Using Platelet-Rich Fibrin in Combination With Allograft Bone Particles Can Induce Bone Formation in Maxillary Sinus Augmentation

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Background: Sinus pneumatization secondary to posterior maxillary tooth extraction can hinder proper implant installation. Maxillary sinus floor augmentation is a surgical procedure that has been proposed to overcome this issue.

Purpose: The aim of this study was to evaluate and compare the histomorphometric outcomes of sinus floor elevation using allograft bone particles with or without platelet-rich fibrin (PRF).

Study design, setting, sample: This randomized clinical trial included patients scheduled for maxillary sinus floor elevation in the Implant Department of Mashhad Dental School. Healthy adults with an edentulous maxilla and residual alveolar bone height of 3 mm or less were eligible to participate and were randomly allocated to intervention (A) or control (B) groups. Bone biopsies were obtained 6 months postoperatively.

Predictor variable: The predictor variable was using a PRF membrane for maxillary sinus augmentation. In group A, sinus floor elevation was performed using PRF combined with bone allografts, while in group B only allograft particles were used.

Main outcome variables: The primary outcome variables were the recorded postoperative histologic parameters, as in the area of newly formed bone, new bone marrow, and residual graft particles (μm^2). The secondary outcome variables were the radiographically measured postoperative bone height and width at the graft site.

Covariates: Age and sex.

Analyses: Independent sample *t*-test was employed to compare the postoperative histomorphometric parameters between groups A and B. *P* value $\leq$ .05 was considered statistically significant.

Results: A total of 20 patients (10 per group) completed the study. The mean rate of new bone formation was 43.25 $\pm$ 5.22% in group A and 38.25 $\pm$ 7.01% in group B. This difference was statistically insignificant (*P* = .087). The mean amount of newly formed bone marrow was significantly more in group A compared

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to group B (6.81 ± 2.19% vs 10.23 ± 4.49%; $P = .044$). The average amount of remaining particles was also significantly less in group A patients (9.35 ± 3.43% vs 13.18 ± 3.67%; $P = .027$).

Conclusion and relevance: Incorporating PRF as an adjunctive grafting material results in fewer residual particles of allograft and in more bone marrow formation and may serve as a treatment option for developing the atrophic posterior maxilla.
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The use of dental implants has become an increasingly popular and well-accepted intervention for replacing missing teeth, restoring esthetics, and re-establishing function in the partially or completely edentulous patient. Despite its versatility and wide-spread application, precise implant placement can sometimes be problematic. Alveolar bone resorption is an inevitable consequence of tooth extraction, resulting in insufficient bone volume which ultimately precludes straightforward implant installation. This matter becomes particularly challenging in the posterior maxilla, due to maxillary sinus pneumatization which restricts adequate vertical ridge dimensions in this region.[1] Maxillary sinus floor elevation has been proposed to overcome these drawbacks and enables appropriate implant placement in atrophic maxillary ridges. This surgical procedure has gained increasing popularity over the past decade.

Sinus floor elevation is a long-established surgical technique; ever since its initial introduction in the 1960s, multiple modifications and variations have been proposed. In 1980, Boyen and James were the first to successfully preform this treatment modality in a patient with largely expanded pneumatic sinus cavities in aim of developing the posterior maxilla for implant placement. In this surgery, a two-stage technique was applied. In the initial phase, autogenous iliac bone was harvested and used as a graft material to elevate the maxillary sinus membrane. Three months postaugmentation, dental implants were placed in the recipient sites. Installed implants were eventually loaded with implant-supported restorations.[2]

At present, several techniques for sinus floor elevation surgery exist, with the lateral window technique being one of the most favored for achieving vertical ridge augmentation.[1,3] After the Schneiderian membrane is elevated and the chosen biomaterial is placed, osteoprogenitor cells migrate from the bone walls to the graft site and enhance matrix bone formation. Osteogenesis initially begins from the periphery and continues toward the central and apical areas.

Platelet-rich fibrin (PRF), a second-generation platelet-rich concentration, was first developed by Choukroun et al in France in 2000. PRF preparation does not demand any sort of additives and simply consists of centrifuged blood; this was considered an important milestone in France due to legal restrictions against reimplantation of blood-derived products.[4] This simple procedure will constitute an autologous fibrin matrix, which contains platelet and leukocyte growth factors and can be applied in bone regeneration procedures.[5,6] It seems that PRF membranes, geared with appropriate handling and repair properties, can potentially serve as alternatives for other grafting materials.

Numerous studies have described the benefits of using fibrin glue in bone regeneration procedures.[7-9] A considerable number of studies have also been conducted on the osteoinductive effect of PRF and its ability to serve as an adjunctive grafting material in maxillary sinus augmentation surgeries;[10,11] however, based on our literature review, few randomized clinical trials and histologic studies have to date investigated this subject using allograft and PRF combination biomaterials for maxillary sinus floor augmentation.

