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Review

β-Tricalcium Phosphate as a Bone Graft Substitute

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ABSTRACT

Autogenous bone grafts are often used to facilitate bone healing in the treatment of musculoskeletal disorders. However, graft tissue is of limited supply and is associated with donor-site morbidity. The search for useful bone-graft substitutes has focused on synthetic, biocompatible materials. Porous β-tricalcium phosphate (β-TCP) is used to treat defects of cortical and cancellous bone owing to its bioresorbability and osteoconductivity. We reviewed animal studies and clinical applications of β-TCP, which show that β-TCP used to fill bone defects is biodegraded, and present evidence of bone regeneration. Gradual replacement of the β-TCP with mature remodeling bone is usually observed. The findings suggest that rapid bone ingrowth into β-TCP porous scaffolding is influenced by its macropores, whereas bioresorption depends on the micropore interconnections. Once β-TCP is incorporated into the bone its mechanical properties are significantly altered, and mechanical strength is increased after the replacement of β-TCP with mature bone.

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Key words : β-tricalcium phosphate, bone graft substitute, synthetic material, bone defect, osteoconduction

INTRODUCTION

Bone grafts are used to facilitate bone healing, with numerous approaches available to replace or reconstruct skeletal defects resulting from trauma, infection, or tumors. Autogenous bone remains the ideal graft material because it is nonantigenic, osteoinductive, and osteogenic[1]. However, autogenous bone grafting is associated with several limitations, including the availability of graft material and donor-site morbidity. These limitations have led to a search for appropriate substitute materials for bone grafts.

Bone-graft substitutes now used include cancellous and cortical allograft bone, demineralized bone matrix (DBM), bone marrow, synthetic materials, such as calcium phosphates, and composite grafts. Most bone allografts are either frozen or freeze-dried, and the main concern with their use is the significant risk of disease transmission.

DBM is formed through acid extraction of bone, which leaves noncollagenous proteins, collagen, and bone growth factors. DBM has been used to promote bone regeneration, mainly within well-supported, stable skeletal defects. The osteoinductive ability of DBM is enhanced by bone morphogenetic protein (BMP), although the amount of BMP within demineralized grafts is far lower than if recombinant BMP were used. Nevertheless, results of clinical trials with DBM grafts have been excellent[2].

Bone marrow contains osteoprogenitor cells (1 per 50,000 nucleated cells) and has been used clinically, either alone or with an inorganic matrix[3,4]. Bone marrow can grow into ceramics, provide a

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grafting bed with osteoprogenitor cells, and augment all other synthetic grafts but would play a more important role if osteoprogenitor cells could be easily propagated.

Calcium phosphate compounds have been studied as synthetic bone fillers for the last 80 years[5]; β-tricalcium phosphate (β-TCP), hydroxyapatite (HA), and their composites have been commonly used in the form of granules or blocks. β-TCP has the same composition as α-TCP but has a different crystallographic structure. β-TCP can be produced by thermal treatment at 650°C or greater and is bioresorbable. Its bioresorption occurs via osteoclastic activity[6] and typically continues for 1 to 2 years. HA is the most stable calcium phosphate in an aqueous solution and is sometimes considered to be the most biocompatible calcium phosphate complex. It is produced by sintering stoichiometric precipitated hydroxyapatite (Ca : P molar ratio, 1.67) at temperatures higher than 700°C. Unlike β-TCP, HA is considered nonbioresorbable, since it is resorbed over decades rather than years. Biphasic calcium phosphate is a composite of β-TCP and HA which has characteristics intermediate between those of its two structural elements.

The desire to incorporate favorable properties of different materials into a single graft compound has led to various types of composite graft. A composite graft can be defined as any combination of materials that includes both osteoconductive matrix and osteogenic or osteoinductive material. For example, in a composite of β-TCP and BMP β-TCP maintains soft-tissue position and provides the osteoconductive matrix environment while BMP stimulates osteoinduction.

Since 1988, highly purified β-TCP has been continually refined, and animal studies and clinical trials with β-TCP as a bone graft substitute have been performed in our institution in collaboration with the Olympus Optical Co., Ltd. (Tokyo, Japan). In the present study, the characteristic features and usefulness of highly purified β-TCP as an alternative to autogenous bone grafting are reviewed.

