ORIGINAL RESEARCH

# Impact of Different Preparations of Tooth Graft vs Xenogeneic Bone Graft on Bone Healing: An Experimental Study

Yousef Ali Abdulrahman Farhan[1], Sally El-Sayed Abdelsameaa[2], Mahitabe Elgamily[3], Sally Awad[4]

## Abstract

**Aim:** This study aims to compare the effect of demineralized xenogeneic tooth graft in its two forms, particulate and block, with bovine xenograft in the healing of a rabbit tibial bone defect model.

**Materials and methods:** Two monocortical bony defects were made in the right tibias of 36 rabbits, and were divided into four groups. Group I defects were left empty, while group II, III, and IV were filled with bovine xenograft, demineralized particulate tooth graft, and demineralized perforated block tooth graft, respectively for evaluation of the bone healing process. Three rabbits from each group were euthanized at 2, 4, and 6 weeks after surgery. The bone specimens were processed and stained with hematoxylin and eosin (H&E) and osteopontin (OPN) immunohistochemical staining. The results were subjected to image analysis and quantitative evaluation.

**Results:** Demineralized particulate tooth graft showed the best bone healing capacity compared to all other groups at all time points tested, as it showed a large amount of the formed bone, rapid closure of the defect with a significant increase in OPN expression, and the least amount of the residual grafted particles.

**Conclusion:** In comparison to bovine xenograft and demineralized dentin block graft, the demineralized particulate tooth grafting material is a promising bone grafting substitute as it proved to be osteoconductive, biocompatible, and bioresorbable.

**Clinical significance:** Demineralized tooth grafting material can aid in the regeneration of large bone defects, leading to improvement in the filling of the bone defects which can help in oral and maxillofacial reconstruction.

**Keywords:** Bone defect, Dentin particulate, Osteopontin, Tooth graft, Xenograft.

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## Introduction

Bone grafting and reconstruction is one of the great challenges in oral and maxillofacial surgery. The filling of bone defects may influence the patient's quality of life. A bone graft is described as a tissue that has the ability to promote bone healing after transplantation into bony defects, either alone or accompanied with other materials.[1] They are categorized as class II devices (bone grafts which fill the bony voids and defects), however Class III represents (bone graft including drugs).[2]

The utilization of bone substitutes in regenerative dentistry has obviously increased due to advancements in dental implantology, periodontal regeneration, and the growing demand for restoration of craniofacial bone defects which may result from trauma, infections, periodontal disease, surgical excision of tumors, or congenital anomalies and malformations.[3]

The ideal graft material should stimulate osteoregeneration via fundamental biological properties such as osteogenesis, osseointegration, osteoinduction, and osteoconduction, as well as ideal biodegradation and resorption rate; these factors are paramount in implementing this role effectively.[4] Osteogenesis is new bone formation via the existent osteoblasts within the grafting material. Osseointegration is the bonding of the graft to the bone surface without the formation of interfering fibrous tissue. Osteoinduction is the recalling of host stem cells to the graft site, and induction of differentiation of these cells into osteoblasts. Osteoconduction is the formation of bioactive scaffold on which the host cells can grow. Biodegradation and absorption rate are important in order to give space for the newly formed bone and not to make interference.[5]

[1,2,4]Department of Oral and Maxillofacial Surgery, Mansoura University, Mansoura, Egypt

[3]Department of Oral Biology, Mansoura University, Mansoura, Egypt

**Corresponding Author:** Mahitabe Elgamily, Department of Oral Biology, Mansoura University, Mansoura, Egypt, Phone: +01000777402, e-mail: mahitabe.fathy@yahoo.com

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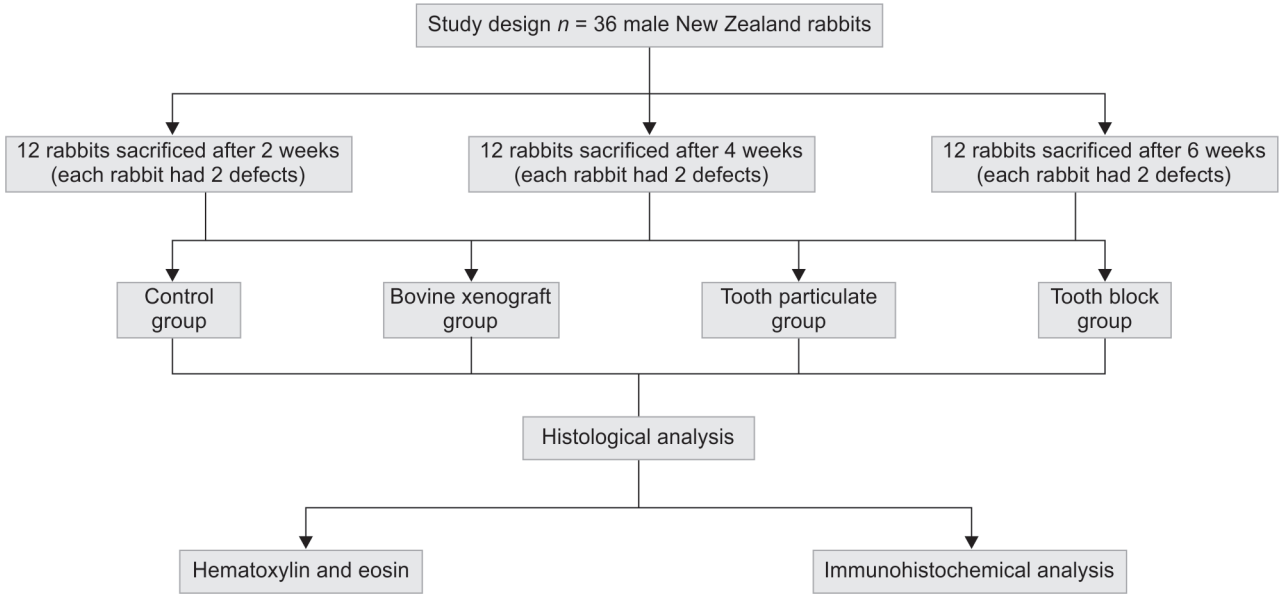
Bone substitute materials recently used in regenerative dentistry have been generally classified into five classes, including natural bone grafts such as allograft, autograft, and xenograft. Synthetic grafts, such as hydroxyapatite and beta-tricalcium phosphate. There are also composite bone substitutes, growth factors based bone substitutes, bone substitutes with infused living osteogenic cells.[3]

