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SOCKET PRESERVATION IN THE MAXILLARY ESTHETIC ZONE USING AUTOGENOUS BONE AND GINGIVAL GRAFT FROM THE MAXILLARY TUBEROSITY (A RANDOMIZED CONTROLLED CLINICAL STUDY)

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ABSTRACT

BACKGROUND: After tooth extraction, bone resorption related to structural modifications and soft tissue composition can be assumed. The socket experiences a natural remodeling process resulting in a new shape of the soft and hard tissues, and hence socket preservation can be performed to maintain bone and soft tissue structure for delayed implant insertion.

AIM OF THE STUDY: This study aims to assess clinically, radiographically, and histologically bone formation following socket preservation using autogenous bone and gingival graft from maxillary tuberosity compared to bone formation without socket preservation.

METHODS: This study was a randomized controlled clinical trial; participants were randomly allocated into 2 groups: in the study group, 8 patients underwent socket preservation in the maxillary esthetic zone utilizing autogenous bone and gingival graft from maxillary tuberosity, while in the control group, 8 patients were left without socket preservation after extraction. All patients obtained immediate (within 24 hours)and six months post-operative CBCT to evaluate bone formation in both groups. Histological assessment was performed 6 months postoperatively.

RESULTS: The collected data were evaluated using SPSS software version 22.0.

CONCLUSION: Despite our research limitations, particularly in the anterior area where the vestibular wall is thin and prone to rapid resorption after tooth extraction, clinical and radiological findings indicate that punch grafting of hard and soft tissues reduces dimensional changes in both hard and soft tissues post-extraction. Furthermore, histological analysis conducted six months after socket preservation (ARP), revealed new bone formation and a decrease in the remaining graft particles when using autogenous tissues.

KEYWORDS: Socket preservation, Autogenous graft, Maxillary tuberosity, Esthetic zone, Dental implants.

RUNNING TITLE: Socket preservation using autogenous bone and gingival graft.

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INTRODUCTION

Implant insertion in the esthetic zone is a difficult process. The healing process causes changes in the ridge dimensions after tooth extraction. A comprehensive study conducted in 2012 found that six months following extraction, 29% to 63% of horizontal bone loss and 11% to 22% of vertical bone loss (or 3.79± 0.23mm and 1.24 ± 0.11mm, accordingly), occur(1).

After tooth extraction there are several possible treatments: immediate or delayed implant insertion with or without bone and/or soft tissue augmentation(2).However, compared to immediately inserted implants, delayed implants placed into healed sockets had a noticeably higher survival rate, according to multiple studies. Socket preservation procedures have been widely utilized to optimize delayed implant insertion in adequate bone, with an optimal implant position, resulting in an appropriate emergence profile for a functional and esthetic prosthesis(3-6).

Many types of grafts, such as autogenous bone grafting, which includes utilization of bone from the same recipient of the graft, can be used in socket preservation. Bone can be harvested from non-essential bones, including the anterior mandibular ramus (coronoid process), mandibular symphysis (chin area), and iliac crest. Autogenous bone is the most preferable option for block grafts since it has a reduced risk of graft rejection as the graft originates from the patient's body. Additionally, growth factors, ceramic-based bone graft substitutes, polymer-based bone graft substitutes, allografts, synthetic variants, alloplastic grafts, and xenografts are used in socket preservation(7).

Because autogenous bone has osteogenic, osteoinductive, and osteoconductive characteristics,

it is still believed to be the 'gold standard' for bone regeneration (8, 9).The osteogenic potential of autogenous bone grafts may be attributed to the presence of bone morphogenic proteins, which attract osteogenic cells from the surrounding tissues and subsequently release additional growth factors necessary for bone repair(10).

Maxillary tuberosity bone grafts have been widely utilized in clinical practice as particulate transplants for augmenting a deficient alveolar ridge or the maxillary sinus, either before or simultaneously with implant placement. According to recent studies, osteoprogenitor cells for bone tissue engineering can be obtained in sufficient quantities from the maxillary tuberosity(11).

Therefore, the current study aimed to evaluate clinically, radiographically, and histologically bone formation following socket preservation using autogenous bone and gingival graft from the maxillary tuberosity(punch technique) compared to bone formation without socket preservation.

The null hypothesis of this study is that there is no statistically significant difference in bone formation between performing socket preservation using autogenous bone and gingival graft from the maxillary tuberosity compared to bone formation without socket preservation.