Hence, the purpose of the present study was therefore to compare the histomorphometric outcomes of the regenerated vital bone in the grafted maxillary sinus with a combination of PRF and allograft or allograft material alone. The authors hypothesized that PRF would influence bone formation when used in conjunction with allograft particles for maxillary sinus floor argumentation. The specific aims of the study were to *1)* evaluate the histological outcomes of newly formed bone, and *2)* radiographically compare the alveolar ridge dimensions after maxillary sinus augmentation with and without a PRF membrane.

Methods and Materials

STUDY DESIGN AND SAMPLE

To address the research objective, a randomized clinical trial was designed and implemented. This study was conducted in the Department of Periodontology and Implant Dentistry of Mashhad School of Dentistry, Mashhad, Iran, from October 2020 till September 2021. The protocol of this randomized clinical trial was approved by the Research and Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.DENTISTRY.REC.1398.128) and was registered in the Iranian Registry of Clinical Trials under the code IRCT20190114042354N1. Guidelines of the Declaration of Helsinki and Consort statement were followed in this research. Patients were only recruited after obtaining fully informed written consent.

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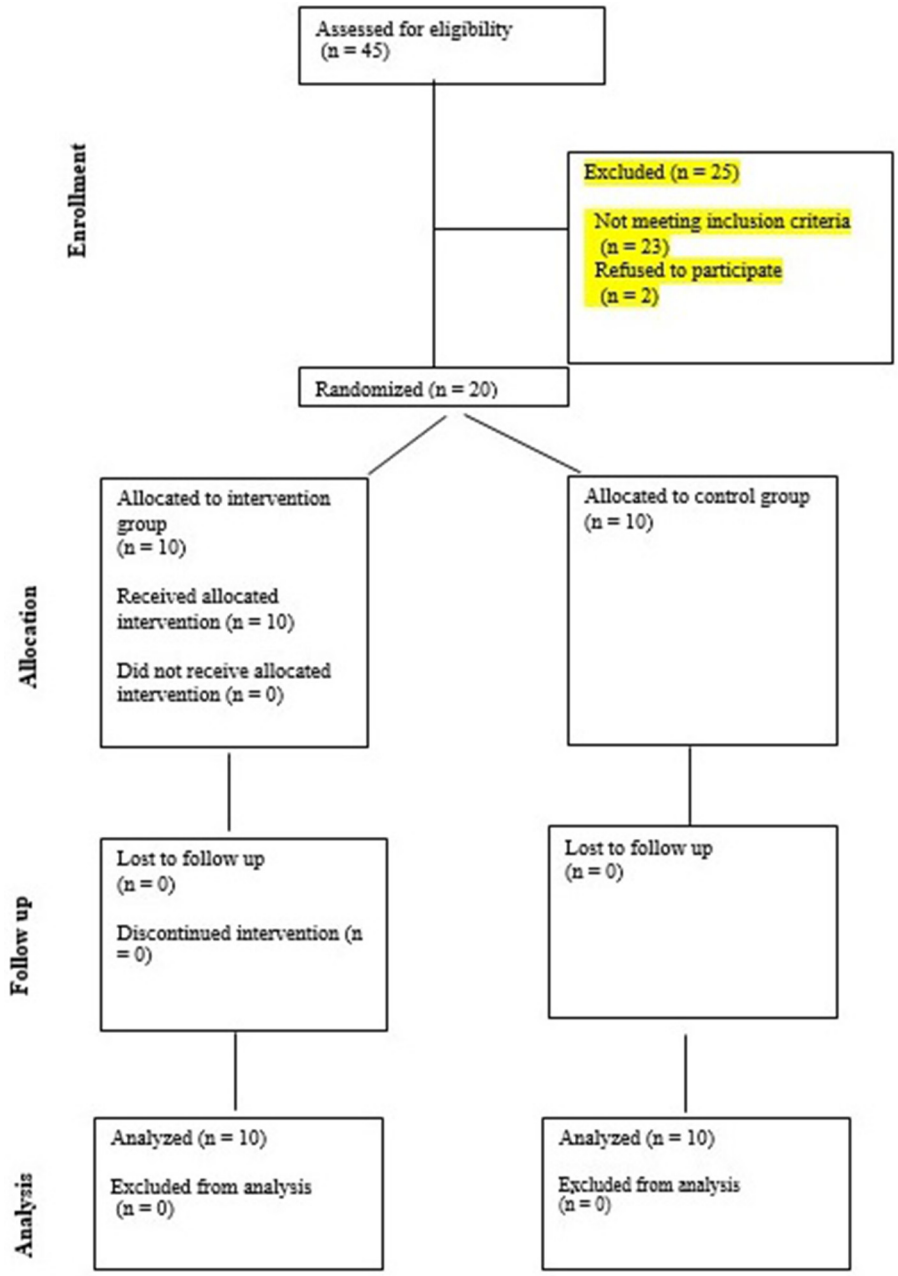

FIGURE 1. Consort flowchart of included patients in this clinical trail.

