Original Article

Evaluation of the Effectiveness of Liquid Platelet-Rich Fibrin and Deproteinized Bovine Bone Mineral Mixture on Newly Formed Bone in Maxillary Sinus Augmentation: A Split-Mouth, Histomorphometric Study

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ABSTRACT

Objective: We aimed to investigate the effectiveness of the liquid PRF-DBBM mixture on new bone formation in maxillary sinus augmentation. **Material and Methods:** Seven patients requiring two-stage bilateral maxillary sinus augmentation were included in the study. The patients were selected according to the criteria of having an alveolar bone height of at least 2 mm in the atrophic region. The elevated sinus cavities were randomly grafted with DBBM + liquid PRF (test) or DBBM alone (control) in a split-mouth design. Bone samples were collected during implant surgery with a trephine bur for histomorphometric evaluation after 4 months. **Results:** In the control group, the newly formed bone was 39.49%, the mature bone was 15.66%, the residual graft was 15.62%, and the fibrous tissue ratio 28.59%, while in the test group, the newly formed bone (NFB) was 45.95%, the mature bone was 14.40%, the residual graft was 10.32%, and the fibrous tissue was 29.31%. No statistically significant difference was found between the groups in terms of the parameters studied ($p > 0.05$). The mean osteocalcin score in the control group was 2.70 ± 0.39, while it was 2.81 ± 0.36 in the test group. There was no statistically significant difference between the averages of osteocalcin scores of the groups ($p > 0.05$). **Conclusion:** The results of our study showed that DBBM is a reliable graft material for maxillary sinus augmentation even in the early period. Combining of DBBM with liquid-PRF contributed to new bone formation over a four-month period, but this contribution was not statistically significant.

Keywords: *Bone grafts, bone regeneration, histomorphometry, platelet-rich fibrin, sinus lift*

Introduction

Alveolar bone volume and density play a key role in osteointegration and long-term function of dental implants. Rapid resorption develops in the alveolar bone both horizontally and vertically due to the lack of intraosseous stimulation of periodontal ligament fibers as a result of tooth loss in the posterior maxilla. The absence of upper molars leads to increased osteoclast activity in the Schneider membrane, causing resorption in the bone and pneumatization of the sinus within a few months.[1,2] In many cases, vertical bone height is limited in the posterior maxilla due to the conditions mentioned above. To overcome this problem, maxillary sinus floor elevation or sinus augmentation procedures have been developed.[1,3] During the maxillary sinus augmentation process, the space obtained after the membrane is

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elevated can be filled with different graft materials such as autogenous, allogenic, xenogenic, alloplastic, structures containing growth factors, or combinations of these grafts.[4]

Deproteinized bovine bone mineral (DBBM) is a biocompatible xenogenic graft material with osteoconductive properties, which is frequently preferred for maxillary sinus augmentation. The advantages of this biomaterial include larger availability than autogenous bone, lower resorption rate, and less morbidity. However, its resorption is slow and maturation of this material in the grafted region takes a long time such as 8-9 months since this graft material has no osteogenic and osteoinductive effects.[5] To shorten this process, it was aimed to accelerate the formation of new bone by adding growth factors to this graft material.[6-8]

Mineralized plasmatic matrix (MPM), defined as growth factors within a fibrin network, is a product of mixing two phases, plasma, and mineral phase. It is obtained by centrifuging for more time in fewer cycles compared to platelet-rich fibrin (PRF) clot. Two layers are seen in the tube after centrifugation. A layer rich in red blood cells at the bottom and plasma rich in white blood cells and growth factors are obtained at the top (plasma phase). The liquid-PRF obtained in the top layer is mixed with an alloplastic or xenogenic graft material (mineral phase). As a result of this merger, a homogeneous, compact, stable, and shapeable graft mass is achieved.