MATERIALS AND METHODS

This study was carried out as a randomized controlled clinical trial with an equal allocation ratio of 1:1. Patients seeking treatment for dental implants, who required teeth extractions in the maxillary esthetic zone, were selected from the outpatient clinic of the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Alexandria University, Egypt.

Sample Allocation

Sixteen eligible participants were randomly allocated into 2 equal groups. The study group consisted of 8 participants who underwent socket preservation utilizing autogenous bone and gingival graft from the maxillary tuberosity after extraction, while the control group included 8 participants who were left without socket preservation after extraction. Patients were allocated to either group by simple randomization using computer-generated random numbers(12).

It was set up and reported following the CONSORT guidelines (http://www.consort-statement.org).

Eligibility criteria

Participants selected for this study were included after fulfilling the following criteria.

Inclusion criteria

Participants selected for this study were included after fulfilling the following criteria:

Patients of both genders aged between 20 to 65 years old.

Patients with unrestorable maxillary teeth in the esthetic zone.

Patients who have sufficient existing bone volume in the tuberosity area.

Patients who have good oral hygiene.

Patients with grade 1 dental socket.

Exclusion criteria

Participants were excluded from the study if they met any of the following criteria:

Heavy smoker patients.

Patients with systemic diseases that directly affect bone remodeling and healing.

Pregnant women.

Patients with alcohol or drug abuse.

Patients with mental retardation.

Immunocompromised patients.

Methods

Pre-Surgical Procedures

History was taken from all patients, including medical, surgical, and dental history, followed by extra-oral and intra-oral clinical examinations.Thebuccopalatal and mesiodistal width of the tooth to be extracted were measured using a calibrated periodontal probe, and a panoramic x-ray was requested to evaluate the tooth to be extracted and the maxillary tuberosity area from which the bone graft will be harvested.(Fig 1A-1D)

Surgical procedures

Epinephrine 1/100,000 (Artpharmadent, Egypt) and 4% Articaine hydrochloride were administered as local infiltration anesthesia. Atraumatic extraction was performed using a periotome (Devemed, Germany), that help in the removal of the tooth with minimal injury to the surrounding alveolar bone. Subsequently, a periodontal probe was utilized to evaluate the socket wall quality.

For the study group:

Alveolar bone width and height were measured to estimate the proper punch dimensions for both soft and hard tissues.Gingival and bone graft that precisely fit the previously prepared recipient site were harvested using a punch trephine bur (Medesy, Italy). The harvested gingival and bone graft were then carefully placed within the socket and fastened with interrupted sutures (polypropylene 3/0, Ghatwary Medical GMS, Egypt).

An absorbable collagen material,CollaPlug(Absorbable Collagen Wound Dressing, Zimmer Dental Inc.) was used as a hemostatic agent. It was applied in the posterior area at the donor area and anchoredin place by interrupted sutures. (Fig 2A-2F)

For the control group:

The teeth were extracted, and the sockets were left without socket preservation measures.

Postoperative Measures:

Patients were instructed to place cold packs extraorally every 10 minutes for 2 hours on the first

day and to avoid hot food, hot drinks and mouthwash for 24 hours.

Medications prescribed:

Oral Antibiotic: Augmentin 1g (875mg Amoxicillin + Clavulanic acid 125mg) GlaxoSmithKline, UK,1 tablet every 12 hours daily for 5 days.

Anti-Inflammatory: Alphintern (Chymotrypsin300mg + Trypsin300mg) AMOUN PHARMACEUTICAL Co., 1 tablet every 8 hours daily for 3 days.

Oral Analgesic: Cataflam(Diclofenac potassium 50mg), Novartis-Switzerland, 1 tablet every 8 hours daily for 5 days.

Mouthwash: Chlorhexidine0.2% (Orovex Mouthwash), Macro Group, Egypt, for 2 weeks starting on the second post-operative day.

Sutures were removed after 10 days.

Follow up phase:

Clinical evaluation

Follow-up appointments were scheduled one week post-procedure and then monthly for six months to assess the presence or absence of postoperative complications. The soft tissue healing index score, based on the scale by Landry et al.(13),was used for evaluation. This scale ranges from 1 (extremely poor healing) to 5 (excellent healing), with ratings of 2, 3, and 4 indicating progressively better healing.

