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ALVEOLAR RIDGE PRESERVATION COMPARING ALLOGRAFT, ALLOPLAST, AND AUTOGENOUS TOOTH GRAFT: A RANDOMIZED CLINICAL TRIAL

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ABSTRACT

Introduction: Following tooth extraction, sequels of events that reduce alveolar crest width and height take place. Alveolar ridge preservation (ARP) after extraction of a tooth is crucial as it maintains ridge dimensions facilitating dental implant placement. Previous systematic reviews and clinical trials recommended allografts, alloplasts, and autogenous tooth bone graft (Auto-BT). However, the results were heterogeneous about the exact superiority of one material over the other. Thus, the herein work aimed to evaluate the efficiency of the different bone graft materials in ARP.

Material and Methods: In this randomized clinical trial, thirty patients were divided into three groups. ARP was performed using allograft, alloplast, and Auto-BT. Changes in alveolar ridge dimensions were evaluated clinically, radiographically, and histomorphometrically after 3 months.

Results: Results of intergroup comparisons for clinical and radiographic measurements showed higher bone loss values in the allograft and alloplast groups than in the Auto-BT group. Histological measurements disclosed that the Auto-BT group had the highest value of mature bone followed by alloplast while allograft presented the lowest value.

Conclusion: In conclusion, tooth graft could be considered a viable alternative to other graft materials in ARP. Autogenous tooth graft is the new concept of graft material. They can be processed and used as an economical, natural, and biocompatible, versatile, and predictable grafting material.

KEYWORDS Alveolar ridge augmentation; Allogenic graft; Alloplastic graft; Tooth graft

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INTRODUCTION

Following tooth extraction, sequence of events eventually occur altering alveolar ridge dimensions and subsequently reducing alveolar crest width and height. The first 6 months after tooth extraction experience the most important changes with an average vertical and horizontal bone resorption of 1.24 mm and 3.79 mm respectively [1,2].

A range of alveolar ridge preservation (ARP) techniques has been suggested to compensate for the variations previously mentioned. Generally, the foundation of these techniques comprises emplacement of bone graft into the tooth socket, instantly after extraction and sealing the socket with a barrier. Evaluation of the efficacy of these interventions has been well studied and, the efficiency of ARP procedures in bone resorption reduction compared to treatment without ARP is well reported [3,4]

Alveolar ridge preservation after tooth extraction is crucial for dental implant success as it maintains dimensions that facilitate dental implant placement. One another goal for ARP is to afford vital osseous tissue at the extracted tooth site, which will eventually hold up the implant and provide osseointegration. Diverse materials and techniques are used to achieve these two goals comprising autogenous tissues, allografts, alloplastics, and xenografts [4-6].

Guided bone regeneration (GBR) aims at preventing gingival epithelial cells and connective tissue from entering the socket by cell occlusive membranes. Resorbable and non-resorbable barrier membranes are sometimes used to keep space for bone formation and growth. Having an advantage over non-resorbable membranes, resorbable membranes show good healing of soft tissues and do not need a second surgery to remove [7]. In addition to the profits of collagen in helping clot organization and stabilization, collagen membranes could be effortlessly manipulated, and adapted to bone [8].

Allograft materials from cadavers are usually obtained through tissue banks. They have both osteoinductive and osteoconductive properties [9]. Having a slower resorption rate, allografts can keep the ridge dimensions stable [2,4]. Small allograft particles may remain up to a year before complete resorption. Creeping substitution at the recipient place and connective tissue is the method of revascularization of freeze-dried bone allografts (FDBA) [10]. The quantity of the newly formed bone and the dimensional stability of both cortical and cancellous FDBA were similar when used in ARP [11].

Alloplastics such as beta tricalcium phosphate (β-TCP) represent a group of largely available synthetic bone substitutes. They are osteoconductive, biocompatible, and do not carry any risk of infection or disease transmission [12, 13]. They act as biologic fillers with restricted periodontal regeneration [10, 14].

