Comparison of Autologous Blood, Advanced Platelet-Rich Fibrin, and Graftless Approach on New Bone Formation in Sinus Floor Elevation Supported by a Resorbable Membrane and Ultrasonic-Activated Pin System: A Controlled Experimental Study

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Purpose: To evaluate the histologic and radiologic effects of autologous blood (AB), advanced platelet-rich fibrin (A-PRF), and a graftless approach in sinus floor elevation supported by a resorbable membrane and ultrasonic-activated pin system. ***Materials and Methods:*** A total of 21 sinus floor elevation procedures were performed in 11 New Zealand rabbits that were divided into three groups. Each group planned to include seven maxillary sinus regions. Resorbable membranes and ultrasonic-activated pins (SonicWeld Rx, KLS Martin) were used to support the elevated sinus floor in all groups. After elevation of the sinus floor, bone defects were supported with either AB (Group 1), A-PRF (Group 2), or no biomaterial (control; Group 3). The rabbits were sacrificed 8 weeks after surgery. Specimens were obtained from the upper and lower regions of the sinuses. Radiologic and histologic examinations were performed on all samples. The radiologic examination evaluated new bone formation based on density difference (grayscale [GS]) in axial, coronal, and sagittal planes of CBCT sections. Histologic evaluation was performed by analyzing new bone tissue percentage/score, inflammation, and fibrous tissue parameters. ***Results:*** In the radiologic evaluation, the differences in GS values obtained in Group 3 in all planes were significantly lower than in Group 1 and Group 2, indicating significantly higher bone formation in the control group ($P < .05$). In the histologic evaluation, the percentage and score of newly formed bone were higher for Group 3; however, that difference was only significant between Group 3 and Group 1 for the specimens taken from the upper regions ($P = .005$ and $P = .018$, respectively). Fibrous tissue formation was lower in Group 3; the difference was significant between Group 3 and Group 1 for the specimens taken from the upper regions ($P = .037$). Inflammation values were similar between the groups. ***Conclusions:*** New bone formation after graftless sinus floor elevation was associated with more successful outcomes than the groups using AB or A-PRF in terms of histologic and radiologic parameters. *Int J Oral Maxillofac Implants 2026;41:451–458. doi: 10.11607/jomi.11391*

Keywords: advanced platelet-rich fibrin, autografts, autologous blood, bone tissue, graftless approach, maxillary sinus, oral surgical procedures, osteogenesis, resorbable membrane, ultrasonic-activated pins

Dental implants are considered a validated method for the rehabilitation of edentulism.[1,2] However, using dental implants in the posterior maxilla can be challenging due to various complicating factors such as maxillary sinus pneumatization and alveolar resorption following tooth loss, insufficient bone quality, limited visibility, and difficulty in accessing the region.[3–6]

Several surgical treatment approaches are proposed for the rehabilitation of the edentulous posterior maxilla, including maxillary sinus augmentation, short implants, zygomatic implants, and tilted implants.[7–10] Maxillary sinus augmentation is a predictable procedure for the partially or fully edentulous atrophic posterior maxilla to support dental implants.[11] With this surgical approach, the floor of the maxillary sinus is elevated to create sufficient bone height to support the stable placement of dental implants.[12] Transalveolar or single-/two-stage lateral sinus floor elevation approaches have primarily been described in the literature.[13] Autogenous, alloplastic, allogeneic, and xenogeneic graft materials have been widely used in lateral sinus floor elevation techniques.[11,12] In recent years, an