FORMATION OF HIGHLY PURIFIED β-TCP

β-TCP powder was synthesized with the following wet-milling method. A slurry of calcium carbonate, calcium hydrogenphosphate powder (at a molar ratio of 1 : 2), and distilled water was prepared in a pot mill for 24 hours. By drying the slurry at 80°C, a calcium-deficient HA powder was obtained. Calcining this powder at 750°C to 900°C allowed conversion of the crystal phase to β-TCP. Next, a porous body was formed. A foaming slurry was prepared by mixing β-TCP powder, pure water, and a foaming agent. A green body of a porous structure was then formed by drying the foaming slurry at 40°C for 24 hours, and a porous body of β-TCP (Ca : P molar ratio, 1.50) was obtained by sintering the green body at 1,050°C for 1 hour.

Porous β-TCP has a homogenous and fenestrated macropore distribution with a pore size of 100 to 400 μm, a porosity of 75%, and numerous micropore interconnections about 1 μm in diameter (Fig. 1).

ANIMAL STUDIES WITH β-TCP

The dog cancellous defect model : Cylindrical cancellous bone defects were created at the proximal tibial metaphyses in beagles, after which granular β-TCP was implanted into the defects[7]. Dog tibiae were harvested at 2, 6, 12, and 24 weeks after im-

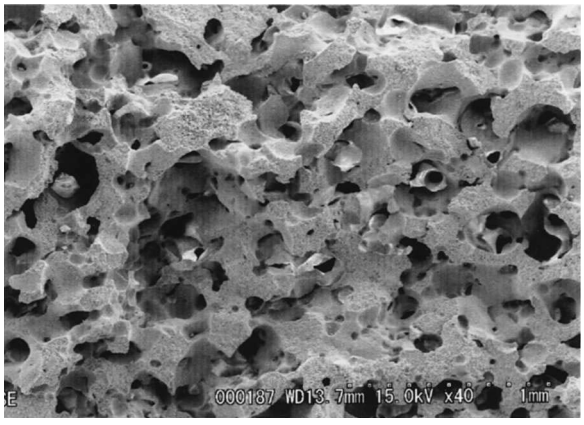

Fig. 1. Scanning electron micrograph of β-TCP showing fenestrated macropore distribution with a pore size range of 100 to 400 μm and micropore interconnections with diameter of about 1 μm (×40).

plantation, and microradiographic and histologic studies were carried out. A large volume of bone ingrowth into the porous β-TCP was observed 6 weeks after implantation, whereas bone regeneration had decreased and mature bone trabeculae were detected 12 weeks after implantation. The volume of implanted β-TCP decreased with time. The degradation rate was high at early stages but decreased once β-TCP had become surrounded by newly formed bone. The bond between the regenerated bone and β-TCP was tight, without fibrous tissue or inflammatory cells, indicating successful incorporation of the synthetic β-TCP into cancellous bone defects. Bone regeneration and bioresorption of β-TCP occurred during bone remodeling, and β-TCP was finally replaced by new bone.

The rabbit cortical defect model: Blocks of β-TCP and HA were implanted into cortical bone defects in the diaphyses of rabbit tibiae[8]. The tibiae were harvested 8 to 24 weeks after implantation and examined histologically and biomechanically. The β-TCP had been partially bioresorbed and replaced by regenerated bone tissue 8 weeks after implantation. Sixteen weeks after implantation, the β-TCP had largely degraded, with only 7.8% of the original implant volume remaining. In contrast, although bone ingrowth into HA pores was observed, HA was poorly biodegraded, with 44.6% of the original implant volume remaining. Twelve to 16 weeks after implantation, bone defects filled with β-TCP had a higher maximum crack-strength on bending tests and occupied larger areas under the strain-stress curve (equal to destructive energy) than did defects filled with HA. Maximum endurable crack-strength of a cortical defect filled with β-TCP was as much as 87% of that of normal tibiae 24 weeks after implantation. Thus, β-TCP with its porous scaffolding permitted rapid bone ingrowth and was gradually resorbed and replaced by mature remodeling bone. Bone defects filled with β-TCP were mechanically stronger than were defects filled with HA. Initially, the porous β-TCP was mechanically weaker than cancellous bone; however, after implantation in the tibial diaphysis, β-TCP and the regenerated bone exhibited significantly different mechanical properties. The changes in mechanical strength were probably due to replacement of β-TCP in mature bone and further remodeling of the bone into a denser scaffolding. β-TCP should thus be considered a useful bone substitute for repairing cortical bone defects that must bear weight.