There are few studies on MPM in the literature. However, it was seen that beta-tricalcium phosphate particles were used as a graft mixture in these studies.[9-11] In this histomorphometric study, we wanted to evaluate the effect of MPM combination obtained with DBBM on new bone formation in the early period after sinus augmentation procedure. For this purpose, we selected cases with sufficient bone height to provide primary implant stabilization.

Material and Methods

Patients

A total of 7 patients (five females and two males) had bilateral maxillary sinus atrophy were included in this study. However, care was taken to ensure that residual alveolar bone height was less than 5 mm and more than 2 mm in these patients. Our aim was to consider the primary stabilization of the implants as the effectiveness of the grafts will be examined in the 4th month. The mean age of the patients was 50,57 ± 11,73 and the range of age was 31-63. The clinical and demographic data of the patients were shown in Table 1. The study was conducted in accordance with the standards of the Declaration of Helsinki (2002) and was approved by the Ethical Board of Selcuk University, Faculty of Dentistry (Nr. 201507). Exclusion criteria included maxillary sinusitis, blood platelet disorders, prolonged use of corticosteroids, diabetes, other metabolic diseases, smoking, periodontal disorders, radiotherapy, and previously performing any operations in the maxillary sinus.

Preparation of MPM

Venous blood samples obtained from the patients were taken into four 9 ml tubes without anticoagulation. The tubes were placed symmetrically in the centrifuge device (Ample Scientific Champion F-33D, Georgia, USA) and centrifuged at 2300 rpm for 15 minutes. After centrifugation, two separate layers were formed inside the tubes, yellow at the top and red at the bottom. The upper yellow part was taken with an injector and poured onto DBBM (Bio-Oss, Geistlich Pharma AG, Wolhusen, Switzerland) previously wetted with serum in a plastic sterile cup. Then it was mixed with a spatula for a few minutes. After completion of the coagulation reaction, a solid, viscous, and homogenous bloc containing all graft particles (MPM) was obtained, which could be manually shaped.

Surgical procedures

Before the operation, all patients were gargled with 0.2% chlorhexidine gluconate solution for 1 minute for oral antisepsis. Surgical procedures were performed under local anesthesia in all patients. Lateral wall protocol was preferred for the sinus augmentation procedure. The sinus membrane was raised in superior. The prepared graft material was then filled into the space between the sinus floor and the Schneiderian membrane. Sinus cavities on the right and left sides of the patients were randomly determined for the test and control groups. While DBBM and plasma mixture was placed into the sinus cavity of the test group, only DBBM was filled into the sinus cavity in the control group. Following this procedure, the lateral window was covered with a resorbable collagen membrane (Bio-Gide, Geistlich Pharma AG, Wolhusen, Switzerland), and mucoperiosteal flaps were sutured with 3-0 silk suture (Troge, Hamburg, Germany) [Figure 1]. After the operation, all patients were prescribed antibiotics, painkillers, decongestants, and mouthwashes. The patients were followed clinically and radiographically in the first week, first month and fourth months.

Dental implants were placed 4 months after sinus augmentation. In this second stage of the surgical procedure, biopsy materials were taken using a trephine bur with a diameter of 3 mm. The preparation depth was defined by considering the planned implant length. During this procedure, it was paid attention that biopsy samples included parts of residual alveolar crest bone

as well as previously grafted areas of the sinus. Dental implants of appropriate diameter and length were then inserted in biopsy sites. The samples were then fixed immediately in buffered 10% formalin at 4°C for histological preparation.

