Biological Response to β-Tricalcium Phosphate/Calcium Sulfate Synthetic Graft Material: An Experimental Study

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Purpose: *The aim of this study was to evaluate the effect of a biphasic synthetic bone graft material composed of β-tricalcium phosphate (β-TCP) and calcium sulfate (CS) in 12 New Zealand rabbits.*

Materials and Methods: *A circular bicortical critical-size cranial defect was created in each of 12 rabbits. The defects were grafted with β-TCP/CS. Animals were euthanized at 3 and 6 weeks. Harvested tissue specimens were evaluated histologically and histomorphometrically. Parameters associated with new bone formation and graft resorption were measured and calculated. The results were statistically analyzed using the Mann-Whitney test.*

Results: *Our data demonstrated the biocompatibility of synthetic β-TCP/CS, as no inflammatory response was observed, and no fibrosis was developed between the graft particles and the newly formed bone. Moreover, β-TCP/CS acted as an osteoconductive scaffold that allowed a significant bone regeneration and graft biodegradation with time.*

Conclusion: *In this animal model, synthetic β-TCP/CS proved to be a biocompatible, osteoconductive, and bioresorbable bone graft substitute. (Implant Dent 2014;23:37–43)*

Key Words: β-TCP, calcium sulfate, osteoconduction, synthetic grafts

Clinical and experimental studies have shown that tooth extraction inevitably leads to atrophic changes of the alveolar ridge.[1,2] An average of 40% to 60% of original height and width is expected to be lost, with the greatest loss occurring within the initial 3 months.[1,3] This may render difficult, or sometimes impossible, subsequent rehabilitation with dental implants as the residual bone volume may be insufficient for the placement of an implant in an ideal 3-dimensional position.

The most predictable way to maintain the alveolar bone and the architecture of the residual ridge is preservation at the time of tooth extraction by grafting the postextraction socket with a bone grafting material[4] (socket grafting) or immediate implant placement with or without grafting.[5] This concept led to the development of many techniques[3,4,6–8] during the past 2 decades, and today a large number of grafting materials are available. Among bone grafts, autogenous bone is still considered to be the gold standard.[9] Autografts possess osteogenetic, osteoinductive, and osteoconductive properties, they do not transmit diseases, they do not cause immune reactions, whereas they are gradually absorbed and replaced by newly formed osseous tissue. However, restricted availability, postoperative complications at the donor site, and extended operating time are the drawbacks that limit their scope of application.[10,11] As an alternative solution, bone grafting substitutes such as allografts, alloplasts, and xenografts are widely used and documented.[12–18] It is of great clinical importance that these bone substitutes vary in terms of origin, composition, and biological mechanism of function regarding graft resorption and new bone formation, each having its own advantages and disadvantages.

Alloplasts represent a group of synthetic biocompatible bone substitutes that are free of any risk of

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transmitting infections or diseases by themselves, and their availability is unlimited.[18,19] One of the most promising groups of synthetic bone substitutes are calcium phosphate ceramics, and among them hydroxyapatite (HA) and tricalcium phosphate (TCP) are the most commonly used. Both these materials had been used in the 1970s in dental applications, but there was little real understanding of their properties and great variation in function.[20] de Groot,[21,22] in the 1980s began the search to understand β-TCP more with extensive research into particle size and porosity. Beta TCP has a compressive strength similar to that of cancellous bone and undergoes resorption over a 6 to 18-month period and replacement by newly formed vital bone[9] dependant on variation in patient physiology. Several clinical and experimental studies have confirmed the osteoconductive potential of β-TCP.[11,19,23–31] Zhao et al,[32] evaluating the molecular mechanisms and cellular events associated with bone formation, bioresorption, regeneration, and healing of β-TCP after its implantation showed that β-TCP enhances bone healing processes and stimulates the coordinated actions of osteoblasts and osteoclasts, leading to bone regeneration.