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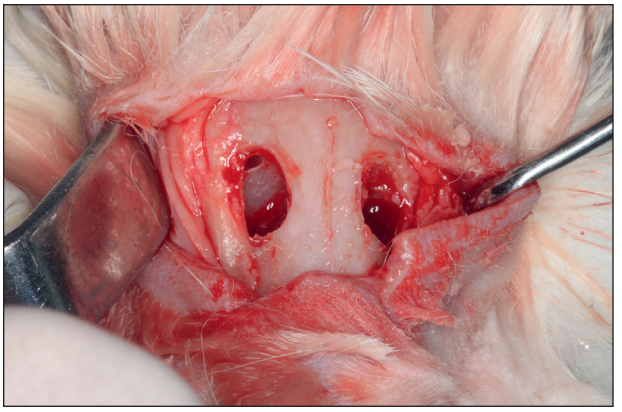


Fig 1 Elevation of the sinus membrane.

increasing number of studies have reported promising results for graftless maxillary sinus floor elevation.[14–16] In addition, the use of autologous blood (AB) products has been shown to benefit healing and bone formation.[17–19] However, there is currently no clear consensus on whether lateral sinus floor elevation surgery should be performed with a grafted or graftless approach and with or without biomaterials.[14–16]

Technologic advances enable the support of bone defects with space-maintaining applications that can improve bone healing in lateral sinus floor elevation.[20] Space-maintaining applications such as resorbable polymeric thermoplastic gels, titanium bone fixation devices, titanium mesh, and resorbable meshes have been reported in the literature.[19–22] To the best of our knowledge, there are no controlled experimental studies using resorbable membranes and ultrasonic-activated pin systems in lateral sinus floor elevation comparing AB products.

It has been hypothesized that advanced platelet-rich fibrin (A-PRF) could result in better histologic and radiologic outcomes regarding bone healing. This study therefore aimed to evaluate the histologic and radiologic effects of AB, A-PRF, and a graftless approach in sinus floor elevation supported by a resorbable membrane and ultrasonic-activated pin system.

MATERIALS AND METHODS

Study Design

This study was approved by the Kırıkkale University Ethic Committee for Animal Experiments (registration date and number: 2014, 14/04) and conducted at the Kırıkkale University Research Laboratory of Experimental Animals. Eleven mature New Zealand rabbits (aged 9 to 12 months) were used for the experiments. Three experimental groups were designed according to graft material: AB (Group 1, n = 7 sites), A-PRF (Group 2, n = 7 sites), and no biomaterial/control (Group 3, n = 7 sites). A resorbable membrane and ultrasonic-activated pin system (SonicWeld Rx, KLS Martin) were used to support the elevated sinus floor in all groups. All rabbits were cared for according to the consideration of welfare conditions in separate cages with appropriate space, adequate feed and water supply, and appropriate light and temperature conditions. Regular controls were carried out daily. Rabbits were sacrificed 8 weeks after surgery.

Surgical Procedure

General anesthesia was performed with 50 mg/kg ketamine hydrochloride (Alfamine, 10%, Eczacıbaşı) and 2.5 mg/kg xylazine hydrochloride (Rompun, Bayer). The surgical area was shaved and disinfected with povidone-iodine (Batticon). Then 1 mL of 4% articaine hydrochloride (contains 0.006 mg/mL epinephrin (Ultracaine DS, Sanofi Aventis) was injected to provide local anesthesia. Following the skin incision along the midline of the frontonasal suture region, a mucoperiosteal flap was elevated to approach the medial wall of the maxillary sinus. A 10-mm-diameter bone window was created with round steel burs on the upper wall of the maxillary sinus using a micromotor under sterile saline solution cooling. The bone window was located 10 mm lateral to the midline and approximately 20 mm below the frontonasal suture. The sinus membrane was elevated carefully with sinus elevators (Fig 1). This procedure was applied to 21 sinuses (bilaterally in 10 rabbits and unilaterally in 1 rabbit).