Characteristics of β-TCP

β-TCP is a synthetic material composed of tricalcium phosphate ($Ca_3[PO_4]_2$). It is produced as porous blocks and granular particles with pores (Osferion; Olympus; Fig. 2). Porous β-TCP is created with a high-temperature process called sintering. A pore size of greater than 100 μm is thought to be necessary for bony ingrowth[9]; pore interconnections are believed to facilitate the invasion of blood vessels[10,11] and subsequent bioresorption of β-TCP[12]. Material factors, such as surface area, also affect bioresorption; in general, the larger the surface area, the greater the bioresorption. Therefore, dense blocks with a small surface area biodegrade more slowly than do porous implants. Thus, the shape and architecture of a material have a profound effect on resorption rate.

Early in the bone regeneration process initiated by cancellous bone, undifferentiated osteoprogenitor cells are recruited. These cells later differentiate into chondrocytes and osteoblasts by osteoinduction, and, finally, a suitable scaffold is established, on which active osteoprogenitor cells produce new bone (osteoconduction)[1]. In other words, three elements are ideally required for bone regeneration: osteogenic cells, osteoinduction, and osteoconduction.

Osteogenic cells: Osteogenic cells can differentiate and facilitate various stages of bone regeneration. Within the living cancellous bone, bone marrow contains osteoprogenitor cells and numerous

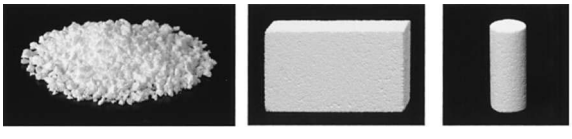

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Fig. 2. Various types of porous β-TCP. A. Granular type. B. Block type. C. Cylinder type.

stromal cells, which are indispensable for osteogenesis.

Osteoinduction : Osteoinductive factors are chemical agents that induce various stages of bone regeneration and repair. The process is mediated by numerous growth factors produced by the bone matrix itself. The most notable of these growth factors are BMPs, which initiate and augment bone regeneration, leading to completion of the bone formation process. Transforming growth factor β, which is closely related to BMP, appears to stimulate bone formation in a similar manner. Fibroblast growth factors are angiogenic factors important in neovascularization, and platelet-derived growth factor acts as a local tissue-growth regulator. Other important growth factors produced by the bone matrix include insulin-like growth factors and microglobulin β[13].

Osteoconduction : The osteoconductive matrix is a nonviable scaffolding that creates an appropriate environment for bone ingrowth ; HA and collagen are well suited to serve as an osteoconductive framework.

The three factors mentioned above (osteogenic cells, osteoinduction, and osteoconduction) are provided by autogenous cancellous bone grafts but are somewhat limited in autogenous cortical bone grafts ; the latter also provide structural integrity important for reconstruction of larger bone defects.

The first successful clinical trials with β-TCP were reported in dentistry and reconstructive craniofacial surgery. In orthopedics, Bucholz et al.[14] demonstrated that tricalcium phosphate is comparable to autogenous bone for filling defects due to trauma, benign tumors, and cysts. Studies performed by other authors, including Altermatt et al.[15], have shown that granular HA and β-TCP are effective, particularly when used in bone defects. However, β-TCP undergoes biologic degradation 10 to 20 times faster than does HA[16]. The porous scaffolding of β-TCP permits rapid bone ingrowth, and gradual replacement of the ceramic with mature remodeled bone occurs simultaneously with bioresorption. Thus, β-TCP serves as an excellent framework for incorporation of connective tissue and, subsequently, regenerated bone. It is entirely biocompatible and allows direct bonding between itself and ingrowing bone without an intervening fibrous capsule.

β-TCP appears to have no early adverse effects, and foreign-body responses to ceramics are rare when the structure is preserved[14,15,17]. However, small granules of β-TCP may elicit a foreign body-giant cell reaction and partial resorption[6].