To further enhance its biological and mechanical performance, β-TCP has been combined with other compounds such as calcium sulfate (CS).[33,34] CS is of great benefit being bacteriostatic.[35] It also increases the porosity of the grafting material by its early resorption, which facilitates the circulation of biological fluids and growth factors. It has been shown that by mixing CS with other bone grafting materials, osteogenesis is accelerated by accomplishing increased calcification and quantity of new bone in a shorter period of time.[11] CS acts as a bioabsorbable barrier that prevents epithelial down growth during new bone formation, which makes it ideal for using as an adjunct with other graft materials in guided bone regeneration.[36–40] Moreover, it binds and enhances graft containment, making the mixture more stable and pressure resistant.[11] The improved stability throughout the graft material seems to further improve the quality of the bone to be regenerated due to reduced micromotion of the material, which may lead to mesenchymal differentiation to fibroblasts instead of osteoblasts.[41]

Table 1. Histomorphometric Parameters

Parameter	Abbreviation	Units
Tissue area	TAr	mm^2
Bone area	BAr	mm^2
Graft area	GrAr	mm^2
Fibrous tissue area	FbAr	mm^2
Osteoid area	OAr	mm^2
Osteoid perimeter	OPm	mm
Osteoblast perimeter	ObPm	mm
Osteoclast perimeter	OcPm	mm
Number of osteoblasts	NOb	Cardinal number
Number of osteoclasts	NOc	Cardinal number
Bone volume/tissue volume	BV/TV	%
Osteoid volume/bone volume	OV/BV	%
Graft volume/tissue volume	GrV/TV	%

The table shows the histomorphometric parameters measured, quantified and calculated, and their abbreviations. These parameters are associated with new bone formation or graft resorption.

Fortoss Vital (Biocomposites, Staffordshire, United Kingdom) is a biphasic synthetic bone graft consisting of β-TCP in a CS matrix. This novel graft material has an increased negative isoelectric charge [zeta potential charge {ZPC}] in an aqueous solution which has been shown to upregulate the host response by attracting significantly increased presence of positively charged host bone morphogenetic proteins to the site. These in turn result in the increased presence of osteoblasts to the site for improved early bone regeneration.[42] In our previous study,[43] we suggested that Fortoss Vital possesses osteogenic activity and can support new bone formation in surgically prepared rabbit mandibular osseous defects. Our histological findings demonstrated the gradual resorption of the material and by the end of the 5th week, the osseous cavities grafted with Fortoss Vital were occupied mostly by newly-formed woven bone and in some areas by mature lamellar bone. At this stage, masses of the material were still apparent. These granules seemed to be gradually limited in favor of the ingrowth of bone tissue and by the end of the observation period after 6 weeks, only few residual traces of Fortoss Vital could be detected, whereas most of the material was replaced by creeping lamellar mature bone.

To our knowledge there are no reports of the biologic activity of β-TCP/CS for the treatment of osseous defects using the rabbit cranial model. The aim of this study was to investigate the biocompatibility and the dynamics

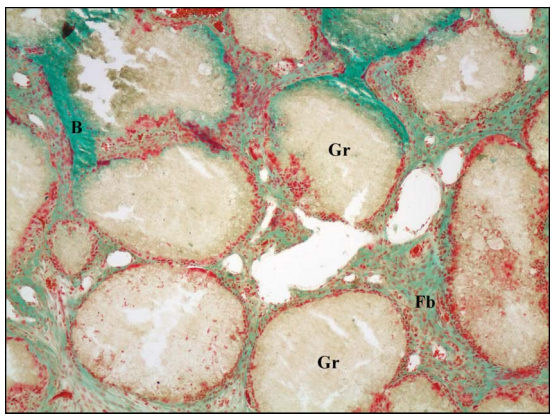

Fig. 1. Representative histologic microphotograph of defect filled with β-TCP/CS at 3 weeks. Goldner's trichrome staining showing newly formed mineralized bone (B), remaining graft particles (Gr), and fibrous connective tissue (Fb). Original magnification ×10.

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of resorption and bone replacement of a synthetic bone graft material made up of β-TCP and CS (Fortoss Vital), placed in critical-size cranial bone defects in rabbits.

MATERIALS AND METHODS

Animals

Twelve adult male New Zealand white rabbits, each weighing 3 kg (±250 g), were used in this study with the approval of the Institutional Animal Care and Use Committee of the Veterinary Department of Athens Prefecture. The animals were fed a balanced rabbit diet and caged individually in a standard manner at the animal research facility "N. S. Christeas", Medical School, University of Athens, Athens, Greece. All animals were allowed 7 days from their arrival to the facility to be acclimatized to their new environment.