Resorbable membranes (Resorb-X, KLS Martin) were applied with 2.1-mm ultrasonic-activated pins (SonicPins Rx, SonicWeld Rx) to the maxillary sinuses in all groups to support the elevation of the sinus membrane (Fig 2). Resorbable membranes were shaped to dimensions of 3 × 6 mm according to the region, then placed in a water tank at 62°C and adapted to the region. Half of the membrane was adapted to remain on the surface of the nasal bone above the bone window, and the other half was directed inward to support the elevated sinus membrane (see Fig 2a). To fix the resorbable membrane, drilling was performed on the nasal bone and resorbable membrane (see Fig 2b), and resorbable ultrasonic-activated pins were applied to the drilled area (see Figs 2c and 2d).

Following the application of the membrane and pins, the sinus augmentation procedure was performed in the three groups as follows: For Group 1, autologous venous blood obtained from the ear vein was applied to the right maxillary sinus of seven rabbits (n = 7) (Fig 3a). For Group 2, A-PRF obtained from autologous venous blood taken from the ear vein was applied to the left maxillary sinuses of seven rabbits (n = 7) (see Fig

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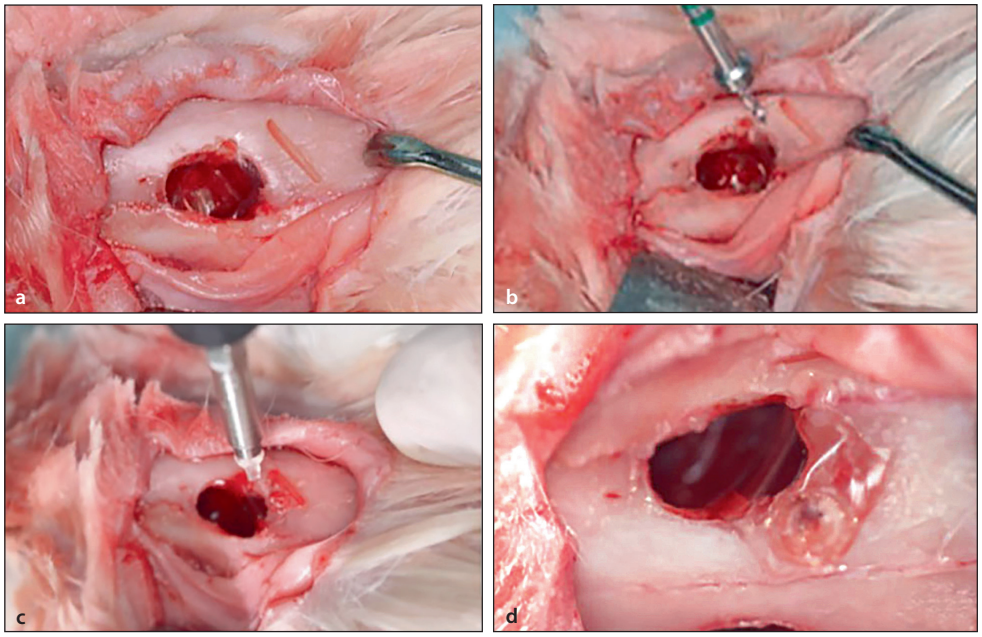


Fig 2 *(a to d)* Application of the resorbable membrane by ultrasonic-activated pins.

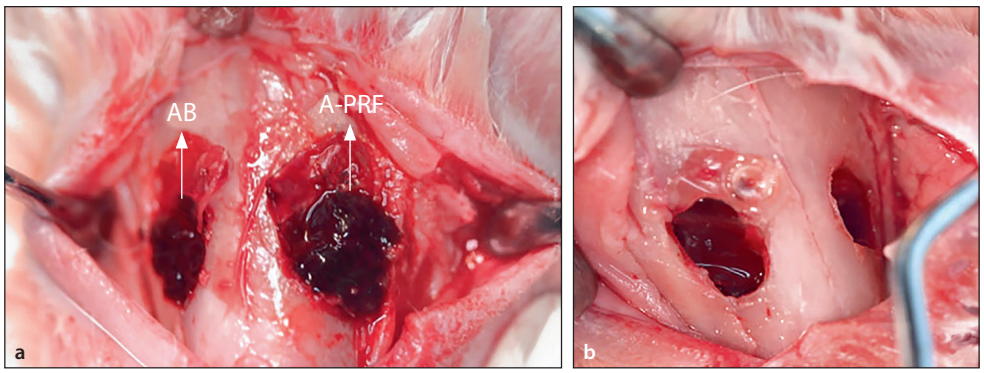


Fig 3 *(a)* Application of AB and A-PRF. *(b)* No graft material was used for Group 3.