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Twenty healthy adults with an American Society of Anesthesiology status I or II, with edentulism in the posterior maxilla and candidates for sinus floor augmentation prior to implant placement, were enrolled in this study. Original residual bone height prior to augmentation was measured to be less than 3 mm. Patients' medical histories were evaluated and in case of any systematic contraindications to therapy or sinus pathologies, the patient was subsequently excluded from the study (Fig 1) After enrollment, patients were randomly divided into groups A and B according to the utilized grafting material and applied treatment protocol. In the control group (group B), sinus augmentation was achieved using 1-2 mm particle corticocancellous allograft (DIZG, Berlin, Germany); whereas in the intervention group (group A) patients received a combination of allograft biomaterial and PRF (Figs 2-4). Randomization was accomplished by using block randomization technique. Allocation concealment was performed using sequentially opaque sealed envelopes. While patients were aware of the treatment protocol they were receiving, the outcome assessor was blind to the group the patient was assigned to (single-blind randomized clinical trial).

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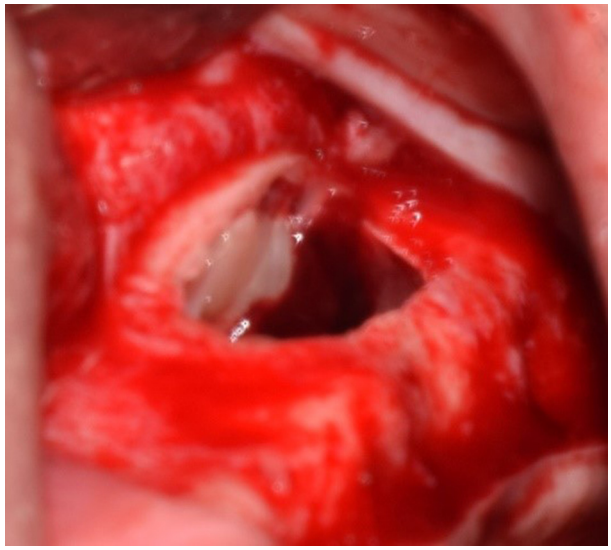

FIGURE 2. Surgical technique for sinus floor augmentation in group A (PRF + allograft).

Shiezadeh et al. Adding Platelet-Rich Fibrin to Sinus Augmentation. J Oral Maxillofac Surg 2023.

Preoperative planning entailed preimplant site measurement using cone beam computed tomography (CBCT) imaging (Fig 5). Measurements of the edentulous ridge were made on CBCT images (sagittal cuts) at 2 mm interval. Residual alveolar bone height was measured from the alveolar crest to the maxillary sinus floor. The distance between the buccal and lingual walls was also measured and defined as buccolingual bone width, respectively. The mean recorded values were presented as alveolar ridge dimensions. PRF was obtained by a technique described by Choukroun et al in 2006.[4] Venous blood samples were drawn from the patient at the beginning of the operation and then converted to empty vacutainers. The blood was then centrifuged at 2,700 rpm for 12 minutes.

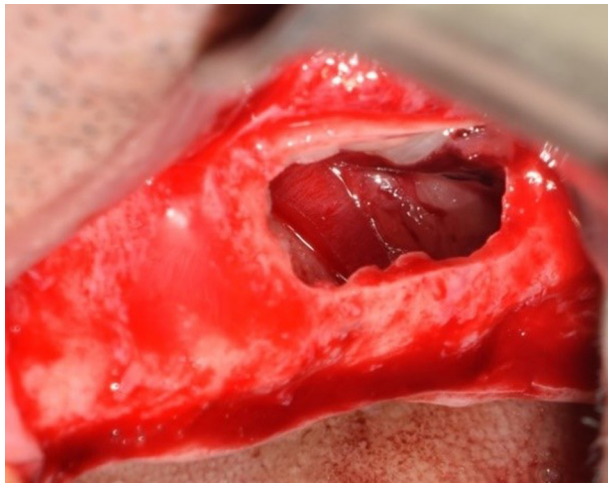

FIGURE 3. Insertion of PRF in the sinus cavity.

