ORIGINAL RESEARCH

Biological Impact of Alloplastic Bone Graft vs Bovine Xenograft and Allograft Materials in Bone Healing: An Experimental Study

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Abstract

Aim: This study aims to compare the performance of beta-tricalcium phosphate with calcium sulfate (β-TCP/CS) vs a bovine xenograft, freeze-dried mineralized allograft, and spontaneous healing in surgically prepared bone defects in rabbit tibia.

Materials and methods: The grafting materials were implanted in three out of four standardized monocortical bony defects, 3-mm diameter and 3-mm deep, in rabbit tibia while one defect was left empty for spontaneous healing as a control group. Twelve rabbits were euthanized at 2 and 6 weeks after surgery. The bone tissue specimens were histologically evaluated using hematoxylin and eosin, Masson's trichrome and osteoprotegrin (OPG) immunohistochemical staining. Results were quantitatively evaluated.

Results: An enhancement of bone healing was noticed in the defects grafted with β-TCP/CS compared with all other groups at 2 and 6 weeks after surgery as it showed significant increase in OPG expression and a significant decrease in the amount of collagen at 6 weeks after surgery. The residual grafted particles were the least with β-TCP/CS at 6 weeks after surgery.

Conclusion: The β-TCP/CS grafting material is a promising bioactive alloplastic bone substitute as it proved to be biocompatible, osteoconductive, and bioresorbable bone substitute.

Clinical significance: The β-TCP/CS grafting material can be used for guided bone regeneration resulting in pronounced high-quality bone which aids in oral and maxillofacial reconstruction.

Keywords: Allograft, Alloplast, Bone defect, Bone healing, Xenograft.

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Introduction

Biomaterials have been developed in the recent years. They are used for bone regeneration and reconstructive surgeries. A bone graft is the material of choice when a defect in shape and/or volume needs to be repaired.[1]

Autograft is the gold standard bone graft material. It is osteogenic, osteoinductive, and osteoconductive. Moreover, it does not cause immunologic reactions or disease transmission. It is gradually absorbed and replaced by high quality bone. Although autogenous bone is the best choice, it has some disadvantages such as second surgical site, lengthy procedures, limited quantity, and increase morbidity.[2]

As an alternative, other bone graft types have been developed, including allograft, xenograft, and alloplast, having different properties according to their embryologic origin, histologic architecture, structure, and rate of graft resorption and new bone formation.[3]

Allograft is processed aggressively to lessen immune response and prevent disease transmission, then is preserved in bone banks. Allograft is osteoinductive and osteoconductive.[3]

In the xenograft category, the most commonly used xenograft is deproteinized bovine mineral bone. They are osteoconductive and do not cause immunological reaction. Xenografts do not enter the bone remodeling process, but are surrounded by newly formed bone because they are poorly or slowly resorbed.[4,5]

Alloplastic materials have been developed to be used as an alternative to xenograft. They are available in unlimited quantity and different sizes and forms. Alloplastic materials do not cause disease transmission or any immunological reaction. They are similar to natural bone to a great extent because of cutting-edge technology that causes improvement in surface texture, mineral formation, and biocompatibility. Alloplastic materials are osteoconductive and in some cases, they are osteoinductive.[6] The beta-tricalcium phosphate (β-TCP) is the most widely used alloplastic material. Its compressive strength is similar to cancellous bone and it is resorbed by hydrolysis, enzymatic, and phagocytic processes.[7]

Chemical bonding between host bone and bone graft is called bioactivity. Calcium sulfate (CS) has bioactivity properties, gradual resorption and finally replaced by new bone.[1] By mixing CS with β-TCP, an alloplastic compound biomaterial that hardens in place and adheres to the host bone is created. This compound biomaterial

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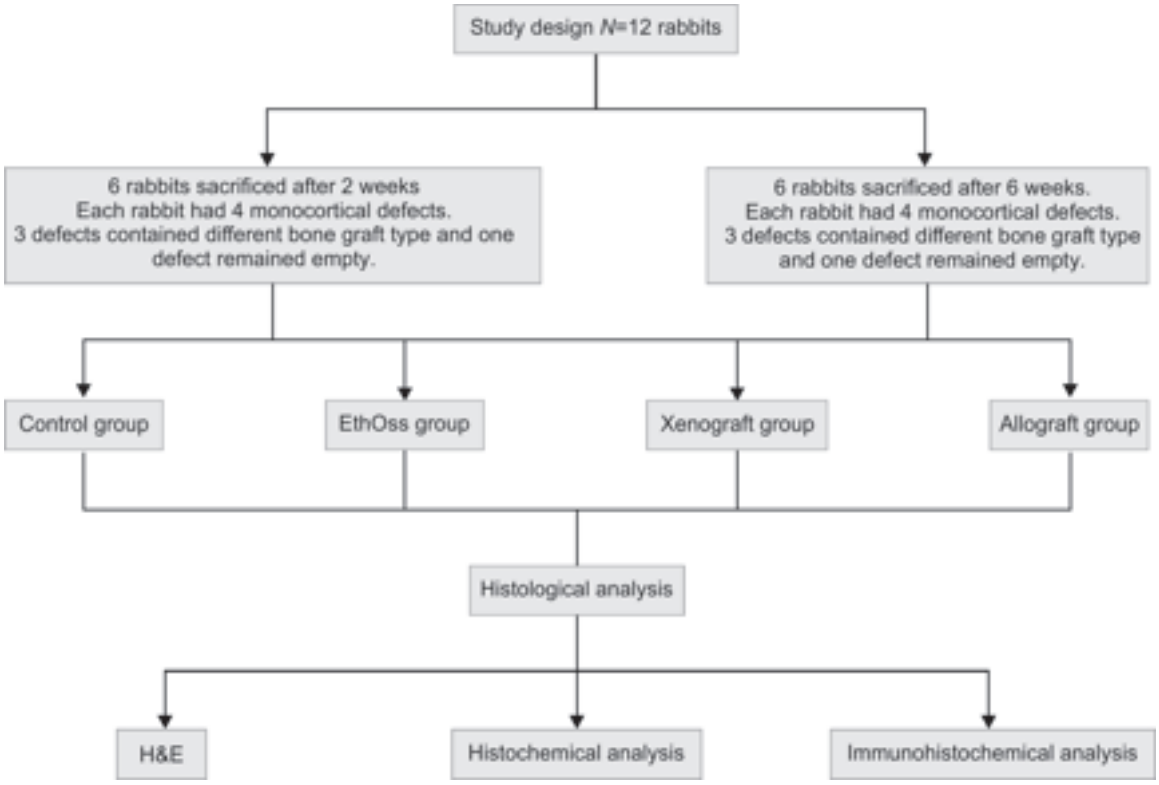
Flowchart 1: Flowchart representation of groups distribution for the various histological analyses conducted in the study