3a). A-PRF was prepared according to the procedure described by Ghanaati et al[23] (1,500 rpm, 14 minutes). For Group 3, no graft material was applied to the surgical cavity of 7 maxillary sinuses, bilaterally in three rabbits and unilaterally in one rabbit (n = 7) (Fig 3b).

Following the suturing of the submucosal tissues with 4/0 Vicryl (Vicryl-Katsan), 4/0 silk (Doğsan) sutures were used to close the skin. The wound site was disinfected with povidone-iodine. After the operation, enrofloxacin (Baytril-K, Bayer, 2.5 mg/kg intramuscularly [IM]) and meloxicam (Maxicam, Sanovel, 1 mg/kg IM) were injected daily for 5 days to prevent postsurgical infection.

Postoperative Period

At the end of the eighth week, the rabbits were sacrificed by intracardiac injection of a lethal dose of xylazine hydrochloride (30 mg/kg Rompun IM) and 10% ketamine hydrochloride (70 mg/kg Alfamine IM). The heads of the rabbits were then decapitated, and skin and subcutaneous tissues were removed and washed with saline. Specimens were fixed in 10% formaldehyde. The specimens were sent to Kırıkkale University, Faculty of Dentistry, Department of Oral and Maxillofacial Radiology for CBCT examinations.

Radiologic Examinations

CBCT images of all specimens were obtained with PaxUni 3D (Vatech), operating at 85 kVp and 4.8 mA, with an exposure time of 12 seconds, 80 × 80–mm field of view (FOV), and 0.2-mm slice thickness. DICOM images were transferred to the Ez3D program (Vatech), and volumetric data was obtained. In the Ez3D software, the "Profile" tool was used to assess minimum and maximum grayscale (GS) values, which represented a numerical description of the densities of the areas appearing in gray for the augmented area and nonaugmented areas in the sagittal, coronal, and axial directions.[24] Due to the lack of GS value standardization, which is a significant problem in CBCT images,[25] only differences in GS values

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Table 1 GS Value Differences in All Groups for Coronal, Sagittal, and Axial Planes

	Group 1 (AB)	Group 2 (A-PRF)	Group 3 (control)	Kruskal-Wallis statistic			
	Median Mean ± SD	Median Mean ± SD	Median Mean ± SD	X^2	Group 3–1 *P*	Group 3–2 *P*	Group 1–2 *P*
Coronal GS difference	2378 2339.3 ± 117.4	2208 2175.3 ± 185.4	1684 1718.6 ± 114.8	14.9	< .001	.033	.635
Sagittal GS difference	2376 2352.3 ± 164.3	2200 2174.8 ± 145.4	1620 1704.3 ± 122.2	15.1	< .001	.037	.545
Axial GS difference	2300 2319.6 ± 178.1	2136 2140.9 ± 153.4	1664 1701.9 ± 121.4	14.5	.001	.026	.844
Mean GS difference	2377.3 2338.1 ± 148.1	2214.7 2163.9 ± 155.8	1657.3 1663.6 ± 115.3	15.6	< .001	.048	.395

were recorded and compared to obtain more reliable results. The smaller the difference between the two GS values assumed the closer the new bone formation density was to the untreated bone density. All radiologic examinations were performed by a maxillofacial radiology specialist (M.Z.A.).

