Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 27 (2006) 1542–1549
Biomaterials
www.elsevier.com/locate/biomaterials

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

Akira Ogose[a,*], Naoki Kondo[a], Hajime Umezu[b], Tetsuo Hotta[a], Hiroyuki Kawashima[a], Kunihiko Tokunaga[a], Tomoyuki Ito[a], Naoko Kudo[a], Makiko Hoshino[a], Wenguang Gu[a], Naoto Endo[a]

[a]*Division of Orthopedic Surgery, Graduate School of Medical and Dental Sciences, Niigata University, Asahimachi 1-751, Niigata 951-8510, Japan*
[b]*Division of Pathology, Niigata University Hospital, Niigata, Japan*

Received 17 June 2005; accepted 23 August 2005
Available online 13 September 2005

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®.

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].

*Corresponding author. Tel.: +81 25 227 2272; fax: +81 25 227 0782.
E-mail address: aogose@med.niigata-u.ac.jp (A. Ogose).

0142-9612/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2005.08.034

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