helps preserve the space and shape of the augmented site and serves as a stable scaffold. In general, the best grafting material should serve as a substrate for bone ingrowth into the defect and eventually be completely replaced by host bone with an acceptable resorption rate in relation to new bone formation.[8]

Osteoprotegrin is a soluble secretory glycoprotein of the tumor necrosis factor receptor (TNFR) superfamily, which is mainly produced by osteoblasts lineage cells. It acts as a soluble decoy receptor which binds the receptor activator of NF-kB ligand (RANKL) and prevents osteoclast differentiation, activation, and induction of apoptosis. Osteoprotegrin has long been considered as a critical factor in bone healing as it regulates the osteoclast function and the bone density alterations. The RANKL/OPG ratio provides an indication whether tissue response tends to bone formation with a predominance of OPG or bone resorption with increase of RANKL.[9]

The aim of this study was to compare the performance of a novel alloplastic bone material β-TCP/CS vs bovine xenograft, freeze-dried bone allograft (FDBA) and spontaneous healing in tibia bone of rabbits, histologically and immunohistochemically.

Materials and Methods

Animal Selection

Twelve clinically healthy adult male New Zealand white rabbits (Oryctolagus cuniculus) (weight, 2–3 kg) were used in this study. The rabbits were kept in a separate standard cage with free access to water and food in Medical Experimental Research Center in Faculty of Medicine, Mansoura University. All experimental procedures were done from July 2021 till August 2021 under the accepted protocol with Registration No. (A17060421) of the ethical committee of the Faculty of Dentistry, Mansoura University, Egypt.

Experimental Design and Sample Distribution (Flowchart 1)

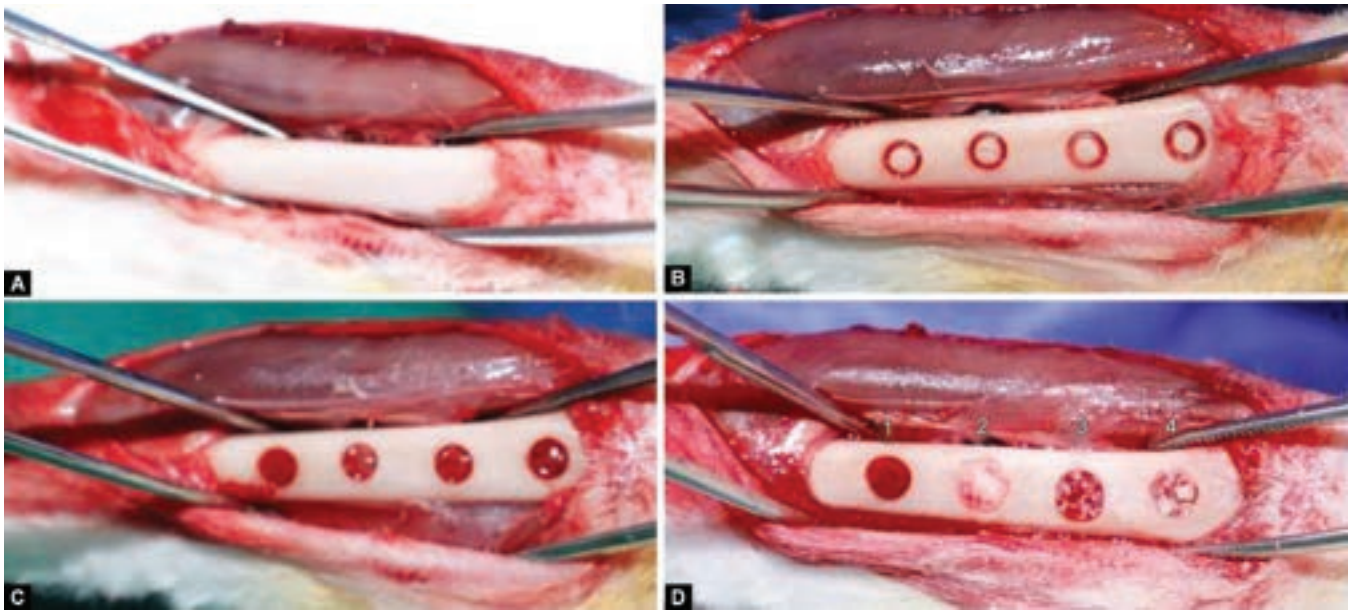
This study involved a total of 12 rabbits. Four monocortical bony defects in the right tibia were made in each rabbit. Various materials used to treat the defects were as follows:

- **Group I (Control group):** The defect remained unfilled.
- **Group II (EthOss group):** The defect was filled with an alloplastic bone substitute, (EthOss, EthOss Regeneration Ltd., Silsden, UK) consists of 65% β-TCP and 35% CS.
- **Group III (Xenograft group)**: The defect was filled with bovine xenograft (Creos xenogain®, Nobel Biocare, Zürich–Flughafen, Switzerland), which was deproteinized bovine bone mineral matrix with a low crystalline structure and large specific surface area with 0.2–1-mm granule size.
- **Group IV (Allograft group):** The defect was filled with allograft (FDBA, Hamanand Saz Baft Tissue Regeneration Corporation, Iran) is mineralized cortical cancellous powder of CenoBone, 150–2,000 μm in size.

Surgical Procedures

All rabbits included in this study were anesthetized using intramuscular injection of Diazepam (0.5-mg/kg body weight), Ketamine hydrochloride (20-mg/kg body weight), and xylazine (ADWIA Co. S.A.E 10 of Ramadan city, Cairo, Egypt) (25-mg/kg body weight). Also, the region of surgery which was the proximal right tibia, was injected locally with an anesthetic solution (Mepivacaine HCL 2% with Levonordefrin 1:20,000. Alexandria Co. for Pharmaceuticals and Chemical Ind., Alexandria, Egypt).

After anesthesia, the hair that covers the skin at the tibia was shaved and cautiously scrubbed with a disinfecting agent



Figs 1A to D: (A) Reflection of the periosteum and exposure of the tibial bone; (B) Preparation of four monocortical bony defects; (C) The four surgical cavities after removing cylindrical bone segments; (D) Filling the bony defects with different type of bone grafting materials. 1, Empty defect; 2, EthOss; 3, Xenograft; 4, Allograft

(povidone–iodine) then the animals were draped in sterile towels. A 6-cm incision in the skin of the right tibia was done, 2 cm below the knee joint. The superficial and deep fasciae were also incised. The periosteum of the tibia was then incised and reflected till reaching the bone (Fig. 1A).

In each rabbit, four monocortical bony defects 3-mm diameter and 3-mm deep were created. The size and depth of the bony defects were standardized by using the same size of trephine bur head (MCTBIO Co., Ltd, Korea) at the same speed (250 rpm) mounted to a contra-angle handpiece attached on a low speed micromotor device with cooling by normal saline (Fig. 1B).

A thin chisel was used to mobilize and detach the bone segments (Fig. 1C). The four surgical cavities were then thoroughly irrigated with normal saline solution using a sterile 5 mL plastic syringe to wash away any debris from inside the holes. They were then carefully dried using small sterile cotton pellets. The first bony defect remained unfilled to act as a control group. The second one grafted by using alloplastic bone substitute (EthOss). The third bony defect was grafted by using xenograft. The fourth bony defect was grafted using by allograft material (Fig. 1D).

Before inserting the alloplastic bone substitute into the defect, the material was mixed in the syringe with sterile saline, then a bone plunger was used to slightly compress graft particles to fill the defect up to the level of the surrounding bone. For more compaction of the graft particles and acceleration of the hardening of CS element of the graft, a saline-wet gauze was used.

For the xenograft and allograft materials, they were inserted into the bone defect after being mixed with sterile saline. The wound edges were then approximated using 4/0 black silk. The skin was then scrubbed with iodine after suturing and the sutures were removed after 9 days to prevent infection.

Postoperative Medication

After the surgery, the animals received antibiotics (150 mg/kg of Cefotaxime, Egyptian International Pharmaceutical Industries Co. E.I.P.I.Co., Egypt) injected every 12 hours for 5 days and analgesics (75 mg of Voltaren, Novartis Pharma S.A.E., Cairo, Egypt) every 8 hours for 2 days postoperatively.

Sacrifices of Animals

Twelve rabbits were randomly divided into two groups (six rabbits per group). They were scarified by overdose of diethyl ether at 2 and 6 weeks after surgery, respectively, to dissect out right tibia immediately after scarification.

Histological Analysis

After scarification, the specimens were properly fixed, decalcified in 10% neutral EDTA and then they were dehydrated and paraffin embedded, finally six longitudinal sections from each specimen of 4 µm (totally 36 samples for each time interval) were made for subsequent staining with hematoxylin and eosin (H&E) for general histological observations, Masson's trichrome to detect collagen fibers and to observe new bone formation and OPG immunohistochemistry.

Regarding immunohistochemical staining, the sections were blocked in 10% normal goat serum then overnight incubation with primary antibodies against OPG (1:100) (Novus Biologicals, USA) was done at 4°C; and then stained with HRP-conjugated secondary antibodies (ZSJQ-BIO, Beijing, China). The utilized substrate for color development was Diaminobenzidine and counterstaining with hematoxylin.[10] Finally, digital morphometric and statistical analysis were performed for the Masson's trichrome and OPG immunohistochemistry results.

Statistical Analysis

Analysis of the data was done using Statistical Package for Social Science software, version 26, with computer program (SPSS, Inc., Chicago, IL, USA). The data were presented as means and standard deviations for parametric data after testing normality using Shapiro–Wilk test. One-way analysis of variance (ANOVA) and *post hoc* test, Tukey test, were used for comparing the quantitative parametric data of more than two different groups while Paired

t-test was used to compare between two different groups. Also, $p < 0.05$ was considered statistically significant.