Recently, autogenous tooth bone graft material (Auto-BT) has gained much attention in dental implant augmentation through GBR [15]. The production of Auto-BT from the teeth after extraction has been approached in a number of ways. The fillings, soft tissues, and carious parts should be removed following tooth extraction [16]. Some protocols advise using the extracted tooth's root alone [17], while others support using both the crown and root [18]. Some research has confirmed the excellent clinical and histological outcomes of the deciduous and permanent tooth- based Auto-BT graft materials [19, 20].

The superiority of Auto-BT lies in its resemblance to autogenous bone in both its histological structure and components [21,22]. The composition of both human dentin and bone is 65% inorganic and 35% organic [23]. Auto-BT ensures excellent biocompatibility with no fear of immune rejection. In addition, many studies have reported that Auto-BT exhibits not only osteoconductive but also osteoinductive capability [20, 24, 25]. The osteoconductivity is linked to the inorganic proportions [26] while the organic

matrix of mineralized dentin is responsible for its osteoinductive characteristics [27, 28]. The hydroxyapatite in dentin is in the form of calcium phosphate with low crystal content making it easily degradable by the osteoclastic activity. However, the organic content consists of type I collagen network (90%), non-collagenous proteins (10%) (osteocalcin, osteonectin, sialoprotein, and phosphoprotein) which aid in calcification of bone, and some growth factors (bone morphogenetic proteins, and insulin-like growth factor) which give the tooth its osteoinductive properties [29].

Due to the paucity of studies specifying the superiority of one material over the other in ARP, the present study aimed to compare the efficiency of allograft, alloplast and Auto-BT graft materials on ARP subsequent to tooth extraction.

MATERIAL AND METHODS

This study received an approval from the ethics committee of the Faculty of Dentistry, Ain Shams University, Cairo, Egypt (IRB no: FDASU-Rec IR092210) and registered in Clinical Trials (ID: NCT05812872). It was performed according to the Declaration of Helsinki

Thirty patients were selected from the outpatient clinic of Oral Medicine, Periodontology, and Oral Diagnosis department, Faculty of Dentistry, Ain Shams University. A power analysis was designed to have adequate power to apply a statistical test of the null hypothesis that there is no difference would be detected between tested groups. By adopting an alpha (α) level of (0.05), a beta (β) level of (0.2) (i.e. power=80%), and an effect size (f) of (0.637) calculated based on the results of a previous study [30]; the minimal required sample size (n) was found to be (27) cases (i.e. 9 cases per group). Sample size calculation was performed using G*Power version 3.1.9.7 [31].

Inclusion Criteria

a) Healthy adult patients as evidenced by Burket's oral medicine health history questionnaire [32].

b) Both genders

c) Age ranging from 20 – 40 years old

d) Having at least one hopeless tooth (traumatized, badly broken, unrestorable, etc.) indicated for extraction upper / lower (Anterior or premolar area)

e) Sockets type I or II.

Consent was written by patients after explaining the nature of the study.

Exclusion Criteria

a) Smokers

b) Pregnant and breast-feeding females

c) Mentally retarded patients

d) Handicapped patients and prisoners

e) Teeth with periodontal or periapical infections

f) Patients with malocclusion

g) Patients with parafunctional habits

h) Patients receiving drugs that may influence bone metabolism

Study Design and Patient Grouping

This study is a clinical comparative prospective study. Patients who met the eligibility criteria were allocated into: allograft group, alloplast group and autogenous tooth graft group.

The randomization of patients into the three groups was performed by a computer-generated randomization list. Allocation blindness was attained by putting the subject's treatment in a sealed envelope. The patients, outcome examiners and the statistician were blinded.

Each group included 10 patients who had undergone single tooth extraction, then the socket

was filled by either mineralized cortico-cancellous allograft (Maxgraft, botiss dental GmbH, Berlin–Germany), β-TCP bone graft (Bioresorb, Implant direct, CA, USA) or Auto-BT. All three materials in each group were loaded with collagen membrane (Hyprosorb-F Atelo collagen membrane).

