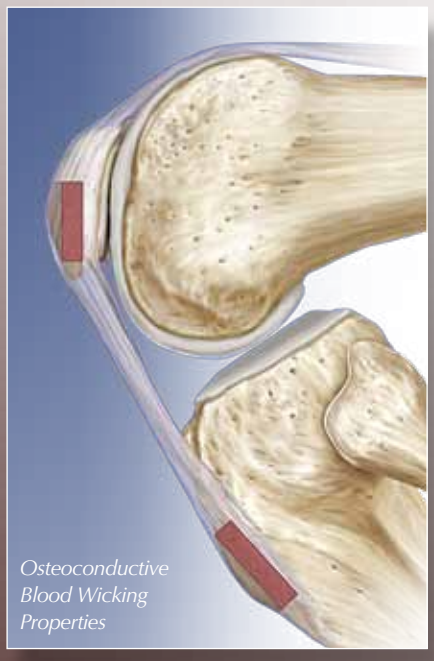
OSferion

Porous Trapezoid ß-TCP Synthetic Grafting of BTB Autograft Harvest Sites

Pretreat with autologous blood, bone marrow PRP and ACP products

OSferion Trapezoid



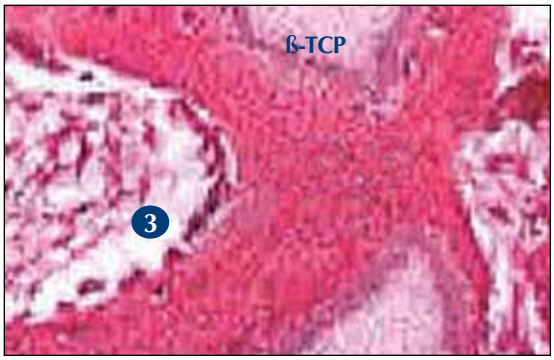
Osteoconductive Blood Wicking Properties

Arthrex®

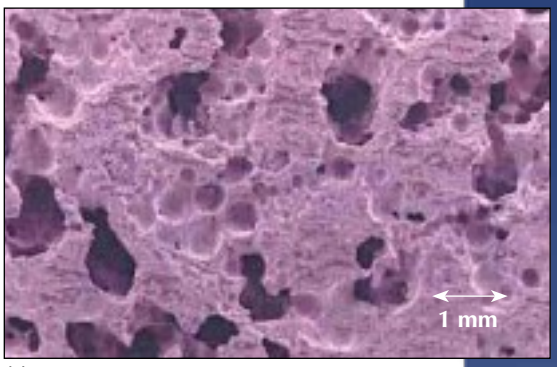
OSferion Features and Benefits:

OSferion is an osteoconductive bone graft substitute and bone void filler consisting of 100% high purity Beta-tricalcium phosphate (ß-TCP).

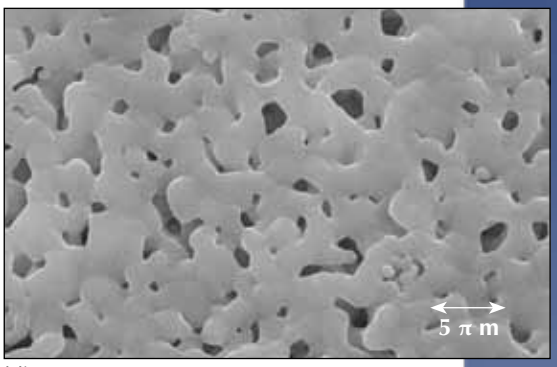
- Allows for simultaneous controlled absorption and promotion of osteogenesis
- Micro and macro porous structure allows for excellent cell communication to promote vascularization
- Optimum pore diameter facilitates maximum cell infiltration
- Revolutionary material has a compressive force of 15 - 20 MPa (2900 pounds/inch^2)



New bone containing osteocytes and surrounded by osteoblasts adjacent to the OSferion (3)



Macrostructure

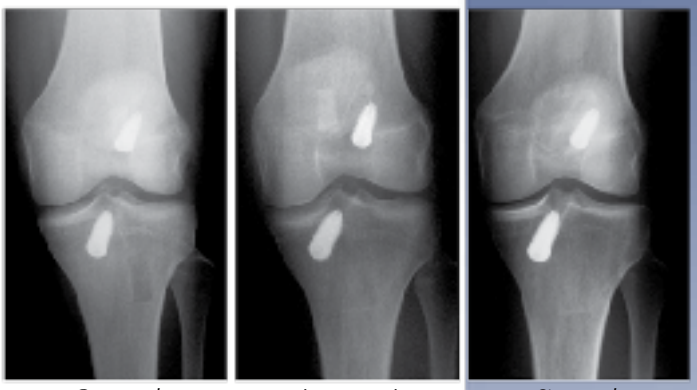


Microstructure

OSferion Trapezoids

- Intended to be used as a bone-patellar tendon-bone graft harvest site bone void filler in the patella and tibia
- OSferion naturally wicks up autologous blood, ACP, or bone marrow aspirate that may enhance healing
- Can easily be custom trimmed to size using a rongeur or oscillating saw
- Clinical studies have shown that the bone-patellar tendon-bone graft harvest site bone void filling reduces the incidence of patella fracture, anterior knee pain, and postoperative kneeling pain.

Serial postoperative x-rays



One week *Three months* *Six months*

ORDERING INFORMATION

OSferion Trapezoid, 8 mm x 25 mm x 7 mm x 75˚	AR-13372- 1
OSferion Trapezoid, 9 mm x 25 mm x 7 mm x 75˚	AR-13372- 2
OSferion Trapezoid, 10 mm x 25 mm x 7 mm x 75˚	AR-13372- 3

ACP™ Autologous Conditioned Plasma for OSferion Preparation

Autologous **C**onditioned **P**lasma (ACP) has created a growing interest for use in a number of orthopaedic therapies. The healing effects of plasma are supported by growth factors released by platelets. These growth factors may induce a healing process wherever they are applied. OSferion Trapezoids can be impregnated with ACP prior to implantation by soaking for several minutes in a sterile dish.

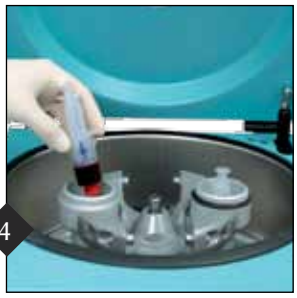
Features and Benefits:

- The ACP System is a simple, cost-effective method of concentrating growth factors for therapeutic use.
- Producing ACP with the ACP System can be performed within minutes. Typical platelet rich plasma (PRP) systems take up to 45 minutes to process the blood for application, thereby delaying treatment and increasing the cost of the procedure.
- The ACP System can be used under sterile conditions in an OR setting. The unique double syringe design allows for convenient and safe handling, as the whole preparation process takes place in a sterile, closed system.
- The cost of the ACP System is significantly less than conventional PRP devices.

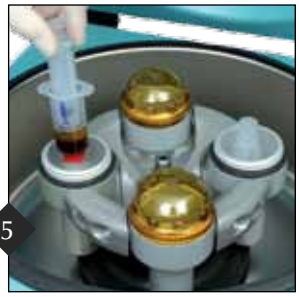
Prior to withdrawing ACD-A, prime the innermost syringe by pulling it back and pushing it forward completely before starting the process. Withdraw approximately 1 mL ACD-A into the syringe.
Caution: Draw back only the plunger of the outer syringe.

Withdraw 9 mL of venous blood and seal the syringe with the red cap.

Gently rotate the syringe in order to mix the blood and the ACD-A.

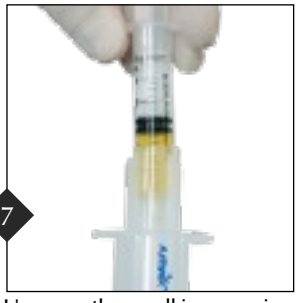


Place the syringe into one bucket and an appropriate counter balance in the opposite bucket. Two syringes may be used to increase dosage.



Run the centrifuge at 1500 rpm for 5 minutes. Remove the syringe, taking care to keep it in an upright position to avoid mixing the plasma and red blood cells.

In order to transfer 2 - 4 mL of supernatant (ACP) from the larger outer syringe into the small inner syringe, slowly push down on the outer syringe while slowly pulling up the plunger of the small inner syringe.



Unscrew the small inner syringe.

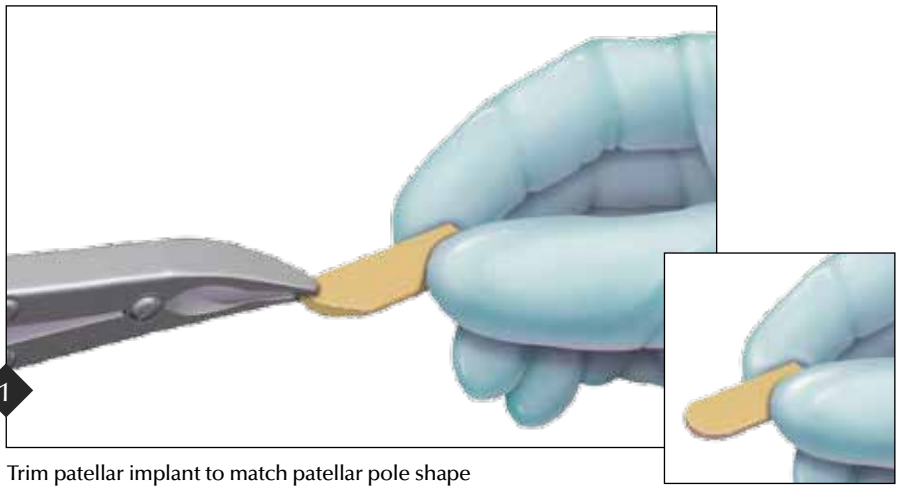
The ACP is ready for use or to pretreat OSferion bone void filler prior to implantation.

