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Comparing Autograft, Allograft, and Tricalcium Phosphate Ceramic in a Goat Instrumented Posterolateral Fusion Model

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The most common application of bone grafts is spinal fusion surgery, in which the use of iliac crest autograft is the gold standard. Harvesting of autograft, however, requires an extra surgical procedure, which is associated with additional morbidity. Allograft is the well-known alternative, but it is generally considered less effective in posterior fusions. Therefore, the need for an effective alternative remains. Recently, it was shown that ceramics can be endowed with biologically instructive properties by changing the basic parameters of the material. In this study, we compared a novel tricalcium phosphate ceramic (TCP) to iliac crest autograft and allograft, in instrumented posterolateral fusions in a goat model.

A total of nine goats were included, who underwent a two-level lumbar fusion. Each side of the spine was randomized into one type of graft: iliac crest autograft; fresh-frozen allograft; TCP alone; or TCP combined with local autograft (50:50). The fusion rates after 16 weeks were comparable between the groups (autograft 3/8, allograft 4/8, TCP 4/8, and TCP/local autograft 5/8). Calculation of the fusion volume on computed tomography images, showed significantly greater volume in the control groups (autograft 7.8 mL and allograft 8.9 mL) compared with the groups with TCP (TCP 6.1 mL and TCP/local autograft 6.0 mL). No adverse tissue response was seen on histological analysis and TCP was almost completely resorbed. The results demonstrate that TCP is capable of achieving fusion at a similar rate to iliac crest autograft in posterolateral fusions, while almost completely resorbing within 16 weeks. Despite the lower fusion volume, the TCP is a promising alternative circumventing the disadvantages of autograft and allograft.

Introduction

Each year over 2.2 million bone grafting procedures are performed worldwide, making bone the second most common transplantation tissue, with blood being most common.[1,2] The most common application of bone grafts is spinal fusion surgery, especially posterolateral fusions. The current gold standard is to use autologous bone harvested from the iliac crest to create a bony bridge between the vertebrae of the affected segments. This iliac crest autograft provides all three mechanisms associated with bone regeneration: osteoconduction, osteoinduction, and osteogenic cells.[3] Harvesting of bone graft from the iliac crest, however, requires an extra surgical procedure in healthy bone, which is associated with additional morbidity, including donor site pain, infections, and neurovascular damage.[4–7] To circumvent the disadvantages of iliac crest autograft the quest for an alternative bone graft substitute remains. An ideal bone graft should exhibit osteoconductive, osteogenic, and osteoinductive properties; be able to degrade; provide a favorable environment for invading blood vessels and bone forming cells; and should be biomechanical stabile. This does not imply, however, that all characteristics are mandatory in each indication for a bone graft substitute to be effective.

Allograft from bone banks is a frequently chosen alternative,[8] which eliminates donor site morbidity and is available in large quantities. Allografts are osteoconductive, but they are considered to have weak osteoinductive capabilities and contain no viable cells for osteogenesis.[1] Despite extensive use, allograft is generally considered less effective than autologous bone graft,[9] which may be a result of the absence of viable cells or the host immune response.[10–12] Finally, the

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risk of disease transmission continues to be questioned.[13–15] Therefore, the need for an effective bone graft alternative remains.

Bone tissue engineering or regenerative medicine strategies have shown to be a promising technology to develop such an alternative. The concept of these techniques is to develop biologically active implants that restore, maintain, or improve tissue function.[16] A well-known example of clinical application of this technology is the use of bone morphogenetic proteins (BMPs). For spinal fusions, BMP application has exponentially increased in the United States during the last decade.[17,18] Recently, however, serious concerns have been expressed regarding underreporting of complications and product-related adverse events,[19,20] which may be related to the high doses of recombinant proteins that are administered.