Histologic Evaluations

Specimens were obtained from the sinus elevation areas with a trephine bur (diameter of 3.5 mm for the upper regions and 2.5 mm for the lower regions) and sent to Gazi University Faculty of Dentistry, Department of Oral Pathology, for histopathologic evaluation. Following the fixation and decalcification process, all samples were embedded in paraffin blocks and then sliced into sections approximately 4 to 5 µm thick in the sagittal plane using a microtome. The sections were stained with routine hematoxylin-eosin (H&E), Periodic Acid–Schiff (PAS), and Masson trichrome for histopathologic examination. Histopathologic evaluation was performed under a Leica DM 4000B light microscope in combination with the Leica QWin Plus v3.3.1 image analysis software. Specimens were scored regarding the newly formed bone tissue, inflammation, and amount of fibrous tissue.[26] The histologic evaluation process was performed by an oral pathology specialist (B.S.T.)

Measurements obtained from each central histologic section included the total defect area, newly formed bone area, and residual graft material area. These measurements were used to determine defect fill percentage (DF%) and new bone percentage (NB%) using the following formulas[27]:

$$\textit{New bone (NB\%)} = (\textit{New bone area} / \textit{Total defect area}) \times 100$$

$$\textit{Defect fill (DF\%)} = [(\textit{New bone area} + \textit{Residual graft material area}) / \textit{Total defect area}] \times 100$$

The degree of inflammation was assessed by counting mononuclear inflammatory cells within the defect area and surrounding connective tissue under high-power magnification. Inflammatory density was categorized into four grades based on cell count per field. H&E-stained sections were used to identify and quantify inflammatory cells within the defect site. The severity of inflammation was then evaluated using a four-grade scoring system as described by Hirshberg et al[28]:

- Grade 0: No inflammation
- Grade 1: Fewer than 15 cells per field
- Grade 2: Between 15 and 50 cells per field
- Grade 3: More than 50 cells per field

Fibrous tissue formation was evaluated based on its presence and extent within the defect area. The scoring criteria were as follows[29]:

- No fibrous tissue formation
- 0% to 25% fibrous tissue formation
- 25% to 50% fibrous tissue formation
- 50% to 75% fibrous tissue formation
- Complete fibrous tissue formation

Statistical Analyses

Statistical analysis was performed using IBM SPSS Statistics version 21 for Windows. Descriptive statistics were given as median, mean, and standard deviation. The difference of the variables in the groups was analyzed by the nonparametric Kruskal-Wallis test. The level of significance was set at $P < .05$.

RESULTS

During the study, it was observed that the animals tolerated the surgical procedure, and there were no complications related to the surgery.

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Table 2 Newly Formed Bone (in Percentage and Scores) in All Groups

		Group 1 (AB)	Group 2 (A-PRF)	Group 3 (control)	Kruskal-Wallis statistic			
		Median Mean ± SD	Median Mean ± SD	Median Mean ± SD	X^2	Group 3–1 *P*	Group 3–2 *P*	Group 1–2 *P*
New bone formation percentage	Upper region	50 47.14 ± 17.9	70 62.86 ± 14.7	70 78.57 ± 14.3	9.83	.005	.453	.269
	Lower region	40 35 ± 25	40 40 ± 20	70 55.71 ± 39.5	1.50	.472	.352	.400
New bone formation score	Upper region	1 1.43 ± 0.53	2 2.00 ± 0.577	2 2.14 ± 0.37	8.00	.018	.744	.290
	Lower region	1 1.00 ± 0.57	1 1.28 ± 0.48	2 1.57 ± 0.95	1.83	.399	.635	.345

Radiologic Outcomes

GS value differences for coronal, sagittal, and axial planes and the mean of the planes were calculated for the groups. The GS value differences obtained in Group 3 (control) were significantly lower than Group 1 (AB) and Group 2 (A-PRF) ($P < .05$). Additionally, mean GS value differences calculated for Group 3 were significantly lower than Groups 1 and Group 2 ($P < .001$ and $P = .048$, respectively). No significant differences were found between Group 1 and Group 2 for all planes. GS value differences in all three planes in the groups are presented in Table 1.

