Histologic and histomorphometric assessment of sinus-floor augmentation with beta-tricalcium phosphate alone or in combination with pure-platelet-rich plasma or platelet-rich fibrin: A randomized clinical trial

Songül Cömert Kiliç, DDS, PhD1 | Metin Güngörmüş, DDS, PhD2 | Seçil Nazife Parlak, PhD Student3

1Ministry of Health, Department of Oral and Maxillofacial Surgery, Center for Oral and Dental Health, Erzurum, Turkey
2Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Gaziantep University, Gaziantep, Turkey
3Department of Histology and Embryology, Faculty of Medicine, Ataturk University, Erzurum, Turkey

Correspondence
Songül Cömert Kiliç, Ministry of Health, Department of Oral and Maxillofacial Surgery, Center for Oral and Dental Health, Erzurum, Turkey.
Email: drmckilic@yahoo.com.tr

Funding information
Scientific Research Project Fund of Ataturk University, Funding Number: 2011/33

Abstract
Background: The potential effects of adding pure platelet-rich plasma (P-PRP) or platelet-rich fibrin (PRF) to beta-tricalcium phosphate (β-TCP) graft substitute on bone formation and regeneration after maxillary sinus-floor elevation remains unclear.

Purpose: To compare the histologic and histomorphometric outcomes of maxillary sinus-floor augmentation among β-TCP alone, P-PRP-mixed β-TCP, and PRF-mixed β-TCP.

Material and Methods: In this randomized clinical trial, elevated sinus cavities were grafted with β-TCP (the control group), P-PRP-mixed β-TCP (the P-PRP group), and PRF-mixed β-TCP (PRF group). The sample was composed of 26 patients: 9 subjects in control and P-PRP groups, and 8 subjects in PRF group. After a 6-month, healing period, bone graft biopsies were harvested prior to implant placement, and the specimens were analyzed. The main outcome variables included findings of histologic and histomorphometric analyses of the bone graft biopsies. The data were analyzed by ANOVA and Tukey HSD tests.

Results: The mean percentages of new bone formations were 33.40 ± 10.43%, 34.83 ± 10.12%, and 32.03 ± 6.34% in control, P-PRP, and PRF groups, respectively, with no significant differences (P > .05). Mean percentages of residual graft particle area were 30.39 ± 10.29%, 28.98 ± 7.94%, and 32.66 ± 7.46% in control, P-PRP, and PRF groups, respectively, with no significant differences (P > .05). The mean percentages of soft-tissue area were 36.21 ± 10.59%, 36.19 ± 13.94%, and 35.31 ± 10.81% in control, P-PRP, and PRF groups, respectively, with no significant differences (P > .05). Mean densities of osteoblasts, osteoclasts, osteocytes, and capillary vessels showed insignificant difference between groups (P > .05), but osteoprogenitor cells were lower and inflammatory cells were higher in the PRF group than those in other groups (P < .01). Biopsies of P-PRP, PRF, and control groups showed similar composition and distribution of histologic structures.

Conclusion: These findings suggested that adding P-PRP or PRF to β-TCP graft substitute was not beneficial on new bone formation and regeneration, and P-PRP plus β-TCP or PRF plus β-TCP is not superior to β-TCP alone.

Keywords
beta-tricalcium phosphate, histomorphometry, platelet-rich fibrin, pure platelet-rich plasma, sinus-floor augmentation
The idea and technique for reconstruction and elevation of the alveolar crest via sinus-floor augmentation with a lateral window approach was introduced about 40–50 years ago by Boyne and James and Tatum, and this approach is recommended when the residual alveolar crest bone height is 5–7 mm or less for implant rehabilitation. For this purpose, several bone grafts, barrier membranes and some proteins have been used so as to provide healing, formation and maturation of new bone.

Beta-tricalcium phosphate (β-TCP) is a porous type of calcium phosphate. The rates of calcium and phosphate agents in β-TCP were similar or close to their rates within cancellous bone. β-TCP has biocompatible and osteoconductive properties, and it also supports attachment, proliferation and differentiation of osteoblasts and mesenchymal cells, as well as bone growth.

Platelet-rich plasma (PRP) is defined as a three to eightfold concentrated autologous solution of platelets obtained by sequential and concentrating autologous blood by gradient density centrifugation. Platelet-rich fibrin (PRF)—which includes many growth factors and platelet concentrates, fibrin and activated cellular elements, such as leukocytes—is an optimized blood clot. PRF is obtained by centrifugation of autologous blood from a subject without adding anticoagulants. PRP and PRF include many growth factors. PRP and PRF can promote chemotaxis, cell migration, angiogenesis, proliferation, differentiation, and matrix production, and it can accelerate new bone formation, bone remodeling, and wound healing.