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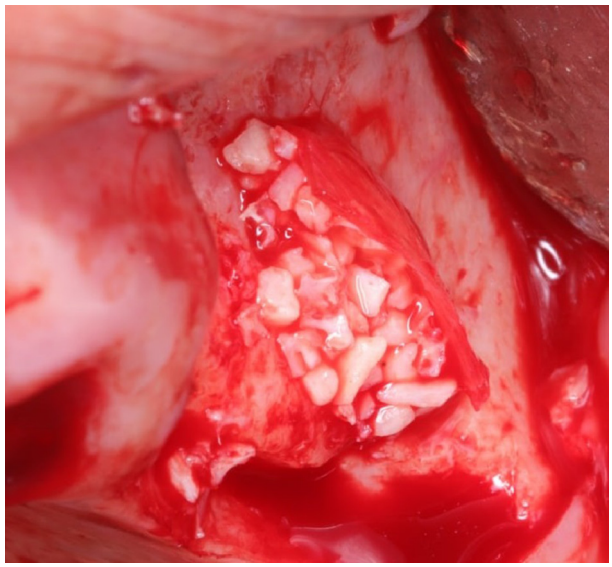

FIGURE 4. Insertion of allograft particles into sinus cavity (PRF inserted under the schneiderian and medial wall of sinus).

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All maxillary sinus augmentation surgeries were performed by the same surgeon and under local anesthesia. The lateral window technique was used. In brief, a crestal incision was made in the posterior edentulous maxilla and the lateral wall of the maxillary sinus. Mesial and distal releasing incisions were made in sufficient length to allow adequate exposure of the surgical site. The full-thickness mucoperiosteal flap was then carefully reflected and the lateral sinus wall was exposed. A 4-mm rounded bur with irrigation (physiologic saline) was used to prepare an osteotomy in the lateral wall of the maxillary sinus. After establishing ideal vision and access, the Schneiderian membrane was gently elevated using a curette. The membrane was elevated at least 14 mm to provide adequate space for proceeding implant placement but was never elevated beyond the sinus ostium to avoid the risk for medial meatus obstruction. Subsequently, depending on the study group, either a mixture of allograft and PRF (PRF membrane first inserted in the sinus and covered the schneiderian and medial wall of the sinus and then allograft inserted in the sinus cavity) or solitary allograft was introduced into the maxillary sinus and then covered by a collagen bio-absorbable barrier (Regene, Tehran, Iran) membrane. The mucoperiosteal flap was repositioned and finally sutured with 4-0 polyglactin 910 sutures (Supa Medical, Tehran, Iran). Cases which required a greater amount of sinus augmentation or those who experienced intraoperative sinus membrane perforation were omitted from the study. Patients were placed

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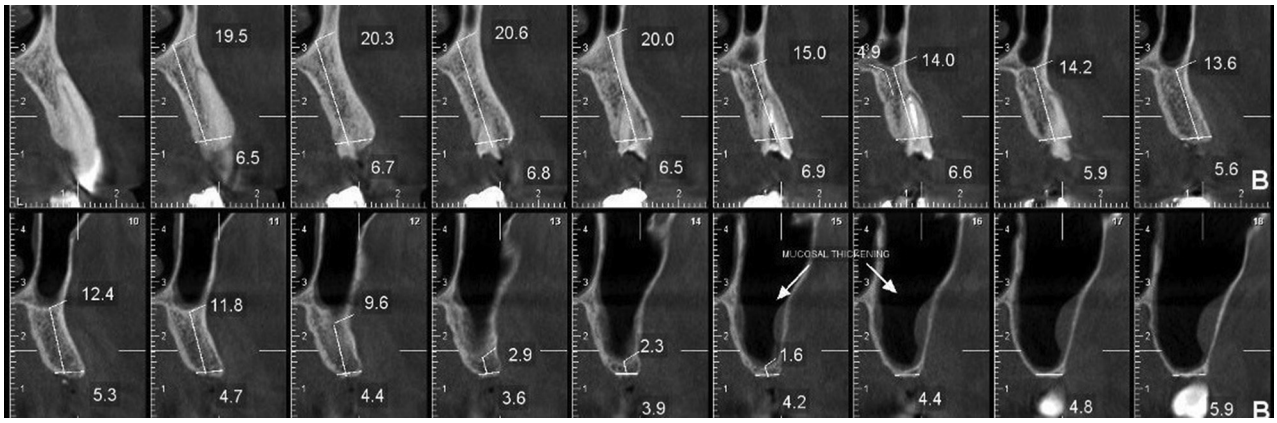

FIGURE 5. Presurgical CBCT of sinus cavity for test group.

Shiezadeh et al. Adding Platelet-Rich Fibrin to Sinus Augmentation. J Oral Maxillofac Surg 2023.

on amoxicillin-clavulanic acid 625 mg (Farabi, Tehran, Iran) 3 times a day for 1 week postoperatively and the prescribed analgesic of choice was acetaminophen-codeine 320 mg (Arian, Tehran, Iran). Patients were also advised to use an antibacterial rinse of chlorohexidine (Nazho, Tehran, Iran) 2 times a day for 2 weeks. Postoperative instructions and sinus precautions were thoroughly explained for patients.