Surgical Procedure

***Extraction and Socket Augmentation Procedure* (Fig.1)**

a) Baseline cone beam computed tomography (CBCT) was taken on the extraction day for socket type evaluation.

b) Patients were injected with anesthesia (Artinibsa 40mg/0.01 mg/ml solution injectable-inisba-Spain), extraction was performed atraumatically, using periotome (Nordent – Germany), to preserve bone and soft tissue, then extraction was completed using forceps (Martin-Nelson-Germany).

c) Curettage of socket was carried out by bone curettes (Reicodent-Germany).

d) Clinical measurements of bone height and width were performed as follows:

Bone height

Bone height was assessed using a periodontal probe (Hu-Friedy UNC 15 Co., LLC-USA) till reaching the bone. A stent of thickness one mm was fabricated before extraction using a cast. The tooth to be extracted was removed from the cast [33]. Six holes were made in the resin plate in the following positions: mesio buccal, mid buccal, disto buccal, mesio palatal, mid palatal, and disto palatal. Measurements were taken after tooth extraction (baseline) and 3 months after extraction before placement of the implant.

Bone Width

Alveolar ridge width was measured immediately after extraction and after 3 months using a caliper clamp (Lwebinger GmbH.Mulheim, Germany), the width was measured perpendicular to the tangent of the dental arch at the mid-point of the extraction site approximately 4 mm apical to the marginal gingiva of the adjacent teeth [34].

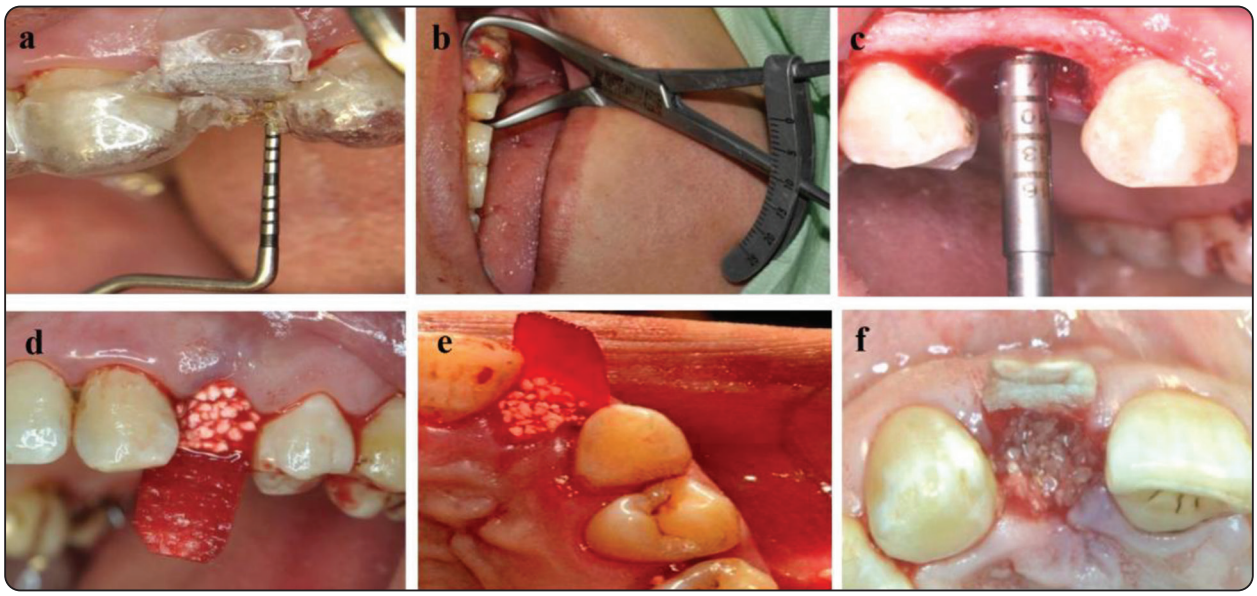

Fig. (1) Photomicrographs showing: (a) Periodontal probe measuring mesiobuccal bone height, (b) Measuring bone width immediately after extraction using a bone caliper. (c) Socket filled with mineralized allograft, (d) Socket filled with beta tricalcium phosphate alloplast, (e) Socket filled with autogenous tooth graft.

