks, small amounts of graft were observed in both the loosely-packed and impacted cages. In one impacted cage at 8 weeks, a large amount of graft was observed without any sign of new bone apposition, even though the graft was surrounded by vascular fibrotic stroma. Similar developments have been described in the literature, where autograft was impacted from the pedicle to the vertebra in order to treat a lumbar fracture, after which necrotic graft appeared 18–20 months later (Tägil et al. 1999). The reasons behind the presence of large amounts of graft bone in the present study are not clear, since the phenomenon was not found in other cages at either 8 or 16 weeks. Prediction of whether or not these grafts would ultimately be incorporated was not feasible. Togawa et al. (2001) investigated bone graft incorporation in human intervertebral fusion cages by radiologically aided needle biopsy. Necrotic bone grafts were seen in association with viable bone after 8–72 months. It would be interesting to see whether the presence of unincorporated graft can alter the biomechanical behavior of the fusion segment, but unfortunately, in the present study, we did not perform mechanical testing due to the initial aim of preserving the bone-cage interface.

ß-TCP has been used clinically as a bone graft extender in spinal fusions, and satisfactory results have been achieved (Muschik et al. 2001, Linovitz and Peppers 2002, Meadows 2002). Applying only ß-TCP in spinal fusion cages, a study using sheep resulted in good osteointegration as early as 8 weeks, but fibrocartilage lines could be seen to extend across the cages even after 32 weeks (Steffen et al. 2001). In the present study, ß-TCP granules were completely absorbed after 16 weeks with variable bone ingrowth. Most of the cages filled with ß-TCP particles were largely occupied by fibrous tissue. Suboptimal packing of the cages with ß-TCP, which was mentioned by the authors in the above study on sheep, was also a factor in our study. The small particle size can also lead to rapid absorption before bone formation occurs.

In the pig model we used, the true intervertebral disc space was only about 4–5 mm. We removed the end-plates together with the epiphyseal plates in order to avoid rapid vertebral growth and to create the 'critical distance' for preclusion of spontaneous fusion that may occur when an empty cage and fixation are employed. In one of our previous studies in which empty cages and staples were used in the same model, none of the 10 pigs achieved fusion with the empty cage after 12 weeks (Xue et al. 2003). On the other hand, implant subsidence could be expected to occur without the preservation of end-plates. It is not surprising that subsidence occurred in a loosely-packed cage, but based on the present study design, we were not able to confirm that impacted cages reduce subsidence.

In summary, manual impaction of autologous bone graft within Brantigan cages did not produce superior histological results, nor did it affect the bone ingrowth and final fusion in this pig model. However, impaction of autologous bone graft increased bone remodelling, which may be explained by increased load sharing. β-TCP alone could not produce fusion after 16 weeks in this model.

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No competing interests declared.

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