ORIGINAL PAPER

© 2023 Polish Dental Association

EVALUATION OF AUTOGENOUS TOOTH GRAFT IN REGENERATION OF PERIODONTAL VERTICAL ALVEOLAR DEFECTS: CLINICAL, RADIOGRAPHIC, AND EXPERIMENTAL STUDY

Fatma S. Elsherbini, Mohamed Abdulrahman, Mohamed Mostafa El-Shennawi

Faculty of Dentistry, Mansoura University, 35516, Mansoura, Dakahlia, Egypt

ABSTRACT

Introduction: Periodontitis is a chronic inflammatory disease, in which destruction of the periodontium occurs. Not well-treated periodontitis causes teeth loss and problems regarding speech and appearance.

Objectives: To evaluate the effectiveness of tooth particles as a graft material in the regeneration of periodontal vertical alveolar defects clinically and regeneration of bone defect experimentally.

Material and methods: A total of 21 patients with stage III severe periodontitis, aged 25 to 45 years, having periodontal vertical defects were selected for the study. They were randomly divided into 3 groups, in which split-mouth study design was used. In group 1, right side was treated by open flap debridement (OFD) alone, and left side with autogenous tooth graft particles. In group 2, control side treated by OFD, and left side using beta-tricalcium phosphate (β-TCP) graft material. In group 3, left side was treated using autogenous tooth graft, and right side using β-TCP graft. For the experimental part, a total of 21 rats had an intended surgical bony defect at diastema area, and were randomly divided into 3 groups. In group 1, defects were created without addition of any graft material. In group 2, defects were filled with β-TCP graft material. In group 3, autogenous tooth particles were used. Clinical data, including plaque index (PI), gingival index (GI), periodontal probing depth (PPD), clinical attachment level (CAL), and radiographic parameters were recorded at baseline and 3 and 6 months post-surgically. For the experimental part, animals were sacrificed one month later. Tissue specimens were prepared for histological analysis.

Results: After 3 and 6 months post-treatment, there was an improvement in PI and GI in all the groups. Also PPD, CAL gain, and radiographic bone formation were significant for autogenous tooth-grafted sites and β-TCP-grafted sites, with a significant improvement in PPD and CAL for tooth-grafted sites compared with β-TCP-grafted sites. Histologically, the amount of new bone formation was higher in group 3 compared with group 1 and 2.

Conclusions: It can be concluded that autogenous tooth particles can be considered as an effective and suitable bone graft material used in the treatment of intra-bony defects in patients with chronic severe periodontitis.

Key words: severe periodontitis, autogenous tooth graft, regeneration, vertical alveolar defects.

J Stoma 2023; 76, 2: 84-93
DOI: https://doi.org/10.5114/jos.2023.128774

INTRODUCTION

Periodontitis is a chronic inflammatory disease, in which destruction of the periodontium occurs. Not well-treated periodontitis causes teeth loss and problems regarding speech and appearance. Classic methods used in treating periodontal diseases include scaling, root planning (SRP), and open flap debridement (OFD). However,

JOURNAL OF STOMATOLOGY
CZASOPISMO STOMATOLOGICZNE
OFFICIAL JOURNAL OF THE POLISH DENTAL ASSOCIATION | ORGAN POLSKIEGO TOWARZYSTWA STOMATOLOGICZNEGO

Address for correspondence: Fatma S. Elsherbini, Faculty of Dentistry, 60 El-Gomhouria St., 35511, Mansoura, Egypt,
e-mail: elsherbinifatma@mans.edu.eg

Received: 17.12.2022 • **Accepted:** 16.03.2023 • **Published:** 20.06.2023

using specific biological materials was shown to be more beneficial than flap surgery in attachment level enhancement in periodontal defects [1-3]. These surgical approaches, such as OFD, provide good accessibility for root surface evaluation and detoxification besides establishing better periodontal form and architecture. Unfortunately, such surgical approaches have limited ability to restore or regenerate lost periodontal tissue. Grafts used for replacing lost bone are widely used in clinical practice to assist the formation and regeneration of destructed bone and periodontium [3]. Bone grafts play partial role as structural scaffolding materials and matrixes for osteoblastic attachment and proliferation. Broad spectrum of bone grafts and bony substitutes have been applied and evaluated in clinical situations, including autogenous, allogenic, and xenogenic grafts as well as alloplastic materials [3]. Bone autografts are considered the gold standard because they have osteogenic, osteoinductive, and osteoconductive potentials [4]. Regardless of these merits, the use of autogenous bone as a graft material is hindered due to limited availability, donor site morbidity, and patients' discomfort, if large amount of bone is needed [5-7]. However, sources of bone graft materials, other than autogenous, present with multiple disadvantages. Allogenic grafts are considered expensive and might have a risk of infection, because information given by the donor is limited or sometimes insufficient [8, 9]. Besides these disadvantages of allografts, xenografts might pose a risk of zoonotic diseases transmission [8]. Alloplastic materials act primarily as bone fillers, and are available at reasonable cost and unlimited quantity, without the risk of disease transmission or need for additional surgery [10]. One of the most widely used alloplastic materials is the beta form of tricalcium phosphate material (β-TCP), which is partially resorbable material that acts primarily as a scaffold for bone formation. Although synthetic materials lacks osteogenic or osteoinductive properties, their use in periodontal reconstruction has been proven beneficial [9]. As a result of restrictions of non-autogenous sources of bone, human teeth could be used as a source of autogenous bone graft. Tooth extraction is a relatively common procedure in dentistry; extracted teeth are considered clinical waste and therefore discarded [11]. Teeth and bone have almost the same biochemical constituents, and almost similar organic and inorganic components [11].