e) Sockets were filled with mineralized cortico-cancellous allograft, β-TCP bone graft (500-1000μm), and Auto-BT.

f) All sockets were covered by collagen membrane to cover the graft and stabilize the blood clot.

g) Socket approximation was performed using 5/0 reverse cutting 3/8th vicryl suture.

h) Amoxicillin (Amoxil-GlaxoSmithKline, medical union pharmaceuticals, Egypt) 500 mg t.d.s, Metronidazole Flagyl, Sanofi Aventis, Egypt) 500 mg twice/day and antiseptic (Hexitol, Arab drug company, Egypt) mouth wash for 1 week) were prescribed.

i) Post-operative instructions were given to the patients; the patients were instructed not to wear any prosthetic restoration.

Preparation of Autogenous Tooth Graft

Method: from extraction to grafting particulate dentin

Extraction of teeth without any root canal restorations was done and prepared for immediate grafting.

a) Crowns or restorations were removed. Caries, areas of dentin discoloration, calculus and periodontal ligament (PDL) remnants were reduced, and multi-rooted teeth were split.

b) Air syringe was used to dry clean teeth and grinded into the grinding sterile chamber of the Smart Dentin Grinder (SDG) (Kometa Bio ltd., London, United Kingdom) [35].

c) The roots were ground in 3 seconds by the SDG. The vibrating movement of the grinding chamber then filtered and collected particles between 300μm and 1,200μm into a lower chamber. Smaller particles usually fell into a waste drawer, as this fine particulate is incompatible for bone grafting. This protocol was repeated to grind the remaining teeth particles. The collected particulate dentin was immersed in basic alcohol (0.5M of NaOH and 30% alcohol (v/v)) for 10 minutes for dissolving any fats, organic debris or bacteria. The particulate was then washed twice in sterile phosphate-buffered saline (PBS) leaving wet particulate dentin that was grafted into the extracted sockets.

Implant Placement and Core Biopsy Procedure

a) After 3 months, another CBCT was done. Changes in the width and height measurements at the center of the extraction socket were evaluated in merged axial and sagittal views using the Romexis superimposition system besides measuring the changes in density (**Fig.2**).

b) Before implant placement, clinical measurements of height and width were repeated.

c) Reflection of an open flap was done for taking a core biopsy using trephine bur (Hu-Friedy trephine bur TREO20), and placement of a submerged implant (SIC Invevt AG Birmannsgasse 3 CH-4055 Basel, Germany) and then flaps were closed.

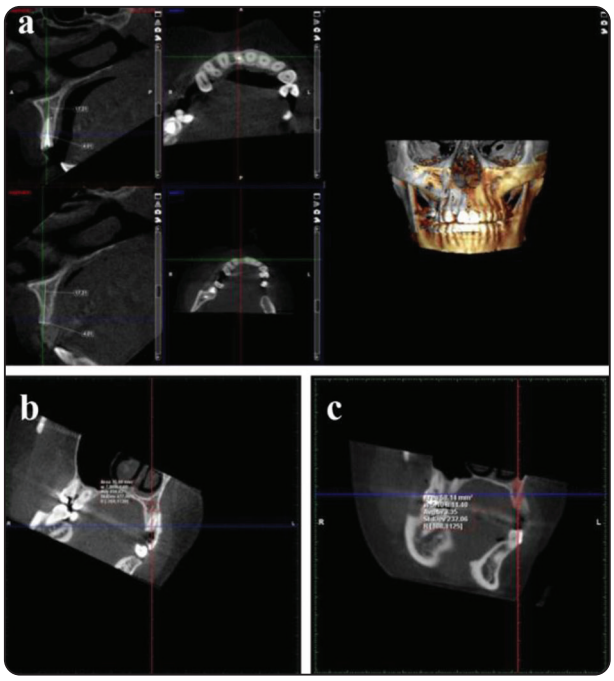

Fig. (2) Photomicrographs showing: (a) superimposition of CBCT base line and after three months to detect bone height and width, (b,c) measurements of preoperative and postoperative bone density, respectively.