Histology and histomorphometry

Two blinded examiners performed the histomorphometric analysis. The biopsy samples were subjected to a slow, long-term decalcification procedure in a 10% EDTA (Ethylene diamine tetra acetic acid) solution to ensure the efficiency and continuity of the fixation process during decalcification after at least 48-96 hours of pre-fixation. The solution was renewed at one-week intervals and the decalcification efficiency of EDTA was tried to be achieved. The tissues were embedded in paraffin blocks, sliced into 4-mm-thick specimens, and stained with Masson's trichrome stains. In addition, osteocalcin antibody (FL- 100) (sc-30044, Santa Cruz Biotechnology) was used to examine osteoblast activity. The preparations were evaluated with a light microscope (Olympus BX51, Tokyo, Japan) and were photographed with a camera (Olympus DP72, Tokyo, Japan) attached to a light microscope during this evaluation. In the analysis process, the presence of new bone formation, mature bone, graft particles, and fibrous tissue were evaluated by two researchers as double-blind at different times. In addition, the samples were stained with osteocalcin antibody that binds to receptors on the osteoblast surface and examined under fluorescent light.

Statistical analysis

In the statistical analysis paired t-test was performed. to compare the normally distributed parameters between groups. To compare the non-normally distributed parameters between groups, the Mann-Whitney U test was used. The significance level was accepted as $P < 0.05$.

Results

Of the patients who underwent sinus augmentation, 5 were partial and 2 were total edentulous. A total of 14 sinus augmentation procedures were performed in 7 patients. The average residual crest height was

Table 1: Patient demographics and clinical data

Patient	Age (Years)	Gender	Partially/totally edentulous	Maxillary sinus		Residual alveolar bone height	
				Test	Control	Right	Left
1	31	F	Partially	R	L	4,20 mm	5,37 mm
2	63	M	Totally	R	L	3,80 mm	4,00 mm
3	55	M	Partially	L	R	5,40 mm	4,90 mm
4	52	F	Partially	R	L	4,50 mm	2,10 mm
5	38	F	Partially	L	R	3,60 mm	2.20 mm
6	55	F	Partially	L	R	2,10 mm	2,20 mm
7	60	F	Totally	R	L	3,10 mm	2,90 mm

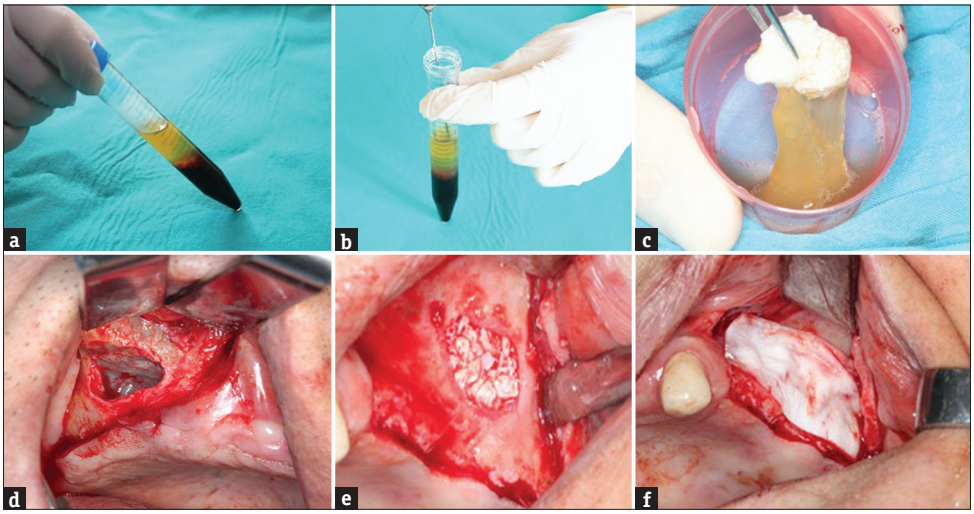

Figure 1: (a) After centrifugation, two separate layers were obtained inside the tubes, yellow at the top and red at the bottom (b) The upper yellow part was taken with an injector and poured onto DBBM previously wetted with serum in a plastic sterile cup (c) After completion of the coagulation reaction, a solid, viscous, and homogenous bloc containing all graft particles was obtained, which could be manually shaped (d) Lateral wall protocol was preferred for the sinus augmentation procedure. Elevation was performed to the superior without damaging the sinus membrane (e) The prepared graft material was filled into the space between the sinus floor and the Schneiderian membrane (f) The lateral window was covered with a resorbable collagen membrane