OBJECTIVES

The objective of the present study was to evaluate the effectiveness of tooth particles as a graft material in the regeneration of periodontal vertical alveolar defects clinically and regeneration of bone defect experimentally.

MATERIAL AND METHODS

ANIMAL STUDY

EXPERIMENTAL MODEL

A total of twenty-one healthy adult male Albino rats, weighing from 200 to 220 grams, were included in this study. The procedures were conducted at the Mansoura Experimental Research Centre (MERC), Faculty of Medicine, Mansoura University.

RANDOMIZATION AND STUDY GROUPS

Animals were allocated randomly into three groups, seven rats each group. In group 1, the defect was left for healing without any implanted material. In group 2, autogenous tooth graft material was inserted into the defect, and in group 3, β-TCP graft material was inserted into the defect.

TOOTH BONE GRAFT PARTICLES PREPARATION

Extraction of one of two upper central incisors of the rat intended for graft was performed. Crowns of extracted teeth were decapitated at CEJ using high-speed diamond disc under gentle water cooling; the pulp was extirpated using nerve broaches. Then, the remaining root structure was disinfected with 10% oxygenated water for 1-3 minutes, and rinsed with sterile saline. The remaining roots were dried with air syringe and were grounded using a grinder (De'Longhi KG40 170 Watt, electric coffee bean grinder, Italy).

SURGICAL PROCEDURES

Surgical procedures were made under complete aseptic conditions. All surgical steps and animals' follow-ups were conducted under supervision of MERC veterinary members. Surgical procedures were performed under general anesthesia using intra-peritoneal injection of xylazine 0.1 mg/kg body weight (Xyla-Ject® Adwia Pharmaceutical Co., Cairo, Egypt) and ketamine 75 mg/kg body weight (Ketamax-50®, Troikaa Pharmaceutical Ltd., India). Skin hair was shaved in the area of surgery and disinfected with povidone-iodine. A clean cut horizontal incision was done using scalpel number 15, along lower border of the mandible till exposure of the bone at the area of diastema. Critical-sized bone defects were made at the diastema area of all rats in the right side of mandible using surgical trephine bur with 4 mm diameter and 1 mm depth, at low-speed and under normal saline irrigation [12, 13]. Different materials were inserted into the cavities according to grouping. Flaps were repositioned carefully and sutured with 6/0 polypropylene sutures. All animals were maintained on a soft diet for two weeks after surgery, and antibiotics were prescribed (amoxicillin 250 mg®, EIPI Co., 10th of Ramadan City, Cairo, Egypt; IM injection twice daily for 3 days) and analgesic (diclofenac sodium 75 mg/3 ml®, PHARCO, Alexandria, Egypt; IM injection once daily for 3 days). The surgical site was then painted with povidone-iodine (povidone-iodine solution®, Pharmaplast, Alexandria, Egypt) externally. All drugs were administered by veterinaries.

SACRIFICE AND HISTOLOGICAL EXAMINATION

The rats were sacrificed 4 weeks post-surgically using an overdose of anesthesia (halothane). Then, all mandibles were dissected and collected. The right side of mandible was fixed in 10% natural buffered formalin and de-calcified in 10% EDTA. Then, the samples were processed for hematoxylin and eosin staining (H&E).

HISTOMETRIC ANALYSIS

Calculating the amount of new bone formation on H&E-stained slides was done by virtual slide system microscopy, and surface area of computer-assisted digital image analysis was obtained using an automated image analysis system coupled with a video camera on a light microscope. Sections were examined at 200× magnification.

CLINICAL STUDY

STUDY DESIGN

The present study included twenty-one male participants selected from the Department of Oral Medicine and Periodontology Outpatient Clinic, Faculty of Dentistry, Mansoura University (study flow chart shown in Figure 1). Inclusion criteria were participants aged between 25 and 45 years with one or more radiographically detectable intra-bony defects with clinical periodontal pocket depth ≥ 5 mm, clinical attachment loss ≥ 5 mm, and stage 3 severe periodontitis. Participants' exclusion criteria were patients with significant comorbidities, such as recent heart attack or coagulation disorder, smokers, pregnant and lactating women, and patients with poor oral hygiene. Patients with acute infection, periapical lesion, root fracture, and root caries of teeth to be extracted [14] were also excluded.

SAMPLE SIZE CALCULATION

Calculating of the sample size was based on evaluation of new bone formation between studied groups and was retrieved from a previous research (Lee *et al.*, 2011). Using G*power version 3.0.10 to calculate sample size, two-tailed, α error = 0.05, and power = 80.0% were based on effect size = 1.17, and total sample size was 14 in each group.