Despite the prevalent global usage of bone replacement materials, there are still some limitations which include the usage of allografts that involves the transfer of grafting materials in between two genetically irrelevant subjects with the risk of graft rejection by the recipient's immune system and concerns with disease transmission. Autografts, in which the grafting material is transferred from one site in the body to another site within the same person, with subsequent pain and morbidity at the donor site.[6]

**Flowchart 1:** Flowchart showing study design and groups distribution




None of the currently present products in the market introduces all of the ideal characteristics for a bone replacement material comprising low patient mortality, ease of handling, reduced immunogenicity, reasonable cost, and angiogenic potential. Thus, there is a pronounced increase in demand for the formulation of novel grafts used for bone substitution operations.[7]

Teeth and bone have many similarities. Teeth are a composite structure consisting mainly of calcium hydroxyapatite and non-collagenous matrix proteins like OPN, osteocalcin, type I collagen, and dentin matrix protein. Because these proteins stimulate bone formation, demineralized teeth may be considered a valuable alternative to bone graft due to its osteoinductivity and biocompatibility.[8] Block and particles are the two known forms of the tooth graft. Root dentin was used to create the block form of the tooth graft, it is considered to be a biomimetic of cortical bone, with innate micropores measuring between 3 and 5 m[9], and it has been broadly used in ridge and sinus enlargement.[10]

Given its autogenous origin and content of growth factors, we hypothesize that demineralized tooth-derived graft may act as a valuable alternative bone graft when teeth extraction is necessary. As a result, a comparison with xenogenic bone substitute, which is commercially available and considered osteoconductive and serves primarily as space creators while also preserving volume, may be valuable. Osteopontin is a major non-collagenous protein found in the extracellular matrix of bones; it is produced by differentiated osteoclasts, osteoblasts, and osteocytes; it is involved in bone resorption and formation.[11] Osteopontin is found in various human cell types in numerous tissues, including bone, cementum, dentin, and cartilage. It is considered one of the important markers in bone formation.[12] So, this study aimed to compare the histological and immunological effects of demineralized xenogeneic tooth graft with its two forms (particles and block) and bovine xenograft.

## Materials and Methods

### Animal Selection

In this study, 36 adult healthy male white New Zealand rabbits (Oryctolagus cuniculus) weighing 2 to 3 kg were used. Unhealthy, female, previously treated, and low-weight animals were excluded. After housing the animals in separate cages, they were allowed to access food and water freely. The procedures were performed at the Medical Experimental Research Center, Faculty of Medicine, Mansoura University, from August 2021 to September 2021, under an accepted protocol from Mansoura University's ethical review board; the Faculty of Dentistry, Egypt, with Registration No. (A09010021).

### Sample Size Calculation for Experiment

The current research aimed to compare and evaluate various bone graft preparations. ANOVA was used for analysis of variance; according to Cohen, a minimum total sample size of 36 samples was sufficient to detect the effect size of 0.89 at a power (1-$\beta = 0.90$) of 80% at a significance probability level of $p < 0.05$. A total sample size of 36 was used, according to sample size calculations, each group would be represented by a minimum of six samples. The sample size was calculated according to G*Power software version 3.1.9.6 (Cohen),[13] where $f$ is the effect size = 0.89; $\alpha = 0.05$; $\beta = 0.1$; Power = 1-$\beta = 0.90$

$$f = \frac{\sigma_\mu}{\sigma}$$

$$\sigma_\mu^2 = \frac{\sum_{i=1}^{k} n_j (\mu_i - \mu)^2}{N}$$

### Experimental Design and Sample Distribution (Flowchart 1)

The animals were randomly divided into four groups of nine animals each. Two monocortical bony defects were made in the right tibia of each rabbit. Various grafts were inserted inside the defects as follows:

- Group I (control group): The defects were left empty.
- Group II: The defects were filled with bovine xenograft (particle size <2.0 mm) (OneXeno Graft, OneGraft, Germany).
- Group III: The defects were filled with demineralized particulate tooth graft (particle size 500 µm).

- Group IV: The defects were filled with demineralized perforated block tooth graft (block dimension 3 mm in diameter × 3 mm thickness).

Three rabbits from each group were sacrificed by an overdose of diethyl ether 2, 4, and 6 weeks after surgery, followed by dissection of the tibial bone, which included the surgical sites.