measured as 3.77 mm in the test group and 3.88 mm in the control group. No statistically significant difference was found between the residual crest heights of both groups ($p > 0.05$). In the postoperative period, no complication was observed except oedema and mild pain. In panoramic X-rays taken after a four-month recovery period, the improvement in both groups looked similar [Figure 2]. In the second stage surgery, a total of 47 implants were placed and 14 of them were inserted to the augmentation region. No problems were observed in any of the implants during a 2-year follow-up period.

Graft particles were observed in the samples at the end of 4 months in both groups. No foreign body reaction or a significant inflammatory reaction was detected around the graft material. The NFB tissues were in dense appearance. Mature bone areas were seen in the residual alveolar crest regions on the upper part. In the middle region, fibrous matrix, NBF, and graft materials were intertwined, while in the apical regions, NBF areas and graft materials were observed. In both groups, the new bone that progresses into the graft area was rich in cells and its lamellar lines were observed in some areas, while in other areas it was seen as woven bone structures.

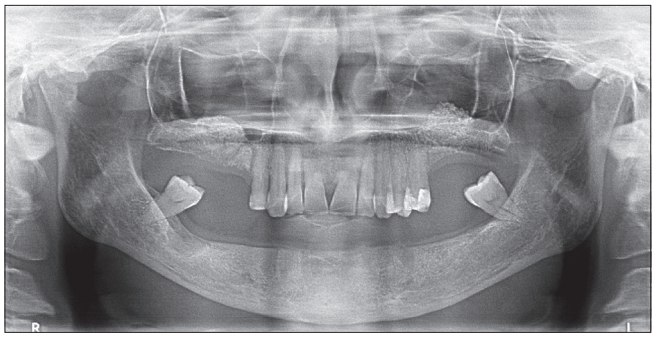

Figure 2: After 4 months, sufficient bone height was achieved on both sides. Right side is test group, left side is control group

When the groups were evaluated individually, large resorption areas of the graft material were observed in histology sections obtained from the control group. In some of these resorbed regions, graft particles surrounded by fibrous tissue and osteoclastic giant cells and howship lacunas were found. In some regions, it was observed that the NBF surrounded the graft particles. In the areas adjacent to the alveolar bone of the biopsy material, bone trabeculae of the natural structure were observed, in which fibrotic tissues were also visible, and vascularity was detected in these fibrous tissues [Figure 3].

In the histological evaluation of the test group, it was observed that the graft materials were replaced by NBF in the middle part of the sections. The NFB was surrounding the graft materials and was separated from the graft materials by resorption-apposition lines. It was intertwined with new bone and graft material in many places. In some areas, osteoclastic giant cells and Howship lacunaes were found around the graft particles. A vein-rich fibrous tissue was observed between the graft and the new bone in some sections [Figure 4].

Considering the histomorphometric evaluation results of the groups, it was seen that the NFB ratio 39.49%, the mature bone ratio 15.66%, residual graft ratio 15.62%,