Six months after graft surgery, CBCT images were obtained and the dental implants were installed (Figs 5 and 6).

VARIABLES AND DATA COLLECTION METHOD

The primary predictor was the use of PRF for sinus augmentation and subjects were randomly divided into groups A and B as per the used grafting material and applied treatment protocol. In the control group (group B), sinus augmentation was achieved using 1-2 mm particle corticocancellous allograft (DIZG, Berlin, Germany), whereas in the intervention group (group A) patients received a combination of allograft biomaterial and PRF (Figs 2-4). The primary outcome variable was the histologic parameters of newly formed bone in the graft site, bone tissue area, bone marrow area, and total tissue area. The secondary outcome variables were the radiographic height and width of the augmented bone. Age and gender were the investigated covariates in this study.

A 2-mm trephine bur with 12 mm length was used to obtain bony tissue biopsies from each surgical site. Biopsies harvested from planned implant site of crestal table and then implant inserted in these sites. Specimens were fixed in 10% formalin for 24 hours, incubated in 20% formic acid solution for 3 days, dehydrated in a graded series of alcohol baths, cleared in xylene, and finally embedded in paraffin. Histological ground sections 6-μm thick were prepared, stained with hematoxylin/eosin, and examined under a light microscope (Olympus BX51, Japan). ImageJ software (US National Institutes of Health, Bethesda, Maryland) was used to carry out histomorphometric analyses.

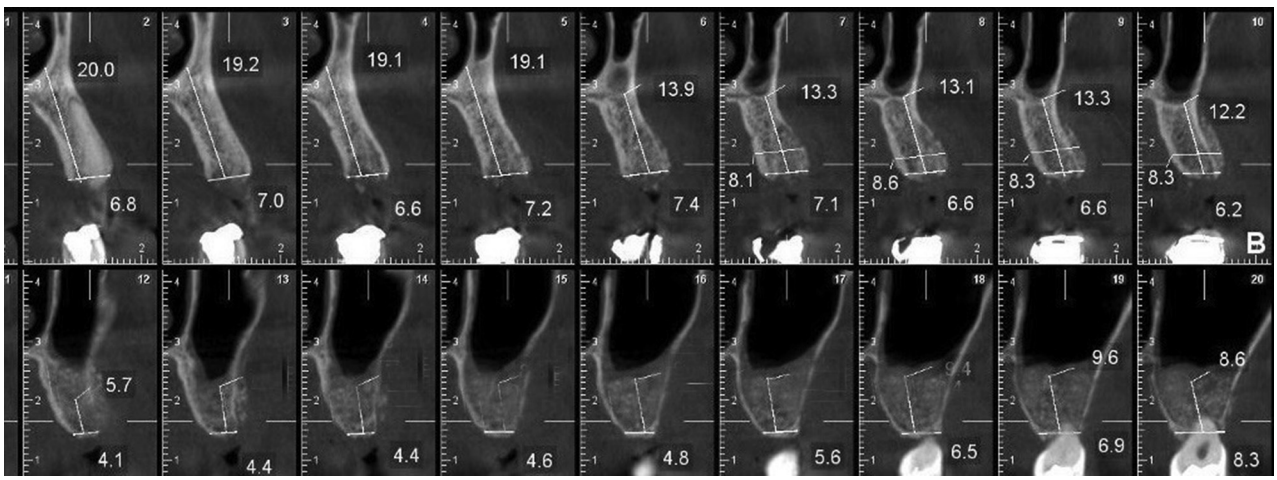

FIGURE 6. Postsurgical CBCT of sinus cavity of test group.

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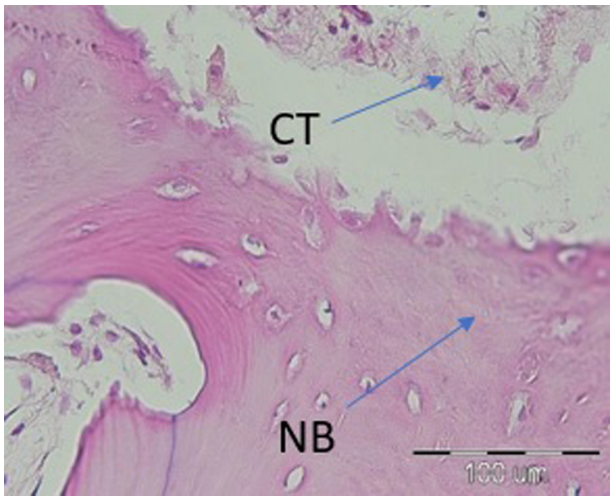

FIGURE 7. Histomorphometric images and analysis of PRF + allograft with new bone and connective tissue.