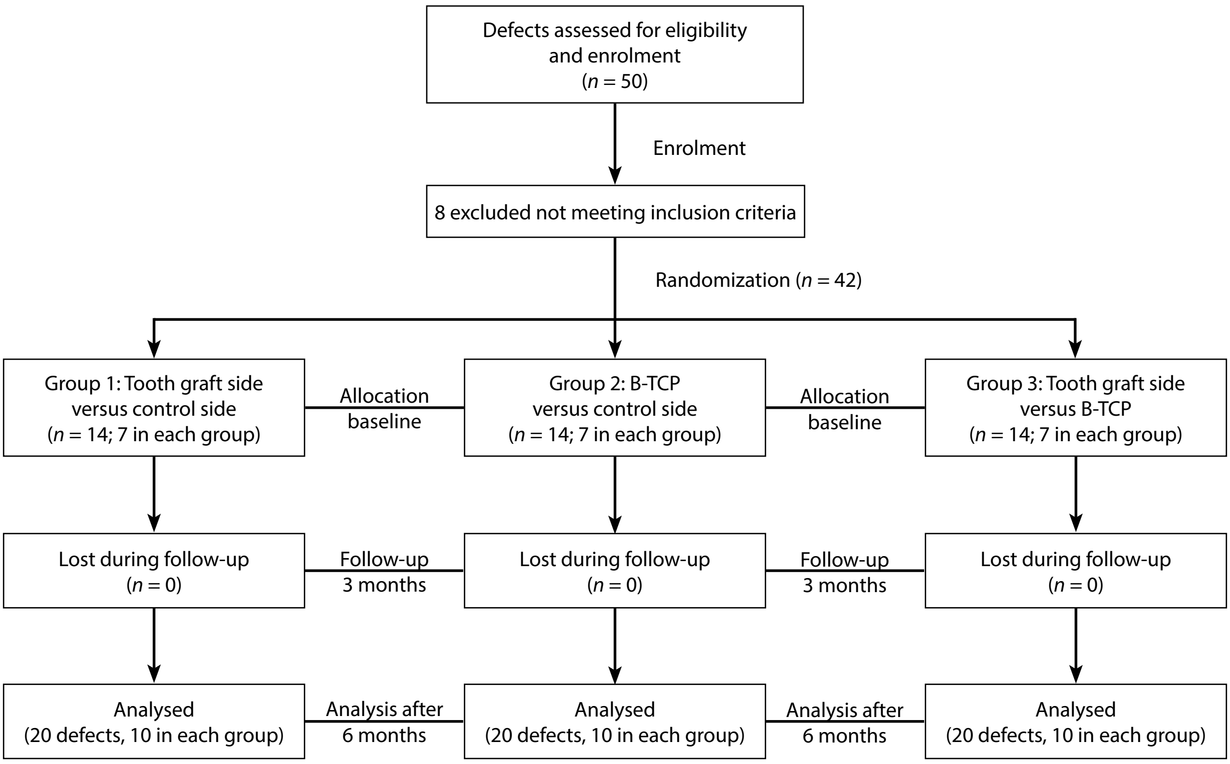

FIGURE 1. Study flow chart

ETHICAL CONSIDERATIONS

This study was conducted following guidelines regulating research work on human subjects of the Faculty of Dentistry, Mansoura University, and the study protocol was reviewed and approved by Research Ethics Committee of the Faculty of Dentistry, Mansoura University, Egypt (number: A02110220).

PATIENTS' CONSENT

A written informed consent was obtained from all patients who were enrolled into the study at baseline. They were informed about the purpose of the study, the treatment received, and the steps performed, including surgical procedures, possible risks, side effects, time of treatment, and appointment schedule according to rules of Ethical Committee of the Faculty of Dentistry, Mansoura University.

PHASE I THERAPY

All participants underwent perfect scaling using ultrasonic device with special tip, and root planning was performed using Gracey curettes. Finally, all participants were re-evaluated at four weeks following phase one periodontal therapy to assess suitability for surgery. Also, patient cooperation in maintaining oral hygiene was assessed.

CLINICAL RECORDINGS

Periodontal clinical parameters (PI [15], GI [16], PPD [17], and CAL [18]) were obtained immediately before surgery, and repeated three and six months after surgery with periodontal probe (a graduated 1 mm UNC 15 probe). Periapical paralleling radiographs (Soredex, Digora®, Optime, Tuusula, Finland; info@soredex.com) were used to measure the vertical depth of bony defect. Customized bite blocks were applied in patients and used during pre- and post-operative exposures. Bone fill (BF) and change in the height of alveolar crest (ACH) were assessed at baseline, and 3 and 6 months post-operatively.

RANDOMIZATION AND STUDY GROUPS

A split-mouth study was conducted in all patients who had bilateral intra-bony defects, with a total of forty two bony defects involved in the study. Patients were randomly allocated into three study groups, seven patients in each group, with fourteen bony defects. In group 1, bony defects were treated using open flap debridement (OFD) alone in the right side, and autogenous tooth graft particles were used with OFD in the left side. In group 2, bony defects were treated using open flap debridement (OFD) alone in the right side, while β-TCP was applied in the left side. In group 3, autogenous tooth particles were used in the right side (prepared from extracted tooth/ teeth at the time of surgery), and β-TCP graft was used in the left side.

PREPARATION OF AUTOGENOUS TOOTH BONE GRAFT

Extraction of the selected tooth/ teeth for each patient was performed. All extracted teeth had their crowns decapitated at CEJ using high-speed diamond disc under gentle water cooling; the pulp was extirpated and diseased cementum was scrapped away from the root surface using sharp curette; the remaining root structure was disinfected with 10% oxygenated water (hydrogen peroxide 10% solution®, Luna Perfumes & Cosmetics, A.R.E., Cairo, Egypt) for 1-3 minutes, rinsed with sterile saline [13], and placed in 0.1% chlorohexidine (Antiseptol 0.1% solution®, Kahira Pharmaceuticals & Chemical Industries Co., Cairo, Egypt) for 5 minutes. Then, it was dried with air syringe and was grounded with a grinder.