Results

Hematoxylin and Eosin Results

The histological results revealed a higher new bone formation in EthOss group than the other experimental groups. Figure 2 shows representative histological sections of the four experimental groups at 2 and 6 weeks after the surgery.

At 2 weeks after surgery, the bone defect in control group revealed dense layer of fibrocellular tissue containing finger-like thin irregular woven bone trabeculae, fibroblasts, dilated blood vessels with little inflammatory cells (Fig. 2A). In all other experimental groups, different grafting materials demonstrated very low level of inflammation. The analyzed specimens histologically revealed newly formed woven bone trabeculae extended from the defect margins with a tendency toward the center, surrounded by vascularized and non-inflamed connective tissue. Particularly, the newly formed woven bone trabeculae were thicker in EthOss group when compared to the other groups. In all other experimental groups, the residual grafted materials were embedded in newly formed interstitial connective tissues and surrounded by newly formed bone trabeculae. The interstitial connective tissue contains numerous mesenchymal cells (Figs 2B to D).

At 6 weeks after surgery, the defect area in control group was filled with denser and thicker formed woven than that of the previous period. Moreover, large bone marrow cavities were observed (Fig. 2E). There was increased in bone formation in all other experimental groups. The newly formed compact bone characterized by Haversian system which composed of concentric layers of bone lamellae surrounding centrally placed Haversian canals (osteon) with osteocytes entrapped inside the formed bone trabeculae were seen. However, in EthOss group numerous osteons were presents when compared to xenograft and allograft groups, indicating high remodeling activity of the new bone. Also, the newly formed bone was thicker, denser, and more mature than all other groups.

Furthermore, the grafted materials were surrounded by or in contact with lamellar bone indicating good osteoconduction. Particularly, most of β-TCP/CS particles in EthOss group were resorbed and small sized residual appeared while xenograft and allograft materials were still apparent in the defect areas with partially resorbed areas. The residual materials often occupied space that may have impeded newly forming bone and prevented establishment of its normal architecture (Figs 2F to H).

Histochemical Results

Masson's trichrome stain was performed to further confirm bone maturation. The collagen fibers and a new bone matrix are stained blue; however, the well-calcified bone is stained red. In tibial bone defects, collagen deposition was prominent at 2 weeks, indicating that bone matrix is actively synthesized from an early time. At 6 weeks after surgery, the newly formed bone turned to red color, indicating that the new bone had undergone maturation (Fig. 3).

At 2 weeks after surgery, the control group displayed reasonable blue stain reaction (Fig. 3A) followed by allograft group (Fig. 3D) with the least blue stain for xenograft group (Fig. 3C) and the EthOss group (Fig. 3B).

At 6 weeks after surgery, the bone tissue showed continuous bone remodeling with properly arranged bone trabeculae comparable between all experimental groups. A small amount of blue stain was observed in the EthOss group indicated the higher bone maturity (Fig. 3F) followed by xenograft (Fig. 3G) and allograft groups (Fig. 3H) with the least bone maturity in control group (Fig. 3E).

One-way ANOVA comparing the quantitative analysis of collagen deposition between different groups at the same time periods showed that at 2 weeks after surgery; there was no statistically significant difference between studied groups ($p = 0.545$). At 6 weeks after surgery, a statistically significant difference was detected between control group and all other experimental groups. Similarly, a statistically significant difference between EthOss group and all other studied groups was detected ($p < 0.001$). However, no statistically significant difference was detected between allograft and xenograft. Analyzing the quantitative data using paired t test displayed significantly significant decrease of collagen among experimental groups from 2 to 6 weeks ($p < 0.001$) (Table 1).

Immunohistochemical Results

Immunohistochemical analysis of OPG protein at 2 and 6 weeks after surgery are shown in (Fig. 4). Positive expression of OPG protein was visualized in connective tissue cells, osteoblasts, osteocytes, and new bone matrix. At 2 and 6 weeks after surgery, the OPG positive expression in the defect area were significantly more pronounced in EthOss group than the other experimental groups (Figs 4B and F). In OPG expression, there was no significance between control, xenograft and allograft groups while OPG expression was significantly higher in EthOss group than the other experimental groups especially in bone matrix at 2 and 6 weeks after surgery. The paired *t*-test illustrated statistically significant increase in OPG expression from 2 to 6 weeks after surgery in EthOss group ($p < 0.001$). However, no significant difference was observed in other experimental groups (Table 2).