## Tooth Graft Preparation

The tooth graft was prepared in the manner described by Kim et al.[14] Extracted human permanent teeth free from carious lesions or filling were collected at the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Mansoura University. The teeth were cleaned from any soft tissue or calculus using diamond burs followed by extirpation of the pulp tissue and cementum removal. The teeth were decapitated at cementoenamel junction. Teeth were sterilized in 1% chlorhexidine for 10 minutes then two types of tooth graft were constructed.

### *Xenogenous Block Tooth Graft*

To accommodate the size of the bone defect, the root dentin was sectioned and standardized dentin blocks 3 mm in diameter and 3 mm thickness were formed, with one small microhole (0.5 mm wide) made in each block using a small round bur.[15,16] Irrigation with saline solution was performed with all the cutting procedures to prevent possible denaturation of the proteins in dentin with heat.

### *Xenogenous Particulate Tooth Graft*

The teeth were cut into small pieces to facilitate the grinding process,[17] then they were transformed into powder using a conventional grinder with a motor (Mienta - Grinder Presto Co. France). The crushed particles were passed through autoclaved stainless-steel sieve to obtain graft with particle size measuring 500 µm.[18]

Both graft types were sterilized with a peracetic acid ethanol solution 70% (El-Gomhouria Co. Egypt) then demineralization was done using 0.6-N hydrochloride for 70 minutes, washing process was performed two times, the first one with phosphate-buffered saline for 10 minutes and then with distilled water for a further 10 minutes.[16]

### *Xenogeneic Bone Graft (OneXeno Graft)*

It is extracted from bovine bone tissue using a novel chemical-physical enzymatic deantigenation technique. The particle size was <2.0 mm. The use of digestive enzymes functioning at physiological temperature (37°C) resulted in the complete removal of the antigenic component of the tissue without affecting the mineral composition.

## Surgical Procedures

In a clean and sterile environment, the rabbits were anesthetized with intraperitoneal injection of xylazine (25 mg/kg body weight) and ketamine hydrochloride (ADWIA Co. SAE 10th of Ramadan city_ Egypt) (20 mg/kg body weight).[11] A local anesthetic solution containing 2% mepivacaine HCl (Mepecaine-L Alexandria Co. Egypt) was injected into the area of surgery (proximal right tibia) to improve local homeostasis and postoperative analgesia.[12]

After anesthesia was secured, shaving of the tibial skin and disinfection was performed with a sterile cotton pellet soaked with betadine and alcohol. A sharp-cut longitudinal incision of about 6 cm was made along the proximal side of the right tibia. The periosteum was detached to expose the bone and carefully pushed in the lateral direction using a mucoperiosteal elevator.[19]

In each rabbit, two monocortical bony defects were standardized using trephine bur (Bosco Co. Pakistan) 3 mm in diameter and 3 mm in depth mounted to a low-speed micromotor device coupled to a contra-angle hand piece. The drilling was done at constant speed (250 rpm), with cooling by normal saline.

Detachment and mobilization of the bone segments was done using a thin chisel. For irrigation of the cavities, a sterile 5 mL plastic syringe filled with normal saline solution was used, followed by careful drying with small sterile cotton pellets. The graft materials were inserted into the cavities, while the cavities of the control group were left empty. In order to fill the defects to the same height as the surrounding bone, a bone plunger was utilized to gently compress the graft particles. A saline-soaked gauze was employed to further compress the transplant particles (Figs 1A to E). The wound edges were stitched together with 4/0 black silk. The sutures were taken out after nine days, and the skin was sterilized with iodine.

## Postoperative Medication

After surgery, the animals were treated with antibiotics gentamicin 5 mg/kg injected intramuscularly every 12 hours for 5 days (International Egyptian Pharmaceutical Industries Co.10 of Ramadan city_ Egypt). They also received analgesic (100 mg of ketofan, AMRIYA pharmaceutical Co. Egypt) for 48 hours after surgery, every 8 hours.[20]