SURGICAL PROCEDURES

Surgery for each individual patient was performed under local anesthesia. The incision performed was sulcular incision with elevation of full thickness mucoperiosteal buccal and lingual flaps. Different materials were inserted into bony defects according to a group. Finally, the surgical flap was closed and sutured in a simple interrupted manner using 4/0 polypropylene sutures. Instructions were provided to each individual patient regarding antibiotic treatment (amoxicillin and clavulanic acid) (Augmentin TM 1 gm tablets, El Salam City, Cairo, A.R.E, Egypt): 1 gm per day beginning one day before the surgery and continued for six days, and an analgesic (ibuprofen 400 mg, Brufen 400 mg; Kahira Pharm. & Chem. Ind. Co., Cairo, Egypt) two times per day for three days. Mouth rinse composed of 0.2% chlorhexidine digluconate (Orovex® mouth wash, Macro Group Pharmaceuticals, Macro Capital, Cairo, Egypt) was prescribed to the patients for four weeks after surgery. Sutures were removed usually after 10-14 days [19]. At follow-up appointments (at the 3rd and 6th months post-treatment), clinical parameters and periapical paralleling radiographs were taken for the defect site, and measurements of the defect were recorded.

STATISTICAL ANALYSIS AND DATA INTERPRETATION

Analyzing of the data was done using IBM SPSS Corp., 2013. IBM SPSS Statistics for Windows, version 22.0. (Armonk, NY IBM Corp.). Qualitative data were described in number as well as percentage. Quantitative data were depicted using mean, standard deviation for parametric data after testing normality with Shapiro-Wilk test. Significance of the obtained results was established at 0.05 level. Tests used included Student's *t*-test, one-way ANOVA test with post-hoc Tukey's test to detect pair-wise comparison, paired *t*-test, and repeated measures ANOVA test with post-hoc Tukey's test.

RESULTS

ANIMAL STUDY RESULTS

HISTOLOGICAL RESULTS

In group 1 (control group), the results showed large amount of granulation tissue interspersed with minute amount of bone pieces inside. Blood vessels were seen dispersed within this granulation tissue. Moreover, there was inflammatory cell infiltrate surrounding the newly formed bone. The border of the wound cavity was lined by osteoblasts (Figure 2). In group 2 (β-TCP), the results showed that a number of non-connected bony trabeculae were surrounded by granulation tissue. The newly formed bony trabeculae differed in size, and the intervening connective tissue indicated newly originating bone marrow cavities (Figure 3). In group 3 (tooth graft), the results showed newly formed bony trabeculae surrounding pieces of dentin. Non-resorbed dentin pieces were present within the center of the cavity, with evident osteoblastic activity at the borders of dentin pieces. There was apparent formation of bone tissue radiating from the cavity borders towards the cavity center. Reversal lines were present between newly formed bone and old bone. Blood vessels were present, surrounded by granulation tissue (Figure 4).

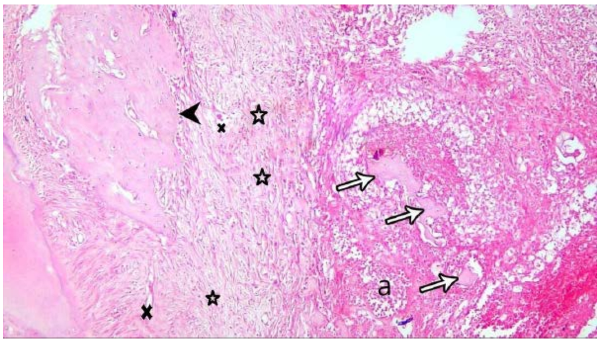

FIGURE 2. A photomicrograph of group I specimen showing large amount of granulation tissue (star) interspersed with minute amount of bone pieces inside (arrows). Inflammatory cell infiltrate could be seen surrounding the newly formed bone (a). The border of the wound cavity was lined by osteoblasts (arrow head). Blood vessels could be seen dispersed within this granulation tissue (cross)

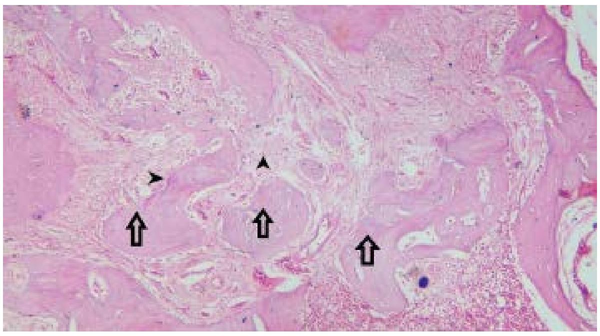

FIGURE 3. A photomicrograph of group II specimen showing non connected bony trabeculae suurounded by granulation tissue (arrows). The newly formed bony trabeculae differ in size. The intervening connective tissue denoting newly originating bone marrow cavities (arrow head)

STATISTICAL ANALYSIS OF HISTOLOGICAL RESULTS

Table 1 shows that there was statistically significant difference in the amount of bone formation between different groups. Post-hoc Tukey's test showed that group 3 exhibited a higher significant difference in the amount of bone formation compared with that of group 2. However, group 2 showed statistically significant difference in the amount of bone formation than that of group 1, with p-value > 0.001.

CLINICAL STUDY RESULTS

Clinical results showed statistically significant decrease of both plaque and gingival indices at follow-up intervals (after 3 and 6 months) in all treatment groups.