Discussion

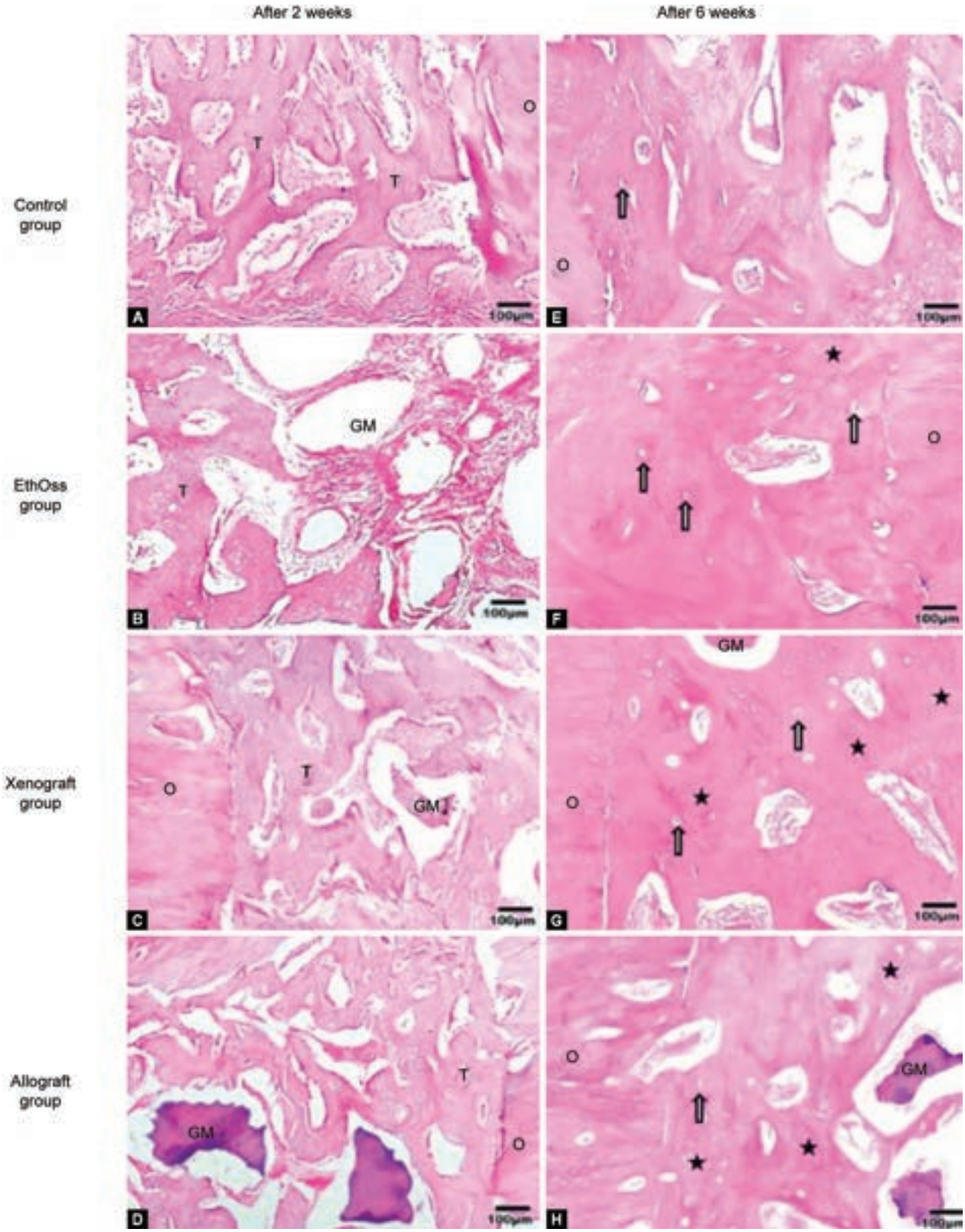
In oral and maxillofacial surgery, regeneration of large osseous lesions is still a challenge. The bone graft materials have been developed for adequate bone regeneration. Autogenous bone remains the gold standard in bone healing procedures, but its postoperative morbidity and low availability necessitate the development of alternative products for it.[11]

The aim of this study was to evaluate the performance of three commercially available bone grafting materials in tibial bone defects in rabbits. In detail, novel alloplastic bone substitutes composed of β-TCP/CS (EthOss), bovine xenograft, allograft, and an empty bone cavity left for spontaneous healing.

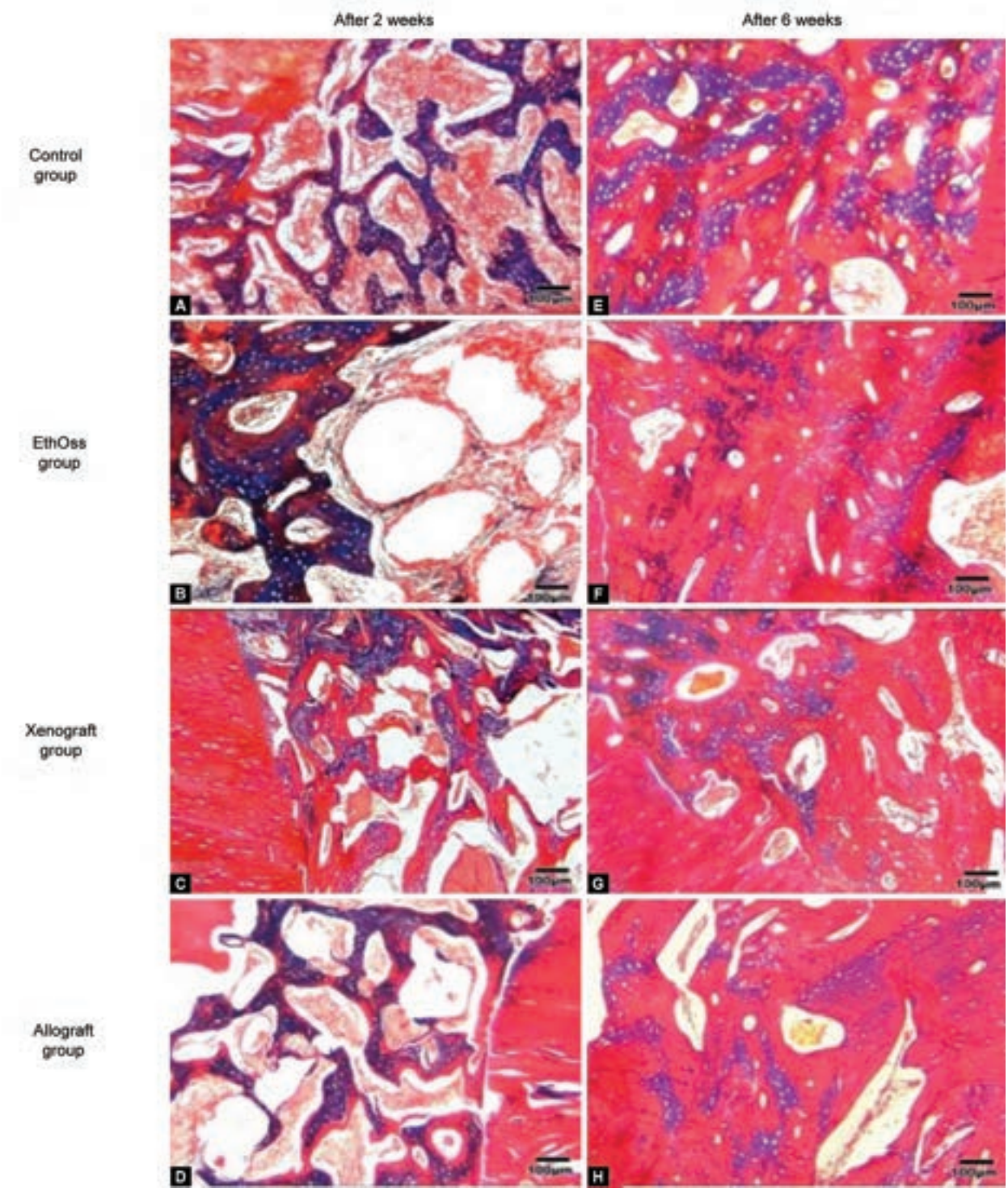
The New Zealand white rabbits are the most popular research breed. These calm and easily handled animal were chosen as an animal model in this study. Adults display some Haversian remodeling and their bone metabolism is somewhat similar to humans thus exhibit desirable traits for bone research as mentioned by Schafrum Macedo et al.[12] and Stübinger et al.[13]