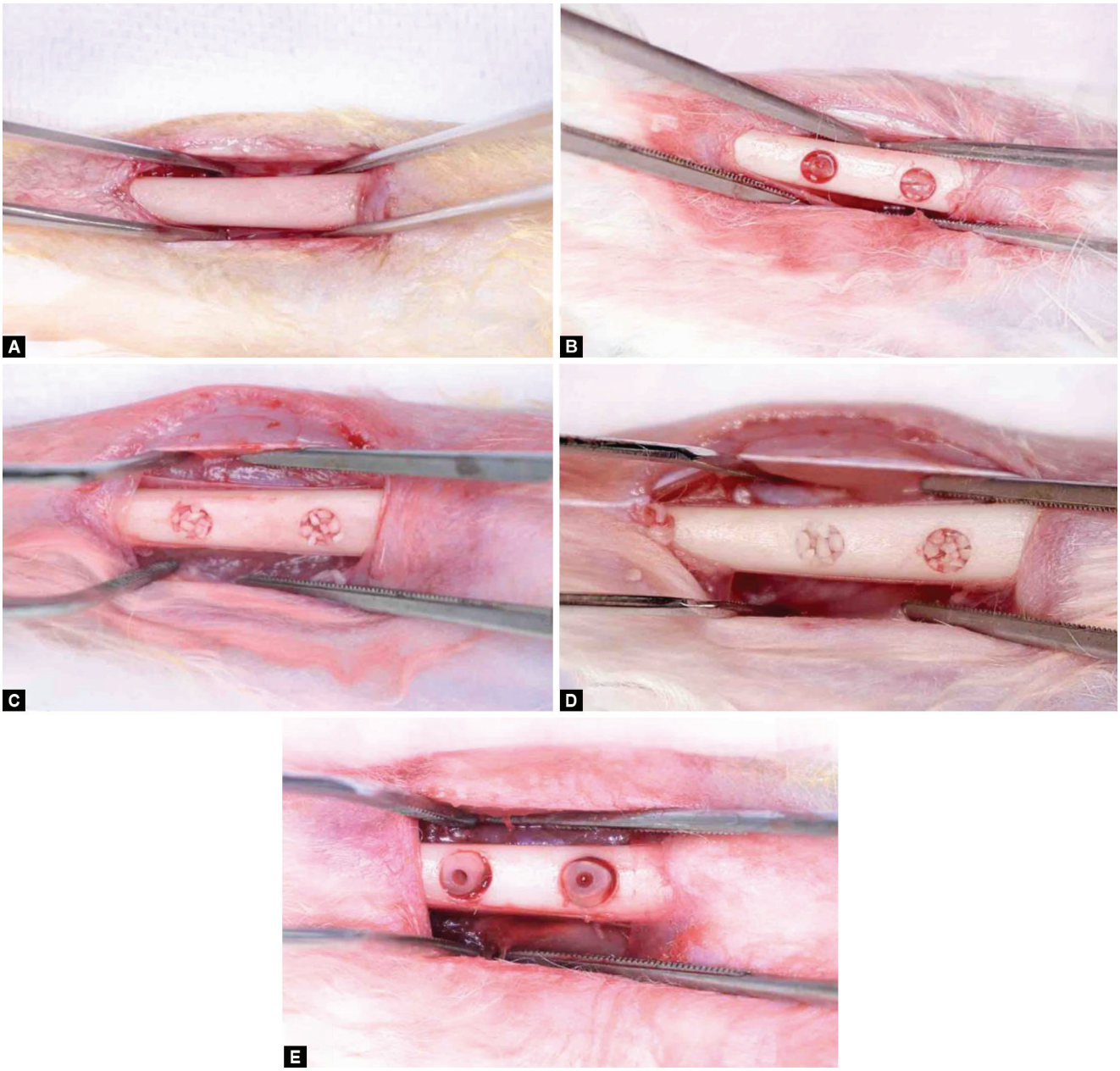
## Histological Analysis

The specimens were carefully dissected immediately after the animals were sacrificed, and then immediately fixed in 10% formalin. Decalcification was performed using 10% ethylenediaminetetraacetic acid (EDTA). After proper decalcification, tissue blocks were routinely processed and paraffin embedded. Six longitudinal serial sections of 4 µm thickness were made (a total of 36 samples per time point). The sections were stained with H&E for general histological observations and OPN immunohistochemical stain.

Regarding immunohistochemistry staining procedures, the sections were blocked in 10% normal goat serum then incubated with the anti-OPN primary antibody (1B20, NB110-89062, Novus Biologicals) at 4°C overnight; and conjugated secondary antibodies HRP (ZSJQ-BIO, Beijing, China) was applied. Diaminobenzidine served as chromogen and slices were counterstained with hematoxylin.[21] The slides were given numbers to be examined blindly without knowing the type of graft used. Finally, digital morphometry was performed for the OPN results and the data were statistically analyzed.

## Statistical Analysis

The data was analyzed using the Statistical Package for Social Science software, version 26, with computer program [statistical package for the social sciences (SPSS), Inc., Chicago, IL, USA]. After testing normality using Shapiro–Wilk test, the data were presented as means ± standard deviations (SDs) for normally distributed data. A one-way analysis of variance was used to compare more than two independent groups, with a *post hoc* Tukey test to detect pairwise comparison while repeated measures ANOVA test to compare




**Figs 1A to E:** (A) Exposure of the tibial bone after periosteal reflection. Filling of the bony defects with different types of grafting materials; (B) Empty defect; (C) Bovine xenograft; (D) Particulate tooth graft; and (E) Perforated block tooth graft