Histologic Outcomes

Samples taken from the upper and lower regions of the surgical sites of each group were evaluated regarding newly formed bone tissue (in percentage and score), inflammation, and amount of fibrous tissue. All specimens were suitable for histopathologic examination, and none showed giant cells or chronic inflammation indicating a foreign body reaction.

Regarding newly formed bone percentage and score, the values in Group 3 were significantly higher than Group 1 for the specimens taken from the upper regions ($P = .005$, $P = .0018$, respectively). The results were similar between the other groups and regions. The results of the newly formed bone in percentage and scores within and between groups are presented in Table 2. Differences in histologic appearances between the groups are shown in Figs 4 to 6.

Regarding the amount of fibrous tissue formation score, the values in Group 3 were significantly lower than Group 1 for the specimens taken from the upper regions ($P = .037$). The results were similar between the other groups and regions. The results of the amount of fibrous tissue formation scores within and between groups are presented in Table 3.

Inflammation scores calculated for the specimens obtained from upper and lower regions were similar between all regions and groups (Table 4).

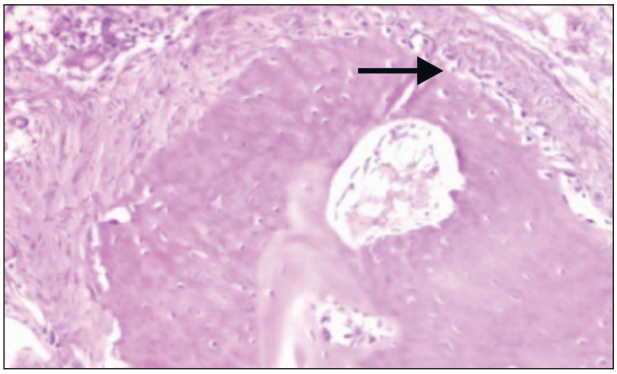


Fig 4 Osteoblastic alignment at the end of 8 weeks in Group 3 (PAS ×200 magnification).

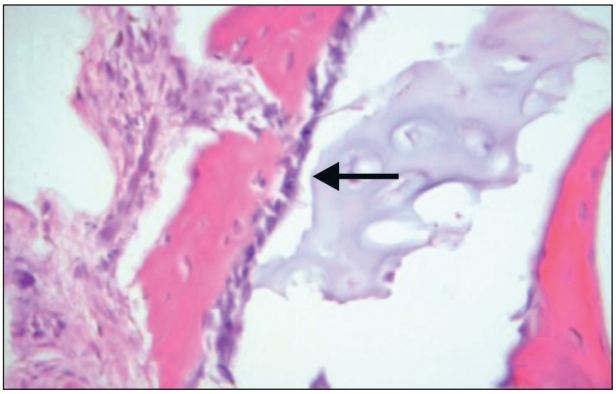


Fig 5 Osteoblastic alignment at the end of 8 weeks in Group 2 (HE ×400 magnification).

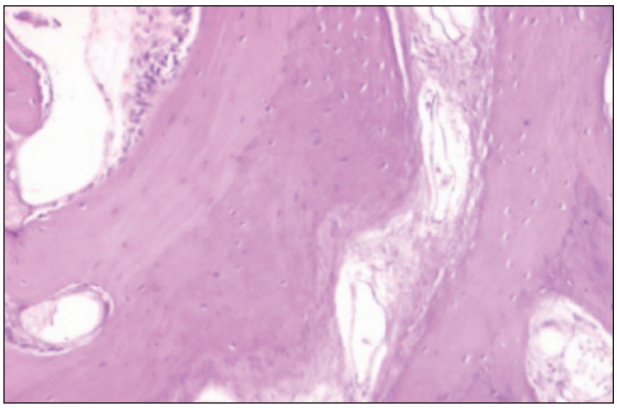


Fig 6 Mature and newly formed bone tissue at the end of 8 weeks in Group 1 (PAS ×200 magnification).