In this study, the histologically stained sections (H&E and Masson's trichrome) clearly showed the difference in bone healing among all different experimental groups.

Regarding the control group, the histological results in this study revealed that the osseous defects filled with granulation tissue with dense inflammatory infiltrates surrounded by thin, newly formed woven bone trabeculae at 2 weeks after surgery. These



Figs 2A to H: Representative histological photomicrographs of bone defect areas at 2 and 6 weeks after surgery in control, EthOss, xenograft and allograft groups. (T) new woven bone trabeculae, (O) old bone, (GM) grafted material. Darkly stained woven bone (star) is surrounded by mature lightly stained trabecular bone consisted of Haversian canals (arrow) (H&E X100)

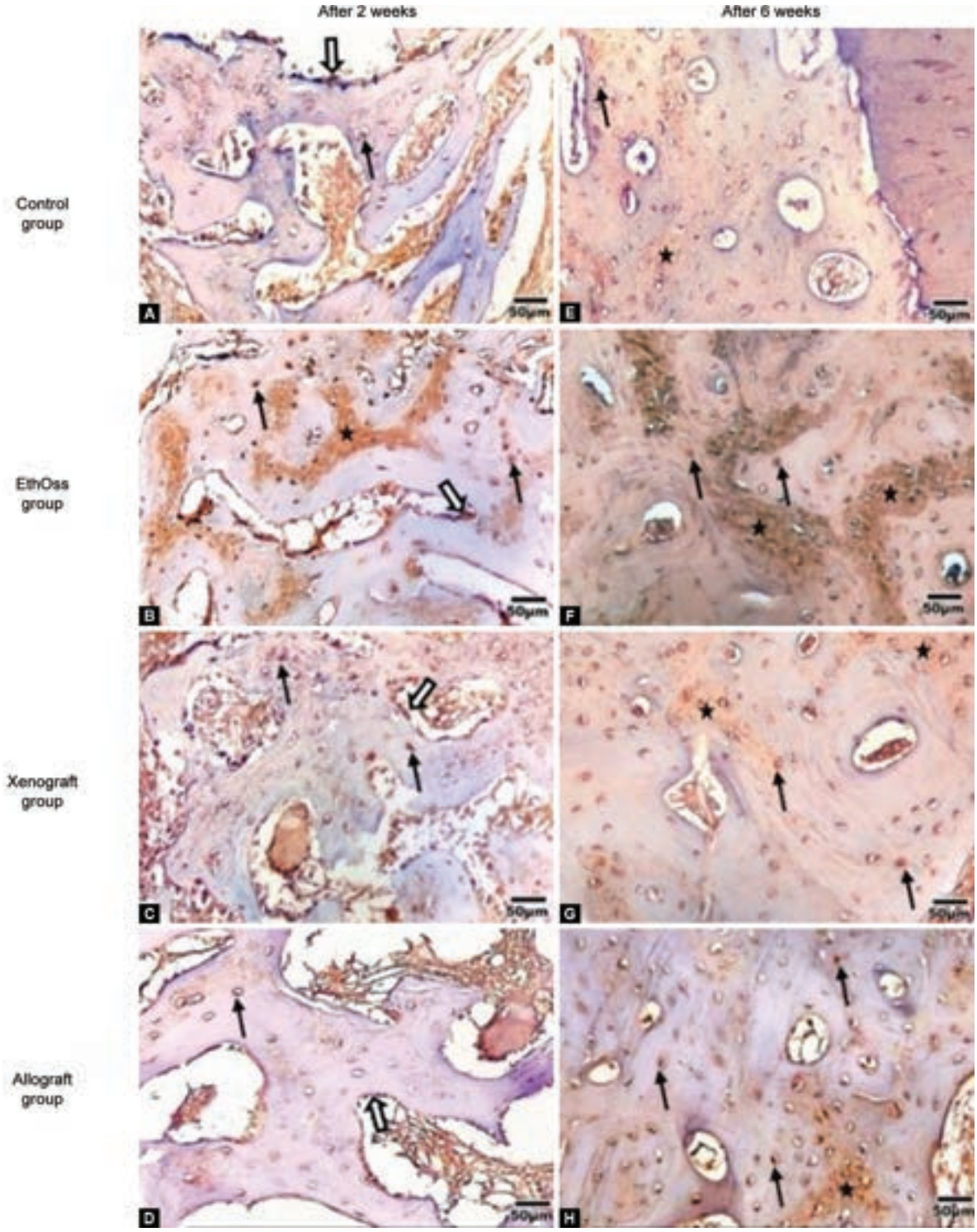


Figs 3A to H: Representative histological photomicrographs of bone defect areas at 2 and 6 weeks after surgery in control, EthOss, xenograft, and allograft groups show the amount of collagen deposition. The blue color of collagen is indicated for the immature woven bone formation while the marked red coloration is indicated for bone maturation and less amount of collagen fibers (Masson's trichrome, ×100)

Table 1: Comparison between the experimental groups regarding the collagen deposition (% area) in newly formed bone (mean ± SD)

	Control	*EthOss*	*Xenograft*	*Allograft*	*One-way ANOVA*
2 weeks	4.19 ± 0.268	3.65 ± 0.15	3.7 ± 0.376	3.99 ± 0.16	$p = 0.545$
6 weeks	2.90 ± 0.009	0.893 ± 0.012	1.44 ± 0.012[a]	1.50 ± 0.012[a]	$p < 0.001$
Paired *t*-test	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	

Similar superscripted letters denote non-significant difference between groups



Figs 4A to H: Representative histological photomicrographs of bone defect areas at 2 and 6 weeks after surgery in control, EthOss, xenograft and allograft groups show the OPG expression in osteoblasts (arrows), osteocytes (black arrows), bone matrix (stars) (OPG, ×200)

Table 2: Comparison of OPG expression in the bone defect area in all experimental groups after 2 and 6 weeks after surgery (mean ± SD)

	Control	*EthOss*	*Xenograft*	*Allograft*	*One-way ANOVA*
2 weeks	3.50 ± 0.435^{a}	4.25 ± 0.14^{abc}	3.51 ± 0.39^{b}	3.48 ± 035^{c}	$p = 0.002$
6 weeks	4.01 ± 0.51^{a}	7.24 ± 0.96^{abc}	4.49 ± 1.41^{b}	3.91 ± 0.63^{c}	$p < 0.001$
Paired *t*-test	0.051	<0.001	0.08	0.211	

Similar superscripted letters denote significant difference between groups

results is in line with El-bahrawy et al.[14] who reported that the bone defect contained loose granulation tissue with inflammatory cells infiltration 2 weeks after surgery. Moreover, at 6 weeks after surgery we revealed relatively thick bone trabeculae began to coalesce with each other. These events were comparable to findings that were reported by AlNashar et al.[15]