Radiographic evaluation

Cone Beam Computed Tomography (CBCT) was performed immediately post-operatively for both the study and control groups (T0) and repeated after six months (T1) to assess vertical and horizontal dimensional changes of the alveolar bone (Fig 4A-4D).

CBCT cuts(cross-sectional, axial and sagittal view) analyzed using Dentascan software; 3D reconstruction cuts (para-axial cuts) evaluated at T0 and T1. The radiographic evaluation based on the radiological parameters, using reference points and lines at T0 and T1 as described by Das et al.,(14)

Histological evaluation

After 6 months, biopsies were obtained using a trephine bur, labeled, and preserved in 10% neutral buffered formalin. Following fixation, specimens underwent decalcification with 8% trichloroacetic acid, were washed, dehydrated in increasing concentrations of ethanol, cleared with xylene, infiltrated, and embedded in paraffin wax. Thin sections of 5 µm thickness were cut apico-coronally using a rotary microtome. Sections were stained with Hematoxylin and Eosin (H&E) for general histological evaluation. Qualitative histological examination was performed using a light microscope (OPTIKA Microscopes B-290 series, Ponteranica, Italy).

Histomorphometric Analysis

Morphometric evaluation aimed to determine the proportion of total bone surface area within the defects. This was conducted on H&E-stained sections using ImageJ software (ImageJ 1.53k, NIH, Bethesda, MD, USA). Three sections were taken from each sample for quantification by two blinded researchers. Each section was photographed under a light microscope (OPTIKA Microscopes B-290 series, Ponteranica, Italy) equipped with a camera (OPTIKA Microscopes C-B10, Ponteranica, Italy) and Optika Proview software (version 3.7, OPTIKA Microscopes, Ponteranica, Italy) at a consistent magnification (400X).

The surface area of the region of interest (ROI) in each digital image was measured as the total surface area. Measurements were also taken of the area occupied by bone marrow and other tissue spaces. The bone-filled surface area was calculated by subtracting the latter from the former, and a proportion of the entire region was determined accordingly.For statistical analysis, the average measurements from the three images were used.

Statistical analysis

All data collected were analyzed statistically and presented in tables and graphs using IBM Statistical Package for Social Sciences (SPSS) Software version 22.0.

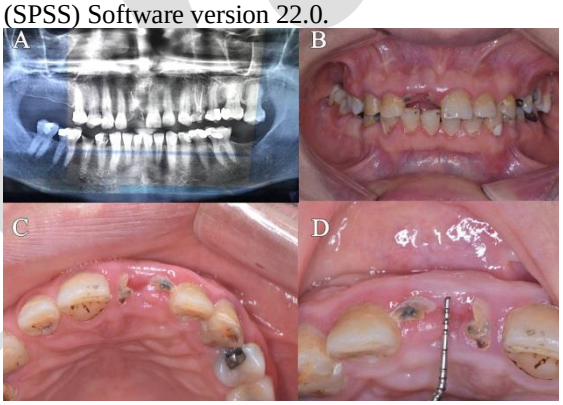

Figure 1: (A) Preoperative panorama x-ray. **(B)**Preoperative clinical (frontal). **(C)** Preoperative clinical (occlusal). **(D)**Measuring tooth width using calibrated periodontal probe.

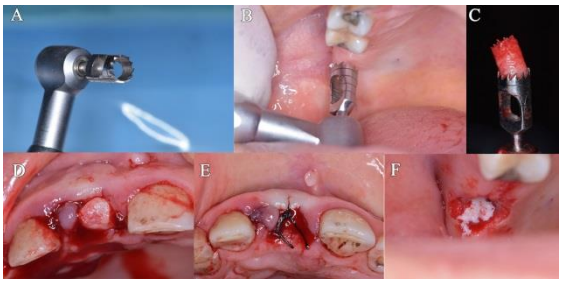

Figure 2: Clinical procedures.**(A)** Trephine bur.**(B)**Harvesting graft from maxillary tuberosity.**(C)**Graft harvested. **(D)**Graft inserted in socket after extraction.**(E)**Suturing socket after graft insertion.**(F)**Absorbable collagen material applied at donor area.

RESULTS

A total of sixteen patients (5 males, 11 females; average age 45 years, range 27 to 65) were selected for this randomized controlled clinical trial. All participants in both the study and control groups showed no signs of infection or swelling, and all sockets healed without complications or failure.