The potential of synthetic bone grafts, such as calcium phosphate ceramics, has been investigated for over four decades.[21] These materials have the advantage of unlimited supply, low costs, and ease of sterilization and storage. Of special interest are the recently introduced tricalcium phosphate (TCP) ceramics, due to their good bioabsorbability compared with hydroxyapatite and biphasic calcium phosphate, and thereby not interfering with physiological bone remodeling. Preclinical studies[22–25] and clinical studies,[26–33] have generated encouraging data when using TCP in lumbar posterolateral fusions, but almost exclusively in combination with local bone, bone marrow aspirate, and/or BMPs. Recently, it was shown that ceramics can be endowed with biologically instructive properties by changing basic physicochemical parameters of the materials, such as surface textures.[34] In this way TCP was capable of possessing an intrinsic osteoinductive capacity comparable to autograft or BMPs. In a comparative study, osteoinductivity of calcium phosphate ceramics appeared to be advantageous for bone defect healing when compared with nonosteoinductive phosphate ceramics.[35]

The purpose of this study was to determine whether this TCP, alone or in combination with local autograft, is a suitable bone graft substitute for instrumented posterolateral fusion in a large animal model compared with the currently most-used grafts, iliac crest autograft and allograft. Fusion was quantitatively and qualitatively assessed using computed tomography (CT), and histological evaluations.

Materials and Methods

Experimental design

After approval by the animal care committee, nine Dutch milk goats (60–70 kg, age 23–28 months) underwent a two-level (L2-L3 and L4-L5) pedicle screw instrumented posterolateral fusion. We choose this model based on our experience with goats[36,37] and its resemblance to the human vertebral size. Each side of the spine was randomized into one type of graft (10 mL), making a total of four grafts per goat. The groups were as defined below:

Group 1: autograft from the iliac crest;
Group 2: fresh-frozen allograft;
Group 3: TCP with local autograft;
Group 4: TCP alone.

All animals were sacrificed 16 weeks after the surgery. At necropsy, the specific spinal levels were harvested *en bloc* and cleaned from soft tissue, and the instrumentation was removed. The primary outcome was the presence/absence of fusion based on CT scans. In addition, the volume of the newly formed bone was semi-automatically calculated using custom software program. Finally, the spinal segments were processed for histological analysis.

Surgical technique

The procedures were performed under general inhalation anesthesia of an isoflurane in air gas mixture (Abbott Laboratories, AST Pharma) preceded by dexmedotomidine sedation (Pfizer). After shaving and disinfection of the dorsal thoracolumbar area, a midline incision between T12-L5 was made to expose the muscle fascia. The paraspinal muscles were subperiosteally stripped from the spinous processes and retracted laterally. The laminae, posterolateral aspect of the pars, facet joints, and transverse processes were denuded of all soft tissue and thoroughly decorticated with a rasp. The pedicle screws were inserted after probing the pedicles and interconnected with rods (BWM-system; Stryker Howmedica Osteonics). Iliac crest bone graft was obtained using the same midline incision and by dissecting a subcutaneous flap free from the underlying lumbodorsal fascia until the iliac crest. An incision was made parallel to the iliac crest and the musculature was stripped off the outer surface of the ilium so that a large enough graft could be obtained. Using a rongeur, 10 mL of tricortical bone was harvested. From the spinous processes of the level receiving TCP, 5 mL of local autograft was obtained using a rongeur. In a randomized fashion, the experimental grafts were placed at one side of the spine in the decorticated lateral gutter against the lamina and the dorsal base of transverse process. The muscle fascia, subcutaneous tissues, and skin were subsequently closed in layers. Postoperative pain relief was provided by Buprenorphin (Schering-Plough). After 16 weeks, the animals were killed using an overdose of pentobarbital (Organon).

Bone grafts

A total of 10 mL of graft was used for each experimental condition. The amount of graft was determined by filling a syringe with graft material until 10 mL of graft was obtained with careful hand-pressing. Care was taken not to crush or break the material. The iliac crest bone graft and local autograft was cleaned from soft tissue and morselized into corticocancellous granules of 1–3 mm. For the TCP/local autograft group, 5 mL of local autograft from the spinous processes was morselized to granules of 1–3 mm and uniformly mixed with 5 mL of TCP. Allograft was obtained from the femoral heads of nine Dutch milks goats from a previous experiment, which could not have affected the bone quality,[38] under sterile conditions in the operation room. On harvesting of each of the femoral heads, swabs from the surface were taken and tested for contamination. Subsequently, the femoral heads were frozen to −80°C. If the allograft specimens were clear of bacterial contamination, the allograft was thawed at room temperature in its sterile container prior to surgery. Under sterile conditions, remnants of the femoral neck were resected to leave only the sphere of the femoral head. With the use of a rongeur the allograft was morselized into corticocancellous granules (1–3 mm).