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Table 3 Fibrous Tissue Formation Scores in All Groups

	Group 1 (AB)	Group 2 (A-PRF)	Group 3 (control)	Kruskal-Wallis statistic			
	Median Mean ± SD	Median Mean ± SD	Median Mean ± SD	X^2	Group 3–1 *P*	Group 3–2 *P*	Group 1–2 *P*
Upper region	1 1.42 ± 0.53	1 1.14 ± 0.37	1 0.85 ± 0.37	6.61	.037	.595	.595
Lower region	2 1.85 ± 0.69	2 1.71 ± 0.48	1 1.42 ± 0.97	1.36	.507	.535	.445

Table 4 Inflammation Scores in All Groups

	Group 1 (AB)	Group 2 (A-PRF)	Group 3 (control)	Kruskal-Wallis statistic			
	Median Mean ± SD	Median Mean ± SD	Median Mean ± SD	X^2	Group 3–1 *P*	Group 3–2 *P*	Group 1–2 *P*
Upper region	0 0.28 ± 0.48	1 1.14 ± 0.37	0 0.28 ± 0.48	5.39	.067	.197	.396
Lower region	0 0.14 ± 0.37	1 1.00 ± 0.57	0 0.14 ± 0.37	0.74	.689	.548	.345

DISCUSSION

An increasing number of studies have recently reported promising outcomes for graftless maxillary sinus floor elevation surgery.[14–16] In addition, it has been stated that using AB products or space-maintenance applications could contribute to the healing period and bone formation in sinus elevation procedures.[17–22] However, there is currently no clear consensus on whether lateral sinus floor elevation surgery should be performed with a grafted or graftless approach and with or without biomaterials.[14–16]

The present study aimed to evaluate the histologic and radiologic effects of three different approaches—AB, A-PRF, and graftless (no biomaterials)—on new bone formation after lateral sinus floor elevation supported by a resorbable membrane and ultrasonic-activated pins to maintain space. According to the radiologic results, the differences in GS values obtained for the control (graftless) group in all planes were significantly lower than those obtained in AB and A-PRF groups. GS values derived from CBCT units are not certain to be consistently representative of the actual density values, and even further, it is not certain if they are similar to the Hounsfield units derived from medical CT scanners.[25] In this study, to overcome this situation, the GS value differences in the areas of new bone formation and untreated bone were calculated instead of the numerical input values obtained. In this respect, this study also presents a different method for using GS values for comparing bone densities in CBCT images.

Several different types of animals could be selected for experimental studies. However, rabbit sinuses have the advantage of an ostium opening into the nasal cavity that creates similar air pressure changes to humans.[30,31] Rabbits have been previously used in experimental maxillary sinus floor augmentation studies. Therefore, rabbits were preferred for this study. In previous studies, it has been shown that bone healing is accomplished in 6 to 8 weeks in rabbits. Therefore, our study duration was set at 8 weeks, considering that bone healing in rabbits is completed between 50 and 60 days.[32,33]

In the present study, the histologic findings aligned with the radiologic findings to indicate significantly higher bone formation for the graftless approach (ie, no biomaterials used). The percentage and score of newly formed bone for the specimens taken from the upper regions were significantly higher in the graftless group than in the AB group. Fibrous tissue formation scores were also lower in the graftless group. Inflammation scores for specimens obtained from the upper and lower regions were similar between the groups. It could be assumed that graftless sinus floor elevation supported by a resorbable membrane and ultrasonic-activated pins has been associated with more successful radiologic and histologic outcomes. However, that result could be associated with several factors and needs to be negotiated.