CLINICAL APPLICATION OF β-TCP

In 2000, Ozawa et al.[18] reported 167 cases in which β-TCP was implanted as a bone graft substitute. The patients' average age at surgery was 40 years (range, 3 to 78 years). All patients had osseous defects caused by benign bone tumor (87 cases), fracture (40 cases), had undergone revision of total hip arthroplasty (34 cases), or had surgery for other reasons (6 cases). Supplemental autogenous bone was grafted with a mixture of β-TCP in 50 cases, and supplemental allografts were applied in 15 cases. In 2001, Morikawa et al.[19] reported 190 cases in which β-TCP was implanted to defects of weight-bearing bones. Defects were located in the femur (71 cases), acetabulum (62 cases), tibia (36 cases), or foot (21 cases). The patients had had hip osteoarthritis (69 cases : revision total hip arthroplasty in 47 cases and shelf operation or osteotomy in 22 cases), benign bone tumor (51 cases), fracture or nonunion (38 cases), avascular necrosis of the femoral head (30 cases), or osteomyelitis (2 cases). Supplemental autogenous bone grafts were performed with a β-TCP mixture in 134 cases. Sai et al.[20] reported 13 cases of distal radius fractures with comminution of both the dorsal and palmar bone cortices. The patients, 7 men and 6 women with an average age of 53 years (range, 27 to 71 years), had large metaphyseal bone defects complicated by extremely unstable distal radius fractures. The large bone defects were filled with only β-TCP following stabilization with an external fixtator and percutaneous Kirshner-wire fixation. In 2002, Asanuma et al.[21] reported on 101 patient in whom β-TCP was implanted into defects after curettage of benign tumors. The patients, 53 male and 48 female, with an average age of 34 years (range, 5 to 70 years), had enchondroma (38 cases), solitary bone cyst (13 cases), fibrous dysplasia (12 cases), giant cell tumor of

bone (10 cases), chondroblastoma (4 cases), or other tumors (24 cases). In other studies, β-TCP was used to treat tibial plateau fractures (21 cases)[22] and applied to rotational acetabular osteotomy (5 cases)[23]. In all cases, fracture union, bone regeneration, and resorption of β-TCP was monitored radiologically. Radiologic evidence of β-TCP biodegradation was present in all cases, and β-TCP remained radiodense for several months. The rate of radiographic disappearance of β-TCP was variable and seemed to depend upon the size of the bone defect, the volume of β-TCP implanted, and the density of the β-TCP grafts. The β-TCP-regenerated bone composite blended with the surrounding medullary and cortical bone without evidence of radiolucent lines at the interface. Finally, β-TCP was bioresorbed and replaced by mature bone followed by bone remodeling. A morphologic gradient was evident, with more osteonic regenerated bone within the cortical portion and trabecular bone within the medullary portion (Fig. 3).

Functional results were comparable to those of autogenous bone grafts that were used as controls (Fig. 4). No adverse reactions to β-TCP, such as excessive postoperative drainage, erythema, and other wound problems, were found in the early postoperative period. Six major complications occurred in these clinical series : 4 recurrences of bone tumors (2 bone cysts, 1 giant cell tumor, 1 desmoplastic fibroma) and 2 infections. None of these complications was attributable to β-TCP. When β-TCP was placed outside the periosteum or in subcutaneous tissue, it showed no bone ingrowth. Biodegradation appeared quickly and occurred by both passive dissolution and osteoclastic resorption.

β-TCP Resorption and Bone Formation

The bioresorption and osteoconduction of β-TCP depend greatly on its physical and chemical properties, but also on its structural parameters, such as the size and width of pores and the integrity of partition walls. Bhaskar et al.[24] first reported β-TCP biodegradation, and their results were confirmed by others[25-27]. Resorption rates depend on the manufacturing method, porosity, implantation site, and animal species used in the experiments. Peelen et al.[27] reported a new aspect of bioresorption by describing the formation of micropores (micrometers in diameter) resulting from the incomplete fusion of particles during the sintering process. These micropores were suggested to determine the rate of degradation independently of the chemical composition of the ceramic and thus were considered ultimately responsible for biodegradability[12]. The pore interconnections seem to promote vascular and nutritional supply