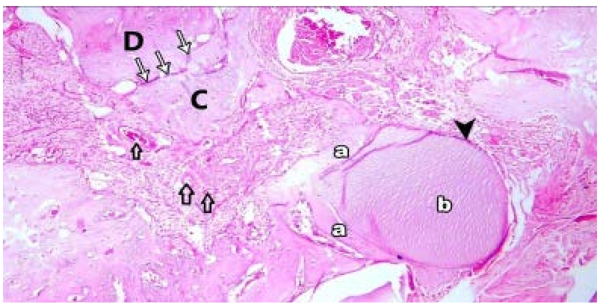

FIGURE 4. A photomicrograph of group III specimen showing newly formed bony trabeculae (a) surrounding pieces of dentin (b). The non-resorbed dentin pieces could be seen easily in the center of the cavity. Reversal lines (arrows) could be seen between newly formed bone (C) and old bone (D). There was an apparent formation of bone tissue radiating from the cavity borders towards the cavity center. Osteoblastic activity at the boders of dentin pieces (arrow head). Blood vessels were present surrounded by granulation tissue (upside arrow)

TABLE 1. The amount of new bone formation between the study groups with different materials used

Groups	Group 1, *n* = 7	Group 2, *n* = 7	Group 3, *n* = 7	*F*-value	*p*-value
Amount of bone formation	1.26 ± 0.07^{ac}	17.15 ± 2.90^{bc}	19.51 ± 2.75^{ab}	184.72	< 0.001*

**Statistically significant; ^{a,b,c} – superscripted letters denote significant difference between groups by post-hoc Tukey's test; F – one-way ANOVA test, parameters described as mean ± SD*

TABLE 2. Comparison between different parameters pre and post treatment between tooth graft and control sides in group I

		Group 1		Student's *t*-test	*p*-value
		Tooth graft side, *n* = 7	Control side, *n* = 7		
PPD	Baseline	5.07 ± 0.61	5.54 ± 0.76	1.28	0.225
	After 3 months	3.77 ± 0.53	4.39 ± 0.46	2.31	0.039*
	After 6 months	2.64 ± 0.419	3.14 ± 0.25	2.71	0.019*
F-value		57.18	52.35		
p-value		< 0.001*	< 0.001*		
CAL	Baseline	5.33 ± 0.32	5.76 ± 0.65	1.57	0.142
	After 3 months	4.07 ± 0.52	4.39 ± 0.56	1.08	0.302
	After 6 months	3.16 ± 0.51	3.73 ± 0.46	2.18	0.049*
F-value		42.91	78.81		
p-value		< 0.001*	< 0.001*		
ACH	Baseline	5.60 ± 0.60	5.53 ± 0.53	0.242	0.813
	After 3 months	4.59 ± 0.56	4.90 ± 0.47	1.14	0.278
	After 6 months	3.357 ± 0.60	4.26 ± 0.38	3.33	0.006*
F-value		201.87	180.03		
p-value		< 0.001*	< 0.001*		
BF	After 3 months	1.10 ± 0.24	0.629 ± 0.19	4.03	0.002*
	After 6 months	2.24 ± 0.14	1.271 ± 0.16	12.17	< 0.001*
Paired *t*-test		11.38	9.38		
p-value		< 0.001*	< 0.001*		

F – repeated measures ANOVA test
**Statistically significant parameters described as mean ± SD*

In Table 2, different parameters for comparing tooth graft and control sides in group 1 are presented, where PPD, CAL, and ACH show statistically significant decrease in both treatment sides after 3 and 6 months. Also, a statistically significant increase of BF is shown, with statistically significant higher mean among control side, as compared with tooth graft side.

Table 3 demonstrates comparison of β-TCP and control sides in group 2, where PPD, CAL, and ACH showed statistically significant decrease with a higher mean among control side, as compared with β-TCP side in CAL and ACH. BF indicated significant increase after 3 and 6 months post-treatment. Moreover, the results showed statistically significant decrease in PPD, CAL, and ACH after 3 and 6 months in group 3 (as shown in Table 4), with a higher mean among β-TCP side, as compared with tooth graft side in PPD and CAL. Also, a significant increase of BF after 3 and 6 months was observed, with a higher mean BF among tooth graft compared with β-TCP side at 6-month interval.

DISCUSSION

The key of using human tooth as a graft material is that it resembles the alveolar bone, which made many researchers to attempt utilizing it as an autogenous source of bone graft. Teeth and bone have almost the same biochemical constituents. Almost all the proteins, which are present in the alveolar bone have been reported to be present in dentin, including osteopontin (OPN), osteocalcin (OC), and bone sialoprotein (BSP). These made the tooth an efficient substitution for other available bone grafting materials [20-22].

The histological results of group 2 showed an apparent and significant amount of bone formed around

TABLE 3. Comparison between different parameters pre- and post-treatment between β-TCP graft and control sides in group II

		Group 2		Student's *t*-test	*p*-value
		β-TCP side, *n* = 7	Control side, *n* = 7		
PPD	Baseline	5.10 ± 0.60	5.11 ± 0.75	0.04	0.969
	After 3 months	4.07 ± 0.75	4.46 ± 0.29	1.26	0.23
	After 6 months	3.25 ± 0.59	3.31 ± 0.31	0.282	0.782
F-value		431.32	51.50		
p-value		< 0.001*	< 0.001*		
CAL	Baseline	5.44 ± 0.63	5.80 ± 0.53	1.14	0.275
	After 3 months	4.33 ± 0.69	4.64 ± 0.42	1.02	0.327
	After 6 months	3.53 ± 0.55	4.29 ± 2.54	3.32	0.006*
F-value		145.85	135.79		
p-value		< 0.001*	< 0.001*		
ACH	Baseline	5.62 ± 0.33	5.77 ± 0.53	0.629	0.541
	After 3 months	4.53 ± 0.34	4.99 ± 0.45	2.16	0.052
	After 6 months	3.54 ± 0.38	4.64 ± 0.37	5.51	< 0.001*
F-value		658.25	87.87		
p-value		< 0.001*	< 0.001*		
BF	After 3 months	1.124 ± 0.125	0.800 ± 0.238	3.19	0.008*
	After 6 months	2.07 ± 0.11	1.04 ± 0.139	15.24	< 0.001*
Paired *t*-test		12.55	3.07		
p-value		< 0.001*	0.02*		

