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The Effects of β-TCP and β-TCP/CS (EthOss®) Bone Substitutes on Rabbit Tibia Bone Healing: A Histological Comparative Study

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ABSTRACT

Bioactive alloplastic materials are a branch of bone grafts used in bone regeneration and augmentation. EthOss® bone graft and β-TCP are the most common alloplastic materials and are thoroughly researched. This study aimed to compare the biological performance of EthOss®, β-TCP, and spontaneous healing. Each grafting material was used to fill an 8 mm diameter artificial bone defect in the tibia bone of rabbits, a third bone defect in each rabbit where left empty to heal spontaneously. Ten New Zealand White rabbits were used. After a healing period of two months, a histological biopsy where taken to be analyzed under a light microscope. Defects grafted with (β-TCP/CS) showed greater newly formed bone compared to (β-TCP) and the control group. There were statistically significant differences between the three groups ($p<0.05$). Connective tissue was higher at sites that healed spontaneously ($p<0.05$) compared to sites treated with (β-TCP/CS) or (β-TCP). At sites that were filled with (β-TCP), the residual graft particles were greater compared to other sites ($p<0.05$). The artificial bone defects in rabbit tibia bone treated with the novel alloplastic bone graft (β-TCP/CS) displayed good bone growth and biocompatibility after a two-month healing period.

1. Introduction

Dental implants are a popular treatment option, and demand for this type of treatment is expected to increase over the coming decades [1]. As a result, numerous bone replacement materials and grafting techniques have been developed to augment the bone around dental implants, preserve the alveolar ridge after extraction, and manage osseous defects of the alveolar bone [2- 4].

Since it has so many benefits, autogenous bone has long been regarded as the gold standard material for

bone grafting. It is the only grafting material with all three osteogenesis, osteoconduction, and osteoinduction properties, as well as favorable bone quality, rapid healing, and no risk of disease transmission. This form of bone graft has drawbacks, such as increased morbidity, the necessity for additional surgical sites, restricted availability, and prolonged operating time [5- 8].

Therefore, efforts have been made to find new grafting materials that might overcome these drawbacks and bone substitutes have been proposed as an alternative. By definition, bone substitutes are “a synthetic, inorganic or biologically organic combination – biomaterial – which can be inserted for the treatment of a bone defect instead of autogenous or allogenous bone” [9], [10]. There are two primary categories of bone substitutes under this definition: those based on "natural" precursors and those made of synthetic materials. In recent years, a very wide range of synthetic materials have been invented, however, due to their chemical similarity to the mineral component of natural bone, the majority of materials utilized in bone augmentation in daily practice are based on Calcium Phosphate (CP), such as beta-tricalcium phosphate (β-TCP) [11- 13].

However, there is considerable evidence that synthetic materials' regenerative capabilities are limited, especially when compared to autogenous bone, as the majority of synthetic materials can only act as osteoconductive materials [14], [15]. As a feature of the chemical interaction between bone grafts and host biological tissues, bioactivity is a significant factor of synthetic materials since it provides the capacity for the bone graft to be completely replaced with the host's natural bone structure [14], [16].

The most prominent bioactive synthetic materials utilized in augmentation operations are β-TCP and Calcium Sulfate (CS). β-TCP has an interconnected porous structure that enhances vascularization and enables rapid bone regeneration in bone defects. [17]. Furthermore, β-TCP appears radiopaque on radiographs, which can significantly enhance the evaluation of the healing process. The degradation of β-TCP is compatible with the growth rate of newly formed bone [14], [18]. The degradation of the material releases Ca^{+2} ions that can affect the cell activity enhancement due to the alkaline environment that is created when the material releases ions [19].

Calcium Sulfate (CS), discovered by Dreesmann, is the other most promising calcium compound. Calcium sulfate appears to have the capacity to affect angiogenesis since it possesses mechanical qualities that are stronger than cancellous bone, as well as the ability to release Ca^{+2} ions similarly to β-TCP. However, it has a significant disadvantage as it undergoes resorption in a short period (3 – 6 weeks) [20], [21].