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The quality and quantity of the newly formed bone were evaluated and compared between the 2 treatment groups. The following histologic parameters were measured: bone tissue area, bone marrow area, and total tissue area (unit of measurement: μm^2) (Figs 7-10). The primary predictor variable was the used grafting material (either PRF + allograft particles or allograft particles alone).

DATA ANALYSES

The sample size was set at 20 patients, 10 per each group. All data were subjected to statistical analysis using SPSS software (V.21, SPSS Inc, Chicago, Illinois). T-test and linear regression analysis were also incorporated in the statistical analysis process. The statistical significance level was set at *P* value = .05.

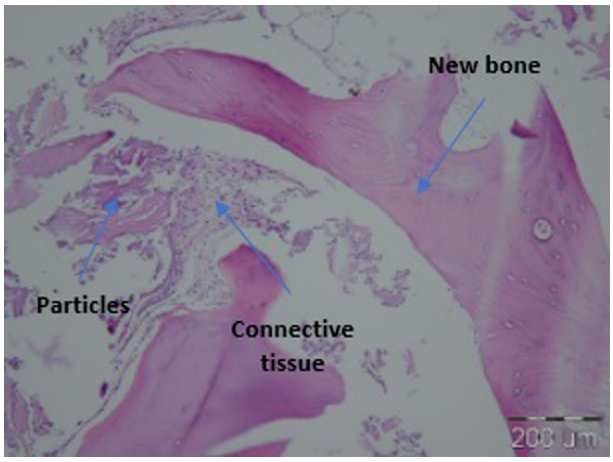

FIGURE 8. Histomorphometric image of (allograft) with 200 micron view with particles new bone and connective tissue.

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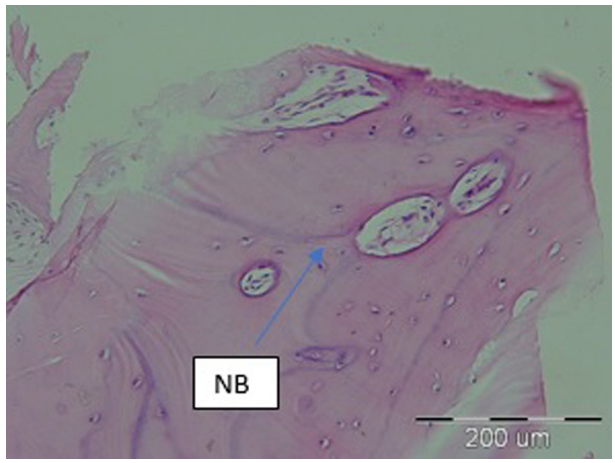

FIGURE 9. The amount of new bone and bone healing process in PRF + allograft group.

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Results

A total of 20 patients who were treated with maxillary sinus floor elevation surgery prior to dental implantation participated in this randomized clinical trial. Subjects were randomly divided into PRF + allograft (A) and allograft (B) groups, 10 patients per group. Patient distribution frequency consisted of 4 males and 6 females in group A and 6 males and 4 females in group B. Although the mean age of patients in group A (42.7 ± 5.79 years) was slightly higher than that in group B (40.3 ± 4.83 years), as per independent *t*-test this difference was statistically insignificant (*P* = .328).

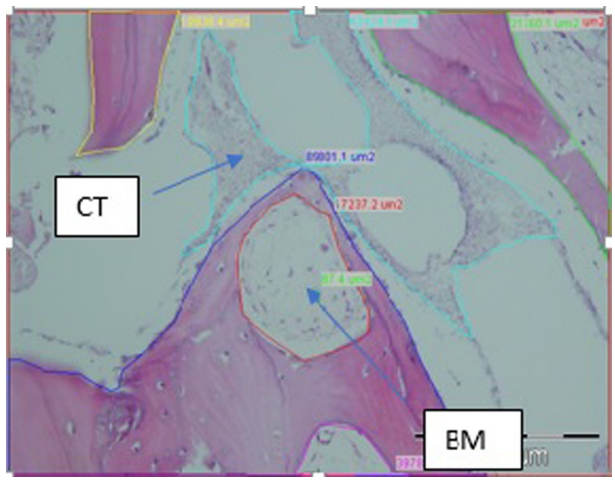

FIGURE 10. Histomorphometric analysis of allograft group with measuring area of new bone and connective tissue and bone marrow space.

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Table 1. HISTOLOGIC PARAMETERS VERSES PRF STATUS (PRF + ALLOGRAFT [A] AND ALLOGRAFT [B] GROUP)

Variables	Group	Patients	Mean	SD	Min	Max	*P* Value
New bone	A	10	43.25	5.22	33.27	50.33	*P* = .087
	B	10	38.25	7.01	29.48	48.78	
New bone marrow	A	10	6.81	2.19	3.37	10.67	*P* = .044
	B	10	10.23	4.49	2.83	17.68	
Remnant Particles	A	10	9.35	3.43	3.25	15.45	*P* = .027
	B	10	13.18	3.67	7.38	17.46	

Abbreviations: Max, Maximum; Min, Minimum; SD, standard deviation.