F – repeated measures ANOVA test
**Statistically significant parameters described as mean ± SD*

tooth graft particles with osteoblastic activity, which could promote bone healing. These results agree with a research performed by Saleh *et al.* [23], who evaluated the effect of autogenous dentin graft with platelet-rich plasma on alveolar bone healing after tooth extraction. The study group exhibited greater bone formation when compared with the control socket. In the present study, a significant difference of new bone formation between group 2 and 3 was observed ($p < 0.001$). This result agree with a study by Calvo-Guirado *et al.* [24], who reported that the extraction sockets could be grafted with autogenous tooth graft particles obtained by grinding the extracted teeth. They found that more immature as well as mature bone were found at the test sites than the controls at different study times showing that new bone replaced the resorbed tooth particles [24].

However, the histological findings of group 2 disagrees with findings of Mordenfeld *et al.* [25]. They reported that bone formation was not observed using non-demineralized dentin, but it required longer time. Also, results showed that more inflammatory cellular infiltrate seen in group 3 rather than group 2 were not observed in group 1. This could be interpreted by the antigenicity of β-TCP. These findings contradicts with those of Leventis *et al.*, who reported no inflammatory response observed between graft material and bone [26]. Additionally, a study of Su-Gwan *et al.* [27] disagrees with these finding. The authors found that there was more inflammation in dentin particles group than Bio-Oss group.

In the present study, there was significant difference in bone formation between group 1 and 3. This coincides with a study by Rejab *et al.* [28], who reported that histopathological analysis of applying TCP in osseous defect showed more organization and osteoblastic proliferation accompanied by early osteoid tissue formation in the form of trabeculae of woven bone with osteoblastic rimming. However, the histological findings of group 2 in the present study contradicts with a research performed by Eleftheriads *et al.* [29], who found that the defect of rabbits' mandibles in control group had completely filled with mature bone earlier than in β-TCP group. Also, Hirn *et al.* [30] reported that bone defects that are left empty heal only when filled with a bone substitute material. Moreover, the results revealed a significant improvement in all clinical indices of the three groups, which could be due to the fact that SRP is considered the gold standard for mechanical therapy in the treatment of periodontitis [31]. However, the improvement was better for both tooth-grafted sites and β-TCP-grafted sites in PPD and CAL. Regarding tooth-grafted sites, the

TABLE 4. Comparison between different parameters pre and post treatment between tooth graft and β-TCP graft sides in group III

		Group 3		Student's *t*-test	*p*-value
		Tooth graft side, *n* = 7	β-TCP side, *n* = 7		
PPD	Baseline	5.90 ± 0.48	6.07 ± 0.65	0.562	0.585
	After 3 months	3.87 ± 0.48	4.93 ± 0.67	3.41	0.005*
	After 6 months	3.25 ± 0.59	3.91 ± 0.64	3.39	0.005*
F-value		???1395???	521.45		
p-value		< 0.001*	< 0.001*		
CAL	Baseline	6.41 ± 0.69	6.43 ± 0.49	0.04	0.965
	After 3 months	3.89 ± 0.56	5.21 ± 0.67	4.01	0.002*
	After 6 months	3.06 ± 0.47	4.17 ± 0.48	4.44	0.001*
F-value		661.16	296.98		
p-value		< 0.001*	< 0.001*		
ACH	Baseline	6.67 ± 0.44	6.43 ± 0.53	0.922	0.375
	After 3 months	5.11 ± 0.40	5.04 ± 0.54	0.281	0.783
	After 6 months	4.39 ± 0.39	4.33 ± 0.55	0.224	0.826
F-value		484.20	482.90		
p-value		< 0.001*	< 0.001*		
BF	After 3 months	1.60 ± 0.183	1.39 ± 0.19	2.12	0.056
	After 6 months	2.29 ± 0.10	2.10 ± 0.09	3.39	0.005*
Paired *t*-test		7.95	8.08		
p-value		< 0.001*	< 0.001*		

F – repeated measures ANOVA test
**Statistically significant parameters described as mean ± SD*

results could be due to osteoconductive and potentially osteoinductive effects of tooth graft particles. This was in agreement with both Wushou *et al.* [32] and Sánchez-Labrador *et al.* [33], who conducted a split-mouth study in the treatment of osseous defects after extraction of impacted mandibular third molar using autogenous tooth graft powder and autogenous dentin graft, respectively. They found that tooth-grafted sites exhibited progressive bone filling and ossification after six months as well as lower probing pocket depths compared with that of control sites. A study performed by Mazzucchi *et al.* [34] supports these findings.