ORDERING INFORMATION

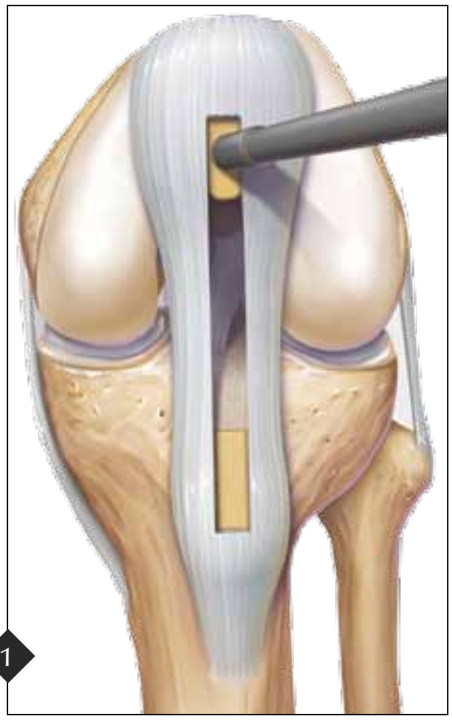
ACP/ Double Syringe w/Cap	ABS-10010
Anticoagulant ACD-A, 4 mL	ABS-10007
Anticoagulant ACD-A, 50 mL	ABS-10008
Centrifuge w/o rotor	ABS-10020
Swing-out Rotor	ABS-10021
Bucket	ABS-10022
Bucket Cap	ABS-10023

Directions for Use

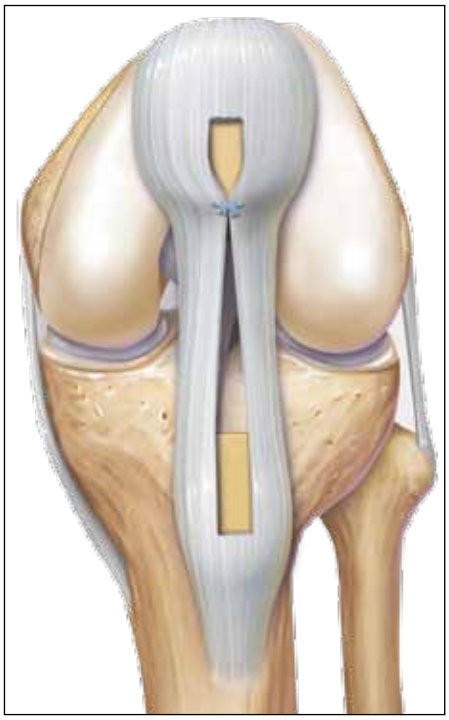
1 Soak OSferion in ACP for 3-5 minutes



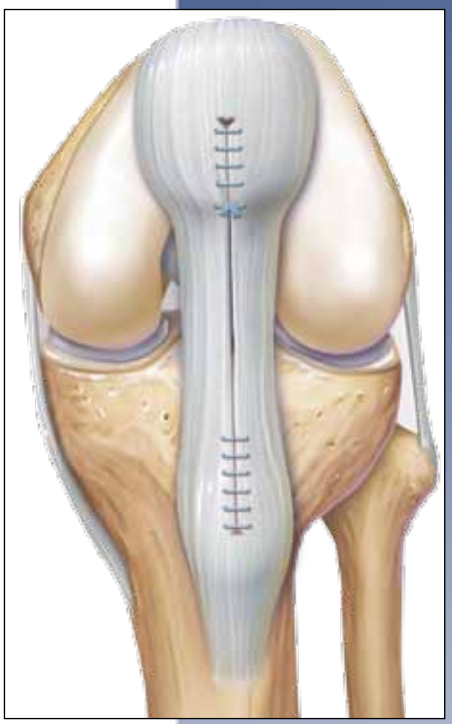
1 Trim patellar implant to match patellar pole shape



1 Gently press fit or tamp into place



Oversew patellar tendon to fixate OSferion implants



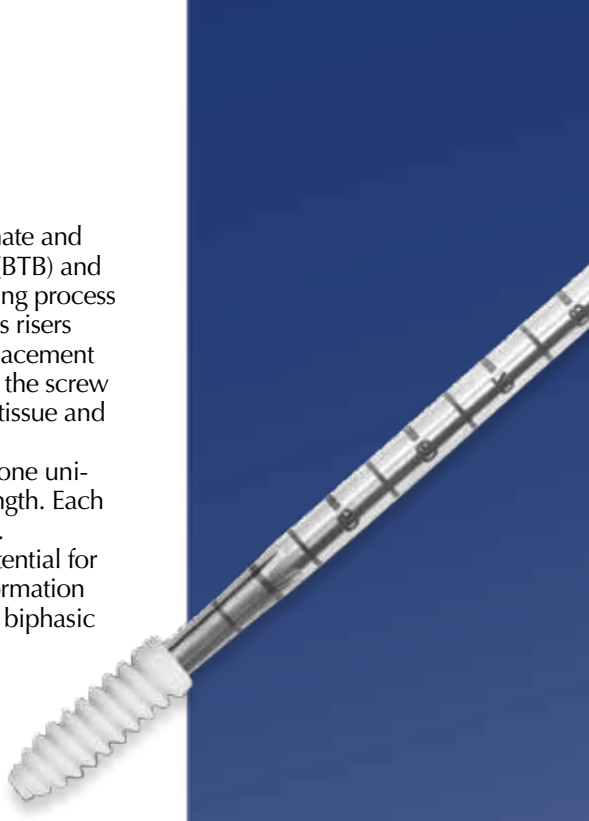
Complete

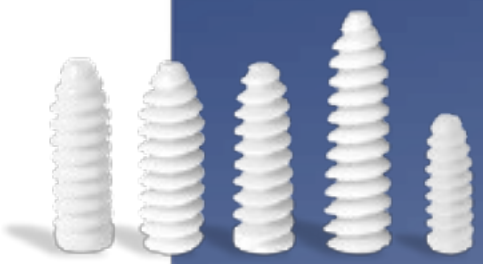
Soft Tissue and BTB Graft Fixation with BioComposite Interference Screws

The BioComposite Interference Screw is comprised of 30% biphasic calcium phosphate and 70% PLDLA and is intended for use as a fixation device for bone-patellar tendon-bone (BTB) and soft tissue grafts during ACL and PCL reconstruction procedures. The blending and binding process of the two materials adds significant strength to the implant by virtually eliminating stress risers while creating a macro and micro porous matrix to aid in the bone remodeling and replacement process. Each screw has a stepped tapered design which maximizes insertion torque, as the screw is fully seated. The thread form has been optimized to ease insertion and maximize soft tissue and bone fixation in cortical and cancellous bone.

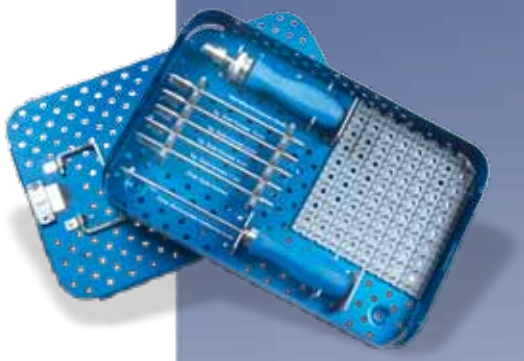
The new cannulated hexalobe drive system enhances the screw family by providing one universal drive system for all screws and significantly improved torsional and insertion strength. Each screw fully seats on and is completely supported along the entire length of the driver tip.

Clinical reports suggest that biphasic calcium phosphate is safe and has excellent potential for orthopaedic applications. As the focus of many bone replacement studies, early bone formation can be connected to the favorable osteoconductive and bioresorbable properties within biphasic calcium phosphates.





BioComposite Interference Screw, w/disposable sheath, 6 mm x 23 mm	AR-1360C
BioComposite Interference Screw, w/disposable sheath, 7 mm x 23 mm	AR-1370C
BioComposite Interference Screw, w/disposable sheath, 8 mm x 23 mm	AR-1380C
BioComposite Interference Screw, w/disposable sheath, 9 mm x 23 mm	AR-1390C
BioComposite Interference Screw, w/disposable sheath, 10 mm x 23 mm	AR-1400C
BioComposite Interference Screw, Full Thread, 8 mm x 28 mm	AR-1380TC
BioComposite Interference Screw, Full Thread, 9 mm x 28 mm	AR-1390TC
BioComposite Interference Screw, Full Thread, 10 mm x 28 mm	AR-1400TC
BioComposite Interference Screw, Full Thread, 11 mm x 28 mm	AR-1403TC
BioComposite Interference Screw, Full Thread, 12 mm x 28 mm	AR-1404TC
BioComposite Interference Screw, Round Delta Tapered, 8 mm x 28 mm	AR-5028C-08
BioComposite Interference Screw, Round Delta Tapered, 9 mm x 28 mm	AR-5028C-09
BioComposite Interference Screw, Round Delta Tapered, 10 mm x 28 mm	AR-5028C-10
BioComposite Interference Screw, Round Delta Tapered, 11 mm x 28 mm	AR-5028C-11
BioComposite Interference Screw, Delta Tapered, 9 mm x 35 mm	AR-5035TC-09
BioComposite Interference Screw, Delta Tapered, 10 mm x 35 mm	AR-5035TC-10
BioComposite Interference Screw, Delta Tapered, 11 mm x 35 mm	AR-5035TC-11
BioComposite Interference Screw, Delta Tapered, 12 mm x 35 mm	AR-5035TC-12
BioComposite RetroScrew, 6 mm	AR-1586RC-06
BioComposite RetroScrew, 7 mm	AR-1586RC-07
BioComposite RetroScrew, 8 mm	AR-1586RC-08
BioComposite RetroScrew, 9 mm	AR-1586RC-09
BioComposite RetroScrew, 10 mm	AR-1586RC-10



BioComposite Interference Screw Instrumentation Set (AR-1996S) includes:	
Driver, BioComposite Interference Screw	AR-1996CD
Driver, BioComposite Interference Screw, quick connect	AR-1996CD-1
Ratcheting Screwdriver Handle	AR-1999
Tap, BioComposite Interference Screw, quick connect, 6 mm	AR-1998CT-06
Tap, BioComposite Interference Screw, quick connect, 7 mm	AR-1998CT-07
Tap, BioComposite Interference Screw, quick connect, 8 mm	AR-1998CT-08
Tap, BioComposite Interference Screw, quick connect, 9 mm	AR-1998CT-09
Tap, BioComposite Interference Screw, quick connect, 10 mm	AR-1998CT-10
BioComposite Interference Screw Instrumentation Case	AR-1996C

Optional Instrumentation:	
Tunnel Notcher for Bio-Interference Screw	AR-1845
Non-Ratcheting Screwdriver Handle	AR-1999NR
Universal Dilator	AR-1377M
BioComposite Interference Screw Dilator, 6 mm	AR-1377C-06
BioComposite Interference Screw Dilator, 7 mm	AR-1377C-07
BioComposite Interference Screw Dilator, 8 mm	AR-1377C-08
Screw Tap, 7 mm	AR-1387
Screw Tap, 8 mm	AR-1388
Screw Tap, 9 mm	AR-1389
Screw Tap, 10 mm	AR-1389-10

Disposable Accessories:	
Transtibial ACL Disposables Kit with Hall Style Blade, qty. 5	AR-1897S
Transtibial ACL Disposables Kit without Saw Blade, qty. 5	AR-1898S

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Histological assessment in grafts of highly purified beta-tricalcium phosphate (OSferion®) in human bones

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Abstract

Prominent osteoconductive activity and the biodegradable nature of commercially available beta-tricalcium phosphate (β-TCP, OSferion®) have been documented in animal experiments. We analyzed four cases of involving grafted OSferion® in human bone with respect to histological features by routine hematoxylin and eosin staining, silver impregnation, immunohistochemistry and in situ hybridization. OSferion® affords early bioresorption by osteoclasts, vascular invasion of macropores and osteoblastic cell attachment on the surface on the ceramic surface 14 days after grafting. Prominent bone formation and direct bone connection between preexisting bone and OSferion® were evident 28 days after grafting. Nearly the entire TCP surface was covered by lamellar bone; additionally, active osteoblastic lining and attachment of the osteoclast-like giant cells were not observed 72 weeks after grafting. Silver impregnation revealed the presence of collagen fibrils within probable micropores of OSferion®.
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Keywords: Bioactive ceramic; Hydroxyapatite; Tricalcium phosphate; Bone graft; Osteoconduction

1. Introduction

Numerous basic studies have been demonstrated that calcium phosphate ceramics are biocompatible, bioactive, and osteoconductive. A variety of synthetic bone grafts have been utilized to fill bone defects. Hydroxyapatite (HA), which is prepared by precipitation and subsequent sintering at temperatures above 1000 °C, displays a Ca-to-P molar ratio of 1.67. Beta-tricalcium phosphate (β-TCP), which possesses stoichimetry similar to amorphous biologic precursors to bone mineral, exhibits a Ca-to-P molar ratio of 1.5. Calcium phosphate ceramics have been considered for use as synthetic bone graft substitutes for over 30 years; furthermore, commercial HA and β-TCP have been examined in terms of suitability as a as bone substitute in the clinical setting. Radiological evaluation in clinical investigation of implanted HA and TCP in human has revealed satisfactory osteoconductive qualities in both materials [1,2]. Many reports have suggested that greater extent and faster rate of bone penetration are correlated with increasing macroporosity (i.e. pores > 50 μm in size) in calcium phosphate ceramics. Recent experiments indicated that manipulation of the level of microporosity within calcium phosphate ceramics can lead to acceleration of bone formation and elevation of the equilibrium volume of bone [1–5].