TCP granules

Calcium phosphate ceramics were produced from TCP powder (Plasma Biotal) with the H_2O_2 foaming method, as previously described.[39] In brief, the green bodies formed with diluted H_2O_2 were sintered at 1100°C for 8 h. The resulting blocks were crushed and sieved to select the specified granules (1–2 mm) (Fig. 1A). X-ray diffraction showed that the ceramic contained phase-pure β-TCP with peaks according to the Joint Committee on Powder Diffraction Standards (JCPD) (card) (Fig. 1B). The material showed an interconnected porosity of around 80%, including abundant micropores as shown with scanning electron microscopy (Fig. 1C). The interconnected porosity of the material was measured with mercury intrusion (AutoPore IV 9500; Micromeritics GmbH). The distribution of the micropores was homogeneous with a size range of 0.4–2.2 μm (average at 1.2 μm (Fig. 1D). The specific surface area was 1.3485 m^2/g measured with mercury intrusion (Micrometrics Instrument Incorporation). The ceramic particles were steam sterilized at 121°C for 30 min before use.

Computed tomography

After harvesting of the spinal segments and removal of the instrumentation, CT scans with sagittal and coronal reconstruction were used to evaluate the presence/absence of fusion. The CT imaging protocol consisted of 0.6-mm thick and 0.3-mm overlapping axial slices that were taken without bone filter. Scans were made using a Philips Tomoscan AVE (Philips CT Secura; Philips Medical Systems). The window and level settings were set to optimize trabecular bone detail. Two observers (D.D. and F.C.O) reviewed all CT scans in a blinded fashion. In case of conflicting findings consensus had to be reached. A modified classification system of Christensen *et al.*[40] was used to determine the fusion rate. Each side was judged separately and categorized in:

—"Fusion" was defined as a continuous bony bridge from the base of the pedicle or transverse process from one vertebra to the other. If the fusion was doubtful in any way, the case was not classified as "fused";

—"Doubtful fusion" indicated suboptimal quality of the bone bridging or some doubtful discontinuity;

—"Nonunion" indicated definite discontinuity of the fusion mass.

Quantification of bone volume

Original software developed at the Image Sciences Institute of the University Medical Center Utrecht was used to quantify the fusion volume. This was done by intensity-based selection of all bone on blurred CT images, and subtracting the vertebrae by manually defining the borders (Fig. 2). This was done for all axial slides of each fused level, which was limited to both transverse processes and the intertransverse space. A line in the middle of, and parallel to, the spinous process was used to divide the spine into a left and right side. The selected area of all images was summed to obtain the total fusion volume per level and side of the spine. TCP particle remnants could not be detected with the CT scan and were considered negligible. The selection of new bone was done by a single investigator (D.D.) blinded to the treatment group.

Histologic processing

After the CT scans, the spinal segments were split in the sagittal plane in the middle of, and parallel to, the spinous processes. Each side of the spine was then fixed in 4% formalin, dehydrated by ethanol series, and embedded in polymethylmethacrylate. Sections (20–30 μm thick) were sawed in the axial plane at the middle of the segments using the Leica® SP1600 Saw Microtome system (Leica). The axial sections were stained with methylene blue and basic fuchsine