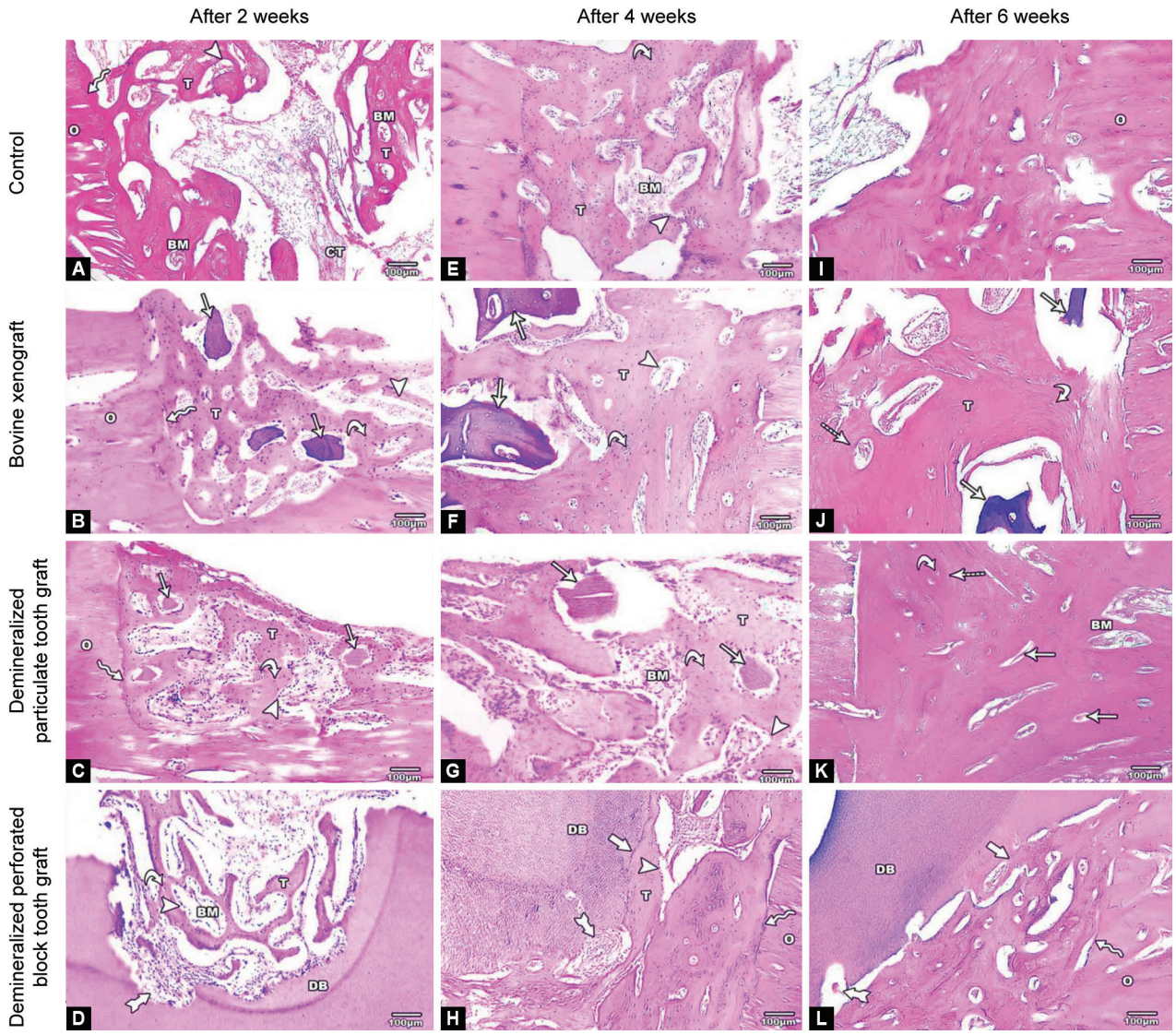
follow-up data. The results were considered statistically significant if the *p* value was less than 0.05.

## Result

### H&E Stain Results (Fig. 2)

The histological specimens showed that healing of the osseous defects filled with tooth particulate was better than all experimental groups and even the control group, which showed a normal healing process with inflammatory cells infiltration, granulation tissue formation, and thin bony trabeculae radiating from the periphery of the defect at the second week (Fig. 2A). These trabeculae became progressively larger at the fourth week enclosing more osteocytes with progressive maturation and osteon formation with large marrow spaces in between these trabeculae (Fig. 2E). When reaching the sixth week the cavity was almost filled except its central area (Fig. 2I).

The healing of the tooth particulate group was better than the other groups regarding the amount of formed bone and the rate of graft resorption in all time intervals tested as the radiating bony trabeculae that were seen at the second week (Fig. 2C) became progressively larger and more mature with increased thickness of bone trabeculae and more amount of osteon formation accompanied by decrease in the marrow spaces throughout the fourth and sixth week and on the contrary the graft material became progressively smaller. By the end of the