In the study group, the soft tissue index score was 3 (good) in 2 patients, 4 (very good) in 5 patients, and 5 (excellent) in 1 patient. In contrast, in the control group, the soft tissue index score was 4 (very good) in 6 patients and 5 (excellent) in 2 patients(Fig. 3A-3C).

Analysis of Radiographic outcomes revealed significant differences in dimensional changes between ridge preservation with autogenous graft and without this graft after six months of healing.

Analysis of radiographic outcomes revealed significant differences in dimensional changes between ridge preservation with autogenous graft and without this graft after six months of healing. In the study group, the mean decrease in bone width was 0.875 ± 0.63 mm, whereas in the control group, it was 3.25 ± 0.42 mm. Additionally, the mean decrease in bone height was 1.5 ± 0.61 mm in the study group and 2.90 ± 1.22 mm in the control group (Table1) (Fig. 6).

Histological Findings

Microscopic evaluation of the H&E-stained sections in the study group demonstrated a smooth, regular surface of the newly formed bone trabeculae with active osteoblasts lining the bone surface. Regularly arranged osteocytes were entrapped with remodeling lines visible. Fragments of grafting material were found in close proximity to the bone surface with plump osteoblast cell lines (Figs 5A,5B).

On the other hand, microscopic evaluation of the H&E-stained sections in the control group demonstrated an irregular trabecular surface of newly formed bone. A discontinuous layer of osteoblasts, involving large, irregular osteocytes, lined the developed bone trabeculae(Figs 5C,D).

Histomorphometric Findings

Histomorphometric analysis in Table 2 revealed a statistically significant increase in total bone surface area in the study group compared to the control group, with means of 69.56 ± 8.46 and 47.87 ± 5.51, respectively.

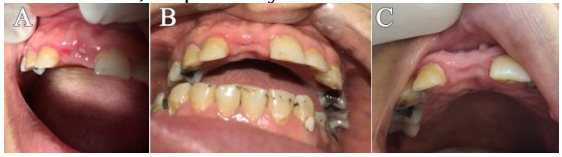

Figure 3: Clinical follow up.**(A)** Clinical one week postoperatively. **(B)**Clinical 3 months postoperatively. **(C)**Clinical 6 months postoperatively.

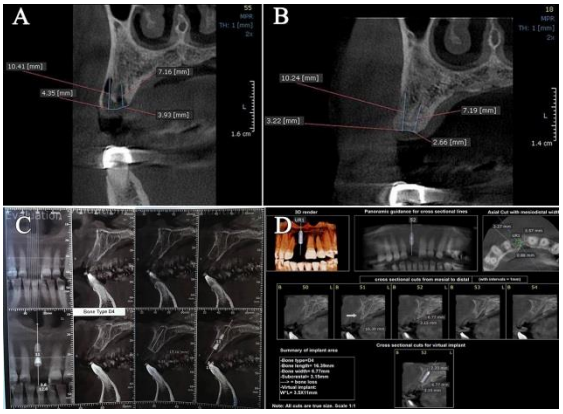

Figure 4: Radiographic follow up CBCT.**(A)** CBCT (T0) Control Group patient. **(B)** CBCT (T1) Control Group patient. **(C)** CBCT (T0) Study Group patient. **(D)** CBCT (T1) Study Group patient.

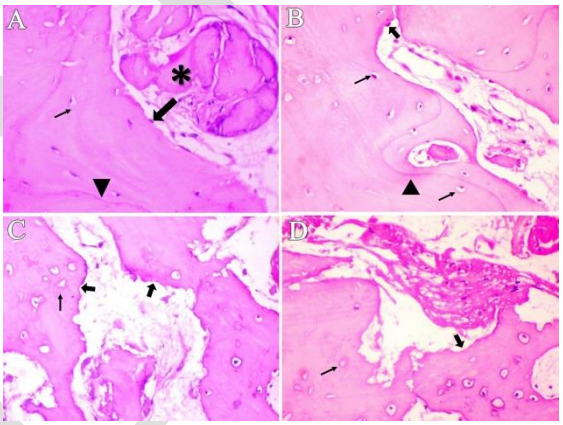

Figure 5: Light micrographs (LM) of H&E-stained decalcified sections. A and B study group revealing thick and well-organized bone trabeculae with remodeling lines (arrowheads). Active osteoblasts (thick arrows) with regularly arranged osteocytes (thin arrows) are seen. Note Autogenous graft (Matrix) enclosed new bone trabeculae. C and D control group showing irregular trabeculation of the newly formed bone with iscontinuous layer of osteoblasts (thick arrows). Irregular osteocytes lacunae (thin arrows) entrapped in newly formed bone. **A, B, C** and **D** ×400 magnification.