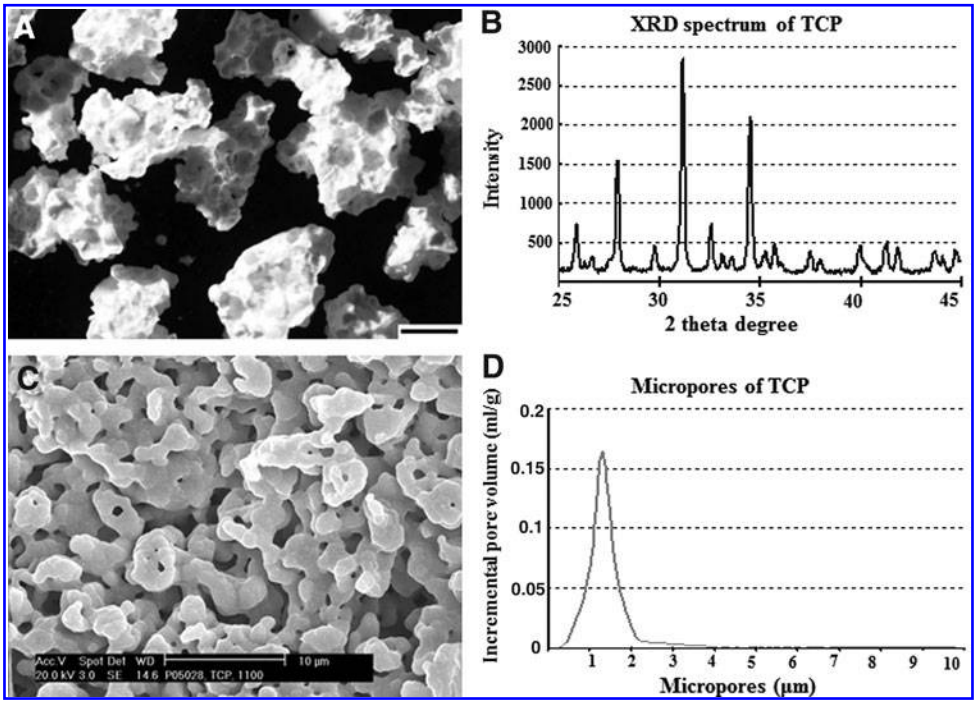

FIG. 1. Characterization of the tricalcium phosphate (TCP) ceramic. **(A)** Microscopic image of the TCP granules (1–2 mm). **(B)** X-ray diffraction (XRD) analysis showing the composition of the material. **(C)** SEM images depicting the microstructure of the TCP. **(D)** Incremental pore volume shows that the micropores were homogeneous with a size range of 0.4–2.2 μm.

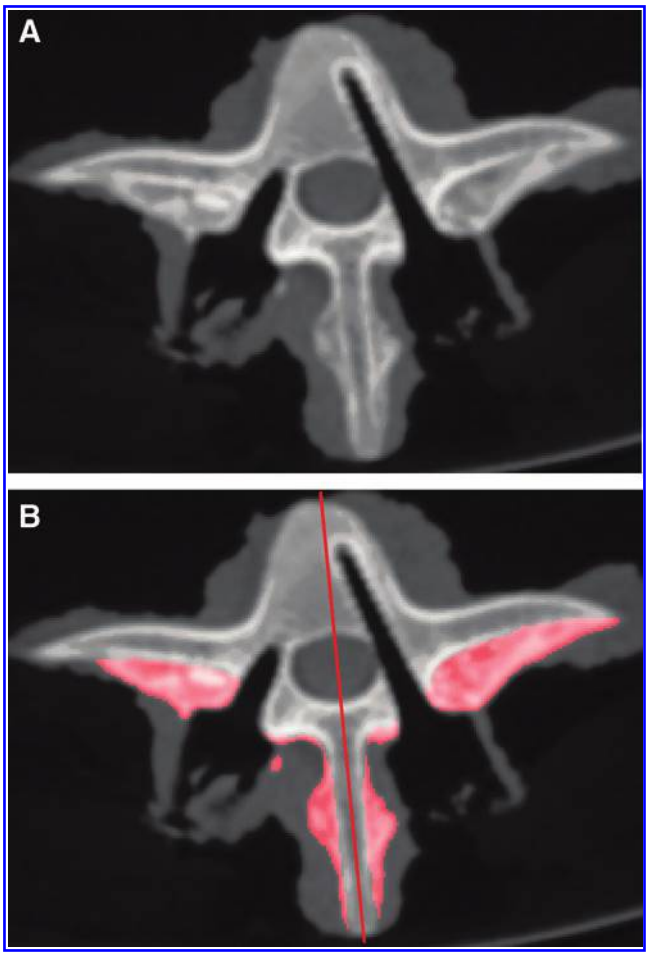

FIG. 2. Image of an axial computed tomography (CT) slide. **(A)** Blurred image on which newly formed bone is visible against the transverse processes and spinous process. The anatomic border of the vertebra can be clearly distinguished from the new bone. Note the position of the removed pedicle screws. **(B)** Semi-automatic selection of the newly formed bone (pink). Red line divides vertebrae into a left and right side. By selecting the newly formed bone on all axial slices, the volume of newly formed bone of each spinal segment was calculated. Color images available online at www.liebertpub.com/tec

and evaluated by regular light microscopy (Olympus-BX51). The purpose of the histology was to assess: (1) the overall morphology of *de novo* bone; (2) the amount of residual TCP; (3) the maturity of the bone; and (4) the presence of any other cell type in the fusion site. Additionally, histomorphometric analysis on high-resolution digital scans was performed to quantify the amount of residual TCP. This was done by pseudo-coloring the TCP remnants and the total area of newly formed bone using Abode Photoshop CS5. The area% of TCP remnants was calculated by dividing the number of pixels.