Histological Examination and Histomorphometric Analysis

The taken bone specimens were fixed in 10% buffered formalin for five days. The specimens were decalcified using a solution containing 12% Ethylene diamine tetra-acetic acid (EDTA) buffered in pH 7.2 PBS for three weeks at 4°C [36], then the specimens were assigned for staining and histomorphometric analysis. Specimens were infiltrated and embedded in the center of paraffin wax blocks after being washed properly under running water, dehydrated by ascending concentrations of alcohol, and transferred to xylol. The embedded specimens were sectioned by microtome (4 microns thick) and stained by hematoxylin and eosin (H &E) and Masson trichrome (MT) stains.

Representative photomicrographs of H &E sections and three microscopic fields of each MT stained section were captured at a magnification of 200X using a digital camera (Canon EOS

650D) mounted on a light microscope (BX60, Olympus, Japan). H &E sections were used for histological evaluation while MT special stain was used to detect areas of immature and mature collagen. The immature collagen appeared blue while areas with mature collagen appeared reddish [37]. The MT images were analyzed to obtain the area percentage of mature and immature collagen of the newly formed bone besides the area percentage of the residual graft materials using image J (1.41a, NIH, USA) software.

Statistical analysis

R statistical analysis software version 4.1.3 for Windows (R Core Team 2022) was used for the statistical analysis. Mean and standard deviation (SD) values of numerical data were calculated. Shapiro- Wilk's test was performed to test for normality. Data were normally distributed and were analyzed using one-way ANOVA followed by Tukey's post hoc test. Unpaired T test was used when comparing 2 groups. The significance level was set at $p<0.05$ within all tests.

RESULTS

Thirty patients were classified equally and randomly into three groups, each group included 10 patients. The allograft group included six males and four females with a mean age of 31.4 years (range 24-40 years). The alloplast group included (four males and six females with a mean age of 29.4 years (range 21-38 years). While, the Auto-BT group included five males and five females with a mean age of 28.5 years (range 24-37 years).

Clinical Measurements

Comparing different groups for clinical measurements presented in **Table. 1** showed that for buccal and palatal bone height loss, there was a significant difference between different groups with the alloplast group having significantly higher bone loss values than Auto-BT ($p<0.05$), while no statistically significant difference was observed between allograft and the other two groups. For

TABLE (1) Clinical analysis (Buccal and Palatal bone height loss and Bone width loss)

Measurement	(Mean±SD) (mm)			f-value	p-value
	Allograft	Alloplast	Autogenous tooth bone graft		
Buccal bone height loss	1.40 ± 0.97^{AB}	1.98 ± 0.55^{A}	0.80 ± 0.67^{B}	6.22	0.006*
Palatal bone height loss	1.20 ± 0.88^{AB}	2.07 ± 0.83^{A}	0.77 ± 0.46^{B}	3.65	0.039*
Bone width loss	1.42 ± 0.94^{A}	1.95 ± 0.10^{A}	1.05 ± 0.83^{A}	1.43	0.256

Means with different superscript letters within the same horizontal row are significantly different *significant (p<0.05)

the loss in bone width, the difference was not statistically significant (p=0.256).

Radiographic Measurements

Radiographic measurements presented in **Table. 2** showed that for bone width loss there was a significant difference between groups, with β-TCP having significantly higher loss value than other groups (p=0.001). For height loss, the difference was also significant, with the β-TCP group having a significantly higher value than the Auto-BT group (p=0.009). For bone density gain, there was a significant difference with the β-TCP group having a significantly lower value than other groups (p<0.001).