Highly purified β-TCP (OSferion® Olympus, Tokyo Japan) has been manufactured and is currently available as a potent bone-grafting substitute for clinical use [6–13]. We recently reported that OSferion® is a suitable bone-filling agent in clinical application [7,9]. Several animal experiments demonstrated satisfactory biocompatibility of OSferion® since both bioresorption and bone formation began at an early stage following implantation [10,14,15]. However, histological studies of β-TCP in human samples are limited [7,11].

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The present investigation describes histological details of the β-TCP, which were grafted in human bones.

2. Patients, materials and methods

2.1. Implants

We have utilized highly purified β-TCP since 1999. β-TCP (OSferion® Olympus, Tokyo Japan) (porosity of 75%, from 100 to 500μm in macropore size with micropore of less than 5 μm, 1050 °C sintering temperature, granules (size 0.5–8.0 mm), porous blocks (size $10 \times 10 \times 10$–$50 \times 10 \times 30$ mm)) was manufactured in an extraordinary high purity [7].

The HA (Bonfil®, Mitsubishi Materials, Chichibu, Japan) in the form of porous cubes (porosity of 70%, from 90 to 200 μm in pore size without micropore, 900 °C sintering temperature, granules (size1.0–5.0 mm), porous blocks (size $5 \times 5 \times 5$ mm to $50 \times 10 \times 10$)) was employed between 1992 and 1998 for the bone filler in large bone defect in orthopedic surgery in our institute [9]. The spatial dimensions of the blocks and granules varied according to the shape and size of the bone defect.

2.2. Patients

The clinical findings are summarized in Table 1.The study group was comprised of our patients with β-TCP and one patient with HA (1 male and 4 females). Patients ranged in age from 18 to 79 years. Reasons for histological evaluation of the grafted materials were as follows: osteosynthesis for fracture of the affected bones (2 cases), additional wide excision (1), autopsy (1), and surgery for tumor recurrence (1), respectively.

2.3. Sample preparation

The resected ceramics were fixed in 10% neutral buffered formalin and decalcified in formic acid and processed for embedding in paraffin in four cases (Case 1, 3, 4, 5). The specimen in case 2 was immersed in 4% paraformaldehyde in 0.1 M phosphate buffer and was decalcified with 0.5 M EDTA 2Na solution for 5 days at room temperature and processed for embedding in paraffin. Each specimen was stained with routine hematoxylin and eosin and silver impregnation to detect the collagen fibers [16]. All specimens and clinical data were evaluated by one surgical pathologist (H, U) and two orthopedic surgeons (A, O; N, K). Histological assessment was conducted via discussion of these three individuals.

2.4. Tartrate-resistant acidic phosphate (TRAP) staining and immunohistochemistry

To detect osteoclasts, TRAP staining was performed out according to Burstone's Azo dye method [15]. Briefly, a mixture of 3 mg of naphthol AS-BI phosphate (Sigma, St. Louis, MO), 18 mg of red violet LB salt (Sigma, St. Louis, MO) and 2.4 mM L(+)-tartaric acid (Wako, Osaka, Japan) diluted in 0.1 M sodium acetate buffer (pH 5.0) was dropped onto the deparaffinized sections. These sections were incubated for 50–60 min at room temperature.

Immunohistochemical staining was conducted with the following primary antibodies: CD68 (Pan macrophage marker) (KP-1; Dako, Glostrup, Denmark), alpha-smooth muscle actin (marker for smooth muscle cells, myofibroblasts and vessels with tunica media) (SMA) (1A4; Dako), CD34 (endothelium marker) (: Dako), and Cathepsin-K (osteoclast marker) (Daiichi Fine Chemical, Takaoka, Japan).

2.5. In situ hybridization

In order to detect the expression of type I collagen mRNA, in situ hybridization was performed as previously described [15,17]. Mouse COL1A1 cDNA was a gift from Life Science Research Institute

Table 1
Summary of the patients

Case no.	Implanted material	Age	Sex	Initial diagnosis	Final diagnosis	Location	Tumor size (cm)	Amount of ceramics (g)	Interval to removal of the ceramics	Reason for the removal of the ceramics	Availability of the specimes for immunohistochemiostry
1	β-TCP	79	F	Fracture of the pelvis after revision hip arthroplasty	Fracture of the pelvis after revision hip arthroplasty	Acetabulum		10	12 days	Reconstruction of the pelvic ring	No
2	β-TCP	65	F	Fibrous dysplasia	Fibrous dysplasia	Distal femur	12	10	14 days	Surgery for fracture	Yes
3	β-TCP	33	F	Fibrous dysplasia	Low grade osteosarcoma	Proximal femur	10	10	4 weeks	Surgery for wide resection	No
4	β-TCP	18	M	Eosinphilic granuloma	Metastatic adrenal cancer	Ilium	5	4	72 weeks	Autopsy	No
5	HA	39	F	Giant cell tumor	Giant cell tumor	Ilium	7	8	160 weeks	Surgery for tumor recurrence	No

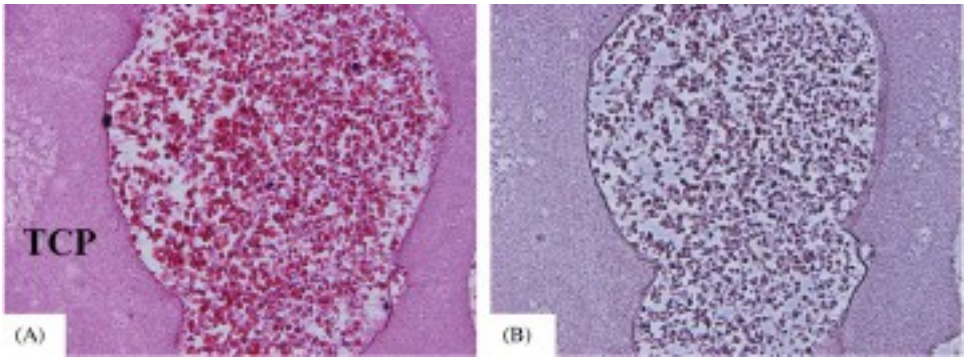

Fig. 1. Case 1. Seventy-nine-year-old female with fracture of the pelvis after revision hip arthroplasty. The specimen was harvested form the acetabulum 12 days after grafting of TCP. (A) HE staining: only red blood cells were present in macropores of β-TCP. (B) Silver impregnation: no collagen fibrils were observed. Original magnification × 200.

(Asahi-Chemical Industry Co., Shizuoka, Japan). After dewaxing in xylene and re-hydrating via a series of graded ethanol treatments, tissue sections were treated with 10 μg/ml proteinase K (Roche Diagnostics, Mannheim, Germany) for 20 min at 37 °C, refixed with 4% PFA solution, immersed in 0.1 M triethanolamine containing 0.25% acetic acid for 10 min, and washed in 0.1 M phosphate buffer (pH 7.4). The samples were then incubated in a hybridization solution (10 mM Tris-HCl (pH 7.6), 1 mM EDTA (pH 8.0), 600 mM NaCl, 0.25% sodium dodecyl sulfate, 1 × Denhart's medium, 50% (v/v) deionized formamide/0.5 μg/ml probe RNA, and 10% dextran sulfate) at 50 °C in a moist chamber for 16 h. Negative controls were incubated with DIG-labeled sense RNA probes. After hybridization, the slides were washed at 55 °C with 50% deionized formamide in 2 × saline-sodium citrate (SSC) (1 × SSC; 0.15 mol/l NaCl, 0.015 mol/l sodium citrate) for 20 min to remove excess riboprobes. The non-specifically hybridized riboprobes were digested with 10 μg/ml of RNase A (Roche Diagnostic) solution at 37 °C for 30 min. The specimens were then washed with 2 × SSC for 15 min and with 0.2 × SSC for 15 min twice. To visualize the hybridized probe, the slides were incubated with alkaline phosphatase-conjugated anti-DIG antibody (Roche Diagnostics) at room temperature for 60 min after blocking with 1.5% blocking reagent (Roche Diagnostics) in 100 mM Tris-HCl (pH 7.5) for 55 min. The specimens were then washed twice with 100 mM Tris-HCl (pH 7.5) for 15 min, and briefly immersed in 100 mM Tris-HCl (pH 9.5) containing 100 mM NaCl and 50 mM $MgCl_2$ for 5 min. The colorimetric reaction was performed with nitro blue tetrazolium salt and bromo-4-chloro-3-indolyl phosphate solution (Roche Diagnostics) in the dark for 20–120 min, and then the reaction was stopped with 10 mM Tris-HCl (pH 7.6) containing 1 mM EDTA. Slides were analyzed under a light microscope with 0.5% methyl green counterstaining.

3. Results

TRAP staining, immunohistochemical staining, and in situ hybridization were performed in all cases. No informative results were obtained, with the exception of case 2. This outcome is probably attributable to hard decalcification of the materials.

3.1. Case 1 (79 yo. Female, 12 days after grafting)

The β-TCP was removed at the surgery for the reconstruction of the pelvic ring. She underwent revision hip arthroplasty with β-TCP graft and had fracture of the pelvic ring. Histologically, there was no nucleated cells were evident in the macropores of β-TCP. Only red blood cells were present. Silver impregnation revealed no collagen fibrils in the pores and within β-TCP (Fig. 1A, B).

3.2. Case 2 (65 yo. Female, 14 days after grafting)

The β-TCP was removed at the surgery for the osteosynthesis of the femur. She underwent curettage of femoral fibrous dysplasia and β-TCP graft. Ten days after initial surgery, the patient fell and the displaced fracture was treated with intramedullary nailing.

Histologically, there was no bone apposition on β-TCP. Prominent capillary proliferation was detected in the macropores of β-TCP. Small oval-shaped mononuclear cells and multinucleated-giant cells were attached to the β-TCP. Silver impregnation demonstrated the presence of massive collagen bundles in the macropores of β-TCP; additionally, collagen bundles were directly connected directly to collagen fibrils within the β-TCP, which were probably present in the micropores (Fig. 2A, B, C, D). TRAP staining revealed that almost all multinucleated cells, which were adhered to β-TCP, were TRAP-positive. Immunohistochemically, multinucleated cells were positive for CD68 and Cathepsin K. Numerous SMA positive cells and CD34 positive cells were present in the macropores of β-TCP (Fig. 3A–F). Fig. 3G illustrates in situ hybridization of COL1A1 mRNA. Considerable numbers of COL1A1 mRNA positive mononuclear cells adhered to β-TCP; moreover, the mRNA was also expressd by a small number of spindle cells in the macropores. Areas of no mesenchymal cells in the macropores were also observed. In these areas, no collagen bundles in macropores and no collagen fibrils within β-TCP were observed.