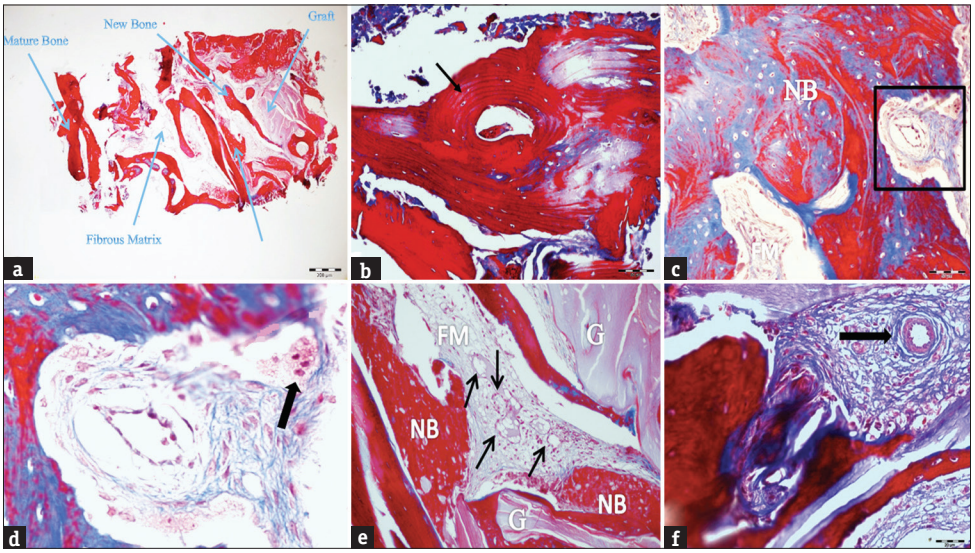

Figure 3: (a) Mature bone areas were seen in the residual alveolar crest regions on the upper part. In the middle region, fibrous matrix, new bone formation, and graft material were intertwined, while in the apical regions, newly formed bone areas and graft materials were observed (b) The new bone tissue that progresses into the graft area was rich in cells and its lamellar lines were observed (Black arrow) (c) Osteocytes were seen in the new bone tissue in the histological view of the Bio-Oss group. There was also fibrous tissue in places (d) The multi-nucleus osteoclast cell and howship lacuna on the new bone were seen (black arrow) (e) The new bone tissue was seen to surround the graft. Vascularity were seen in the fibrous matrix (black arrows) (f) The artery seen in the fibrous matrix (black arrow). NB: New Bone, G: Graft, FM: Fibrous Matrix

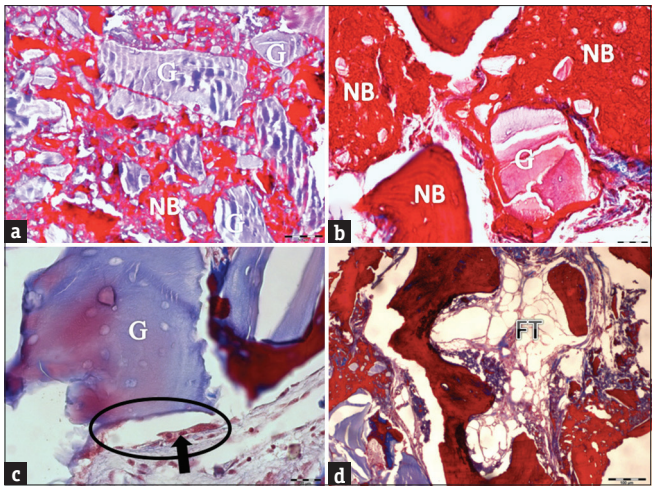

Figure 4: Histological view of the MPM group (a) Although there were some gaps between the graft particles and the new bone, it was observed that in most places it was intertwined with the new bone (b) Graft material embedded in new bone (c) Multi-nucleus osteoclastic cells and Howship lacuna were seen around the graft particles (d) Adipose tissue has been observed in areas where the trabecular bone is weak. NB: New Bone, G: Graft, FM: Fibrous Matrix, FT: Fat Tissue