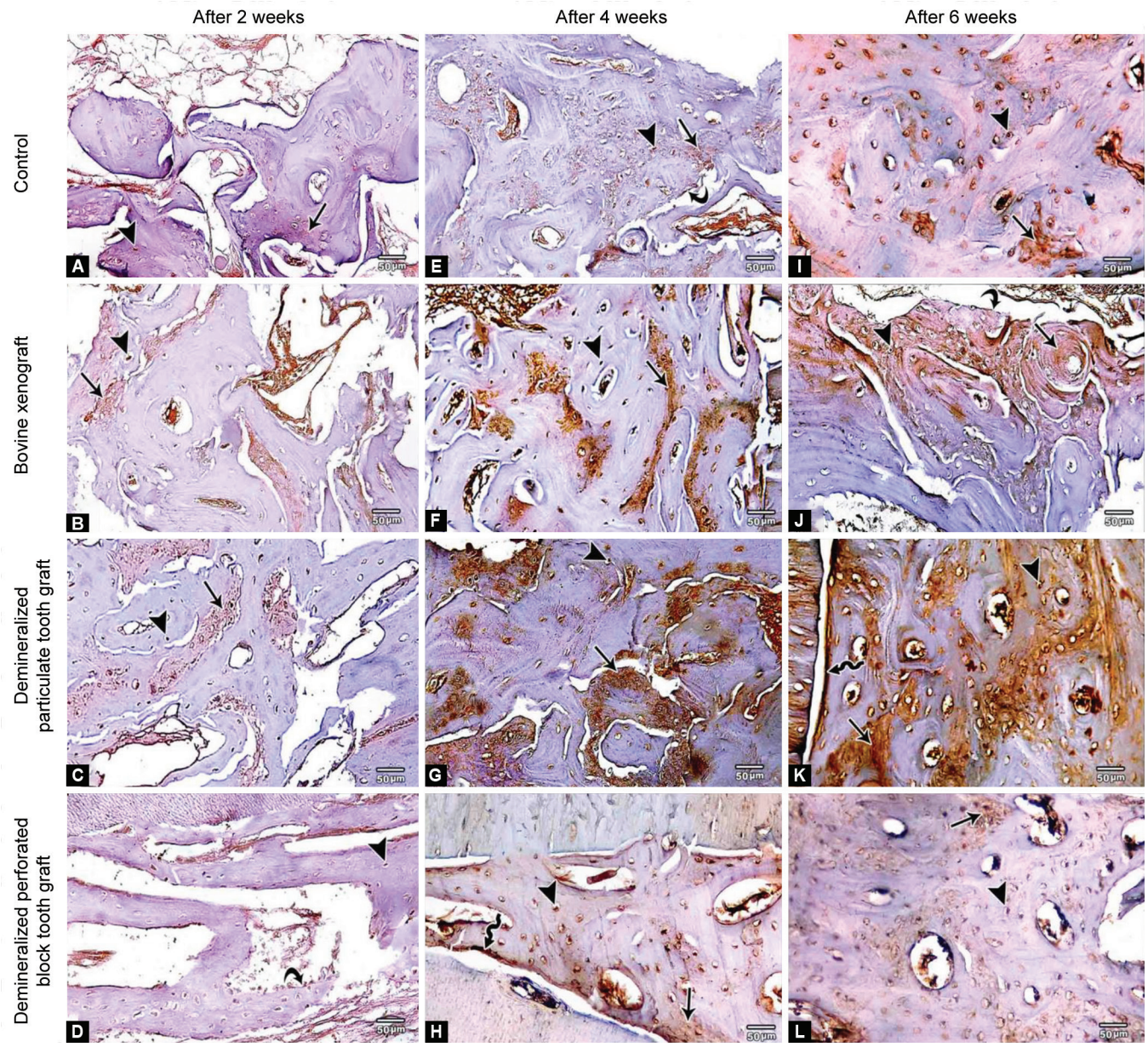

**Figs 2A to L:** Photomicrographs showing the bone defect areas at 2, 4, and 6 weeks following surgery in control, bovine xenograft, tooth particulate, and tooth block groups. (T) new bone trabeculae; (O) old bone; (BM) bone marrow; (thin arrow) grafted material; (CT) connective tissue; (curved arrow) osteocytes; (arrow head) osteoblasts; (wavy arrow) junction between old and new bone; (dashed arrow) osteons; (DB) dentin block; (tailed arrow) resorption lacunae in the dentin block; (thick arrow) cementation line between the dentin block and bone (H&E ×100)

sixth week the cavity was completely filled with bone with mature osteons except for small areas containing minute graft material (Figs 2G and K).

The bovine xenograft group results did not show the same healing capacity as the tooth particulate group, but it was better than the dentin block group because a large amount of bony trabeculae were formed, almost filling the cavity, but the graft remnants were larger than those of the tooth particulate group and remained in the cavity till the sixth week (Figs 2B, F, J) The dentin block showed the least healing capacity as the new bone trabeculae were formed only inside the block perforation and at the periphery of the cavity. Despite the appearance of some areas of adhesion between the newly formed trabeculae and the dentin block and other areas of block resorption, the block seemed to impair the healing process and prevent the establishment of normal architecture as it did not show good resorption rate that allow for rapid replacement of the graft with bone (Figs 2D, H, L).

## Immunohistochemical Stain Results

Osteopontin protein immunohistochemical examination at 2, 4, and 6 weeks after surgery are shown in (Fig. 3). A positive expression of OPN was detected as brown color in osteoblasts as in (Fig. 3D), osteocytes (Figs 3E, I, L), and the newly formed bone matrix (Figs 3A, B, F, J) with higher concentration at the cementation line between the old and new bone (Figs 3K and H). There was a progressive significant increase ($p < 0.001$) in OPN expression from the second till the sixth week in all groups as the mean values of the control group increased from (2.12 ± 0.001) till (4.48 ± 0.007), the bovine xenograft group values increased from (4.63 ± 0.008) to (8.97 ± 0.019), the particulate group from (7.51 ± 0.014) to




**Figs 3A to L:** Immunohistochemical photomicrographs of the bone defect areas at 2, 4, and 6 weeks following surgery in control, bovine xenograft, tooth particulate, and tooth block groups show the OPN expression in osteoblasts (curved arrow), osteocytes (arrow head), bone matrix (arrow), cementation line (wavy arrow) (OPN ×200)

**Table 1:** Comparison of percent area between studied groups during follow-up

| | | *Control* | *Bovine xenograft* | *Tooth particulate* | *Dentin block* | *F* | *p-value* |
|---|---|---|---|---|---|---|---|
| % Area | 2 weeks | 2.12 ± 0.001 | 4.63 ± 0.008 | 7.51 ± 0.014 | 0.990 ± 0.014 | 627 | <0.001* |
| | 4 weeks | 3.04 ± 0.05 | 6.41 ± 0.009 | 12.86 ± 0.004 | 1.12 ± 0.003 | 234 | <0.001* |
| | 6 weeks | 4.48 ± 0.007 | 8.97 ± 0.019 | 16.04 ± 0.056 | 2.21 ± 0.018 | 1875 | <0.001* |
| Repeated measures ANOVA test | | *F* = 9118<br>*p* < 0.001* | *F* = 18235<br>*p* < 0.001* | *F* = 86504<br>*p* < 0.001* | *F* = 13625<br>*p* < 0.001* | | |

*Statistically significant; Parameters described as mean ± SD

(16.04 ± 0.056), and the tooth block group increased from (0.990 ± 0.014) to (2.21 ± 0.018).