The bone healing in control group, in comparison to the other experimental groups, showed a slower rate and minimal extent of bone maturation as observed in the Masson's trichrome stained images, which is a common chemical stain for collagen fibers and immature bone formation. The mean percentage area of collagen fibers was significantly increased than all other experimental groups after 6 weeks (2.90 ± 0.009).

In this study, the results in EthOss group (β-TCP/CS) revealed significant enhancement of bone healing than all other experimental groups at the same healing periods, which declared different degree of bone formation and maturation with a statistically difference at 2 and 6 weeks after surgery.

At 2 weeks after surgery, the histological results demonstrated moderate thick bone trabeculae extended from the defect boundaries toward the center, while at 6 weeks after surgery, well-formed, more compact bone was formed with much less amount of immature bone than all other experimental groups, confirmed a statistically significant decreased of the mean percentage area of collagen fibers; thus, the bone maturation was more than the other experimental groups.

We attributed these results in EthOss group (β-TCP/CS) to the previous speculation by Pabbruwe et al.,[16] which stated that an increase in extracellular calcium concentration from an external source may downregulate the osteoclastic activity without disturbing osteoblastic differentiation; thus, a favorable amount of total bone tissue will be formed.

Moreover, these findings seem to be in agreement with the studies by Leventis et al.,[17] Cai et al.,[18] and Eleftheriadis et al.[19] that described optimum bone regeneration by using alloplastic bone substitutes containing β-TCP/CS as it could promote new bone formation in parallel with graft resorption and acts as an osteoconductive scaffold for bony proliferation. Also, Evaniew et al.[20] and Mazor et al.[21] stated that EthOss (β-TCP/CS), which is characterized by adding CS to β-TCP, makes an excellent compound alloplastic biomaterial that hardens in situ and binds directly to the host bone. It also helped maintain the space and shape of the grafted site. Calcium sulfate act as a binder and making the mixture more stable with a surface that is not susceptible to fracture as well as it increases the porosity of the grafting material by its early resorption, while it facilitates the circulation of biological fluids and growth factors as mentioned by Podaropoulos et al.[22]