3.3. Case 3 (33 yo. Female, 4 weeks after grafting)

The clinical details and histological features of hematoxylin and eosin staining were previously reported [7]. Preoperative and intraoperative diagnosis was benign tumor of the proximal femur; consequently, the patient underwent curettage and β-TCP graft. Four weeks later, wide excision and prosthetic replacement of the proximal

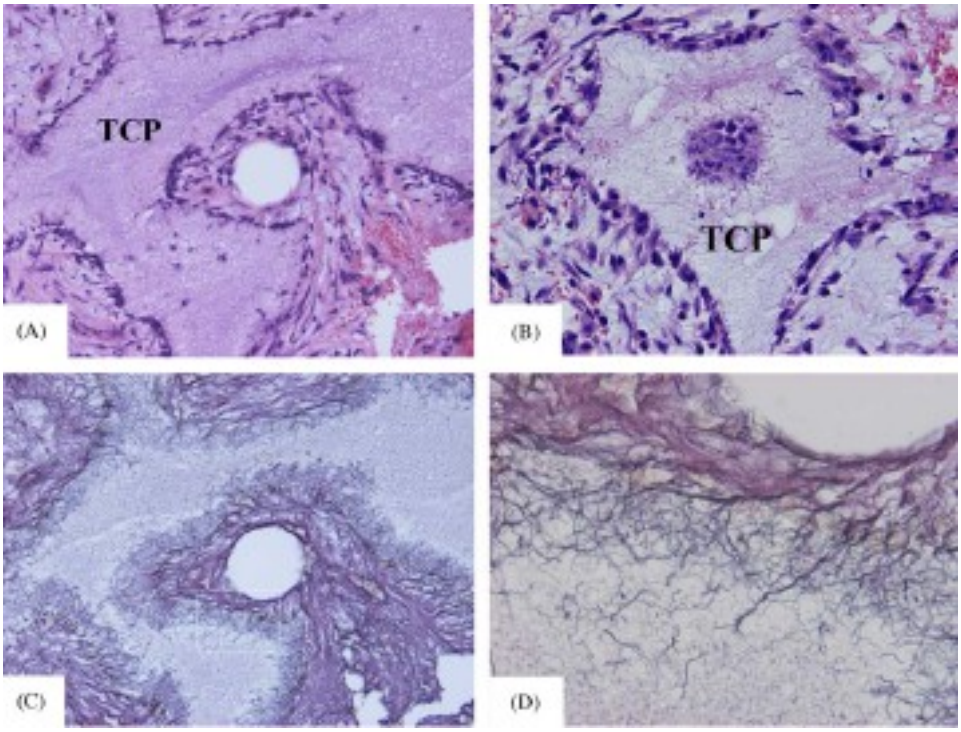

Fig. 2. Case 2. Sixty-five-year-old female with fracture of the femur. The specimen was harvested 14 days after β-TCP grafting for the fibrous dysplasia. (A) HE staining: capillary proliferations and attachment of oval cells on β-TCP were evident. (B) HE staining: high power view showing numerous mononuclear cells on β-TCP. (C) Silver impregnation: serial section (A) exhibiting collagen fibrils in the macropres and micropores of β-TCP. (D) Silver impregnation: high power view displaying abundant collagen fibrils in the micropores of β-TCP. Original magnification (A,C) × 100, (B) × 200, (D) × 400.

femur was performed due to the final diagnosis of osteosarcoma. Histologically abundant new bone formation on β-TCP was observed; furthermore, the newly formed bone was directly connected to preexisting bone trabeculae. Osteoblastic cells lined the surface of newly formed bone and osteoclast-like giant cells were directly attached to β-TCP. Silver impregnation demonstrated that abundant collagen fibrils within the β-TCP were connected to the newly formed bone. The collagen fibrils within the β-TCP displayed reticular arrangement and exhibited sparse distribution in the center of the β-TCP (Fig. 4A–D).

3.4. Case 4(18 yo. Male, 72 weeks after grafting)

The patient presented with multiple osteolytic bone tumors; preoperative diagnosis was eosinophilic granulomas. Open biopsy and β-TCP grafting were performed for the iliac bone tumor. The final diagnosis was metastatic bone tumor arising from adrenal cancer. Although the patient underwent several courses of chemotherapy and radiotherapy, he continued to deteriorate and died of multiple lung metastases 72 weeks after the initial surgery. Autopsy was conducted and grafted β-TCP was removed from the ilium.

Histologically β-TCP was surrounded by lamellar bone. Active osteoblastic lining and attachment of the osteoclast-like giant cells were not observed. Silver impregnation demonstrated the presence of a small amount of collagen fibrils at periphery of the β-TCP (Fig. 5A–C).

3.5. Case 5 (39 yo. Female, 160 weeks after grafting)

The patient displayed giant cell tumor of the ilium; as a result, curettage and HA grafting were performed. The tumor recurred and en bloc resection of the ilium was conducted 160 weeks after the initial surgery.

Histologically, a small amount of direct bone formation on HA was observed. Osteoclast-like giant cells, which were probably a component of the recurrent tumor attached to HA particles. Silver impregnation revealed the absence of collagen fibrils within HA (Fig. 6A–C).

4. Discussion

Previous studies demonstrated that various synthetic calcium phosphate possess osteoconductivity. Moreover, this ability depends on both the species of animal and the type of ceramics in terms of different phasic composition as well as macropore and micropore structure [1,2].

Prominent bioresorbability and osteoconductivity of this TCP (OSferion®) have been documented in animal experiments [10,14,15].

Ozawa et al. [10] described massive new bone formation on OSferion® and bioresorption by osteoclast-like giant cells presented 2 weeks after grafting in dog experiments. In

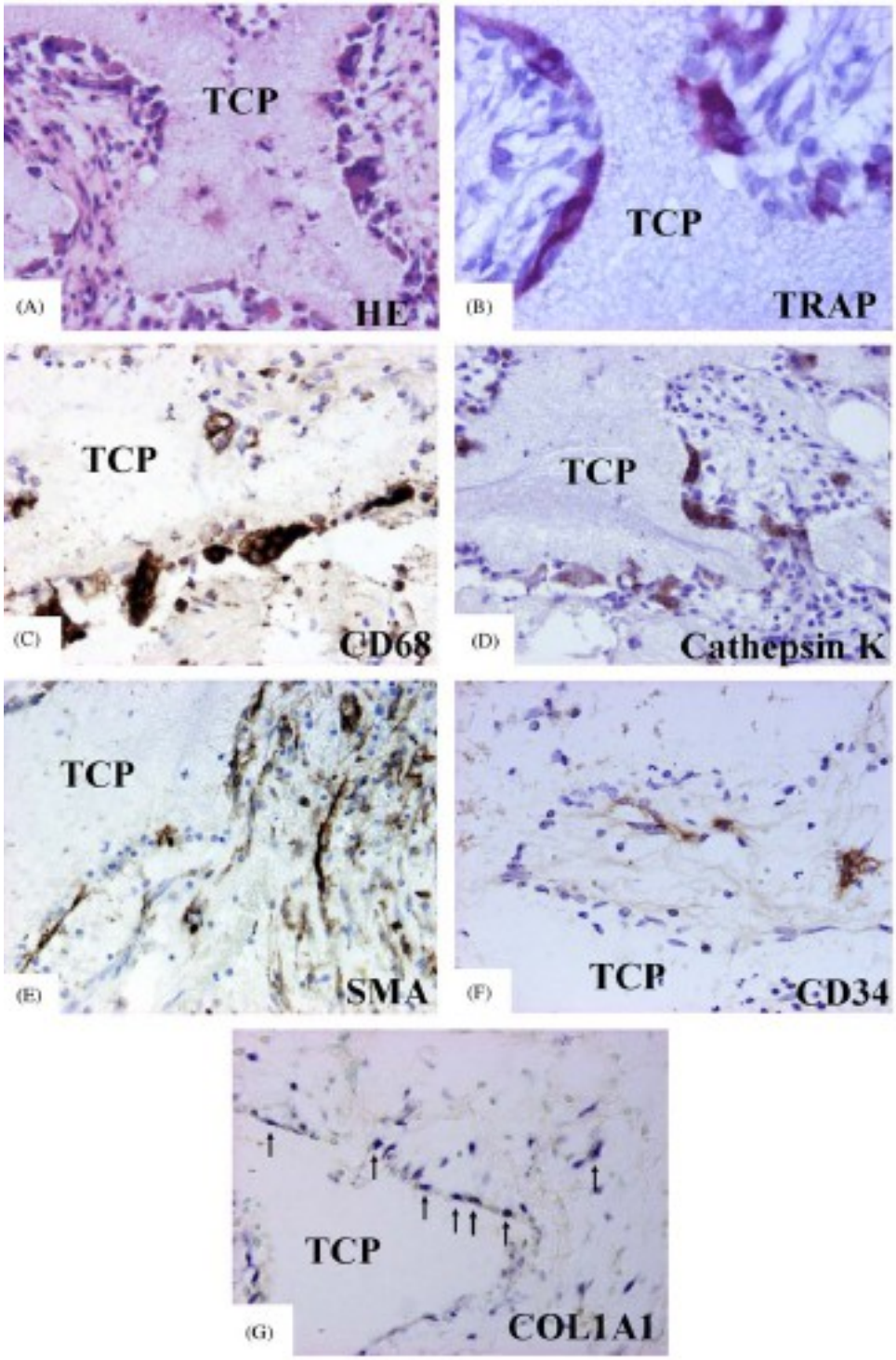

Fig. 3. Case 2. (A) HE staining: multinucleated cells were observed on β-TCP. (B) TRAP staining: TRAP positive multinucleated cells adhered to β-TCP. (C–F) Immunohistochemical staining: multinucleated cells were positive for CD68 (C) and Cathepsine K (D). Smooth muscle actin and CD34 positive vascular tissue were present in the macropores of β-TCP (E, F). (G) In situ hybridization. Mononuclear cells on TCP expressed COL1A1 mRNA (arrows). Original magnification (A, C–G) $\times$ 200, (B) $\times$ 400.

their report, bone formation and resorption of TCP were very active until 6 weeks after grafting, and relatively inactive 12 weeks after grafting.