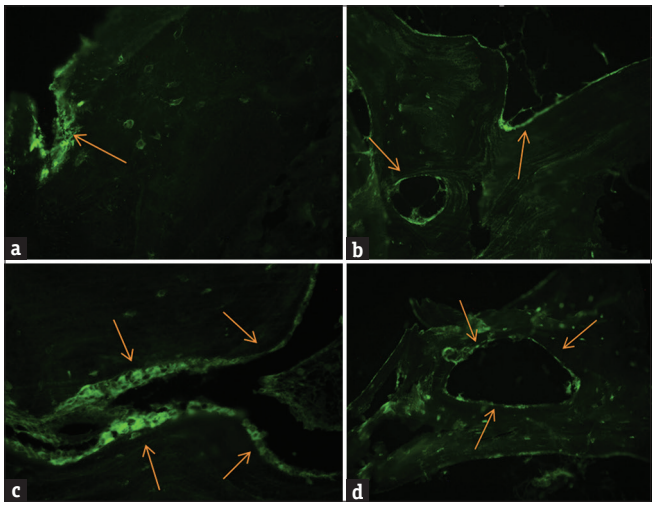

Figure 5: (a) Osteoblasts provide osteoid tissue accumulation on new bone tissue. (Fluorescent staining in the Bio-Oss group) (b) Osteoblast deposition on the new bone surface and around the bone marrow were seen in fluorescent staining of the MPM group (c) Image of osteoblasts extending peripherally along the bone trabecula. (Bio-Oss group) (d) Image of bone marrow and surrounding osteoblast accumulation visible in the bone trabecula. (MPM group)

and fibrous tissue ratio 28.59% in the control group. In the histomorphometric evaluation of the test group, NFB ratio was 45.95%, mature bone ratio was 14.40%, residual graft ratio was 10.32%, and fibrous tissue ratio was 29.31%. No statistically significant difference was found between the groups in terms of the parameters studied ($p > 0.05$).

In the osteocalcin staining results, osteoblast cells were seen in both groups around the new bone [Figure 5]. The mean osteocalcin score in the control group was 2.70 ± 0.39, while it was 2.81 ± 0.36 in the test group. There was no statistically significant difference between the averages of osteocalcin scores of the groups ($p > 0.05$).

Discussion

Factors limiting dental implant applications in the posterior maxilla include lack of good bone quality, physiological resorption due to the tooth loss, and lack of sufficient residual crest height due to pneumatization of the maxillary sinuses. In addition, it is difficult to maintain the primary stability of the implants due to factors mentioned.[12] Today, to overcome this problem in the posterior maxilla, the technique of maxillary sinus augmentation is frequently used and its reliability is accepted. The most important factor determining graft preference and implant placement stage in sinus augmentation is the height and quality of the alveolar bone. Clinical studies have shown that the two-stage surgical approach is generally preferred in the presence of residual alveolar bone less than 5 mm.[13,14]

In the literature, different biomaterials have been proposed to fill the gap obtained by sinus membrane elevation and successful results have been reported for bone regeneration.[15-18] DBBM is one of these materials and is among the most used graft materials for sinus augmentation due to its properties similar to human cancellous bone. DBBM also functions as a scaffold and matrix for osteogenic cell migration from the sinus wall to the graft and enhances the ability to form new bone. Due to these advantages, Bio-Oss is the most preferred material in the literature.[19]

As is known, PRF is considered a biomaterial that contains a strong fibrin matrix that allows the slow release of growth factors. Transforming growth factor (TGF-b1), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) are released from the PRF membrane for 7 days.[20] Especially leukocytes are thought to play a key role in the slow release of TGF-b1 and VEGF.[6] In an *in vitro* study, PRF has been reported to improve the proliferation of human osteoblasts and increase alkaline phosphatase expression in a dose-dependent manner.[21] In tissue engineering, fibrin has been applied as the distribution system of these growth factors.[22] In addition, fibrin has a significant effect on collagen synthesis of osteoblast-like cells.[23] In this study, we investigated the effect of liquid-PRF on new bone formation in sinus augmentation.

According to the histological results of our study, new bone formation was seen in both groups after the healing period of 4 months. However, the amount of NFB increased when liquid-PRF was added to the graft compared to the control group in the histomorphometric

evaluation although we did not find any statistical difference. According to the evaluation results, the residual graft was found in a smaller amount in the liquid-PRF group. One of the possible reasons for this situation is that DBBM resorption occurred faster in the test group. The amount of fibrous tissue was found to be the same in both groups.