Statistical analysis

SPSS version 14.0.0 software (SPSS Incl.) was used to conduct statistical analyses. Frequency and descriptive analyses were conducted on all data sets. Data were expressed as mean ± standard deviation. Comparison of radiological fusion rates between the treatment groups was done by a two-tailed Fisher-Exact test. A two-tailed paired *t*-test was used to assess any differences between the volume measurements between the TCP groups and the control groups. The threshold for statistical significance was set at $p < 0.05$.

Table 1. Fusion Grading by CT

Group	*Fusion*	*Doubtful fusion*	*Nonunion*
Autograft	3/8 (38%)	5/8 (63%)	0/8 (0%)
Allograft	4/8 (50%)	4/8 (50%)	0/8 (0%)
TCP	4/8 (50%)	2/8 (25%)	2/8 (25%)
TCP/Local autograft	5/8 (63%)	1/8 (13%)	2/8 (25%)

No statistical differences in fusion rates between treatment groups ($p = 0.26$).
CT, computed tomography; TCP, tricalcium phosphate.

Results

All animals recovered well without neurological deficiencies from surgery and survived the follow-up period without difficulties. At necropsy, one goat showed signs of surgical infection at both levels and was excluded from further analysis. No instrumentation failure (i.e., breaking of the rod) or loosing of screws was observed.

Radiological fusion

The fusion rates were 4/8 in the TCP group and 5/8 in the TCP/local autograft group, compared with 3/8 in the autograft group and 4/8 in the fresh-frozen allograft group. No statistical differences were found between the treatment groups ($p = 0.26$). The exact classification per treatment is summarized in the Table 1. The newly formed bone was mainly located medial to the connecting rod, appositioned on the lamina (Fig. 3). Notably, in all groups the graft material positioned lateral to the rod had not resulted in new bone formation.

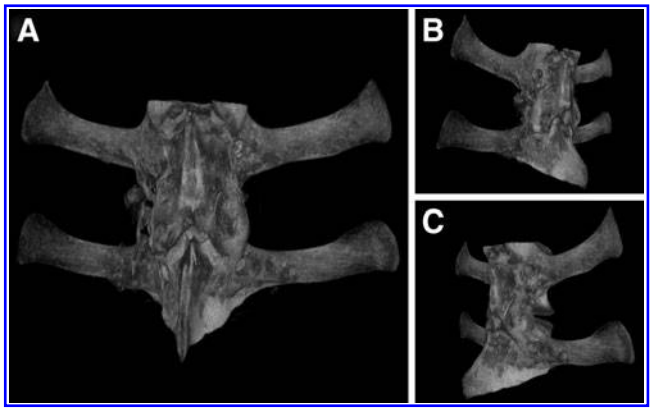

FIG. 3. 3D reconstruction of CT images of the TCP groups. **(A)** Posterior view. Left side received TCP combined with local autograft and right side received only TCP. New formed bone is present against the lamina, medial to the connecting rod (removed before scanning), and bridges the adjecent spinal levels. **(B)** Oblique view of left side (TCP/local autograft). **(C)** Oblique view of the right side (TCP alone).

Volume measurements

The fusion volume was 6.1 ± 1.2 mL in the TCP group and 6.0 ± 1.2 mL in the TCP/local autograft group, compared with 7.8 ± 1.8 mL in the autograft group and 8.9 ± 4.4 mL in the allograft group. There were statistical differences between the autograft group and TCP group ($p = 0.04$), and also between the autograft and TCP/local autograft group ($p = 0.01$). In addition, significant more volume was present in the allograft group compared with the TCP/local autograft group ($p = 0.05$). The volumes per treatment group are shown in Figure 4. The CT method was limited in that it could not differentiate between new bone formation and residual graft materials, which limited the evaluation to only an assessment of total fusion mass volume and not specifically new bone formation.