Surgical Protocol and Experiment Design

Experimental animals received general anesthesia by orotracheal intubation. A semicircular incision was made in the skin over the top of the cranial vault, and a cutaneous-periosteal flap was raised and reflected. A 15-mm bicortical[44] circular critical-size defect was prepared in the calvaria of the rabbits with a round bur under copious irrigation. The use of the round bur preserved the dura mater, which is strong enough to prevent a prolapse of brain tissue into the osseous defect.[45] The defects were grafted with β-TCP/CS (Fortoss Vital). The soft tissues were then sutured in layers. Each experimental animal received antibiotics [30 mg/kg of Zinadol every 24 hours {GlaxoWellcome, Athens, Greece}] and analgesics (15 mg/kg of Depon; Bristol-Myers Squibb, Athens, Greece) for 2 days postoperatively. The postoperative course of all animals was uneventful.

Six animals were killed at 3 and 6 weeks postoperatively with an intravenous injection of sodium thiopental (100 mg/kg of Pentothal; Abbott Hellas, Athens, Greece), and the calvaria bones were excised.

Histological and Histomorphometric Evaluation

The surgically acquired samples were fixed in 10% neutral buffered formalin. After that, specimens were placed in alcohol and methyl methacrylate and plasticized by hot polymerization. Finally, nondecalcified sections were obtained and stained with Goldner's trichrome. Sections were first examined histologically using an optical microscope, under blind conditions. The slides were then placed in a semiautomated histomorphometric measurement system. Histological images were digitized, and histomorphometric parameters regarding the percentage of new-formed bone and the percentage of remaining graft material volume were measured, quantified, and calculated on a computer by means of specialized software (Osteomeasure, Interactive measurement system for bone histomorphometry; Osteometrics, Atlanta, GA). The measurements are presented in Table 1.

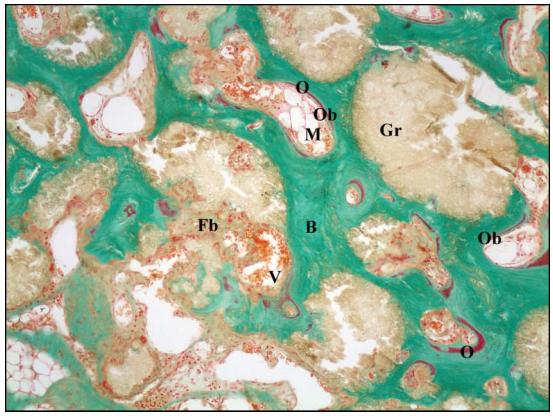

Fig. 2. Representative histologic microphotograph of defect filled with β-TCP/CS at 6 weeks. Goldner's trichrome staining showing newly formed mineralized bone (B), osteoid (O), ream of osteoblasts (Ob), remaining graft particles (Gr), capillary blood vessels (V), marrow (M), and fibrous connective tissue (Fb). Original magnification ×10.

Statistical Analysis

The first step in analyzing the data was to examine the normal distribution of the data (Kolmogorov-Smirnov test and P-P plots). The Mann-Whitney test was used for groups with data that were not normally distributed. All tests were 2-sided. *P*-values of <0.05 were set as the level of statistical significant difference. All analyses were performed using the SPSS version 16.00 (SPSS Inc., Chicago, IL).

RESULTS

Histological Findings

All sections were examined using an optical microscope under "blind"

Table 2. Comparison of Histomorphometric Parameters at 3 and 6 Weeks Postoperatively

Parameter	At 3 wk (N = 6)	At 6 wk (N = 6)	$P < 0.05$
TAr	3.23 (3.23–3.23)	3.23 (3.23–3.23)	NS
BAr	0.85 (0.75–0.95)	1.24 (0.19–1.30)	0.065
GrAr	0.15 (0.14–0.16)	0.05 (0.01–0.10)	0.002
OAr	0.02 (0.02-0.02)	0.09 (0.01–0.21)	0.065
FbAr	0.11 (0.05–0.17)	0.09 (0.03–0.70)	NS
OPm	3.22 (1.70–4.73)	7.63 (0.61–17.89)	0.065
ObPm	4.08 (0.63–7.52)	3.74 (1.25–6.82)	NS
OcPm	1.92 (0.80–3.03)	3.68 (1.86–5.10)	NS
NOb	313.00 (41.0–585.0)	254.00 (98.0–475.0)	NS
NOc	39.50 (17.0–62.0)	73.50 (38.0–98.0)	0.015
BV/TV	26.28 (23.12–29.44)	38.47 (5.98–40.23)	0.065
OV/TV	0.59 (0.56–0.63)	2.70 (0.19–6.37)	0.065
GrV/TV	4.54 (4.25–4.84)	1.67 (0.20–2.97)	0.002

All values are presented as median (min-max). N indicates number of samples; NS, no statistically significant difference. The table shows statistically significant differences in bone regeneration and graft resorption between the 2 time points of observation.