Regarding β-TCP-grafted sites, the improvement could be due to the fact that β-TCP graft has small-sized particles, which improve the regenerative potential through enhancing vascularization of the host site for graft integration. This agrees with Fan *et al.* [36], who reported similar results by studying the response of periodontium to β-TCP. They showed improved results in the reduction of the pocket depth. Also, Saxena *et al.* [35] used β-TCP graft material and reported an improvement in clinical attachment level. However, the improvement in both PPD and CAL were better in autogenous tooth-grafted sites than in β-TCP-grafted sites, which might be due to the presence of cementum particles, where the composition can play a vital role in periodontal regeneration [37]. This was in agreement with data provided by Grzesik and Narayanan [37, 38], who reported that cementum matrix is rich in many growth factors that affect the activities of multiple periodontal cell types. Various chemotactic factors, adhesion molecules, growth factors, and ECM constituents participate together in the recruitment of cemento-blast progenitors, their expansion, and differentiation as well as many of these components may be available during periodontal healing. Cementum consists of many molecules that promote chemotactic migration, adhesion, proliferation, and differentiation of some periodontal cell types, and these molecules are not detectable in other periodontal structures [39, 40].

There was a significant increase in bone levels post-operatively compared with baseline measurements in autogenous tooth-grafted sites, which was due to the superior osteoinductive and osteoconductive proprieties, resulting in better new bone formation [41]. This was in accordance with Upadhyay *et al.* [42], who conducted a study on the treatment of furcation defects using autogenous tooth graft, and found an improvement in clinical and radiographic parameters. Also, bone levels showed remarkable increase after 6 months post-operative in β-TCP-grafted sites, which could be attributed to β-TCP's behavior by acting as a scaffold, on which new bone grew. Franceschini *et al.* [43] agreed with the above by studying the repair of bony defects using β-TCP. They reported improved results.

CONCLUSIONS

According to the present results, it could be suggested that the use of autogenous tooth as a graft material is a viable option for regeneration of periodontal vertical alveolar defects. It was proved to provide greater amount of bone formation in experimental animals than β-TCP graft material. Moreover, it provided comparable amount of bone fill and better soft tissue healing in patients with stage III severe periodontitis.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Trombelli L, Heitz-Mayfield LJA, Needleman I, Moles D, Scabbia A. A systematic review of graft materials and biological agents for periodontal intraosseous defects. J Clin Periodontol 2002; 29: 117-135.
2. Shirakata Y, Setoguchi T, Machigashira M, et al. Comparison of injectable calcium phosphate bone cement grafting and open flap debridement in periodontal intrabony defects: a randomized clinical trial. J Periodontol 2008; 79: 25-32.
3. Reynolds MA, Aichelmann-Reidy ME, Branch-Mays GL, Gunsolley JC. The efficacy of bone replacement grafts in the treatment of periodontal osseous defects. A systematic review. J Ann Periodontol 2003; 8: 227-265.
4. Pandit N, Pandit IK. Autogenous bone grafts in periodontal practice: a literature review. J Int Clin Dent Res Organ 2016; 8: 27. DOI: 10.4103/2231-0754.176247.
5. Bhattacharjya C, Gadicherla S, Kamath AT, Smriti K, Pentapati KC. Tooth derived bone graft material. World J Dent 2016; 7: 32-35.
6. Kim YK. Bone graft material using teeth. J Korean Assoc Oral Maxillofac Surg 2012; 38: 134-138.
7. Kim YK, Lee J, Um IW, et al. Tooth-derived bone graft material. J Korean Assoc Oral Maxillofac Surg 2013; 39: 103-111.
8. Oryan A, Alidadi S, Moshiri A, Maffulli N. Bone regenerative medicine: classic options, novel strategies, and future directions. J Orthop Surg Res 2014; 9: 18. DOI: 10.1186/1749-799X-9-18.
9. Parikh S. Bone graft substitutes: past, present, future. J Postgrad Med 2002; 48: 142-148.
10. Siaili M, Chatzopoulou D, Gillam DG. An overview of periodontal regenerative procedures for the general dental practitioner. Saudi Dent J 2018; 30: 26-37.
11. Khanijou M, Seriwatanachai D, Boonsiriseth K, et al. Bone graft material derived from extracted tooth: a review literature. J Oral Maxillofac Surg Med Pathol 2019; 31: 1-7.
12. Stein JM, Fickl S, Yekta SS, Hoischen U, Ocklenburg Ch, 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.
13. Pakravan A, Yousefnezhad M, Heydarian A, Bamdadian T. Use of autogenous tooth bone graft with dental implants. J Craniomaxillofac Res 2018; 5: 125-130.
14. Del Canto-Díaz A, de Elío-Oliveros J, Del Canto-Díaz M, Alobera-Gracia MA, Del Canto-Pingarrón M, Martínez-González JM. Use of autologous tooth-derived graft material in the post-extraction dental socket. Pilot study. Med Oral Patol Oral Cir Bucal 2019; 24: 53-60.
15. Silness J, Löe H. Periodontal disease in pregnancy. 3. Response to local treatment. Acta Odontol Scand 1966; 24: 747-759.
16. Chaves ES, Wood RC, Jones AA, Newbold DA, Manwell MA, Kornman KS. Relationship of "bleeding on probing" and "gingival index bleeding" as clinical parameters of gingival inflammation. J Clin Periodontol 1993; 20: 139-143.
17. Susin C, Valle P, Oppermann RV, Haugejorden O, Albandar JM. Occurrence and risk indicators of increased probing depth in an adult Brazilian population. J Clin Periodontol 2005; 32: 123-129.
18. Pihlstrom BL. Measurement of attachment level in clinical trials: probing methods. J Periodontol 1992; 63: 1072-1077.
19. Cortellini P, Pini Prato G, Tonetti MS. Periodontal regeneration of human intrabony defects with titanium reinforced membranes. A controlled clinical trial. J Periodontol 1995; 66: 797-803.
20. Choi YS, Lee JY, Suh JS, Lee G, Chung CP, Park YJ. The mineralization inducing peptide derived from dentin sialophosphoprotein for bone regeneration. J Biomed Mater Res A 2013; 101: 590-598.
21. Nampo T, Watahiki J, Enomoto A, et al. A new method for alveolar bone repair using extracted teeth for the graft material. J Periodontol 2010; 81: 1264-1272.
22. Um IW, Kim YK, Mitsugi M. Demineralized dentin matrix scaffolds for alveolar bone engineering. J Indian Prosthodont Soc 2017; 17: 120-127.
23. Saleh A, Abd el Rehim S, Kawana K, Osman S. Effect of autogenous dentin graft combined with platelet rich plasma on alveolar bone healing after tooth extraction in rabbit. Alexandria Dental Journal 2018; 43: 6-10.
24. Calvo-Guirado JL, Maté-Sánchez de Val JE, Ramos-Oltra ML, et al. The use of tooth particles as a biomaterial in post-extraction sockets. Experimental study in dogs. Dent J (Basel) 2018; 6: 12. DOI: 10.3390/dj6020012.
25. Mordenfeld A, Hallman M, Lindskog S. Tissue reactions to subperiosteal onlays of demineralized xenogenous dentin blocks in rats. Dent Traumatol 2011; 27: 446-451.
26. 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.
27. Su-Gwan K, Hak-Kyun K, Sung-Chul L. Combined implantation of particulate dentine, plaster of Paris, and a bone xenograft (Bio-Oss) for bone regeneration in rats. J Craniomaxillofac Surg 2001; 29: 282-288.
28. Rejab A, Minwah BS, Ameen YA. Histological evaluation for the use of β-tricalcium phosphate as a bone substitute in accelerating bone healing: an experimental study on rabbits. Al-Rafidain Dent J 2014; 14: 205-211.
29. Eleftheriadis E, Leventis MD, Tosios KI, Faratzis G, Titsinidis S, Eleftheriadi I, Dontas I. 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.
30. Hirn M, de Silva U, Sidharthan S, et al. Bone defects following curettage do not necessarily need augmentation: a retrospective study of 146 patients. Acta Orthop 2009; 80: 4-8.
31. Cugini M, Haffajee A, Smith C, Kent R Jr, Socransky S. The effect of scaling and root planing on the clinical and microbiological parameters of periodontal diseases: 12-month results. J Clin Periodontol 2000; 27: 30-36.
32. Wushou A, Zheng Y, Han Y, Yang ZC, Han FK. The use of autogenous tooth bone graft powder in the treatment of osseous defects after impacted mandibular third molar extraction: a prospective