The tooth particulate group showed more pronounced significant OPN expression than the other groups at all tested time points. After 2 weeks the values were (7.51 ± 0.014) then reached (12.86 ± 0.004) after 4 weeks, and finally at 6 weeks the values were (16.04 ± 0.056) which was the maximum in this experiment (Figs 3C, G, K). Osteopontin expression values in the tooth particulate group were significantly higher than all other groups at all tested time points ($p < 0.001$) as determined by the *post hoc* Tukey test (Table 1).

## Discussion

The most important goal of oral and maxillofacial surgeons is to restore the architecture and function of bone, especially in large defects regions. The grafting material plays a crucial role in whether or not the bone augmentation surgery is successful. An ideal graft material must have the ability of induction of bone formation and proper biodegradation.[22] The specific goal of the present study was to investigate the influence of different forms of demineralized xenogeneic tooth graft as particles and as blocks vs xenogeneic bone graft in healing of rabbit tibial bone defects.

Tovar et al.,[23] suggested rabbits as the animals of choice because they are larger than mice and rats, and in bone regeneration studies they reveal a better comparability to humans. Rabbits are also less expensive to maintain and house in comparison to larger animal models.

In the current study, the histologically stained sections with H&E obviously revealed the variation in bone healing between all the experimental groups. The control group showed the classical sequence of bone healing, starting with granulation tissue and little radiating bony trabeculae at 2 weeks, followed by a progressive increase in trabeculation with large marrow spaces until the osseous defects revealed more mature osteons and larger trabeculae but the cavity was not completely filled at 6 weeks. These results were in agreement with that of Abdel-Ghany et al.[24] who revealed that in the control group there was no evidence of bone regeneration in the center of the bone defect, although there was evidence of vascular and fibrous tissue arising in the site. The few new bone trabeculae were separated by large areas of marrow 2 weeks post-surgery.

Regarding the experimental groups in this study, we observed differences in the healing of the bone defects as the tooth particulate group showed the best healing capacity, followed by the xenograft group, which showed better bone healing than the perforated block tooth graft and comparable to the control group as remnants of the graft material were still present at 6 weeks. There was a significant difference with the particulate tooth graft group at the same time periods.

The results of the bovine xenograft group were comparable to the results of Sohn and Moon[25] and Brito et al.[26] who assessed the bone repair with different bone substitutes including deproteinized bovine xenograft. The progression of the healing process may be due to the fact that deproteinized bovine xenograft is physically and chemically identical to human bone in the form of cortical granules exhibiting a large mesh interconnecting micro and macropore system that enables angiogenesis and osteoblast migration it also gives stabilization to the coagulum in the first healing phases and act as a scaffold for newly formed bone in the later stages. Also removal of all organic components with maintenance of the natural architecture of bone improved its biocompatibility.

The large remnants of the bovine bone grafted material were obviously seen from the second week, and they were still present at the sixth week, interfering with the complete filling of the defect. This was mentioned by Peng et al.,[27] who demonstrated that the graft material present in the defect was reduced between fourth and sixth weeks. However, it is still present in the sixth week. On the contrary Lee et al.[28] observed histologically resorption of the graft material after six weeks followed by replacement by new bone.

Traini et al.[29] indicate that the difficulty of resorption of bovine bone graft is related to its elevated calcium content and absence of proteins. Besides, a previous study by Ezirganli et al.[30] reported that no sign of deproteinized bovine bone resorption was detected after 30 days and even after 180 days the graft was not resorbed completely. In fact, some authors have considered deproteinized bovine bone as a non-resorbable graft science it requires 3 to 6 years for resorption, while others said that it may be slow to degrade and may not permit sufficient bone healing because of its low osteogenic power.[31]

As an alternative, demineralized tooth was adopted for reconstruction of bony defects and was used in two forms particulate and block. In the present study, the tooth graft in its two forms was demineralized, as it was discovered that demineralization is essential to release growth factors from dentin to promote osteoinduction,[25] these growth factors can induce osteoblast differentiation from the undifferentiated mesenchymal cells and stimulation of bone formation.[32] Calcified dentin showed delayed osteoinduction as the hydroxyapatite may block the release of various growth factors from dentin.[33] Murata et al.,[34] and Park et al.[35] reported that demineralization process reduces the high crystallinity of hydroxyapatite, which improves osteoblast adhesion and enhances the rate of resorption of dentin to permit bone remodeling after grafting, it also decreases its antigenicity making the demineralized dentin to be more active in induction of bone formation than mineralized dentin.

Our findings in the demineralized particulate tooth graft group showed significantly improved bone healing compared to all other groups in all tested time points, whose results indicated varying degrees of bone formation and maturation over the various time periods (2, 4, and 6 weeks). The healing process was progressively improved starting from the second week till the sixth week regarding the amount of the formed trabeculae, their maturation, and also the filling of the defect. The results of this group was superior to all other groups at all the tested time points despite of the presence of remnants of the tooth graft particulates from the second week till the end of the experiment, but these remnants were smaller than those of the bovine xenograft group and they became progressively smaller by time so they did not interfere with the healing process.