As bovine xenograft and allograft were used in this study, less new bone was formed in comparison to β-TCP/CS, most likely as a result of slow resorption of the graft materials. This is in line with the previous studies which found that presence of non-resorbable or slowly resorbable graft particles might adversely affect the bone formation and the remodeling process and result in poor bone quality and quantity.[23]

In this study, EthOss group (β-TCP/CS) had the least remained particles at 6 weeks after surgery, which can be attributed to the addition of CS and the β-phase isomer of TCP (β-TCP), is characterized by physiologic pH, homogeneous microporosity, increased solubility, and a more expectable resorption rate as described by Podaropoulos et al.[22]

Wang et al.[4] mentioned that chemical composition of β-TCP/CS, its porous structure and Ca/P ratio might be the reason for rapid resorption. It is also known that the mechanism of β-TCP bioresorption occurs due to both chemical dissolution in biological fluids and cell-mediated disintegration, as described by Cai et al.[18]

Another study that was in agreement with our observations done by Leventis et al.[17] who found that in grafted rabbit calvaria defects, the percentage of residual graft particles decreased between 3 and 6 weeks (4.54 and 1.67%, respectively).

Regarding xenograft group, in this study, the histological results revealed large bone marrow cavities surrounded by newly formed thin woven bone trabeculae at 2 weeks after surgery, while at 6 weeks after surgery, areas of immature bone rich in osteocytes were present in between mature bone trabeculae. The residual grafted material was surrounded or in contact with the newly formed bone trabeculae. The bone maturation was significantly decreased in comparison to the EthOss group and significantly increased in comparison to the control group while it was nearly as allograft group.

These observations were comparable to studies done by Titsinides et al.[24] and Leventis et al.[25] who evaluated the bone formation in calvaria bone using bovine xenograft. On the other hand, according to Kim et al.,[26] there are still serious worries that using bovine bone graft may transmit prions to patients.

The question of whether this graft is actually resorbable is still up for debate. Human samples that were taken 11 years following sinus floor augmentation with deproteinized bovine underwent histological and histomorphometric analysis as described by Mordenfeld et al.,[27] where they claimed that there were no appreciable changes in particle size and that the xenograft particles were well-integrated in lamellar bone rather than being resorbed.

With respect to allograft group in this study, we used the FDBA that undergoes dehydration and freezing without demineralization, leading to decreased antigenicity and has only osteoconductive potential as described by Titsinides et al.[3] The histological results at 2 weeks after surgery revealed newly formed bone trabeculae which appeared thinner than xenograft group, while at 6 weeks after surgery, moderate thick coalescences bone trabeculae lined by osteoblastic activity with the beginning of osteon formation were observed. The residual grafted material was surrounded or in contact with the newly formed bone trabeculae. The bone maturation was significantly decreased in comparison to the EthOss group.

These findings were in accordance to Titsinides et al.[3] study, who demonstrated decreased bone regeneration with allograft compared to β-TCP, and he attributed this to increase in immune response that could inhibit bone regeneration. According to a recent study done by Steiner et al.,[28] freeze-dried mineralized allografts show slow resorption and remain trapped within the newly formed bone, while resorption capability is suspended after mineralization; thus, it could delay the formation of new bone.

Consistent with our histological results, there were no obvious differences between xenograft and allograft materials regarding the residual grafted materials. A study conducted by Scarano et al.[29] showed similar results. On the contrary, a study by Froum et al.[30] demonstrated the differences in residual grafted materials between allograft and xenograft due to a greater resorption rate in allograft particles.

In this study, the bone formation and maturation did not differ significantly between xenograft and allograft groups. In contrast

to our results, a study by Nappe et al.[31] who demonstrated that the allograft presented a significantly higher amount of newly formed bone percentage than the xenograft, and he speculated that this difference could indicate a different type of biological behavior of the evaluated grafts. On the other hand, disease transmission from the donor to the recipient with allografts, although extremely small, cannot be totally excluded, as mentioned by Shibuya et al.[32]

In this study, the immunohistochemical results confirmed the correspondence between the OPG expression and proper bone healing in our histological results.

The immunohistochemical results revealed different expressions of OPG in osteoblasts, osteocytes, and bone matrix during different healing periods of osseous defects among different groups. OPG expression in EthOss (β-TCP/CS) showed a statistically significant increase when compared to the other groups. However, there were no statistically significant differences between control, xenograft, and allograft groups.

In addition, there was a statistically significant increase during different healing periods which was proportional to an increase of extracellular OPG, which block the bone resorption as stated by Hassumi et al.[33] who performed a study with similar conditions that were in agreement with our results. Also, these findings could be attributed to the fact that the expression of OPG represents the osteoblastic activity and reflects in the quantity of newly formed trabecular bone in elevated periods of bone healing.[34] Also, Silvestrini et al.[9] stated that the presence of OPG in site-specific bone matrix areas of trabeculae has been referred to a protective or preventive action of OPG against the resorbing activity of osteoclasts.

The marked difference between studies may be attributed to the characteristics of the materials used, different healing periods, the type of the animal used as bone healing seems to differ between different species, different types of bone where the defect was created and the type of surgical procedure achieved.

The limitation of this study is the tested grafted materials require further investigations for long period of time to establish their bone forming potential, biodegradation rate, and mechanical strength in critical sized osseous defects.

Conclusion

This study demonstrated the biocompatibility and osteoconductivity of the different grafted materials. The β-TCP/CS grafting material is a promising bioactive alloplastic bone substitute in oral and maxillofacial reconstruction as it showed pronounced bone healing and highly biodegradation rate. The possibility of using β-TCP/CS instead of bovine xenograft and allograft may decrease the risk of disease transmission.

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