The combination of CS and β-TCP can improve the graft material's mechanical characteristics and result in a paste that conforms to the geometry of the bone defect. The porous structure of the graft material can serve as a scaffold for bone regeneration. This new grafting substance showed extremely good healing results, mainly because it made it easier to access the periosteal blood supply [22- 27].

As an alternative to autogenous bone grafts, bioactive materials are progressively coming into focus in recent research, and they may eventually be employed more frequently in dental clinics' day-to-day operations. Therefore, this study aimed to compare the biological performance of a novel synthetic bone substitute synthesized from β-TCP and CS to that of β-TCP used singly and the spontaneous healing in rabbits with artificial bone defects.

2. Material and Methods

This histological comparative Study was approved by the Research Ethics Committee of Damascus University (Approval No. UDDS-676-08072018/SRC-2786).

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Study sample:

The study sample included thirty artificial bone defects in ten rabbits, three of which were in each rabbit's tibia bone. The first defect was filled with beta-tricalcium phosphate (β-TCP), the second defect was filled with a beta-tricalcium phosphate (β-TCP) and calcium sulfate (CS) mixture (EthOss® bone graft), and the third defect was left unfilled. Depending on the type of graft employed, the three equal groups of bone defects in the tissue sample were divided into:

• The control group: included ten artificial bone defects that were left unfilled with any of the grafting materials.

• test group 1: which included ten artificial bone defects that were filled using EthOss® bone graft.

• test group 2: which included ten artificial bone defects that were filled using β-TCP bone graft.

Animals:

The use of ten fully grown New Zealand White rabbits weighing an average of 2.5 kg (±250 g) each in this study. Individually caged animals were given a uniform diet of an appropriate amount of dry food and water whenever they needed it at the faculty of pharmacy Damascus University. From the time they arrived at the institution, the animals had ten days to adjust to their new surroundings.

Surgical procedure:

The ketamine hydrochloride and xylazine mixture solution (Ketalar, Yuhan, Seoul, Korea; Rompun, Bayer Korea, Seoul, Korea) used to anesthetize the animals were administered intramuscularly. Local anesthesia with 2 % lidocaine (lidocaine HCl, Huons, Seoul, Korea) was administered after shaving the surgical site and wiping it with alcohol and povidone-iodine.

To reveal the tibia bone, a 5 cm longitudinal skin incision was made at the anteromedial aspect of the left tibia. The femoral artery was preserved during the elevation of the tissues. The overlying periosteum was then excised, and three distinct and uniform 8-mm-diameter round defects were made in each animal's tibia using a low-speed electric handpiece and a trephine drill with an 8-mm internal diameter (Komet Inc., Lemgo, Germany). The irrigation solution used was 0.9 % sterile physiological saline. The circular bone section was then moved and luxated using a thin peristome.

Each animal had three resulting bone defects that were randomly regarded using the website (www.random.org) as follows:

1. One defect was filled with β-TCP/CS (group 1).
2. One defect was filled with 150 mg of β-TCP bone graft (group 2).
3. One defect was left unfilled (group 3).

β-TCP (65%) and CS (35%) make up the self-hardening biomaterial that serves as the test bone graft substitute in group 1 (EthOss, Ethoss Regeneration Ltd., Silsden, UK). It is preloaded in a sterile plastic syringe. Before inserting the synthetic bone graft into the bone defect, the biomaterial particles were mixed in the syringe with sterile saline in compliance with the manufacturer's instructions. Following application, the moldable graft particles were gently compressed using a bone plunger to fill the full volume of the site up to the level of the adjacent host bone. The graft particles were further compacted and the in situ hardening of the CS component of the graft was sped up by using a saline-wet gauze.

In the second group, the β-TCP bone graft is used as a test bone graft substitute (Bioteck S.p.A., Arcugnano, Italy). Since this material is in powder form, it must be mixed with saline in a metal mixing jar according to the manufacturer's instructions before application. The site was filled with the material to the level of the adjacent host bone after the mixing process was completed.