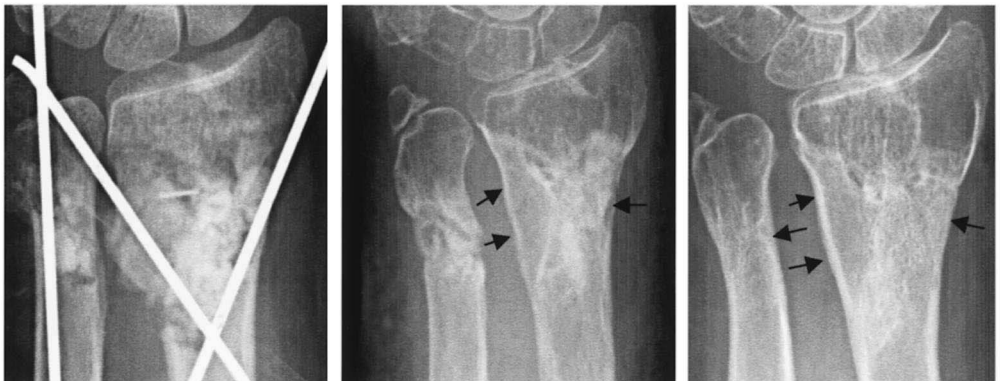

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Fig. 3. Postoperative radiologic evaluation of β-TCP (severely comminuted distal radius fracture in a 78-year-old woman)[20]. A. Immediately after surgery. Both the dorsal and palmar bone cortices were severely comminuted, and β-TCP was used to fill fracture voids of the radius and ulna. B. Radiograph 4 months after surgery shows that the grafted β-TCP has biodegraded from its periphery and that disrupted cortical bone has been restored (arrows). C. The grafted β-TCP was almost completely bioresorbed, and the cortex (arrows) and cancellous bone were reconstructed by bone remodeling 1 year after surgery.