These results seem to be consistent with Sohn et al.,[36] who showed that demineralized particulate tooth graft exhibited more active bone regeneration when compared with anorganic bovine bone. Park et al.[35] attributed the high affinity of tooth graft in stimulating new bone formation to its composition. The main inorganic component is hydroxyapatite, that has an osteoconductive property, in addition to the osteoinductive effect of the organic component.

Regarding the size of the graft remnants, Shapoff et al.[37] reported that the size of the particles affected later bone formation.

Our result showed that the tooth graft remnants were smaller than the bovine xenograft and became progressively small with time, this was in accordance to Sohn et al.[36] who found the density and size of the demineralized particulate human tooth diminished by the end of the sixth week. Moreover, Xu et al.[38] showed that there were significantly lesser amounts of tooth graft material at the eighth week compared to the second week, while in the bovine xenograft group, the decline in the amount and size of the grafted material over time was lesser. Bhaskar et al.[39] revealed that 500 µm is the ideal particle size of the bone graft materials. The recommendation of this size was because resorption requires a prolonged time if the particle size is too large, and if the size is too small early resorption of the particles occur before they are able to function properly as a graft material.

Dentin block is a biomimetic of cortical bone that exhibits slow creeping substitution properties with 3 to 5 µm innate micropores (dentinal tubules) and around 0.2 to 0.3 mm macropores.[9] Regarding the tooth block results, very thin trabeculae began to be formed inside its perforation and surrounding the block periphery. Very little progress in block resorption and in the healing process was noticed by time and at the end of the experiment at 6 weeks the block was still occupying the defect with little amount of bone formed at the periphery. These findings seem to be in agreement with Moon et al.[16] who evaluated new bone formation utilizing human dentin block as a graft on rabbit calvaria. Their results showed that the newly formed bone and fibrovascular tissue were observed in microholes and at the interface between the dentin block and the calvaria after 2 weeks, osteoclasts were still found at the microperforated demineralized dentin block surface after 8 weeks.

Al-Asfour et al.[40] revealed similar findings, they showed that dentin block graft remained in the cavity till 12 weeks. Resorption cavities were seen in the dentin accompanied by bone formation. The graft fused with the underlying bone and was partially resorbed. In some areas, union of bone to dentin was noticed, representing a process of osseous replacement of the dentin by new bone.

Our histological results revealed that the bone defect healing was significantly decreased compared to all previous groups. This can be attributed to the large size of the block which need longer time for resorption and little amount of the constructed perforation as Moon et al.[16] explained that microperforations increased the number of dentinal tubules that transmit growth factors, and permit blood infiltration for carrying of bone-forming cells inside the dentin block which will enhance bone regeneration. Therefore, if a greater number of small holes had been made this could have facilitated its resorption and the new bone formation on the top of the graft material.[41]

In this study, OPN was used as a marker to identify the intensity of new bone formation. Osteopontin is a highly phosphorylated sialoprotein that is considered as an important component of the mineralized bone extracellular matrix. There is a strong evidence supporting that it has close association with osteoblastic differentiation. osteopontin biosynthesis takes place in a various cell types such as odontoblasts, preosteoblasts, osteocytes, and osteoblasts.[42] In this study, the immunohistochemical stain results showed the association between OPN expression and proper bone healing.

Our findings revealed expressions of OPN in osteoblasts, osteocytes, and bone matrix as mentioned by Laçin et al.[43] The expression was weak in the 2 week groups and increased gradually through the fourth and sixth week in all groups with statistically significant difference between the tested time points, this was in accordance with Sakamoto et al.[44] who demonstrated that collagen type I appears in the early stages of osteogenesis, while OPN appears in the middle stage and osteocalcin in the late stages. Sohn et al.[36] also demonstrated that OPN expression increased progressively from 2 weeks till 8 weeks with significant difference.

Osteopontin expression in demineralized particulate tooth graft was higher than all groups in all tested time points with a statistically significant difference. This means larger amount and better quality of the formed bone in comparison with the other groups as the increased expression of major markers of bone differentiation, i.e., OPN, collagen type I and osteocalcin are linked with the maturation of osteoblasts and bone development it was also proved that OPN accumulate in the mineralized matrix binding strongly to hydroxyapatite.[45] The bovine xenograft group came after the tooth particulate in expression of OPN, while the dentin block group was the least one, this result is consistent with the result of the H&E regarding the amount and quality of the formed bone. So, the results of our study confirm the important roles of OPN in bone healing.

Demineralized particulate tooth graft was superior than the bovine xenograft and the demineralized tooth block as it enhanced the bone healing with better filling of the bone defects, appropriate graft resorption rate with little amount of graft remnants, and greater amount of OPN expression in all tested time intervals. Further studies are required with longer periods of follow-up for better tracing of graft resorption and greater number of perforations in the demineralized block tooth graft that may enhance its resorption rate.

## Conclusion

Different bone graft materials were tested for osteoconductivity and found to have varying degrees of biocompatibility. Demineralized tooth particle grafting material revealed the greatest bone healing capacity and highest rate of biodegradation; hence, it could be considered as a viable bioactive bone replacement in oral and maxillofacial reconstruction.

### Clinical Significance

Demineralized tooth grafting material can aid in the regeneration of large bone defects, leading to improved high-quality bone that can help in oral and maxillofacial reconstruction.

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