The soft tissues were repositioned before being sutured with 420 Monosyn (gluconate absorbable monofilament, B-Braun, Aesculap, PA). After surgery, the rabbits were kept caged freely and were given their usual regimen of food and water.

Each test animal received analgesics (25 mg/kg of Dipyrone; HAMA PHARMA; Hama, Syria) for 2 days after surgery and antibiotics (30 mg/kg of Betamox; Norbrook, Hama, Syria) every 24 hours. The animals were sacrificed after two months.

Following immediate storage in 10% buffered formaldehyde, the biopsy was decalcified for three weeks at 4°C in a solution of 12% EDTA buffered in pH 7.2 phosphate buffered saline (PBS). To have a thorough understanding of the quality of the obtained bone, each embedded specimen was sectioned using a microtome at three levels, including the center portion of the specimen, along its longitudinal axis (5 m thickness). The samples were prepared and stained with Hematoxylin and Eosin (H&E) for histological examination in our institute's department of histology and oral pathology. A standard light microscope (Olympus BX60, Olympus Optical Co., Ltd., Japan) was used to examine the specimens. The remaining graft particles, the amount of bone tissue, and the connective fibrous tissue were examined through the color difference between them using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA) to analyze the percentage of the bone tissue to the connective tissue.

Statical analysis:

The data of the present study were analyzed using the statistical package for social sciences software (Version 24, IBM SPSS Inc., Chicago, IL, USA). Data are expressed as mean ± standard deviation (SD). To analyze the parameter normality, the one-way ANOVA test was used to compare variables between the three groups. The Bonferroni model was used to perform pairwise comparisons. P values of < 0.05 were considered statistically significant.

3. Results

Macroscopic analysis:

Throughout duration of this trial, none of the rabbits experienced any post-operative complications. Additionally, there was no animal loss during the experiment, and the surgical site healed uneventfully. At the defect sites, there were no visible clinical signs of infection, or hematoma. The tissues that were in contact with the grafting materials did not show any symptoms of allergic reactions.

Macroscopically, all defect sites were filled with new bone. In contrast to the sites grafted with β- TCP/CS (EthOss), where the graft particles were embedded in newly formed hard tissue, the remaining graft particles were visible at the bone defect site grafted with β - TCP. The circular bone defects that spontaneously healed were covered by a thin layer of newly created hard tissue, according to clinical examination of regions left empty.

Microscopic analysis:

Two months following surgery, histological samples were collected from the surgical site and prepared to be examined under a light microscope. The biopsy showed newly formed bone, and healthy non-inflammatory connective tissue under the microscope. There were no adverse responses to the graft material, nor inflammatory exudates, which is an indication of the biocompatibility of the grafting materials. Figure 1

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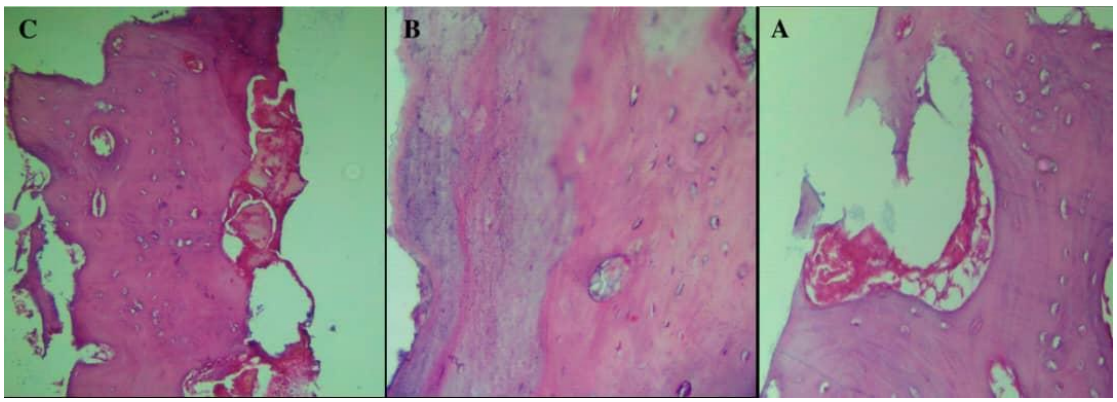