Chazono et al. demonstrated that OSferion® appeared to incorporate with bone, and that osteoblasts deposited osteoid with numerous multinucleated giant cells in the resorptive lacunae in rabbit experiments [14]. A 2-to-4-week time lag was apparent—the number of TRAP-positive multinucleated cells peaked at 2 weeks, whereas the rate of new bone formation peaked at 4 weeks. They

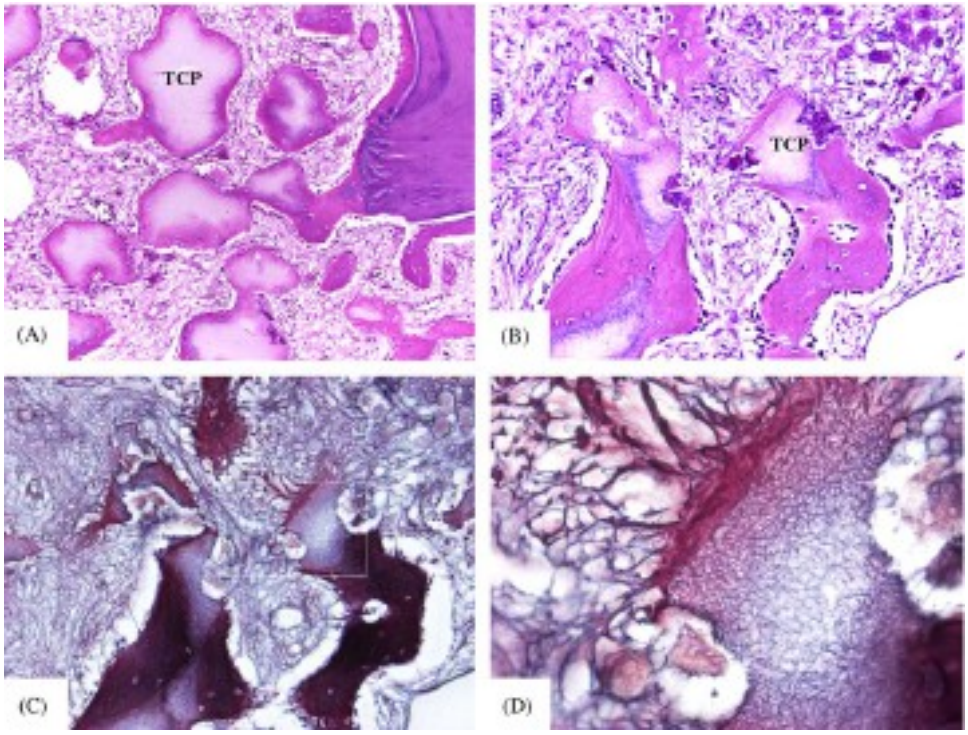

Fig. 4. Case 3. Thirty-three-year-old female with low grade osteosarcoma. The specimen was harvested form the femur 4 weeks after grafting of TCP. (A) HE staining: marked new bone formation on β-TCP, which was connected directly to pre-existing bone with lamellar structure, was apparent. (B) HE staining: middle power view showing prominent new bone formation on β-TCP and osteoblasts arl lining on the new bone. (C) Silver impregnation: serial section of figure (A). (D) Silver impregnation: high power view revealing numerous collagen fibrils within β-TCP characterized by reticular fashion which was directly connected to newly formed bone. Original magnification (A–C) $\times$ 50, (D) $\times$ 200.

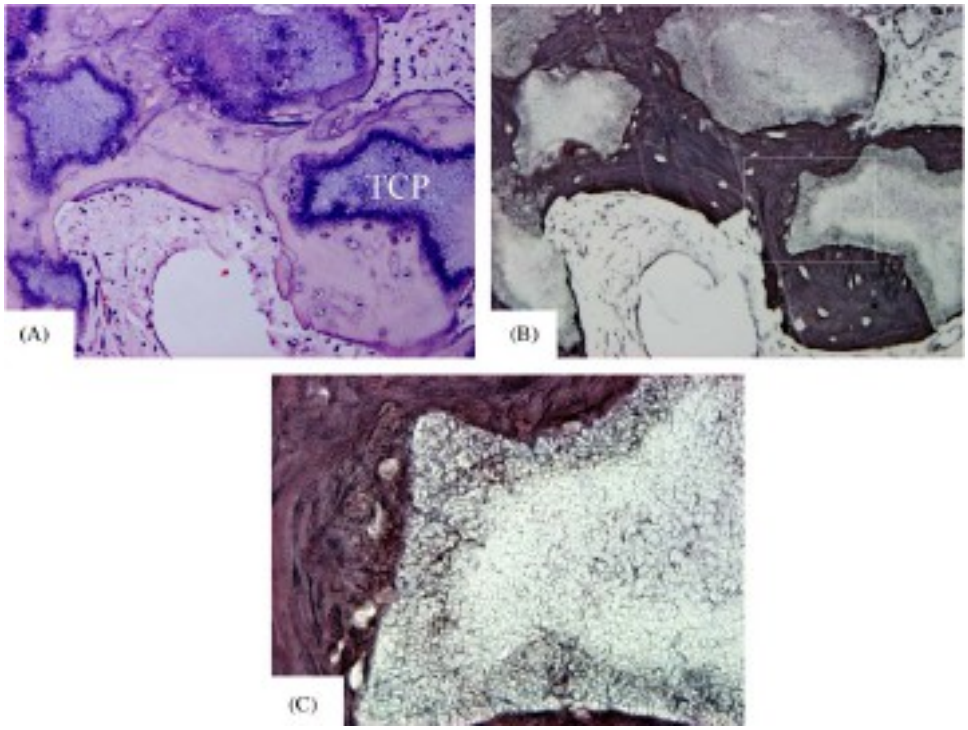

Fig. 5. Case 4. Eighteen-year-old male with multiple metastatic bone tumors. The specimen was harvested from the ilium 72 weeks after grafting of TCP. (A) HE staining: almost the entire β-TCP surface was covered by lamellar bone. (B) Silver impregnation: serial section of figure A. (C) Silver impregnation: high power view showing reticular collagen fibrils in β-TCP which were directly connected to lamellar bone. Original magnification (A,B) $\times$ 100, (C) $\times$ 400.

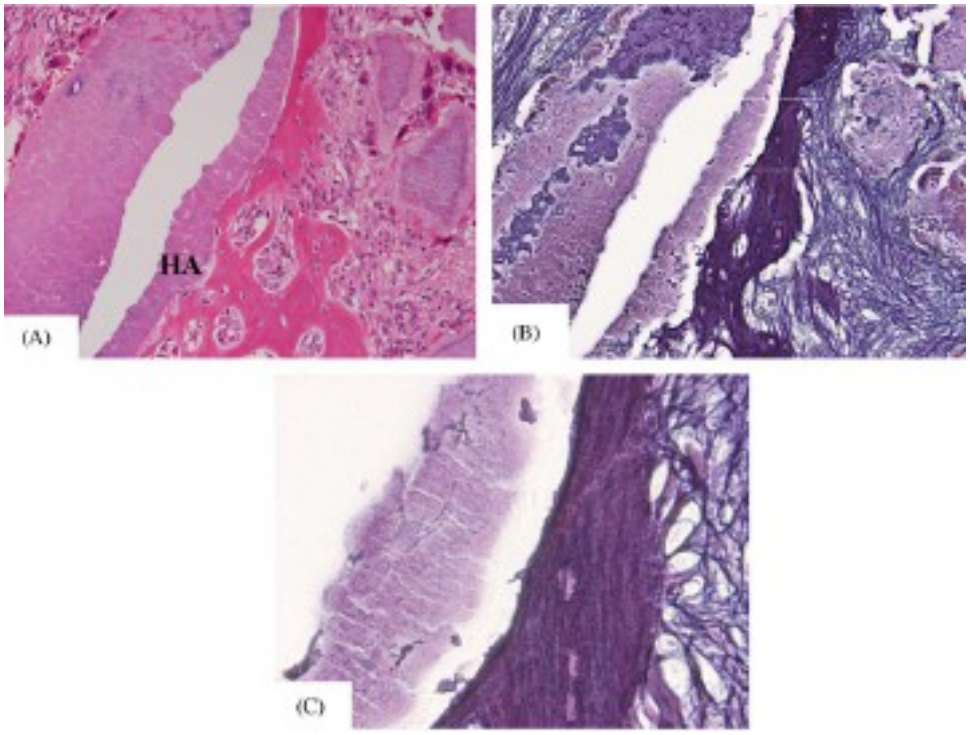

Fig. 6. Case 5. Thirty-nine-year-old female with recurrent giant cell tumor of the ilium. The specimen was harvested from the ilium 160 weeks after grafting of HA. (A) HE staining: direct new bone formation on HA was apparent. (B) Silver impregnation: serial section B. (C) Silver impregnation: high power view showing the absence of collagen fibrils within HA. Original magnification (A,B) × 50, (C) × 200.

hypothesized that new bone formation follows cell-based resorption of β-TCP, suggesting that a coupling like phenomenon occurs in the β-TCP-filled bone defect.

Our recent experiment in rat also demonstrated that OSferion® afforded early bone conduction, followed by bioresorption and the replacement of large portions of the β-TCP with newly formed bone. On day 4, osteoclast-like giant cells adhered to β-TCP and numerous monocyte-macrophage lineage cells proliferated. By day 7, new bone formation occurred in the peripheral region of β-CP. Bone formation and resorption of β-TCP were relatively inactive by day 56 [15].

The current investigation revealed that bone formation and resorption of β-TCP in human in early stage occurred in a manner identical to that of previous animal experiments.

In Case 1 (12 days after grafting), no cellular element was evident in the macropores of β-TCP. In Case 2 (14 days after grafting), prominent capillary proliferation and attachment of small oval-shaped mononuclear cells and multinucleated-giant cells attached to β –TCP were apparent. Areas displayin few cellular elements in the macropores were also observed in case 2; therefore, those differences between case 1 and case 2 appeared to attributable to differences with respect to sampling location. Positive TRAP, CD68, and cathepsin K reactions of multinucleated giant cells indicated the typical osteoclastic natures of these cells. These findings suggested that osteoclasts resorbed β-TCP in human bones in a manner identical to that in animal experiments. On the other hand, considerable numbers of mononuclear cells on β-TCP expressed COL1A1. Although, bone formation on β-TCP was not detected in this case, this in situ hybridization result suggests that bone-forming activity occurred on day 14.

Direct bone formation on β-TCP was observed in cases 3, and 4. In case 3, the bone was immature and active osteoblastic cells lined the surface of newly formed bone. Attachment of osteoclast-like giant cells was also prominent. These findings suggest that bone formation and resorption of β-TCP were very active on day 28. However, no active osteoblasts or osteoclasts were present in case 4. Most of surface of the β-TCP surface was covered with lamellar bone. Thus bone formation and resorption of β-TCP were in active 72 weeks after grafting. In animal experiments, little histological data are available regarding ceramics after a lengthy period following grafting [4].

In silver-impregnated specimens, reticular fibers, which are small bundles of collagen fibrils or individual collagen fibrils [10], are stained dark. In cases 2, 3, and 4, reticular fibers were present within β-TCP of silver impregnated specimens. Reticular fibers were not observed within hydroxyapatite of case 5. This β-TCP possesses numerous micropores with diameters in several microns, in contrast, the HA in case 5 exhibited no micropore structure. The difference in the reticular fibers within ceramics was probably due to difference of micropore structure. If the macropore lacked vivid mesenchymal cells, no reticular

fiber occurred in β-TCP. In case 1, no collagen fibers were detected in macropores and no collagne fibrils within β-TCP. In case 2, no collagen fibrils were detected with in β-TCP where the macropores had few cellular elements. Therefore, silver impregnation in this series appears not to be an artifact. Chazono et al. [18] recently demonstrated the presence of collagen fibrils in the micrpores of OSferion® 2 weeks after grafting in rabbit femur in an electron microscopy study. Our silver impregnation results are consistent with the finding of their electron microscopy study.