Histological Results and Histomorphometric Analysis

Histologically, all groups showed variable amounts of woven bone and lamellar bone with haversian system. Residual graft was detected in which some of the graft remnants were found fused to the newly formed bone especially in the allograft and Auto-BT groups. Osteoblastic rimming was observed lining the graft material and the newly formed bone in the allograft group (**Fig.3**).

TABLE. (2) Radiographic analysis (Width loss, Height loss and Density gain between different study groups)

Measurement	(Mean±SD)			f-value	p-value
	Allograft	Alloplast	Autogenous tooth bone graft		
Width loss (mm)	1.33 ± 0.05^{B}	1.59 ± 0.16^{A}	1.22 ± 0.31^{B}	8.47	0.001*
Height loss (mm)	1.64 ± 0.38^{AB}	2.00 ± 0.94^{A}	0.88 ± 0.84^{B}	5.68	0.009*
Density gain (HU)	77.04 ± 13.18^{A}	52.69 ± 0.75^{B}	85.99 ± 9.62^{A}	33.38	<0.001*

Means with different superscript letters within the same horizontal row are significantly different *significant (p<0.05)

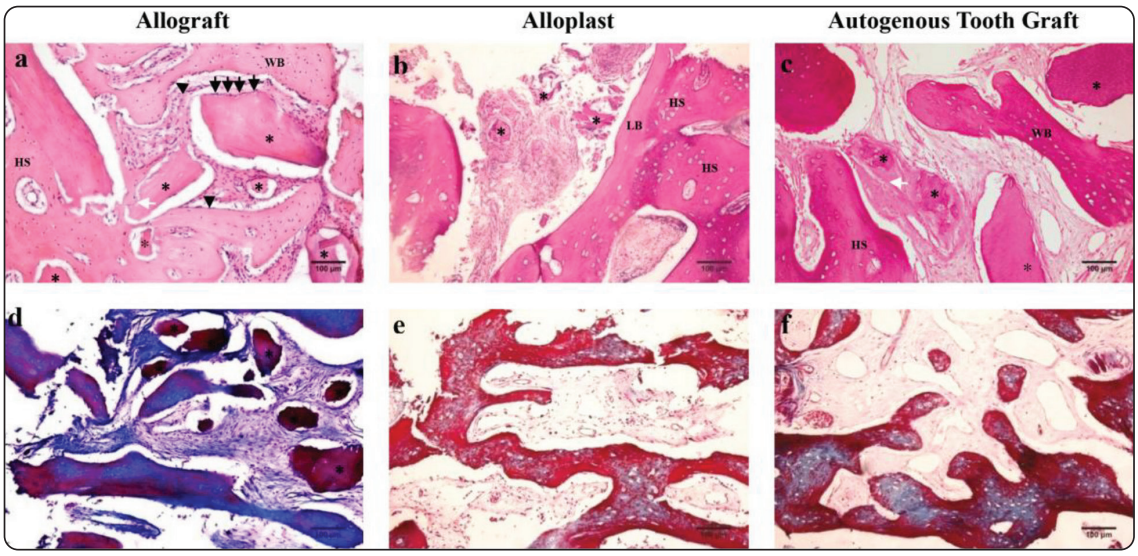

Fig. (3) Photomicrographs of (a,b,c) H& E sections, and (d, e, f) MT sections of biopsy samples (magnification, 200X) showing: Residual graft material in all groups (astrict), (a) Erosion of the surface of allograft residual graft with osteoblastic rimming (black arrows) , osteoblastic rimming of the newly formed woven bone trabeculae (black arrow heads), and Haversian system (b) Lamellar bone with Haversian system and woven bone in alloplast group (c) Woven bone and lamellar bone with Haverian system in autogenous tooth graft group, (a and c) fusion of the newly formed bone with the graft material (white arrows). Bone specimens of experimental groups stained with MT showing immature collagen detected by blue color and mature collagen detected with red color, (d) Allograft group showed more immature blue stained collagen than mature collagen, (e,f) Alloplast and autogenous tooth graft groups showed more red mature than immature blue collagen. WB: Woven bone, LB: Lamellar bone, HS: Haversian system