Figure 1: histological sections (H&E staining) after a two-month healing period, A: control group showing a little newly-formed bone, and non-inflamed connective tissue. B: β-TCP/CS group showing a good bone formation without residual graft particles. C: β-TCP group showing a good bone formation with residual small graft particles. (original magnification 400✕)

The ratio of the newly formed bone was greater in the test grafting groups using (β-TCP/CS) and (β-TCP) compared to the control group. Furthermore, the ratio was greater in the test group 1 (β-TCP/CS) in comparison to the test group 2 (β-TCP). (Table 1).

Table 1. Comparison between of the three groups in terms of newly formed bone ratio. The differences between groups were statically significant (P < 0.05).

Site	N	Mean	SD	*p*-Value
β-TCP	10	49.57	4.94	0.000
β-TCP/CS	10	57.08	5.04	0.000
Control	10	28.17	4.78	0.000

The ratio of the connective tissue was lower in the control group compared to the test grafting groups using (β-TCP/CS) and (β-TCP). Furthermore, the ratio was greater in the test group 2 (β-TCP) in comparison to the test group 1 (β-TCP/CS). (Table 2).

Table 2. Comparison between of the three groups in terms of connective tissue ratio. The differences between groups were statically significant (P < 0.05).

Site	N	Mean	SD	*p*-Value
β-TCP	10	49.73	5.18	0.000
β-TCP/CS	10	42.92	5.04	0.000
Control	10	71.84	4.78	0.000

The ratio of the residual graft particles was greater in test grafting group using (β-TCP) compared to the test grafting group using (β-TCP/CS) and control group. (Table 3).

Table 3. Comparison between of the three groups in terms of residual graft particles ratio. The differences between groups were statically significant (P < 0.05).

Site	N	Mean	SD	*p*-Value
β-TCP	10	0,70	0,58	0,000
β-TCP/CS	10	0	0	0,000
Control	10	0	0	0,000

At two months, there were statistically significant differences between the three groups at confident level of 95% in term of newly formed bone, and the pairwise comparison showed that there were statistically significant differences between the three groups ($p < 0.05$). Regarding the connective tissue, the pairwise comparison showed that there were statistically significant differences between the three groups ($p < 0.05$). Consecutively, in term of residual graft particles, the pairwise comparison showed that there were statistically significant differences between the three groups ($p < 0.05$).

4. Discussion

The aim of this animal study was to evaluate the biological performance of a novel bone substitute synthesized from β-TCP and CS to that of β-TCP alone and to spontaneous healing in rabbits with artificial bone defects created surgically in the left tibia bone of each rabbit.

The EthOss® bone grafting material was compared to the β-TCP bone substitute. β-TCP has largely been used as a bone substitute for more than 25 years, and is considered as the "gold standard" for synthetic bone [28]. It has been well researched and utilized in oral surgery and implantology. Numerous pre-clinical researches, clinical trials, and systematic reviews have demonstrated and detailed its osteoconductive capabilities, as well as its capacity to retain the volume of the augmented site throughout time, along with its angiogenesis abilities [29- 32]. This biomaterial is very biocompatible when implanted in bone since it is significantly more resorbable than other CS based biomaterials; it is resorbable within 6 to 9 months, according to animal and human histological studies [33]. However, due to its low mechanical strength, it should not be utilized in load-bearing regions, and its inadequate mechanical qualities become very clear particularly when facing compressive strength [34], [35]. Zhao et al. concluded that when combined to form BCP, β-TCP appears to reduce the strength of HA [36]. Several researchers investigated the effect of β-TCP on mechanical properties and came to the same conclusion [37].

In our experiment, no inflammatory reaction was seen, and no fibrosis formed between the biomaterial particles and the bone newly regenerated in the area of augmentation, demonstrating the biocompatibility of the β-TCP/CS bone graft. Our findings suggest that β-TCP/CS can aid in the formation of new bone, since it demonstrated considerable new bone formation (57.08%) in circular tibia bone defects in rabbits two months after implantation.