It has been reported that bone formation of biomaterials is material dependent [1–5]. Recent animal experiments suggest that the presence of the micropores in the ceramics is an important factor with respect to bone formation in soft tissue implantation [1–5]. Obvious bone formation was observed in some calcium phosphate ceramics, whereas no bone formation was detected in other materials in animal experiments. Osteoinduction of calcium phosphate ceramics and micropore structure of these ceramics were described simultaneously [3,4,19]. However, how the micropores of calcium phosphate ceramics function remains unclear. In this study, we demonstrated the presence of collagen fibrils in this β-TCP, which displayed micropore structure; however, collagen fibrils were not found in HA which lacked micropore structures. In the clinical setting, this OSferion® appears to possess superior osteoconductivity in comparison to this HA. The presence of micropores and collagen fibrils in this TCP may be correlated with prominent osteoconductivity [5,7].

At early stage after grafting, OSferion® was, at least partially, absorbed and replaced by newly formed bone in clinical use. At late stage after grafting, the bone formation becomes inactive. However, due to the small number of cases, the variable histologic findings and the considerable variability in the study of population in terms of age, indications for the second look procedure, and duration relative to the original procedure, the generalizability of this study is open to debate. Further study should be needed to clarify the bone forming process in ceramics in human.

5. Conclusion

We determined that this β-TCP (OSferion®) affords an early bioresorption by osteoclasts and vascular invasion in macropores and osteoblastic cell attachment on the surface of the ceramic 14 days after grafting in human bone. Prominent bone formation and direct bone connection between prexisisting bone and β-TCP were detected 28 days after grafting. Almost entire β-TCP surface was covered by lamellar bone; furthermore, active osteoblastic lining and attachment of the osteoclast-like giant cells were not observed 72 weeks after grafting. In addition, this investigation showed that collagen fibrils were probably present in micropores of β-TCP.

Acknowledgment

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Histological Examination of β-Tricalcium Phosphate Graft in Human Femur

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Abstract: Prominent osteoconductive activity and the biodegradable nature of beta tricalcium phosphate (β-TCP) for bone grafts in animal experiments has been reported. A new type of β-TCP has been manufactured at extraordinarily high purity and has been available as potent bone grafting substitute for clinical use. The histological features of grafted β-TCP in human bone have been analyzed. A 33-year-old female with a bone tumor of the proximal femur underwent curettage and β-TCP graft under the diagnosis of probable benign fibrous dysplasia. Four weeks later, the proximal femur, including the grafted β-TCP was resected because of the final diagnosis of the curettaged materials was osteosarcoma. The resected specimen revealed abundant direct new bone apposition on β-TCP. There was no cartilaginous tissue or enchondral ossification. Bone formation was more prominent in the periphery of the grafted area than in the center. There was a considerable number of osteoclast-like giant cells surrounding the β-TCP. This case illustrated that highly purified β-TCP had prominent osteoconductive activity and biodegradable nature in human bone. © 2002 Wiley Periodicals, Inc. J Biomed Mater Res (Appl Biomater) 63: 601–604, 2002

Keywords: bioactive ceramic; tricalcium phosphate; bone graft; human

INTRODUCTION

Prominent osteoconductive activity and biodegradable nature of beta tricalcium phosphate (β-TCP) for bone graft in animal experiments has been reported.[1–5] A new type of β-TCP has been manufactured in an extraordinarily high purity and has been available as potent bone-grafting substitute for clinical use.[6] The clinical reports suggest that the β-TCP is safe and has excellent potential for orthopedic applications.[6,7] Radiologically, new bone formation and remodeling around grafted β-TCP was observed in almost all cases.[6,7] However, reports of histological examination of grafted β-TCP in human bone are rare. Here the histological features of β-TCP in a human femur 4 weeks after the grafting are examined.

PATIENT AND METHODS

Case Presentation

A 33-year-old female presented with a 3-month history of the right hip joint pain. Plain radiographs showed relatively well marginated osteolytic lesion in the right femoral neck [Figure 1(a)]. Laboratory tests revealed no abnormality, including serum alkaline phosphatase. Preoperative clinical diagnosis at that time was benign fibrous dysplasia, and curettage and β-TCP graft was scheduled after informed consent was obtained. An open biopsy was performed after the corticotomy of the lateral aspect of the proximal femur and the diagnosis of the frozen section was benign fibro-histiocytic tumor. Following complete removal of the tumor, the defect was irrigated and filled with 10 g of β-TCP (OSferion® Olympus, Osaka Japan) (porosity of 75%, from 100 to 400 μm in pore size, 1050° sintering temperature), a porous-type granules with a particle diameter of 2.8 to 5.0 mm, and two blocks of 10 × 10 × 10 mm size. The nonvascularized autologous fibula was also implanted in the curetted cavity. No cancellous bone chips or bone marrow fluid were grafted [Figure 1(b)]. The diagnosis of the permanent section was osteosarcoma, and wide excision and prosthetic replacement of the proximal femur was performed 4 weeks after β-TCP grafting. The postoperative course was uneventful, and the patient has had no recurrence 8 months after the last surgery.

Histological Preparation

The resected proximal femur was fixed in 10% neutral buffered formalin. The femur was sectioned in a frontal plane,

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No benefit of any kind will be received either directly or indirectly by authors.

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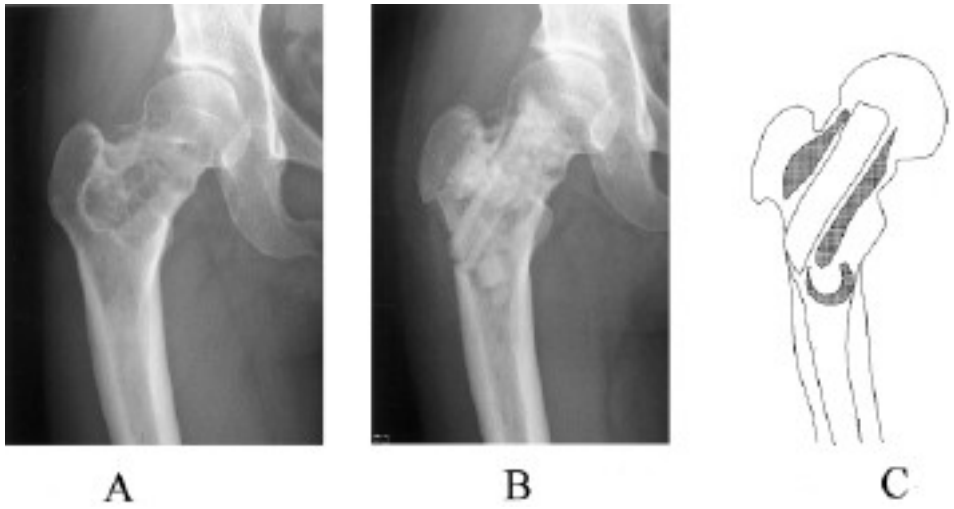

Figure 1. (a) Preoperative radiograph showing a relatively well marginated osteolytic lesion in the right proximal femur. (b) Postoperative radiograph showing grafted β-TCP and the nonvascularized fibula. (c) Scheme of the distribution of the new bone formation in β-TCP in the largest slice of the femur (shaded areas).

and cut off approximately 5 mm thick. The largest cut surface was decalcified and processed for embedding in paraffin. All areas of the largest cut surface were histologically examined. Sections were cut at a 3-μm thickness and stained with hematoxylin and eosin. For the specimens without decalcification, the thin-sliced femur was fixed with 70% ethanol, followed by immersion in Villanueva bone stain solution (Maruto, Tokyo, Japan).[8] The specimens were dehydrated through gradient ethanol and embedded in methylmetacrylate (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and cut into 5-μm-thick sections with a Jung K microtome (Jung, Heidelberg, Germany).

RESULTS

Histologically, there was no remnant osteosarcoma tissue in the resected femur. Nonneoplastic fibroblastic cells were proliferated between the β-TCP granules with capillary formations. Abundant new bone formation was seen on the β-TCP [Figure 1(c)]. The bone formation was more prominent in the periphery of the grafted area than in the center. At the interface between newly formed bone and preexisting bone trabeculae, new bone was directly connected to normal trabeculae and β-TCP. Direct contact between new bone and β-TCP was obvious [Figures 2(a) and 2(b)]. Bone formation surrounding the β-TCP was less frequently observed near the grafted fibula than in the periphery, and no connection between the β-TCP and the cortex of the fibula was observed [Figure 2(c)]. Neovascularization and new bone apposition were also seen in the pore of β-TCP [Figure 2(d), green arrows]. Osteoblastic cells were lining the surface of both newly formed bone and β-TCP [Figures 2(d) and 2(e)]. In some areas, considerable numbers of osteocytes in the newly formed thickened bone on the β-TCP were seen [Figure 2(e)].

There were a considerable number of osteoclast-like giant cells surrounding β-TCP [Figure 2(f)]. There was no cartilaginous tissue or enchondral ossification. Tartrate-resistant acid phosphatase (TRAP) staining and immunohistochemical stainings of CD 68, CD 31, vimentin, and osteopontin were performed. However, no informative results were observed, probably because of hard decalcification of the materials.

DISCUSSION

Different biomaterials have been developed and used as bone grafts. Prominent osteoconductive activity of the biomaterials for bone graft has been reported.[1–5] A new type of β-TCP of extraordinarily high purity has been manufactured, and is available as a potent bone grafting substitute for clinical use.[6,7] However, to the authors' knowledge, histological examination of the β-TCP in human long bone has not been previously reported.

Ozawa demonstrated that highly purified β-TCP was an excellent material that introduced early bone formation. During the bone remodeling process, β-TCP itself gradually degraded and was finally replaced by mature new bone in animal experiments.[5] Active bone apposition and resorption of β-TCP were observed 2 weeks after implantation in the tibia of beagle dog. Renooji et al. also demonstrated that sintered β-TCP was subject to biodegradation, whereas hydroxyapatite was not affected by bioresorption processes 50 weeks after implantation in dog.[9] Oyake et al. reported that abundant bone formation in highly purified β-TCP was seen 4 weeks after implantation, and absorbed gradually over the course of time. However, hydroxyapatite was not absorbed and hindered the formation of new bone at early stage in rabbit model.[10] They concluded that superior bone formation was observed in the grafted β-TCP than in the grafted hy-