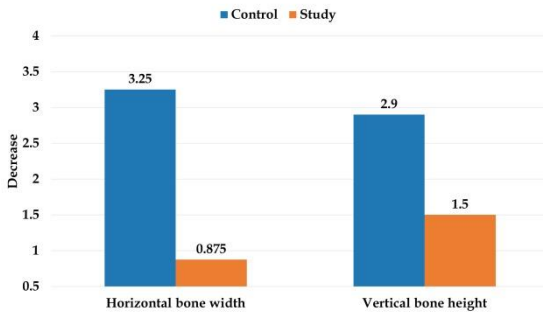

Figure 6: Graph bone loss bet. Study & control group acc. to bone width and height.

Table 1: Comparison between Study group (no. =8) and Control group (no. =8) regarding CBCT differences after six months for the same socket and Bone horizontal width and vertical length on the CBCT.

Differences		Study group No. = 8	Control group No. = 8	Test value	P-value	Sig.
				–	–	–
Decrease in socket horizontal width (mm)	Mean ± SD	0.875 ± 0.63	3.25 ± 0.42	-8.87	0.00005	HS
	Range	-0.22 – 1.9	2.7 – 3.96			
Decrease in socket vertical height (mm)	Mean ± SD	1.5 ± 0.61	2.9 ± 1.22	-2.91	0.023	S
	Range	0.49 – 2.4	-0.4 – 4.03			

Table 2: Histomorphometric Analysis.

Area Percentage		Control group No. = 3	Study group No. = 3	Test value	P-value	Sig.
Area	Mean ± SD	47.87 ± 5.51	69.56 ± 8.46	3.03	0.038	
	Range	40.68 – 54	58.47 – 79			

DISCUSSION

After teeth are extracted, various surgical techniques and several bone substitutes are utilized to fill alveolar defects and maintain bone levels (15-19).Autogenous bone remains the "gold standard" for bone regeneration due to its osteogenic, osteoinductive, and osteoconductive characteristics(20, 21).One of its unique benefits is maintaining cell viability and containing osteoblasts and osteoprogenitor stem cells, which facilitate true osteogenesis (21).In our study, the maxillary tuberosity demonstrated no complications and better accessibility compared to other intraoral donor sites such as the ramus and the chin. In contrast, these other sites are associated with significant post-operative bleeding, discomfort, swelling, and the possibility of nerve injury(22).

Soft tissue augmentation adds more benefits to the grafting material in terms of ridge preservation; a study by Thalmair et al.,(23)comparing sockets with a free gingival graft in addition to xenograft and sites with xenograft alone, found more ridge preservation and less bone reduction when free gingival grafts are used.

For this reason, we chose in our study a soft tissue graft in addition to a hard tissue graft to add more benefits and reduce bone loss

In our study, smokers were excluded because smoking has an unfavorable impact on bone healing. It not only impairs host cell function and alters the inflammatory response but also reduces blood supply, leading to decreased tissue perfusion and ischemia, which in turn impairs healing processes after tooth extraction.Pregnant women were also excluded to avoid the teratogenic impact of high radiation exposure during CBCT scans (24).

Radiographical results in the current study showed that horizontal and vertical bone loss were more prominent in the control group than in the study group. These findings are consistent with those of Houmani et al. (25)who reported significant radiological decreases in horizontal dimensions five months after extraction.

Additionally, Osman et al. (26)analyzed outcomes and found significant differences in dimensional changes and bone density between ridge preservation with SmartBone graft and without this grafting material after six months of healing. In the study group, the mean bone width reduction was 0.94 ± 0.40 mm, while in the control group it was 3.25 ± 0.42 mm. Similarly, the average decrease in bone height was 1.10 ± 0.47 mm in the study group and 2.90 ± 1.22 mm in the control group, which aligns with our study findings regarding the impact of graft material used.