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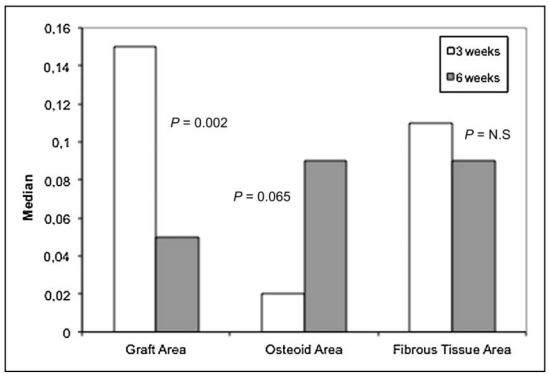

Fig. 3. Median values for graft area, osteoid area, and fibrous tissue area at 3 and 6 weeks of observation. Note the statistically significant material resorption and new bone regeneration.

conditions. At 3 weeks after implantation, the sites were dominated by remaining graft granules that appeared as amorphous masses embedded in newly formed fibrous interstitial connective tissue and newly formed lamellar bone. The interstitial connective tissue (provisional matrix) contained numerous mesenchymal cells. In all specimens, no inflammatory response of note was observed (Fig. 1).

At 6 weeks after implantation, the remaining graft material appeared as surrounded by denser newly formed lamellar bone with osteons. At sites marrow, capillary blood vessels, cellular provisional matrix, reams of osteoblasts, and osteoid were apparent. The residual graft particles were readily apparent as bone-encased or incorporated refractile material. In all specimens, no inflammatory response of note was observed (Fig. 2).

Histomorphometric and Statistical Analysis

Histomorphometric analysis and comparison of parameters at 3 and 6 weeks postoperatively are shown at Table 2 and Figure 3. Between time points of observation, there was statistically significant increase for most histomorphometric parameters associated with new bone (BV/TV, OV/TV, BAr, OAr, OPm). However, a decrease in parameters regarding the number (Nob) and perimeter of osteoblasts (ObPm) was observed, although in a nonstatistically significant matter. Parameters that express the remaining graft material (GrV/TV, GrAr,) showed a statistically significant decrease between 3 and 6 weeks, and the number of osteoclasts was increased in a statistically significant matter. The parameter that express the remaining fibrous connective tissue (FbAr) showed a decrease between 3 and 6 weeks, although in a nonstatistically significant matter.

Based on the above-mentioned findings, it can be concluded that there was an increased amount of newly formed mineralized bone with time with statistically significant differences between the time periods. In parallel, statistical differences were found regarding the gradual resorption of the graft material with time.

DISCUSSION

Bone grafting in implant dentistry arose from clinical need about 30 years ago, and implant surgeons have in their armamentarium a wide variety of grafting materials of biologic and/or synthetic origin.[46] As different types of grafting materials present different resorption characteristics, there is a question as to whether the long-term presence of residual graft particles may affect the quality of the regenerated bone, which is of paramount importance in successful implant therapy.[12,47] The presence of residual graft particles might interfere with normal bone healing and bone remodeling, affects the trabecular architecture of the bone, and it is possible to decrease bone-to-implant contact.[1] In a systematic review by Chan et al,[1] the authors reported conflicting results with the use of xenografts, with changes in the percentage of vital bone ranging from −22% (decrease) to 9.8% (increase), whereas considerable residual HA and xenograft particles (15%–36%) remained at a mean of 5.6 months after socket augmentation procedures. Eggli et al[48] in a case control study reported a TCP resorption of 85% compared with 5.4% for HA 6 months after implantation in cancellous bone in rabbits. Galindo-Moreno et al,[49] evaluating bone core biopsies taken at 6 months after sinus grafting reported minimal xenogeneic graft absorption at this time point, whereas a similar study[50] showed that HA particles of bovine or synthetic origin were observed in sinus biopsy cores 12 months after grafting. In contrast, the pure phase β-TCP seems to be completely resorbed simultaneous with bone formation and is replaced by vital host bone without residual graft particles within 6 to 12 months.[11,13] Artzi et al[51] found total resorption of β-TCP particles compared with inorganic bovine bone 24 months postsurgery in a canine model.