Previous animal studies implying the same (β-TCP/CS) bone substitute reported similar outcomes, consisting of no inflammatory response, no fibrosis development between the graft particles and the newly formed bone, during a healing period varying from 3 to 16 months [25], [38]. Pre-clinical experiments have shown the deterioration of β-TCP/CS bone substitutes. Using histomorphometry, [38] reported a statistically significant decrease in the proportion of residual material in grafted rabbit calvaria defects between 3 and 6 weeks (4.54% and 1.67%, respectively). Four months following implantation of the material in surgically produced bone defects on the iliac crest of Beagle dogs, [25] observed 21.62% residual β-TCP/CS.

Grafting materials used to repair bone defects may have a considerable influence on the volume of regenerated bone tissue, and ration of unresorbed graft particles may change the architecture of newly established bone tissue. The rate of resorption and the ability of a particular grafting material to stimulate bone rebuilding appear to have an effect on the bone healing process and the geometry of newly created tissue. These differences may have an effect on the overall quality of freshly formed bone [39], [40].

[40] studied several grafting materials and their role in the process of bone formation. The researchers

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stated that the presence of unresorbed graft particles might significantly interfere with bone remodeling and healing, affecting the quality and architecture of the bone at the augmented location.

5. Conclusions

Our histology findings indicated that the artificial bone defects in rabbit tibia bone treated with the novel alloplastic bone graft (β-TCP/CS) displayed good bone growth and biocompatibility after a two-month healing period.

6. References

[1] Elani HW, Starr JR, Da Silva JD, Gallucci GO. Trends in Dental Implant Use in the U.S., 1999-2016, and Projections to 2026. J Dent Res 2018; 97: 1424-1430. doi: 10.1177/0022034518792567.

[2] Roca-Millan E, Jané-Salas E, Marí-Roig A, et al. The Application of Beta-Tricalcium Phosphate in Implant Dentistry: A Systematic Evaluation of Clinical Studies. Materials (Basel) 2022; 15: 655. doi: 10.3390/ma15020655.

[3] Wang RE, Lang NP. Ridge preservation after tooth extraction. Clin Oral Implants Res 2012; 23: 147-156. doi: 10.1111/j.1600-0501.2012.02560.x.

[4] Yip I, Ma L, Mattheos N, Dard M, Lang NP. Defect healing with various bone substitutes. Clin Oral Implants Res 2015; 26: 606-614. doi: 10.1111/clr.12395.

[5] El Chaar E, Rutkowski JL. Is Autogenous Bone Still the "Gold Standard" in Oral Bone Grafting?. J Oral Implantol 2022; 48: 1. doi: 10.1563/aaid-joi-D-22-Editorial.4801.

[6] Glenske K, Donkiewicz P, Köwitsch A, et al. Applications of Metals for Bone Regeneration. Int J Mol Sci 2018; 19: 826. doi: 10.3390/ijms19030826.

[7] McKenna GJ, Gjengedal H, Harkin J, Holland N, Moore C, Srinivasan M. Effect of Autogenous Bone Graft Site on Dental Implant Survival and Donor Site Complications: A Systematic Review And Meta-Analysis. J Evid Based Dent Pract 2022; 22: 101731. doi: 10.1016/j.jebdp.2022.101731.

[8] Misch CM. Autogenous bone: is it still the gold standard?. Implant Dent 2010; 19: 361. doi: 10.1097/ID.0b013e3181f8115b.

[9] Giannoudis PV, Dinopoulos H, Tsiridis E. Bone substitutes: an update. Injury 2005; 36: S20-S27. doi: 10.1016/j.injury.2005.07.029.

[10] Schlickewei W, Schlickewei C. The use of bone substitutes in the treatment of bone defects–the clinical view and history. InMacromolecular symposia 2007; 253: 10-23. Weinheim: WILEY-VCH Verlag. doi: 10.1002/masy.200750702.