Another study conducted by Aldini et al. (27)demonstrated that extraction sites without socket preservation showed significantly greater horizontal bone resorption compared to preserved socket sites using xenograft. Both groups experienced reduction in horizontal bone width of the remaining alveolar ridge; however, the extraction group exhibited significantly greater horizontal bone resorption throughout the seven-month period following tooth extraction compared to the ridge-preservation group. These findings are consistent with our study, where preservation was performed using autogenous graft rather than xenograft, highlighting similar trends in bone resorption with different preservation techniques.

Iasella et al. (28)demonstrated average horizontal ridge width values for both the control and preservation groups. Preservation cases initially had a mean alveolar width that decreased

significantly, showing an average loss of 1.2 ± 0.9 mm. In contrast, the control group cases showed a mean decrease in width of 2.6 ± 2.3 mm .These findings are consistent with our study, which found that alveolar ridge preservation is an effective method to reduce dimensional changes following tooth extraction (29-33).

In the current study, the use of autogenous graft was superior to spontaneous healing. After six months, the dimensional changes in the study group were 0.875 ± 0.63 mm in horizontal width and 1.5 ± 0.61 mm in vertical height. In comparison, the control group showed dimensional changes of 3.25 ± 0.42 mm in horizontal width and 2.90 ± 1.22 mm in vertical height. This indicates less than 1 mm and almost 1.5 mm bone loss in horizontal width and vertical height, respectively, in the study group, while the control group experienced more than 3 mm and almost 2.9 mm bone loss in horizontal width and vertical height, respectively. The autogenous technique used in this study met the criteria for alveolar ridge preservation, which include non-traumatic extraction (34),flapless operation (35),
utilization of bone as a filler and soft tissue as a socket sealer, while promoting healing by primary intention. During the procedure, raising the mucoperiosteal flap can lead to separation of blood vessels attached to the bone, resulting in local osteocyte death and reduced blood supply.

Therefore, the surrounding bone walls undergo mineralized tissue necrosis and are eventually removed through surface resorption (36).By inserting the graft without elevating the flap, the procedure preserves the mucogingival junction (MGJ) in its initial position and maintains an intact periosteum.

In the current study, the microscopic results of H&E-stained sections in the study group showed thick and well-organized bone trabeculae with remodeling lines and active osteoblasts, along with regularly arranged osteocytes. In contrast, the control group exhibited irregular trabeculation of the newly developed bone, with a discontinuous layer of osteoblasts and irregular osteocyte lacunae. In a study that conforms with our study conducted by Eldisoky et al. (37)microscopic analysis of the control group revealed that newly developed bone originated from the defect borders and extended a short distance towards the defect center. This bone took the form of irregular trabeculae and was associated with low-density fibrous connective tissue with limited vascularity. The immature bone trabeculae contained several large, enclosed osteocytes and were lined by a discontinuous layer of osteoblasts.

Most sections examined showed high bone marrow vascularity and a regeneration front that is consistent with our histological study findings.

The histomorphometric findings in the present study revealed a statistically significant increase in total bone surface area in the study group compared to the control group, with means of 69.56 ± 8.47 and 47.87 ± 5.51, respectively. This is consistent with findings by Iasella et al. (28), who demonstrated that extraction sites tend to heal with less bone and more trabecular spaces compared to preservation sites, aligning with our study results. Both groups showed a small percentage of amorphous organic material. Additionally, Aldini et al. (27) reported that trabecular bone constituted 25.7% to 9.5% of the extraction-alone group, while connective tissue comprised 59.1% to 10.4% of the total region.

Finally, all the aforementioned results indicate that bone healing in the study group was significantly superior to that in the control group. Based on these findings, the null hypothesis, as stated prior to conducting the study, has been rejected. However, this study had several limitations, including the limited quantity of available bone, which limits its applicability to small or moderate defects. Further studies are needed to evaluate its effectiveness in critical size defects using other grafting materials or growth factors.

CONCLUSION

Despite our research limitations, particularly in the anterior area where the vestibular wall is thin and prone to rapid resorption after tooth extraction, clinical and radiological findings indicate that punch grafting of hard and soft tissues reduces dimensional changes in both hard and soft tissues post-extraction. Furthermore, histological analysis conducted six months after socket preservation (ARP), considered an adequate healing period, revealed new bone formation and a decrease in the remaining graft particles when using autogenous tissues.

CONFLICT OF INTEREST
The authors announce that they have no conflicts of interest.
FUNDING STATEMENT
The authors received no specific funding for this work.

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