Lie et al[15] conducted a systematic review and meta-analysis of randomized controlled trials regarding graftless versus grafted sinus elevation procedures in the atrophic maxilla. Their results reported a high overall implant survival rate in both approaches; however, vertical bone height gain and bone density values were significantly lower in the graftless sinus elevation group. The lower bone gain results related to graftless sinus floor elevation may be due to the absence of the

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scaffold effect created by the graft materials. Another study reported that implant survival was significantly higher following conventional grafted sinus elevation surgery compared to graftless surgery in which a resorbable membrane was used to elevate the sinus membrane.[10] The reduced survival rate could be associated with a low ability of the resorbable membrane to maintain space during the healing period. Graft materials act as a scaffold for bone formation inside the maxillary sinus; therefore, space maintenance of the defect area could optimize conditions for graftless surgery regarding bone healing. A few studies in the literature include data on this topic. Cossellu et al[20] investigated the graftless sinus elevation procedure whereby space was maintained using a resorbable polymeric thermoreversible gel. Sufficient formation of new bone with no fibrous tissue or severe inflammatory cellular infiltration was reported. Their results showed that a graftless sinus elevation procedure using a space-maintaining gel appears to stimulate predictable bone formation; therefore, it could be beneficial for improving bone formation. Similar to that result, Kaneko et al[21] examined graftless sinus elevation supported by a titanium bone fixation device in patients with atrophic posterior maxilla. They concluded that the space maintenance provided by the bone fixation device in a graftless sinus elevation promotes bone formation regarding clinical and radiologic outcomes. However, a second surgery is needed to remove the bone fixation device. Graftless procedures using resorbable membranes and ultrasonic-activated pin systems have several advantages over the techniques described in the studies mentioned above, as the thermoplastic properties of the membrane offer easy application and manipulation in the sinus region, there is no need for a second surgery, and it provides effective space maintenance during the healing period.

An experimental study using autologous periosteum as a barrier membrane placed over graft material in sinus elevation showed that this technique can contribute positively to bone formation with high numbers of osteoblasts.[34] According to the histologic result of the present study, new bone formation percentages and scores were found to be higher in the specimens obtained from upper regions than in lower regions for both groups. Additionally, both experimental groups' fibrous tissue formation scores were lower in the upper regions. It could be assumed that the higher percentage and score of new bone formation and lower fibrous tissue formation in the samples taken from the upper regions in all groups may be related to the osteogenic potential of the sinus membrane, which is closely adjacent to the region.

Limitations

The limitations of this study include the absence of a sham group and the lack of evaluation of early/mid-term histologic examination. However, cost is a significant factor for experimental studies, and the Animal Ethic Committee did not approve the increase in the number of animals. Additionally, in the present study, only histologic and radiologic evaluation was carried out to evaluate the specimens. Immunohistochemical evaluation could contribute investigation of specific biomarkers for bone healing.[35] Further studies with a larger number of animals, including different groups, and an extended time for evaluating bone healing in the early and late periods are warranted.

AB products could be associated with promising results for improving the healing period for maxillary sinus floor elevation.[17–19] However, weak physical characteristics of autologous platelet products restrict the use of a "single" substitute for single-stage lateral sinus floor elevation.[36] Most of the studies underscore the importance of simultaneous implant placement for the tenting effect of the sinus floor when using autologous platelet concentrates such as PRF or A-PRF as the sole substitute during sinus elevation.[37–39] In the present study, a tenting effect was accomplished by resorbable membranes and an ultrasonic-activated pin system, which has been similarly produced by simultaneous implant placement in the studies mentioned above.

CONCLUSIONS

To our knowledge, the present study is the first to compare AB products with the graftless approach in maxillary sinus floor elevation in which a space-maintaining procedure is supported by a resorbable membrane and ultrasonic-activated pin system. Graftless surgery has been associated with more successful outcomes regarding histologic and radiographic parameters than AB and A-PRF. Further clinical and experimental studies with larger sample sizes and more extended periods are needed to confirm these results.

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