[11] Cheah CW, Al-Namnam NM, Lau MN, et al. Synthetic Material for Bone, Periodontal, and Dental Tissue Regeneration: Where Are We Now, and Where Are We Heading Next?. Materials (Basel) 2021; 14: 6123. doi: 10.3390/ma14206123.

[12] Mano JF, Sousa RA, Boesel LF, Neves NM, Reis RL. Bioinert, biodegradable and injectable polymeric

matrix composites for hard tissue replacement: state of the art and recent developments. Composites Science and Technology 2004; 64: 789-817. doi: 10.1016/j.compscitech.2003.09.001.

[13] Le Gars Santoni B, Niggli L, Dolder S, et al M. Effect of minor amounts of β-calcium pyrophosphate and hydroxyapatite on the physico-chemical properties and osteoclastic resorption of β-tricalcium phosphate cylinders. Bioact Mater 2021; 10: 222-235. doi: 10.1016/j.bioactmat.2021.09.003.

[14] Eliaz N, Metoki N. Calcium Phosphate Bioceramics: A Review of Their History, Structure, Properties, Coating Technologies and Biomedical Applications. Materials (Basel) 2017; 10: 334. doi: 10.3390/ma10040334.

[15] Miron RJ, Zhang YF. Osteoinduction: a review of old concepts with new standards. J Dent Res 2012; 91: 736-744. doi: 10.1177/0022034511435260.

[16] Dorozhkin SV. Calcium orthophosphates in nature, biology and medicine. Materials. 2009; 2: 399-498. doi: 10.3390/ma2020399.

[17] Horowitz RA, Leventis MD, Rohrer MD, Prasad HS. Bone grafting: history, rationale, and selection of materials and techniques. Compend Contin Educ Dent 2014; 35: 1-6;quiz7.

[18] Stähli C, Bohner M, Bashoor-Zadeh M, Doebelin N, Baroud G. Aqueous impregnation of porous beta-tricalcium phosphate scaffolds. Acta Biomater 2010; 6: 2760-2772. doi: 10.1016/j.actbio.2010.01.018.

[19] Gao C, Peng S, Feng P, Shuai C. Bone biomaterials and interactions with stem cells. Bone Res 2017; 5:17059. doi: 10.1038/boneres.2017.59.

[20] Mazor Z, Mamidwar S, Ricci JL, Tovar NM. Bone repair in periodontal defect using a composite of allograft and calcium sulfate (DentoGen) and a calcium sulfate barrier. J Oral Implantol 2011; 37: 287-292. doi: 10.1563/AAID-JOI-D-10-00006.1.

[21] Thomas MV, Puleo DA. Calcium sulfate: Properties and clinical applications. J Biomed Mater Res B Appl Biomater 2009; 88: 597-610. doi: 10.1002/jbm.b.31269.

[22] Eleftheriadis E, Leventis MD, Tosios KI, et al. Osteogenic activity of β-tricalcium phosphate in a hydroxyl sulphate matrix and demineralized bone matrix: a histological study in rabbit mandible. J Oral Sci 2010; 52: 377-384. doi: 10.2334/josnusd.52.377.

[23] Fairbairn P, Leventis M. Protocol for Bone Augmentation with Simultaneous Early Implant Placement: A Retrospective Multicenter Clinical Study. Int J Dent 2015; 2015: 589135. doi: 10.1155/2015/589135.

[24] Leventis MD, Fairbairn P, Dontas I, et al. Biological response to β-tricalcium phosphate/calcium sulfate synthetic graft material: an experimental study. Implant Dent 2014; 23: 37-43. doi: 10.1097/ID.0000000000000030.

[25] Podaropoulos L, Veis AA, Papadimitriou S, Alexandridis C, Kalyvas D. Bone regeneration using b-tricalcium phosphate in a calcium sulfate matrix. J Oral Implantol 2009; 35: 28-36. doi: 10.1563/1548-1336-35.1.28.

ISSN: 0005-2523
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[26] Ruga E, Gallesio C, Chiusa L, Boffano P. Clinical and histologic outcomes of calcium sulfate in the treatment of postextraction sockets. J Craniofac Surg 2011; 22: 494-498. doi: 10.1097/SCS.0b013e318208bb21.