The grafting material used in this study was Fortoss Vital, a biphasic synthetic bone graft consisting of β-TCP in a CS matrix. It acts as an osteoconductive scaffold for bony proliferation as it is slowly resorbed by osteoclastic activity and substituted by living bone cells that grow directly in contact with the material. One of the most influential factors in the resorption process of β-TCP has been found to be the macroporosity and microporosity that promotes the ingrowth of blood vessels and enables osteocyte dendrites to infiltrate the micropores. The CS matrix is pyrogen-free and bacteriostatic, creating a nano-porous cell occlusive membrane that prevents the early stage invasion of unwanted soft tissue cells. The product forms a, simple to use, moldable cohesive paste that sets to form a hard, yet resorbable, osteoconductive bone graft material. In this experimental study, this biomaterial displayed excellent handling characteristics as it formed an easy to apply moldable paste that turned into a stable hard form *in situ*. Our study confirms the biocompatibility of Fortoss Vital, as no inflammatory response was observed and no fibrosis was developed between the graft particles and the bone. Moreover, our results showed that β-TCP/CS can promote new bone

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formation in parallel with graft resorption. Studying the histomorphometric parameters, significant results were observed with 26.28% and 38.47% median values for new bone volume at 3 and 6 weeks, respectively after implantation and 4.54% and 1.67% remaining graft volume, respectively at the same time points.

In 2009, Podaropoulos et al[11] evaluated Fortoss Vital in comparison with pure β-TCP in surgically prepared bone defects on the iliac crest of Beagle dogs. The authors reported that the use of β-TCP/CS produced significantly more vital bone fill and preserved bone dimensions compared with the use of β-TCP alone at 4 months after implantation. The remaining graft volume at 4 months in the β-TCP/CS group was measured at 21.62%, which is in contrast to our findings (1.67% at 6 weeks). This marked difference may be attributed to the different animals used, as bone healing seems to vary widely between different species and also to the different type of bone where the defect was created. It is of great importance that this study and the study by Podaropoulos et al[11] evaluated the same β-TCP material provided by the same manufacturer. That means that any differences to the above findings cannot be ascribed to manufacturing variables of β-TCP such as processing, mixing, or sintering that may affect the material's mechanical and biological behavior.[33] It has to be stated that a common confounding factor in generic studies that compare pure β-TCP with other biphasic calcium phosphate materials is that they test grafts from different companies, so that the β-TCP contained in each tested commercially available material may vary in porosity or geometry so that they performed in a different way.

Fortoss Vital has been also evaluated in the surgical treatment of periodontal intrabony defects. Sukumar et al[34] in a clinical study showed that the treatment with β-TCP/CS led to a significantly favorable clinical improvement with reduction of probing depth and gain in clinical attachment level 2 years after surgery. The authors reported that the specific graft material was easy to use and offered the possibility to treat periodontal intrabony defects spanning more than 2 teeth, the use of a membrane was not required, thus reducing surgical time and cost. In a randomized controlled clinical trial, Stein et al[52] showed that the clinical benefits of β-TCP/CS were equivalent to autogenous bone spongiosa and superior to open flap debridement for the treatment of periodontal intrabony defects at 12 months posttreatment.

Other recent studies have also attempted to assess the effectiveness of β-TCP/CS synthetic grafts in bone surgery, reporting positive results. Yang et al[53] evaluated the performance of a synthetic β-TCP/CS graft in a sheep vertebral bone defect model with microcomputer tomography (micro-CT) analysis, histological examination, histomorphometry, and mechanical testing under compression. The authors showed the bone regenerative capacity and the biodegradation properties of the material at 8, 16, and 36 weeks postimplantation. Smeets et al[35] used synthetic β-TCP/CS with a negative surface charge (negative ZPC) for sinus floor augmentation. Six months postoperatively, a core biopsy was taken before implant placement, and the authors analyzed bone neo-formation and graft resorption by histology, bone density by CT, and measured the activity of voltage-activated calcium currents of osteoblasts and surface charge effects. They reported that the β-TCP/CS material was biocompatible and replaced by new mineralized bone after being resorbed completely, whereas the negative surface charge of the graft was found to be favorable for bone regeneration and osseointegration of dental implants.

CONCLUSION

This study demonstrated that Fortoss Vital is biocompatible, possesses osteoconductive properties, and can support new bone formation when implanted in critical-size cranial defects in rabbits. The biodegradation of the material with time is also documented. Our data add to the current knowledge on the dynamics of resorption and bone replacement by synthetic biphasic grafts composed of β-TCP/CS, and this experiment can serve as a pilot study and referral for future experimental and clinical research.

DISCLOSURE

The authors claim to have no financial interest in any company or any of the products mentioned in this article.

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