split-mouth observational clinical pilot study. BMC Oral Health 2022; 22: 433. DOI: https://doi.org/10.21203/rs.3.rs-1489357/v1.
33. Sánchez-Labrador L, Martín-Ares M, Ortega-Aranegui R, López-Quiles J, Martínez-González JM. Autogenous dentin graft in bone defects after lower third molar extraction: a split-mouth clinical trial. Materials (Basel) 2020; 13: 3090. DOI: 10.3390/ma13143090.
34. Mazzucchi G, Lollobrigida M, Lamazza L, et al. Autologous dentin graft after impacted mandibular third molar extraction to prevent periodontal pocket formation – a split-mouth pilot study. Materials (Basel) 2022; 15: 1431. DOI: https://doi.org/10.3390/ma15041431.
35. Saxena S, Chang W, Fakhrzadeh A, et al. Calcium phosphate enriched synthetic tyrosine-derived polycarbonate-dicalcium phosphate dihydrate polymer scaffolds for enhanced bone regeneration. Materialia (Oxf) 2020; 9: 100616. DOI: 10.1016/j.mtla.2020.100616.
36. Fan CJ, Ji Q, Zhang C, Xu S, Sun H, Li Z. TGF-β induces periodontal ligament stem cell senescence through increase of ROS production. Mol Med Rep 2019; 20: 3123-3130.
37. Grzesik WJ, Narayanan AS. Cementum and periodontal wound healing and regeneration. Crit Rev Oral Biol Med 2002; 13: 474-484.
38. Narayanan AS, Bartold PM. Biochemistry of periodontal connective tissues and their regeneration: a current perspective. Connect Tissue Res 1996; 34: 191-201.
39. Saito M, Narayanan S. Signaling reactions induced in human fibroblasts during adhesion to cementum-derived attachment protein. J Bone Miner Res 1999; 14: 65-72.
40. Komaki M, Kang M, Narayanan A. Role of MAP kinases p42erk-2/p44erk-1 in cementum-derived attachment-protein-mediated cell attachment. J Dent Res 2000; 79: 1789-1793.
41. Kim YK, Kim SG, Byeon JH, et al. Development of a novel bone grafting material using autogenous teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010; 109: 496-503.
42. Upadhyay P, Blaggana V, Tripathi P, Jindal M. Treatment of furcation involvement using autogenous tooth graft with 1-year follow-up: a case series. Clin Adv Periodontics 2019; 9: 4-8.
43. Franceschini Neto F, dos Santos Oliveira R, Altheman Lopes AP, dos Santos Ribeiro da Silva CEX. Evaluation of bone repair in the mandible of rabbits using biphasic calcium phosphate micro-macroporous hydroxyapatite bioceramics and beta-tricalcium phosphate. J Pesquisa Brasileira em Odontopediatria e Clínica Integrada 2019; 19. DOI: https://doi.org/10.4034/PBOCI.2019.191.39.