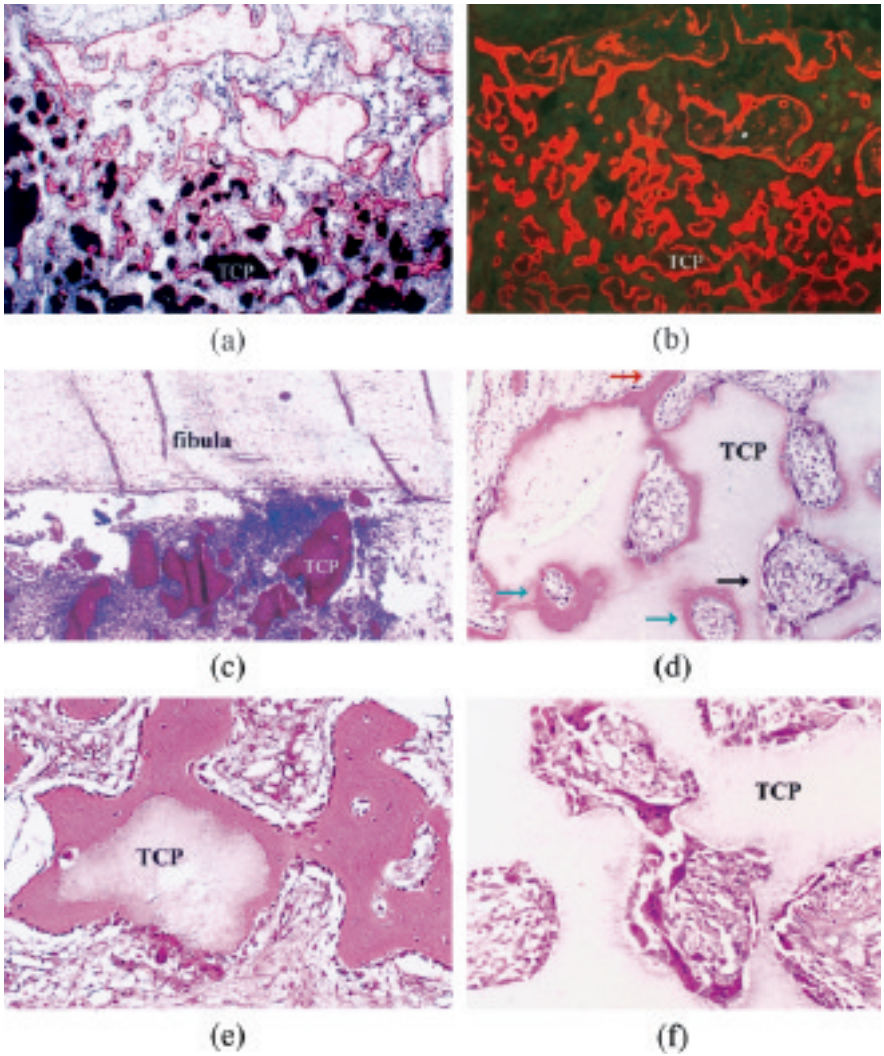

Figure 2. (a) Undecalcified specimen with Villanueva bone staining. There was marked new bone formation on the grafted β-TCP (black), which is directly connected to preexisting bone (PB). (b) Same picture as (a) under fluorescence microscopy showing new bone formation (orange), which is directly connected to preexisting lamellar bone (PB, green) and β-TCP (green). (c) Low power view of interaction between the fibular graft and surrounding tissue. (Undecalcified specimen with Villanueva bone staining) New bone formation was seldom observed around the fibula. (d) Middle power view showing proliferation of osteoclast-like giant cells (black arrow), neovascularization, and new bone formation in the pore of β-TCP (green arrows), and cuboidal osteoblasts are lining on the new bone (red arrows). (e)High-power view showing prominent new bone formation on the β-TCP, osteoblastic lining, and considerable numbers of osteocytes in new bone. There is no fibrous tissue between β-TCP and new bone. (f) High-power view showing considerable numbers of osteoclast-like giant cells, cuboidal osteoblasts on the newly formed bone.

droxyapatite in their experiment. Yamamoto et al. reported histological features of grafted hydroxyapatite in human bone 6 months after the grafting.[11] They reported that hydroxyapatite partially removed by histiocytes and multinucleated giant cells. Uchida et al. reported that new bone formation was rarely seen in the pores of the hydroxyapatite in rat experiments.[3] In the present case, active bone formation, resorption of the β-TCP, neovascularization, and new bone apposition in the pores of the β-TCP were prominent 4 weeks after implantation in human adult bone.

Some clinical studies showed that normal bone trabeculae replaced the β-TCP, and that this process was one of continued remodeling, rather than a biological coating of the implant, as was seen with hydroxyapatite.[6,7,12] The bioresorption and remodeling properties of β-TCP may be an advantage over the hydroxyapatite materials, which appear to remain unremodeled even after long periods of implantation.[9] Hibi et al. reported that 4 of 14 patients who were implanted of hydroxyapatite for bone defect of benign bone tumor, developed fractures probably because of the nonresorbable nature of the grafted material.[13] Ozawa et al, reported 167 patients who were implanted with highly purified β-TCP, and none of them developed fractures or deformities. They also showed marked radiological remodeling around β-TCP in

these patients. Because highly purified β-TCP is well absorbed and osteoconductive in human bone, it has seemed to be a better biomaterial for compensation of the bone defect than hydroxyapatite.[6]

The resorption mechanism of β-TCP is controversial now. Lu et al. suggest that the mechanism is mainly dissolution in biological liquids because of the absence of osteoclasts around the materials in rabbit's experiments.[14] Renooji et al. reported that osteoclast-like giant cells were often observed bordering the implant surface 20 weeks after implantation, and cell-mediated bioresorption might be a predominant factor in the process of biodegradation of β-TCP in dog experiments.[9] In the present case, considerable numbers of osteoclast-like giant cells, neovascularization, and abundant new bone apposition were seen on the β-TCP. These findings suggest that β-TCP have osteoconductive properties in human bone at early stage of implantation, and resorption of β-TCP is, at least partially, mediated by osteoclast-like giant cell resorption. Unfortunately, the cytochemical and immunohistological characteristics of osteoclast-like giant cells could not be clarified, probably because of hard decalcification. Further investigation is needed to examine the mechanism of biological resorption of the grafted materials in bone.

This case highlights that highly purified β-TCP have prominent osteoconductive and bioresorbable properties in human adult bone at early stage. The newly formed bone was directly connected to β-TCP and preexisting bone trabeculae.

The authors thank H. Akazawa for the preparation of histological sections.

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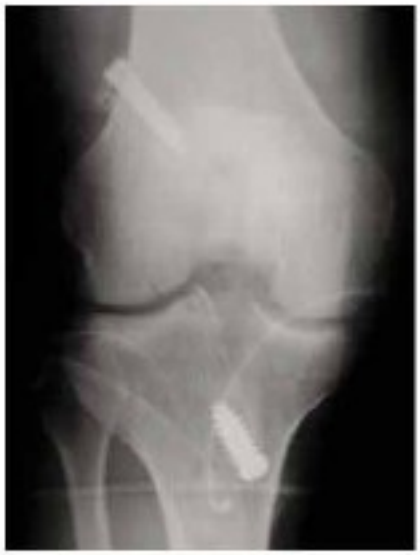
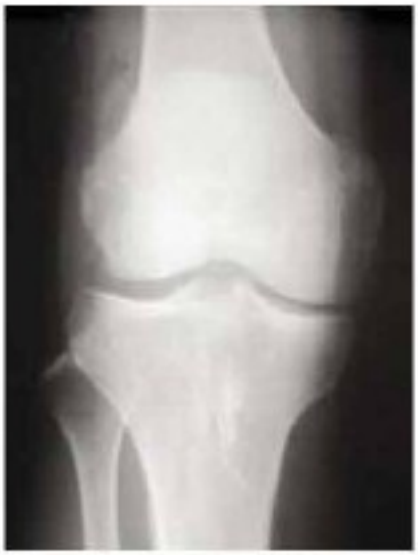
Case

Injury to the cruciate ligament of the right knee, ACL reconstruction, 32 year-old male (Department of Orthopedics, Nara Prefectural Hospital)

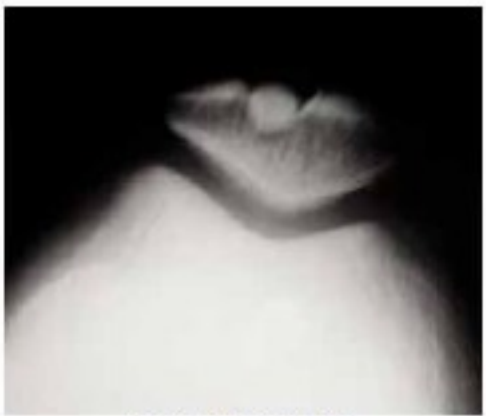
On May 15, 2002, in order to treat the injury to the anterior cruciate ligament, ACL reconstruction was conducted by using BTB grafts. The defect created by collection of the BTB grafts was filled with two pieces of β-TCP (B0820) for the patella and tibia respectively. The X-ray taken at the time of removal of the screw one year later showed complete absorption of the β-TCP and its replacement by new bone. In the previous cases where β-TCP was not implanted in the defect, a marked gap could be seen, especially on the side of the patella. Complications following collection of BTB grafts, such as local pain and fracture of the patella, have been reported. In the case of filling the defect with hydroxyapatite, the fine powder scattered over the soft tissues occasionally causes persistent pain. When implanting β-TCP, the exposed implant and the fine powder scattered over the soft tissues rarely cause pain. The problems resulting from the use of different replacement materials could be successfully solved by implantation using β-TCP. Filling the defect created by collection of BTB grafts with β-TCP (B0820) can be recommended as the optimum technique.



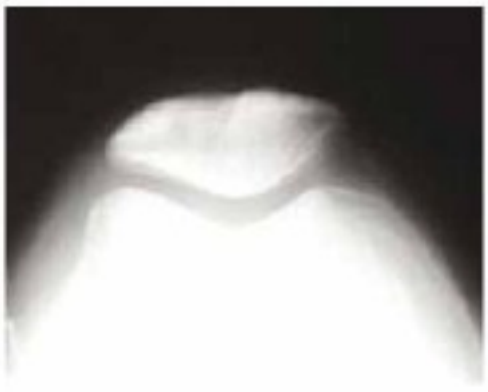
Posteroanterior radiograph of the right knee joint taken immediately after surgery
The implanted β-TCP has achieved close contact with the bone defect.



Posteroanterior radiograph of the right knee joint taken one year after surgery
The implanted β-TCP has been thoroughly absorbed and replaced by new bone.



Axis oblique radiograph of the right knee joint taken three weeks after surgery
Although a triangular bone graft was collected, a circular piece of β-TCP filled the bone defect.



Axis oblique radiograph of the right knee joint taken one year after surgery
The implanted β-TCP was completely absorbed and replaced by new bone. There was no gap at the site of the defect of the patella.

IN VIVO EVALUATION OF β-TCP BONE GRAFT SUBSTITUTES IN A BILATERAL TIBIAL DEFECT MODEL

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INTRODUCTION

A well accepted limitation of many bone graft substitutes is their slow in vivo resorption profiles. Long-term presence of a slowly resorbing bone graft substitute can potentially impede bone formation. Clearly, an ideal bone graft substitute should resorb fully and at a predictable rate but also provide a three dimensional matrix to support bone ingrowth and ongrowth during resorption. The rationale behind more rapid resorption of calcium phosphate based bone graft substitutes is related, in part, to a diagnostic purpose so new bone can be assessed using x-rays. The degradation of the implant also allows for additional space for new bone formation and decreases the load-sharing environment. Ultimate replacement with the bodies own tissue while the implant resorbs needs to be titrated with the rate of new bone ingrowth. This study evaluated the in-vivo response of three β tricalcium phosphate (β-TCP) bone graft substitutes in a bilateral tibial defect model in NZ white rabbits based on radiographic, mechanical, histomorphometry and histology.