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The mean rate of new bone formation was found to be 43.25 ± 5.22% in group A and 38.25 ± 7.01% in group B. Also, the mean rate of bone marrow generation was reported to be 6.81 ± 2.19% and 10.23 ± 4.49% in group A and B, respectively. The mean value of residual particles was 9.35 ± 3.43% in group A and 13.18 ± 3.67% in group B. As per the independent *t*-test, the 2 study groups were significantly different regarding the amount of newly generated bone marrow and residual allograft particles ($P < .05$ for each variable). Table 1 displays these findings in greater detail.

The mean width of sinus bone was 6.24 ± 1.07 mm in group A and 6.25 ± 1.27 mm in group B. The mean bone height values were 2.74 ± 0.88 and 2.72 ± 0.92 mm in group A and B, respectively. Based on the independent *t*-test results, no statistically significant differences emerged between the 2 groups in terms of bone width and ridge height ($P > .05$) (Table 2).

Discussion

This randomized clinical trial attempted to assess the efficacy of using a PRF membrane in addition to allograft bone particles for maxillary sinus augmentation in patients with an atrophic posterior maxilla. In this study, 20 patients requiring sinus floor elevation surgery were enrolled and randomly divided into PRF + allograft and allograft groups. In our study, the mean rates of newly formed bone, newly formed bone marrow, and residual particles were 43.25 ± 5.22%, 6.81 ± 2.19%, and 9.35 ± 3.43% in the PRF + allograft group and 38.25 ± 7.01%, 10.23 ± 4.49%, and 13.18 ± 3.67% in the allograft group, respectively. Based on independent samples *t*-test, there were significant differences between the 2 groups regarding the variables of newly formed bone marrow and residual particles ($P < .05$). As per the obtained results, the initial (null) study hypothesis was accepted.

The PRF protocol was first presented by Dohan et al[12] and incorporated in maxillary sinus augmentation in the year 2006. At present, researchers are mostly focusing on the growth factors produced during this process, which are able to promote the release of autogenous growth factors during the first 7 days[11] and accelerate the maturation period to 28 days. The results of pertaining in vitro studies indicate that PRF tends to demonstrate a more significant and durable effect on the differentiation and proliferation of osteoblasts, compared to platelet-rich plasma.[13] Furthermore, PRF can easily transform into a membrane and act as a matrix facilitating wound healing, enhancing new bone formation, and accelerating the graft healing process. The handling and preparation of PRF are easy and highly cost-effective.[14] The implants placed via sinus lift surgery using PRF technology have shown a 100% survival rate at a mean follow-up period of 33 months.[15] Moreover, Dohan et al[12] state that PRF plays an important role in modulating inflammatory

Table 2. MEAN AND DIFFERENCES FOR BONE WIDTH AND HEIGHT BETWEEN GROUPS FOR SURGICAL SITE IN PRF + ALLOGRAFT (A) AND ALLOGRAFT (B) GROUP

Variables	Group	Patients	Mean	SD	Min	Max	*P* Value
Bone height	A	10	2.74	0.88	1.3	4.3	*P* = .96
	B	10	2.72	0.92	1.6	4.3	
Bone width	A	10	6.24	1.07	4.67	8.46	*P* = .98
	B	10	6.25	1.27	4.36	8.64	

Abbreviations: Max, maximum; Min, minimum; SD, standard deviation.

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reactions and can serve as an immune regulator by inducing the release of anti-inflammatory cytokines. Trisi et al[16] reported that PRF glue in combination with autogenous bone and Biogran can promote a greater increase in bone formation only within 5 to 6 months after sinus augmentation.

Owing to its proven efficacy, PRF is used as a graft material in combination with bone substitutes to augment the maxillary sinus floor. This combination has been used in various clinical and animal studies; nevertheless, its effects remain unconfirmed and questionable to clinicians. In the present study, we aimed to evaluate the impact of using PRF as an adjunct grafting material along with allograft particles on enhancing new bone formation after maxillary sinus floor elevation.

Choukroun et al[17] evaluated the amount of bone formation after sinus floor elevation using a combination of demineralized freeze-dried bone allograft and PRF. They observed that the amount of the newly formed bone in the allograft + PRF group at 4 months after surgery was equal to that of the allograft group at 8 months following the procedure. The amount of newly formed bone in this study was reported to be 20.95% in the experimental group and 20.3% in the control group. These findings suggested that the use of a combination of PRF and allograft to lift the sinus floor is able to enhance bone regeneration and reduce the required time for allograft maturation, such that implantation could be accomplished only 4 months after augmentation. Moreover, the amount of required allograft material was less in the test group compared to the control group, which is also financially beneficial. However, the reported overall rate of new bone formation in a study conducted by Choukroun et al[4] was lower compared to that reported by Kolerman et al,[17] who only used freeze-dried bone allograft (FDBA) and evaluated bone formation after 9 months.