The measurements presented in **Table.3** revealed a significant difference in mature bone area percentage between different groups, with the Auto-BT group having the highest value followed by β-TCP and with allograft group having the lowest value (p<0.001). The percentage of mature to immature bone was found to be statistically significant in alloplast and Auto-BT groups. Moreover, regarding the mean area percentage of the residual graft material, our results showed that there was a significant difference between the tested groups (p<0.001). The highest value was found in the allograft group, followed by the Auto-BT group, while the lowest value was found in the alloplast group. All pairwise comparisons were statistically significant (p<0.001) **Table.4**.

TABLE. (3) Histological analysis (Mean Area % of Mature and immature collagen of bone within and between different study groups)

Measurement	(Mean±SD) (%)			f-value	p-value
	Allograft	Alloplast	Autogenous tooth bone graft		
Mature collagen	11.54±1.14^{C}	21.84±1.81^{B}	24.26±1.95^{A}	162.93	<0.001*
Immature collagen	13.87±3.67^{A}	8.01±0.26^{C}	10.39±1.89^{B}	15.19	<0.001*
t-value	2.00	25.69	25.08		
p-value	0.076	<0.001*	<0.001*		

Means with different superscript letters within the same horizontal row are significantly different *significant (p<0.05)

TABLE (4) Histological analysis (Mean Area % of residual graft material)

Residual graft material (Mean±SD) (%)			f-value	p-value
Allograft	Alloplast	Autogenous tooth bone graft		
8.20±2.48^{A}	1.79±0.35^{C}	4.25±0.75^{B}	69.61	<0.001*

Means with different superscript letters within the same horizontal row are significantly different *significant (p<0.05)

DISCUSSION

The reduction in alveolar ridge dimensions and mucosal thickness after tooth extraction is an inevitable event that could be compensated by ARP [4, 38, 39].

The superiority of flapless extraction of teeth lies in the preservation of the blood supply to the buccal bone, as it does not cause any periosteal detachment. This type of extraction proved to successfully conserve the hard tissue dimensions and keep more healthy keratinized gingival tissue [9, 40].

Collagen membrane was chosen in this study, as it can keep soft tissues away from filling the extraction bony defect, allowing the specialized cells with osteogenic ability to regenerate the defect lost tissues. Collagen membranes are resorbable membranes having the advantage of self-degradation with no need for a second surgery to remove [41-43].

The effectiveness of collagen membranes and bone replacements in ARP has been assessed in a number of trials. One study, for example, found that the absorbable collagen membrane and

deproteinized bovine bone graft helped preserve the alveolar ridge bone while having no negative effects on the osseointegration of delayed implants [44]. Another trial aimed to reduce the dimensional changes in the alveolar bone post-tooth extraction by using an equine collagen membrane and a collagen cone, suggesting the potential of collagen materials in ARP [45].

The 3 months healing period was selected as it was previously demonstrated that the ideal healing time for a tooth socket is 12 weeks [5]. Moreover, after 3 months, the bone formation is adequate for implant placement, as the majority of the graft material is usually substituted by mature bone at that time point [9, 46]. Additionally, Jeong et al. [47] and Kim et al. [48] observed the resorption of the graft in their studies after 3 to 6 months of grafting.

Radiographically, CBCT was chosen for the analysis of changes in osseous dimensions and density. With low radiation doses, CBCT produces a more economical and efficient images [49]. MT stain was used for the histomorphometric analysis as it can differentiate between mature and immature collagen in the newly formed bone [50].