β-TCP has been used as sole bone graft substitute for sinus-floor augmentation. PRP or PRF has been added to β-TCP or other graft materials (autologous bone, freeze-dried bone allograft [FDBA], or deproteinized bovine bone mineral [Bio-Oss]) for sinus-floor augmentation. Consolo et al. and Kassolis and Reynolds reported significant advantages to adding PRP to autologous bone or FDBA such as certain bone regeneration potential or enhanced bone-formation rate during sinus-floor augmentation, whereas Raghoedbar et al. found no beneficial effects of PRP on wound healing and bone formation when it was used with autologous bone grafts for sinus augmentation. Zhang et al. found no an advantage or disadvantage of the applying of PRF in combination with deproteinized bovine bone mineral (Bio-Oss) for sinus-floor augmentation. In contrast, Kim et al. filled round-shaped defects in the maxillary anterior sinus wall of rabbits with three different graft materials (TCP only, PRF-mixed TCP, or rhBMP-2-coated TCP), and reported that PRF-mixed TCP showed more rapid bone healing than the rhBMP-2-coated TCP or the TCP-only control.

The potential effects of adding P-PRP or PRF to β-TCP graft substitute on bone formation and regeneration after maxillary sinus-floor elevation remains unclear. To our knowledge, this is the first study to compare histologic and histomorphometric outcomes of maxillary sinus-floor augmentation among β-TCP alone, P-PRP-mixed β-TCP, and PRF-mixed β-TCP bone graft substitutes.

To address the research purpose, the authors designed and implemented a prospective randomized clinical trial composed of the partially edentulous adults patients with atrophic maxilla who underwent sinus-floor augmentation with 1 of the 3 studied conditions at the Faculty of Dentistry of Ataturk University. All patients underwent preoperative radiographic examination by means of orthopantomographs and were included if they required maxillary sinus-floor augmentation and had less than 7 mm of residual bone crest height in the floor of the maxillary sinus.

The local ethics committee approved this study (Approval Number: 2012.2.35), and all participants signed an informed consent agreement. This study was financially supported by a scientific research project fund of Ataturk University (Funding Number: 2011/33).

The study population was composed of all patients presenting for the evaluation and management of atrophic maxilla (maxillary sinus-floor elevation and implantation) from June 2012 to October 2013.

To be included in the study sample, patients were required to meet the following criteria: (1) athrophic maxilla and previous posterior tooth loss, (2) 7 mm or lesser residual bone crest height measured on orthopantomographs, and (3) age >20 years.

Patients were excluded as study subjects if they had maxillary sinus infection, or hematologic, neurologic, or systemic disorders, had been exposed to radiotherapy and chemotherapy, had inflammatory or connective tissue disease, or had malignant disease in the head and neck region.

The sample was composed of 26 subjects: 9 subjects in the control and P-PRP groups, and 8 subjects in the PRF group: 2 females and 7 males in the control group; 4 females and 5 males in P-PRP group; 3 females and 5 males in PRF group. Their ages ranged from 22 to 51 years.

In this randomized clinical trial, elevated sinus cavities were grafted with β-TCP alone (control group), with P-PRP-mixed β-TCP (P-PRP group and study group A), and with PRF-mixed β-TCP (PRF group and study group B).

The primary predictor variable was the treatment technique (bone graft materials used in each groups). The other variables were subjects’ age and genders, and the healing period of the graft materials (follow-up periods). The age and gender of the subjects were recorded, and the relation of these variables with the predictor variables was considered for statistical analysis.

2.1 | Platelet-rich plasma preparation

PRP can be obtained with many different automated and/or manual methods. In this study, we used the one-step centrifugation procedure. Ten mL of whole blood was drawn from the patients for obtaining PRP. Briefly, 10 mL of whole blood was equally assigned to 2 sterile tubes (5 mL) coated with an anti-coagulant (acid-citrate-dextrose, 3.2% sodium citrate). These tubes were centrifuged in 1000 rpm for 10 minutes at room temperature to obtain three typical layers: red blood cells (RBCs) at the deepest, a “buffy coat” in the middle, and
Platelet-rich plasma (PPP, Platelet-Poor Plasma) in the upper part. The upper part was discarded from each tube by carefully pipetting to avoid creating turbulences. The remaininguffy coat is known as platelet-rich plasma (P-PRP) according to the classification of Dohan Ehrenfest et al. or as Anitua’s platelet-rich in growth factors (PRGF). The buffy coat was collected with a pipette, using “eyeballing” (the act of eyeballing is to measure or weigh something without any tools) as a measuring tool. Each tube containing approximately 1–1.2 mL of P-PRP was activated with CaCl₂. After 15 to 20 minutes a P-PRP gel was formed.