In another study, Kassolis et al[18] used FDBA with concomitant PRF and solitary FDBA as 2 different treatment options for sinus floor augmentation. Histologic analysis was performed 4.5-6 months after augmentation. The rates of new bone formation in the FDBA + PRF group and FDB group were recorded as 33.3 $\pm$ 11.3% and 26.5 $\pm$ 6.8%, respectively. The mean percentage of residual particles was 21.2 $\pm$ 8.3% in the group which received PRF and 37.7 $\pm$ 15.7% in the group which only received FDBA. As per these results, the amount of newly formed bone in both groups in Kassolis et al's study was higher compared to that described by Choukroun et al.[4] On the other hand, the rate of new bone formation was higher in our study compared to both aforementioned studies, although there was no statistically significant difference between the 2 treatment groups. Residual particles were similar in our study and that of Kassolis et al,[18] and there was a statistically significant difference between the 2 groups regarding this variable, showing a lower value in group A compared to group B.

In studies by Inchingolo et al, Zhang et al, Tatullo et al, and Bolukbasi et al,[10,19-21] a combination of Bio-Oss and PRF was employed in sinus lift surgery. A recent meta-analysis study showed that the amount of newly formed bone induced by Bio-Oss grafts (22%) was less than that of autogenous bone (40%). Also, the amount of newly formed bone by the combination of Bio-Oss and autogenous bone was recorded 28% after 4-9 months, which was comparable to that related to autogenous bone after 9 months. Moreover, the slow degradation of xenografts facilitated the preservation of graft height and prevented bone resorption.[22,23]

In the present study after histomorphometric analysis was completed, no significant differences emerged between the 2 groups regarding the amount of newly formed bone; however, there were significant differences comparing the amount of new bone marrow and residual particles of graft materials, presenting lower mean values in patients who received a combination of PRF and allograft particles. Furthermore, the presence of cancellous bone indicated a shorter healing time in the PRF + allograft group compared to the group which only received allograft particles. Thus, the use of PRF in combination with allograft biomaterial in sinus lift surgery could enhance and accelerate bone regeneration. In addition, the use of PRF along with allograft is able to increase graft volume without compromising the quality of bone maturation.

Avila et al[24] investigated the impact of the distance between the medial and lateral sinus walls on sinus lateral augmentation, presenting a relationship between the bone formed and the buco-palatal dimensions of the sinus. In another study, Soardi et al[25] assessed the effects of mineralized allografts on sinus lateral augmentation in severely atrophic maxillary ridges (residual alveolar crest height less than 2 mm) and its relationship with sinus cavity dimensions. They concluded that the larger the maxillary sinus, the longer maturation time necessary for achieving adequate bone formation.

In a retrospective study by Spinato et al,[26] the relationship between maxillary sinus size and the radiographic outcomes of crestal sinus elevation was scrutinized using CBCT images. Better radiographic outcomes were noticed in narrow sinuses with a more prominent sinus membrane thickening in the augmentation area.

These results show that a larger residual ridge could provide greater cellular resources for osteogenesis in the augmentation area. Sinus anatomical features, such as sinus floor morphology, can affect and be in favor of bone regeneration. In this commentary, a larger contact area between native bone and grafting material ameliorates new bone formation. The present

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clinical trial showed the beneficial effects of PRF in combination with allografts on maxillary sinus bone augmentation after maxillary sinus floor elevation.

This study was a clinical trial to assess the effectiveness of using PRF membranes in conjunction with allograft material for maxillary sinus floor augmentation. The relatively small sample size and short postoperative follow-up period can be considered as the shortcomings of the present study. However, within its limitations, this study aimed to highlight the effect of PRF on bone regeneration in augmenting the maxillary sinus. Future clinical trials would work best if a larger sample size was incorporated. Investigating the effect of PRF on bone regeneration when combined with autogenous bone or as a solitary grafting material would be beneficial. We also recommend evaluating other histologic parameters such as the number of osteoclasts, osteocytes, and the angiogenesis properties.

As per the established results, although using adjunct PRF and allograft for maxillary sinus augmentation did not yield a greater induction in bone formation compared to when using allograft alone, this method was able to significantly reduce the amount of residual graft particles and newly formed bone marrow after augmentation. In conclusion, incorporating PRF as an adjunctive grafting material results in fewer residual particles of allograft and in more bone marrow formation and may be clinically advantageous for developing the atrophic posterior maxilla in cases which require sinus membrane elevation.

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