Histological evaluation

Histological analysis confirmed that most new bone was formed at the medial site of the connecting rod, against the lamina. The newly formed bone was mainly lamellar and no differences were seen between the treatment groups (Fig. 5). On the soft tissue side, a layer of fibrous tissue was observed between the fusion mass and the adjacent muscle. Tissue responses surrounding the implants were similar in all groups. The vast majority of TCP was resorbed and no differences were seen between the TCP group and TCP/local autograft group. Almost all unabsorbed TCP remnants were osseointegrated in newly formed bone islands and only few small fragments were seen within the fibrous tissues. Osteoclasts were surrounding the embedded TCP remnants indicating that these were subject to bone remodeling (Fig. 6). Histomorphometric analysis showed that the TCP remnants constituted 1.40% ± 1.01% of the total area of newly formed bone for both TCP groups, which was comparable between the TCP alone (1.56% ± 1.21%) and TCP/local autograft group (1.30% ± 1.03%).

Discussion

Posterolateral fusion is generally believed to be one of the most challenging indications for bone grafts due to the large distance that needs to be bridged, limited contact surface, and the unfavorable biomechanical environment due to the lack of compression forces. The primary outcome in terms of efficacy in this study was based on the ability of achieving bony fusion on CT-scans. This study clearly shows that TCP,

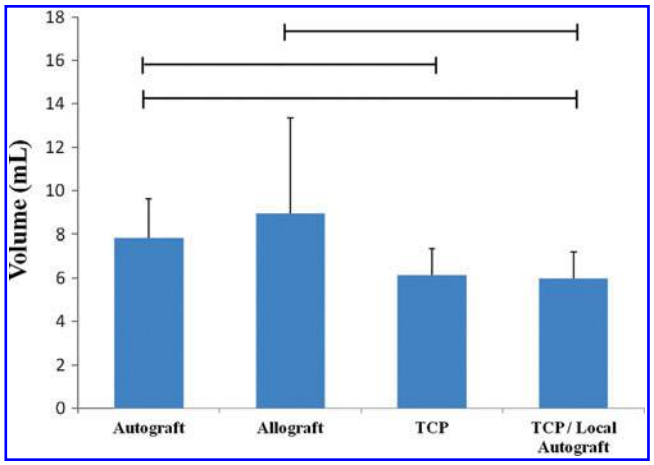

FIG. 4. Volume of the fusion mass from CT. Error bars represent standard deviation. Horizontal bars represent significant differences between treatment groups ($p < 0.05$). Color images available online at www.liebertpub.com/tec

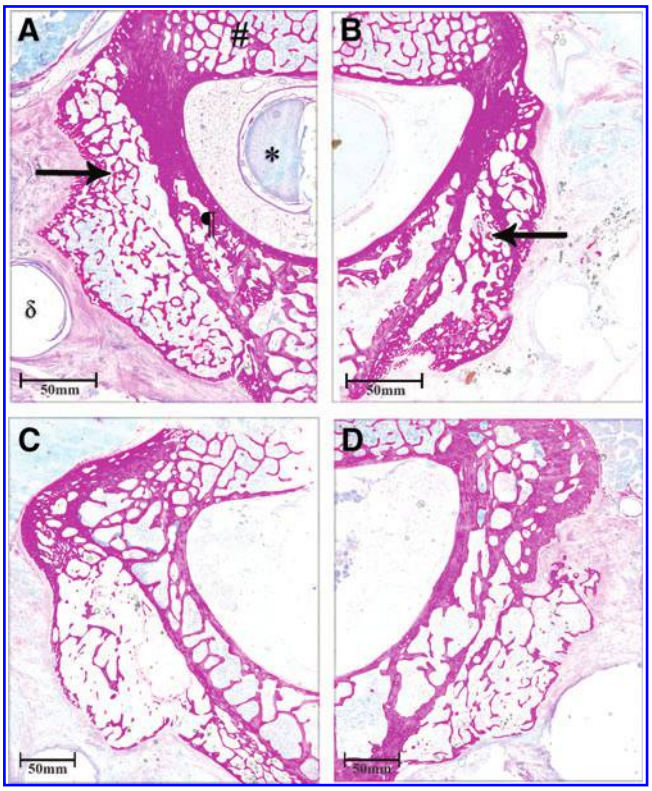

FIG. 5. Overview of histological axial sections in the middle of both the spinal segments in one goat. The upper slides **(A/B)** represent level L2-3, whereas the lower slides **(C/D)** represent level L4-5. Each side of the spine received one type of graft: **(A)** TCP alone **(B)** TCP/local autograft **(C)** Iliac crest autograft **(D)** Fresh-frozen allograft. Please note that the bone is mainly formed against the lamina and is similarly located in all groups. Minimal TCP remnants are present (arrow) *indicates the spinal canal. #represents posterior part of vertebral corpus. ¶represent the lamina. δ is located in the (removed) connecting rod. Color images available online at www.liebertpub.com/tec