Results of the clinical measurements showed that the alloplast group had the highest value of bone height loss than the Auto-BT group, while the Auto-BT group showed the least loss in bone width. In their study Joshi et al., compared ARP using Auto-BT versus β-TCP. After 4 months post-treatment, the vertical and horizontal bone loss was the highest in the ungrafted sites followed by β-TCP sites, and was the least for Auto-BT -grafted sites with a statistically significant difference between the groups [51]. In accordance with our study, Jambhekar et al. [9] reported that allografts resulted in less affection of socket dimensions compared to alloplasts using a cut off healing period of 3 months. This was explained by the ability of the FDBA to regenerate bone or induce new bone formation[9]. The process of freeze –drying lowers the antigenicity[52] and the presence of bone morphogenic proteins exerts an osteoinductive property [5].

Regarding CBCT results, Auto-BT group showed less mean percent decrease in height and width and more density gain with a statistically significant difference between the groups. Similarly, Joshi et al. [51] reported that Auto-BT-grafted sites showed less reduction in ridge height and width, which was significantly lower when compared to β-TCP-grafted sites. In a previous study, Jun et al. [53] claimed no statistically significant difference in bone density gain and bone height change between Auto-BT and bovine bone graft. Thus, they recommended Auto-BT as a good alternative to other bone graft materials in sinus bone grafting.

Histological evaluation and histomorphometric analysis of the core biopsies obtained 3 months after socket grafting showed variable amounts of new bone in the grafted site of all groups. The presence of osteoblastic layer at the newly formed bone surface and at the surface of the resorbing graft particles of the allograft group indicates the presence of active continuous mineralization. The maturation of bone in all groups was indicated by the uniform osteocytic distribution and Haversian canals [54]. Our results revealed the highest value of mature bone in the Auto-BT group followed by alloplast, while the allograft had the lowest value. This agreed with Menetti et al.'s previous histological study where some of the dental granules were resorbed while others were still present. The newly formed bone was closely connected with the Auto-BT material and the granules were totally surrounded in some areas by the new bone [22]. Additionally, our results go along with that of Joshi et al. [51] who detected less bone and less angiogenesis besides more inflammation in β-TCP-grafted sites when compared to Auto-BT. Moreover, a systematic review on socket grafting by allografts, alloplasts and xenografts stated that alloplasts showed the

highest percentage of vital bone formation and the lowest percentage of residual graft material (45.53% , 13.67%, respectively) while allografts presented the lowest percentage of vital bone and the highest remnant graft (29.93%,21.75%, respectively) . This was explained by the osteoconductive potential and the rapid rate of resorption of the graft material owing to the microporosity in β-TCP [9, 55].

The properties of Auto-BT explain the best results of this graft material. First, Auto-BT is similar to bone composition in the presence of 70% hydroxiapatite and other calcium phosphate minerals [56]. Additionally, the bioactive bone morphogenic protein 2 and fibroblast growth factor in tooth structure contribute to the osteoinductive and osteoconductive property of Auto-BT [57]. Moreover, the mechanical stability of dentine particles and their ability to firmly integrate with the new bone creating a rigid anchorage site for implant, give the Auto-BT supremacy over other graft materials [58, 59].

CONCLUSION

Taken together, we conclude that grafts derived from an extracted human tooth could be considered a good alternative to allografts or alloplasts in alveolar ridge preservation as confirmed clinically, radiographically, and histologically. Autogenous tooth graft represents a good model of recycling autogenous tissues instead of discarding them as medical waste.

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Conflict of Interest

The authors declare that they have no competing interest

Abbreviations

ARP, Alveolar ridge preservation; RCT, Randomized controlled trials; GBR, Guided bone regeneration; FDBA, freeze-dried bone allografts; β-TCP, beta tricalcium phosphate;

Auto-BT, autogenous tooth bone graft; PDL, Periodontal ligament; PBS, Phosphate-buffered saline; EDTA, Ethylene diamine tetra-acetic acid; H&E, Hematoxylin and Eosin; MT, Masson trichrome special stain; SDG, Smart Dentin Grinder; CBCT, Cone beam computed tomography

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