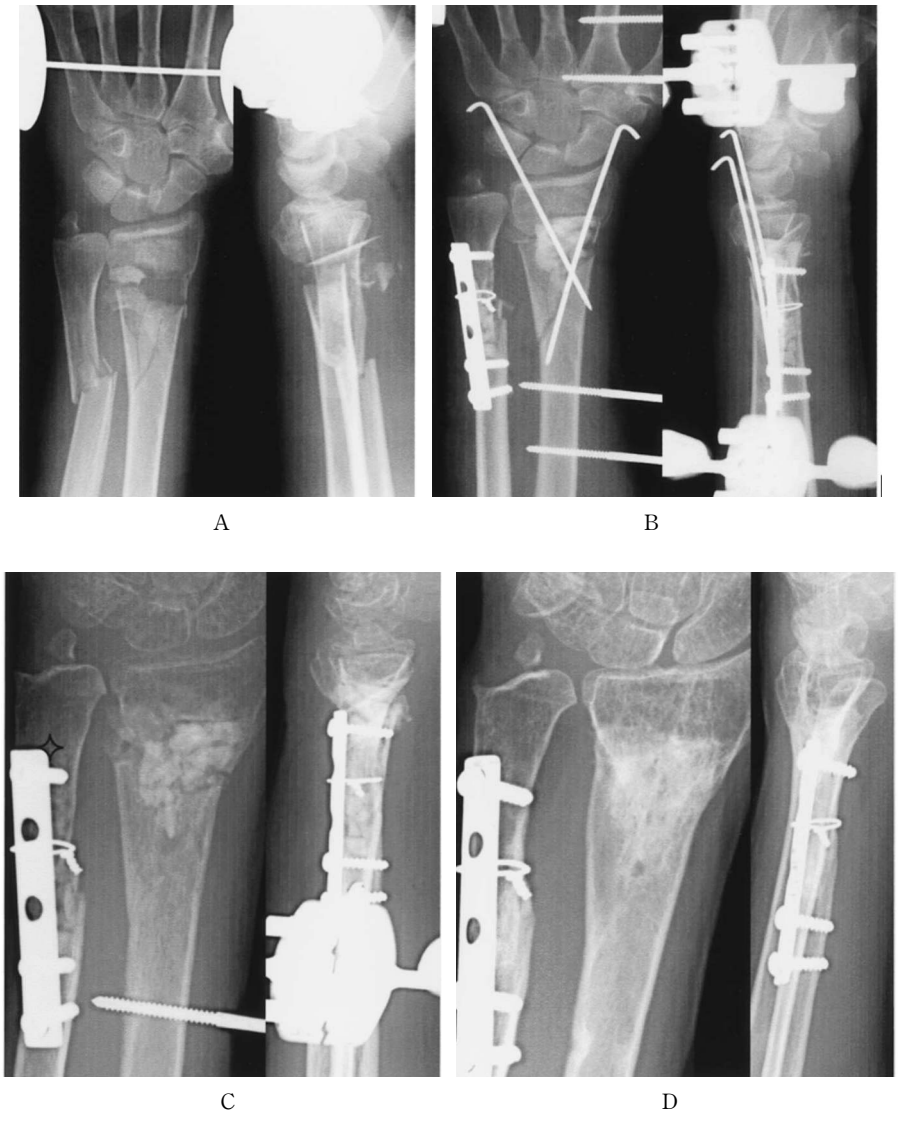

Fig. 4. Distal radius fracture with comminution of both the dorsal and palmar bone cortices in a 71-year-old woman[20]. A. Before surgery. B. Immediately after surgery. Fixation was achieved with an external fixtator, percutaneous Kirshner-wire fixation for the radius fracture and with a plate and wiring for the ulna. Corticocancellous defects were filled with β-TCP. C. After 2 months, partial β-TCP resorption and regeneration of bone trabeculae were seen. D. Radiographs 1 year after surgery demonstrate excellent remodeling of the regenerated bone with bioresorption of β-TCP.

and tissue ingrowth and consequently control the initial rate of implant resorption. In 1988, Eggli et al.[6] reported that the rate of β-TCP degradation is related to the rate of its tissue penetration and, therefore, to the amount of implant surface exposed to invading cells. The authors also found that acid phosphatase-positive osteoclast-like cells by tartrate-resistant acid phosphatase reaction are in contact with the pore wall before bony ingrowth. Studies by Ogose et al.[28] and Chazono et al.[29] confirmed this phenomenon. These osteoclast-like cells invade the tricalcium phosphate matrix together with blood vessels and other tissue elements and initiate active breakdown of the matrix. Small particles detached from the implant are phagocytosed by mononuclear macrophages[6]. These macrophages are seen within the pores or on the ceramic surfaces and are assumed to be able to dissolve the graft material

intracellulary[24]. Therefore, it has become clear that implant resorption involves two different cell types. Acid phosphatase-positve osteoclast-like cells adhere directly to the graft surface. Clusters of macrophages, tightly packed with granular material, gather in pores and along the perimeter of the implant and may play a role in the intracellular degradation of small, detached ceramic particles.

On the other hand, macropores have also been suggested merely to influence bony ingrowth[30]. The rate and range of ingrowth depend mainly on the pore size and conductive properties of the material. Klawitter and Hulbert[31] have reported that bone grows within porous calcium aluminate ceramics only when the interconnecting pore size exceeds 100 μm. However, their later studies indicate that porous polyethylene allows bone growth within pores as small as 40 μm[9]. The β-TCP used in our department was completely replaced by regenerating bone; its macropores ranged in size from 100 to 400 μm, and the micropore interconnections had a diameter of about 1 μm. Bone tissue was observed to be directly deposited upon the β-TCP surface.

Mechanical Properties of β-TCP

β-TCP is brittle and has very less tensile strength. Applications requiring significant impact, torsional, bending, or shear stress seem impractical. However, the mechanical properties of porous β-TCP are comparable to those of cancellous bone once the material has been incorporated and remodeled. β-TCP must be protected from loading forces until bone ingrowth occurs. Rigid stabilization of the surrounding bone and avoidance of weight-bearing are required during this early period because the β-TCP tolerates minimal bending and torque loads. However, once mechanical strength has changed during bone remodeling and β-TCP has been replaced in mature bone, weight-bearing can be permitted.

Osteoinduction and Future Studies

There is no evidence that porous β-TCP is osteoinductive. However, β-TCP is considered osteoconductive, because it allows bone ingrowth from an osseous bed. Bone ingrowth does not occur if an implant is inserted into muscle or subcutaneous tissue. If β-TCP is to serve as more than just an osteoconductive scaffold, adjunctive osteoinductive factors must be provided. Studies of the efficacy of osteoinductive proteins (growth factors) and bone marrow aspirates (osteoprogenitor cells) in enhancing bone regeneration into porous ceramics must follow.

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