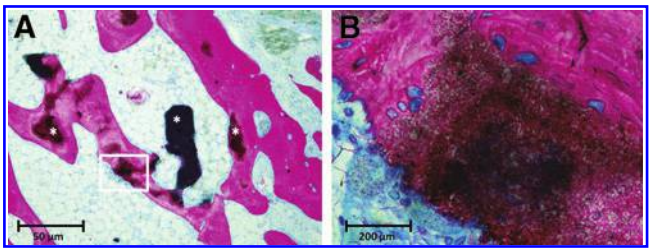

FIG. 6. Histological view of TCP. **(A)** TCP remnants (indicated by *) are incorporated in bone without any foreign body reaction. The box represents the area of Figure B. **(B)** Detailed view of TCP remnant incorporated in bone and surrounded by osteoclastic cells. Color images available online at www.liebertpub.com/tec

alone or in combination with local autograft, was capable of achieving fusion similar to allograft and autograft in instrumented posterolateral fusion in a large animal model. Additionally, no adverse tissue responses were seen and almost all TCP resorbed within 4 months.

Ceramics in posterior fusion are mainly used as bone graft extenders rather than bone graft substitutes as these types of materials are generally considered to be only a template for osteoconduction. Recently, however, the possibilities to improve the bone-forming capacities of ceramics have been recognized and are increasingly investigated. A successful strategy appears to be the addition of trace elements, like silicon.[41,42] In a study that evaluated silicon substituted calcium phosphates, the investigators[43] found promising results in an instrumented sheep posterolateral fusion model. However, it is not precisely clear whether the trace element itself or the resulting microstructure is responsible for the apparent improved performance.[44,45]

The design of the material that was used in the present study was a first generation of ceramic materials with a modified microstructure that can render the material with bone stimulatory properties.[34]

Despite similar fusion rates between all groups, the measured fusion volume was lower in the TCP groups when compared with the iliac crest bone graft or allograft. Potentially, this difference could be due to a less effective bone forming capacity of TCP compared with the controls; however, a limitation of our CT volume measurements is the inability to differentiate between newly formed bone and remnants of graft material. Histological analyses showed that almost all TCP resorbed, but the amount of unabsorbed allograft/autograft remains unclear and may cause overestimation of the newly formed bone volume. Another aspect that should be taken into consideration is that the differences could be based on slower bone formation in the TCP group, which may lead to similar volumes at longer follow-up. In any case, these findings strongly support further evaluation of the current TCP graft.

Fresh-frozen allograft performance is comparable to autograft in our animal model. This is surprising, since in clinical studies the results of allograft in posterior fusions are generally poor.[9] This is especially the case for the shelf freeze-dried allograft, which was not capable of achieving any fusion in a comparative study where fresh-frozen allograft did achieve fusion, although less than iliac crest autograft.[46] Unfortunately, the efficacy of fresh-frozen allograft has never been reported in other large animal models evaluating posterolateral spinal fusion.

There are several limitations to our study. The relatively small number of animals in our study limits statistical power, especially for categorical variables such as fusion. This was partly compensated for by evaluating all conditions in the same animal, and thereby allowing paired measurements. It should also be emphasized that the aim of the study was to evaluate the potential of TCP and not to determine exact differences with autograft/allograft. Another limitation is that we did not include a group who received no graft, so the effect of decortication alone was not determined. However, this was recently investigated by our group in a separate study, where only minimal bone formation was seen and fusion in only one of four segments without any grafts.[47] Finally, the interpretation of internal control studies should be viewed with caution. The effect of grafts placed on the contralateral side of the spine is unknown. This is especially the case for the potential immunological response to allograft. The mechanical effect of fusion at the contralateral side, however, is negligible due to the use of rigid instrumentation.

Despite these limitations, the results of the present study show that the investigated TCP is a promising bone graft alternative circumventing the disadvantages of autograft and allograft. The efficacy can be further optimized by studies addressing volume, microporosity, and grain size. Finally, clinical trials need to determine its applicability as a stand-alone alternative to autograft.

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Disclosure Statement

Two of the authors (J.D.B. and H.Y.) are employees and shareholders in Progentix Orthobiology, who commercialized the TCP material. For the other authors no competing financial interests exist.

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