[27] Stein JM, Fickl S, Yekta SS, Hoischen U, Ocklenburg C, Smeets R. Clinical evaluation of a biphasic calcium composite grafting material in the treatment of human periodontal intrabony defects: a 12-month randomized controlled clinical trial. J Periodontol 2009; 80: 1774-1782. doi: 10.1902/jop.2009.090229.

[28] Galois L, Mainard D, Delagoutte JP. Beta-tricalcium phosphate ceramic as a bone substitute in orthopaedic surgery. Int Orthop 2002; 26: 109-115. doi: 10.1007/s00264-001-0329-x.

[29] Yuan H, De Bruijn JD, Li Y, et al. Bone formation induced by calcium phosphate ceramics in soft tissue of dogs: a comparative study between porous alpha-TCP and beta-TCP. J Mater Sci Mater Med 2001; 12: 7-13. doi: 10.1023/a:1026792615665.

[30] Walsh WR, Vizesi F, Michael D, et al. Beta-TCP bone graft substitutes in a bilateral rabbit tibial defect model. Biomaterials 2008; 29: 266-271. doi: 10.1016/j.biomaterials.2007.09.035.

[31] Kao RT, Nares S, Reynolds MA. Periodontal regeneration - intrabony defects: a systematic review from the AAP Regeneration Workshop. J Periodontol 2015; 86: S77-104. doi: 10.1902/jop.2015.130685.

[32] Kumagai H, Makihara T, Funayama T, et al. Angiogenesis and new bone formation in novel unidirectional porous beta-tricalcium phosphate: a histological study. J Artif Organs 2019; 22: 294-299. doi: 10.1007/s10047-019-01120-8.

[33] Chappard D, Guillaume B, Mallet R, Pascaretti-Grizon F, Baslé MF, Libouban H. Sinus lift augmentation and beta-TCP: a microCT and histologic analysis on human bone biopsies. Micron 2010; 41: 321-326. doi: 10.1016/j.micron.2009.12.005.

[34] Fernandez de Grado G, Keller L, Idoux-Gillet Y, et al. Bone substitutes: a review of their characteristics, clinical use, and perspectives for large bone defects management. J Tissue Eng 2018; 9: 2041731418776819. doi: 10.1177/2041731418776819.

[35] Zhao R, Yang R, Cooper PR, Khurshid Z, Shavandi A, Ratnayake J. Bone Grafts and Substitutes in Dentistry: A Review of Current Trends and Developments. Molecules 2021; 26: 3007. doi: 10.3390/molecules26103007.

[36] Zhao J, Lu X, Duan K, Guo LY, Zhou SB, Weng J. Improving mechanical and biological properties of macroporous HA scaffolds through composite coatings. Colloids Surf B Biointerfaces 2009; 74: 159-166. doi: 10.1016/j.colsurfb.2009.07.012.

[37] Wagoner Johnson AJ, Herschler BA. A review of the mechanical behavior of CaP and CaP/polymer composites for applications in bone replacement and repair. Acta Biomater 2011; 7: 16-30. doi: 10.1016/j.actbio.2010.07.012.

[38] Leventis MD, Fairbairn P, Dontas I, et al. Biological response to β-tricalcium phosphate/calcium sulfate synthetic graft material: an experimental study. Implant Dent 2014; 23: 37-43. doi:

10.1097/ID.0000000000000030.

[39] Froum S, Cho SC, Rosenberg E, Rohrer M, Tarnow D. Histological comparison of healing extraction sockets implanted with bioactive glass or demineralized freeze-dried bone allograft: a pilot study. J Periodontol 2002; 73: 94-102. doi: 10.1902/jop.2002.73.1.94.

[40] Chan HL, Lin GH, Fu JH, Wang HL. Alterations in bone quality after socket preservation with grafting materials: a systematic review. Int J Oral Maxillofac Implants 2013; 28: 710-720. doi: 10.11607/jomi.2913.