METHODS

A bilateral defect model [1] (5 mm wide and 15 mm long) spanning the metaphyseal and diaphyseal region were created 3 mm below the joint line in the anteromedial cortex of the proximal tibia in 66 skeletally mature New Zealand white rabbits following ethical approval. Defects were created using a microburr with a 3 mm diameter tip under saline irrigation. The defects were flushed with sterile saline prior to being filled with the three different β-TCP bone graft substitutes (table 1) to the height of the original cortex. Samples were x-rayed in the A-P and M-L planes using high resolution mammography film. Tibias were embedded in Wood's metal and torsion tested to failure. The tibias were embedded in PMMA, sectioned and examined using back scattered SEM for histomorphometric analysis of bone, implant and void (n=8 per group per time point) [1]. Two samples per group per time point were processed for routine paraffin histology. Time Zero samples were examined with radiographs and SEM histomorphometry only. Historphometry and mechanical data was analysed with a 2-way analysis of variance. Radiographs and histology were qualitative assessed in a blinded fashion for implant resorption and in vivo response.

Table 1 (# of sites examined at each time point)

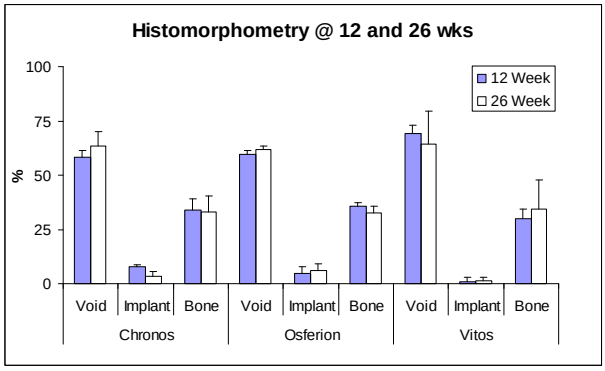
Group	0 wks	2 wks	4 wks	12 wks	26 wks
Osferion	4	10	10	10	10
Vitoss	4	10	10	10	10
Chronos	4	10	10	10	10

RESULTS

Radiographs revealed a progression in new bone formation, implant resorption and healing of the defect over time. There were insignificant changes in all materials between time zero and the 2 week time points with some evidence of new bone formation at the margins of the defect. By 4 weeks new bone formation was observed in all groups with evidence of initial resorption. Implant resorption was notable by 12 weeks in the Osferion and Vitos group and appeared to lag in the Chronos group. All defects appeared well healed by 12 weeks with new bone formation at the site. Implant resorption appeared to be complete in defects filled with Osferion and Vitos at 26 weeks while some evidence of Chronos was noted.

During torsion testing all samples failed in a spiral fashion initiated at the distal margin of the defect. The mechanical properties increased with time as the defects healed and new bone formed within the defect and as the cortex reconstituted. A decrease was noted at 12 weeks where implant resorption was well advanced and the cortex had yet to completely form.

New bone formation within the medullary canal decreased at 12 weeks presumably through a remodelling process. Osferion and Vitos were nearly completely resorbed by 12 weeks in the medullary canal with only residual materials present surround by remodelled cortical bone. Chronos on the other hand was still evident in the medullary canal and confirmed the radiographic findings. The 12 and 26 week SEMS revealed various levels of cortex reconstitution in all groups. Similar to the radiographic findings, the Chronos group appeared to lag behind the Osferion and Vitos groups which were similar.



DISCUSSION

X-rays of the raw materials at time zero revealed marked differences in the appearance and pore structure between Osferion, Vitos and Chronos. Vitos had the most open structure followed by Osferion and Chronos. The Chronos appeared the densest followed by Osferion and Vitos. SEMS from the animals revealed a progression of new bone formation as early as 2 weeks in all materials. At two weeks new bone ingrowth into the porous domains and ongrowth to the surface of the material was evident in the Osferion and Vitos materials. SEM images of Chronos at 2 weeks did not demonstrate the same ingrowth into the porous domains of the material. This may reflect a lack of interconnecting pores in this material compared to the others. The 4 week time point revealed a continued progression of new bone ingrowth and ongrowth in all materials. Implant resorption was noted to begin at this time point compared to the 2 week time point and progressed for all materials at 12 and 26 weeks.

Bone graft substitutes provide surgeons with alternatives for grafting of bony defects. The β-TCP materials examined this study provide a scaffold for new bone formation with the majority of the material resorbed by 26 weeks compared to other calcium phosphate based bone graft materials [1].

REFERENCES

[1] Walsh et al., *J Orthop Res* 21(4): 655-661, 2003.

AFFILIATED INSTITUTIONS

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•HEALING OF A CRITICAL SIZE DEFECT IN SHEEP USING BONE GRAFT SUBSTITUTES IN BLOCK FORM

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INTRODUCTION

Beta Tricalcium phosphate (β-TCP) bone graft substitutes provide a synthetic option for bone graft applications. The osteoconductive behavior of these materials relies upon a complex combination of material chemistry and overall structural makeup. Whilst the porosity of the material is indeed a vital component, the interconnectivity of the pore structure may also play a significant role in the in vivo performance. This study examined the in vivo performance of two β-TCP's with the same chemistry, porosity in a critical size defect model in sheep cancellous bone

METHODS

Bilateral defects (12.5 mm diameter and 25 mm long) were created in the distal femoral and proximal tibia on the medial aspect in 12 adult cross bred wethers. The defects randomly allocated to 3 groups; Empty defect, Osferion (Olympus, Japan) or Chronos blocks (Synthes, Switzerland) (12.5 mm diameter and 25 mm long). Animals were killed at 4, 8 and 16 weeks following surgery (4 animals per time point). The right and left femora and tibae were harvest and fixed in cold phosphate buffered formalin. Radiographic analyses included AP and lateral x-rays using a Faxitron and axial computed tomography (CT) using a Toshiba CT scanner and 1 mm slice thickness. The DICOM data obtained from CT was used to reconstruct 3 dimensional models and assess bone density across the defect based on Hounsfield units using MIMICS software (Materialise, Belgium). Fixed sections were decalcified in 10% formic acid – formalin solution and sectioned perpendicular to the long axis of the implant for paraffin embedding and histology. VEGF, CBFA-1 and PCNA protein expression was examined using immunohistochemistry. The structure of the materials was examined using micro computed tomography using a SkyScan Micro Computed Tomography scanner (SkyScan, Belgium).

RESULTS

All animals recovered uneventfully following surgery. Radiographs did not provide any insight into implant resorption or new bone ingrowth. Computed tomography confirmed the critical nature of the defect with virtually no new bone forming in the defect site at 4, 8 or 16 weeks following surgery. CTs revealed a healing implant bone interface with both graft materials. An increase in Hounsfield values was noted for Osferion at 8 weeks compared to Chronos (Fig 1). Histomorphometyr results are presented in figure 2. Histology confirmed a fibrous tissue response in the Empty defects at 4 and 8 weeks and fatty tissue by 16 weeks (Fig 3). New bone ingrowth was clearly noted in the Osferion defects at 4 weeks with ongrowth and ingrowth through the material (Fig 3). This bone ingrowth increased and matured at 8 weeks and by 16 weeks new marrow spaces had developed. In contrast, the results with Chronos lagged behind that of Osferion. Bone ingrowth was minimal at 4 weeks and increased at 8 weeks (Fig 3). The 16 week time point with Chronos presented some new marrow spaces similar to Osferion.

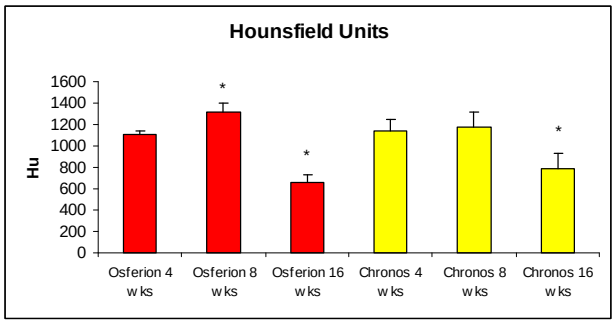

Figure 1: Analysis of the implant based on Hu revealed an increase in density for Osferion compared to Chronos between 4 and 8 week (*P<0.05). Density decreased at 16 weeks compared to 8 weeks (*P<0.05) but did not differ between Osferion and Chronos.

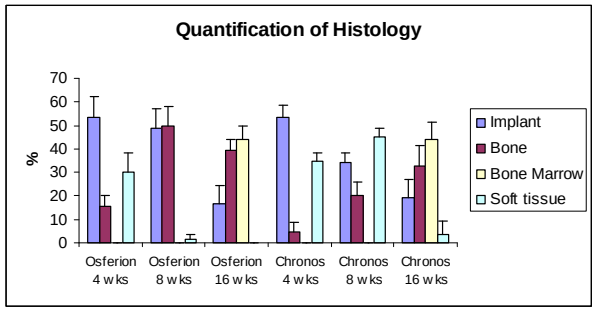

Figure 2: Significantly more bone was found in Osferion groups when compared with ChronOS groups at both 4 and 8 week (P<0.05). Eight week defects revealed significantly bone formation when compared with 4 week defects within each treatment group. Osferion outperformed ChronOS in terms of new bone formation at all time points (P<0.05). The percentage of implant versus time needs to be taken with care considering this was based decalcified histology where the implant is removed during the decalcification process. The presence of bone marrow tissue was only assessed at the 16 week time point where it was clear on the histology.

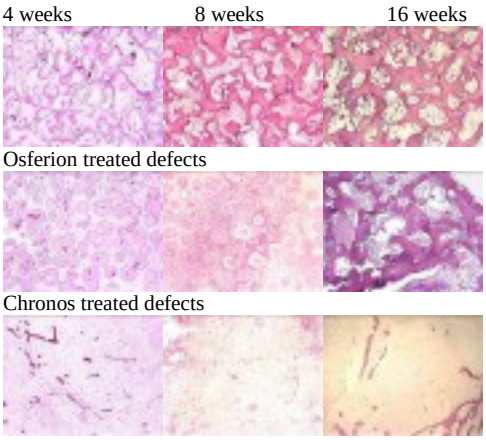

Figure 3: Histology comparison in the center of the defect versus time demonstrates the difference in osteoconductive response.

DISCUSSION

The in vivo performance of two βTCP bone graft substitutes with the same chemistry and similar porosity in block form (Osferion and ChronOS) were evaluated using radiography, computed tomography (CT) and histology endpoints. Micro CT revealed a difference in pore geometry as well as interconnectivity. Faxitron radiographs did not provide any insight into the in vivo performance of these materials and are of limited use in this type of model. Computed tomography confirmed the critical nature of the defect with virtually no new bone forming in the defect site at 4, 8 or 16 weeks following surgery. CTs revealed a healing implant bone interface with both graft materials. Analysis of the Hounsfield values from the scans revealed an increase in density for Osferion between 4 and 8 weeks while no change was detected for Chronos. This finding reflects the new bone formation within the porous domains of Osferion which was observed on histology. New bone integration into Chronos was limited between 4 and 8 weeks based on histology which was reflected in the CT analysis. The 16 week time point demonstrated a reduction in density for both materials which did not differ. Overall biocompatibility of both materials was excellent in the current study in block form with no adverse reactions. Differences in in-vivo performance most like reflects the interconnectivity between the materials and the ability for new bone formation within the porous domains early and mature with time.

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