Osteocalcin is a matrix protein synthesized by osteoblasts. It is the most abundant non-collagenous protein in bone. It appears in the late stages of differentiation, as it is an indicator of mature osteoblast formation. According to the density assessment results in the sections, it was observed that the osteocalcin scores were higher in the liquid-PRF group compared to the Bio-Oss group. Although no statistically significant results were obtained, it was seen that the liquid-PRF application to DBBM minor contributes to osteoblast formation.

When the graft materials are mixed with the normal PRF form, a homogeneous mixture cannot be obtained and does not contribute to the stability of the graft. One of the most important features of this liquid form of PRF and DBBM mixture, also called MPM, is to obtain a homogeneous block that holds the graft particles together when the liquid-PRF combines with the graft. Thus, the dispersion of the graft is prevented. When DBBM particles are mixed in the liquid PRF, an expansion in volume is also obtained. Therefore, more area can be filled with less amount of graft. We used a fewer amount of graft to fill the region in the test group in our study. This can be the probable reason for the small amount of residual graft to be detected in the histomorphometric examination. These physical properties are thought to be due to the fact that PRF has a dense fibrin fiber network. The fibrin fibers also provide adhesion of graft to the surrounding bone wall.[8] In addition, *in vitro* studies have shown that liquid form of PRF can stimulate fibroblast migration and release various growth factors at high concentrations.[24,25] Integration of the fibrin network with the graft material facilitates cell migration, neo-angiogenesis, vascularization, and graft survival. Platelet products such as PDGF, TGF-b1, and IGF-1 in the fibrin network provide a rapid recovery while resorbing the fibrin matrix.[26]

In some clinical studies in the literature, it is seen that the new bone formation obtained in the normal PRF form-DBBM mixture is very close to the new bone formation obtained only in the groups using DBBM and it was concluded that PRF did not contribute to bone formation in the same studies.[6,27] It is noteworthy that in these studies, 6 months is expected as the recovery period. Most of the healing is already over after 6 months. Therefore, it is not known how much new bone formation was obtained in the early period in the test group. In a clinical study, PRF-frozen dried bone allograft (FDBA) mixture was applied to the test group and only FDBA was applied to the control group.[28] In the biopsy samples taken from the test group at the 4th month and the control group at the 8th month, vital bone ratios were found to be 20.3% and 20.95%, respectively. In another split mouth study, the effectiveness of the PRF-DBBM mixture on new bone formation was investigated, biopsy was taken at the 4th month from the test group, and in the 8th month from the Bio-Oss group, and the implants were placed simultaneously.[8] As a result of the study, the NFB amount was 44.58% in the test group and 30.02% in the control group. In this study, we obtained the samples at 4 months to see the effect of graft materials on new bone formation in the early period. Our histomorphometric results showed new bone formation as 45.95% in the test group and 39.49% in the control group. Although we did not find any statistical difference, liquid-PRF seems to trigger new bone formation in the early period. Furthermore, the height of the sub-antral bone was above the average of 3 mm in our patients. Since the osteogenic cell migration to the grafted area comes from the surrounding bone tissues, we think that the higher the alveolar bone height, the better the healing potential of the grafted area. This may have led to a similar improvement in both groups over a four-month period. In addition, the aim of our attention to ensure that the bone height was at least 2 mm in our patients was to provide primary stabilization of the implants in the 4th month. Thus, we were able to evaluate the formation of new bone in the early period in the group where only DBBM was applied.

The results of our study showed that DBBM is a reliable graft material for maxillary sinus augmentation even in the early period. Combining of DBBM with liquid-PRF contributed to new bone formation over a four-month period, but this contribution was not statistically significant.

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Conflicts of interest

There are no conflicts of interest.

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