P-PRP was mixed manually with β-TCP granules. The mixture of P-PRP and β-TCP used as graft material for maxillary sinus-floor elevation in the study group. The time delay between the P-PRP gel formation and the filling of the defect was standardized to 5–10 minutes.

### 2.2 Platelet-rich fibrin preparation

PRF can be obtained with many different automated and/or manual methods. In this study, we used procedure described by Choukroun’s procedure. Briefly, 10 mL of whole blood was drawn from the patients and was equally assigned to 2 sterile tubes (5 mL) coated without an anti-coagulant. The tubes were centrifuged in 3000 rpm for 10 minutes at room temperature. The centrifugation involved circulating blood thrombin turning fibrinogen into fibrin, placed at the center of the test tube. The PRF obtained with this method was used as a filling material. PRF was mixed manually with β-TCP granules. This mixture was used as a graft material for sinus augmentation in the study group.

### 2.3 Surgery technique

A modified Caldwell-Luc technique introduced by Tatum was used for sinus-floor augmentation in all patients. Before sinus surgery, posterior superior alveolar nerve blockage and palatal infiltrative anesthesia were performed with 2 mL of Ultracaine DS-Forte (4% articaine, adrenaline 1/100 000) (Sanofi-Aventis Deutschland GmbH, Industriepark Höchst, Frankfurt, Germany). The skin surface of the perioral region was disinfected with povidone-iodine solution. After proper surgical conditions were provided, crestal incisions were performed from the maxillary tuberosity to the front wall of the maxillary sinus. Vertical releasing incisions were performed in the anterior and posterior vestibule to provide a sufficient point of view.

Full-thickness flap was elevated cautiously to avoid any damage to the periosteum. Four linear osteotomies (anterior, posterior, superior, and inferior) were performed for the lateral bone window in the maxillary sinus-floor elevation. After the lateral bone window was opened, this bone was mobilized with a hammer. The maxillary sinus membrane was then elevated gently and carefully throughout on all osteotomy walls with an open sinus elevation surgery set (Kohler open sinus elevation set, Kohdent Roland Kohler Medizintechnik GmbH & Co. KG, Stockach, Germany). When Schneiderian membrane perforation was occurred during osteotomy or membrane elevation, the perforation area was covered with a resorbable collagen membrane (Collagene AT, Centro di Odontoiatria Operativa s.r.l., Biomaterials and Research Division, Via Guizza, Padova, Italy). The lateral bone window was repositioned under the Schneiderian membrane (Figure 1).

FIGURE 1 Elevated sinus floor after lateral bone window was opened

Three different bone graft substitutes were filled up within the created cavity with membrane elevation. Two cc (2 mL) of β-TCP, a mixture of 2 mL β-TCP plus 2–2.4 mL of P-PRP, and a mixture of 2 mL β-TCP plus 4–5 mL of PRF graft substitutes were placed gently into the sinus cavity in the control, P-PRP, and PRF groups, respectively. β-TCP is commercially available as Suprabone bone graft substitute, 1–2 mm, BMT CALSIS Health Technology, Ankara, Turkey.)

The lateral window was covered with collagen membrane, after bone grafting was completed. The mucoperiosteal flap was covered with silk suture.

Paracetamol (Parol, 500 mg, three times daily), amoxicillin-clavulanate (Augmentin, 1000 mg, twice daily), and chlorhexidine gargle (Kloroben, Benzylamine hydrochloride, four times daily) were prescribed for all patients. Cold-compress application was recommended to all patients. Ten days after from sinus-floor augmentation, all patients were contacted for suture removal.

### 2.4 Outcome variables

The main outcome variables included findings of histologic and histomorphometric analysis of bone graft biopsies harvested prior to implant placement: percentages of new bone formation, residual graft particles, and fibrous tissue (soft tissue) areas. The secondary outcome variables included mean densities of bone cells (osteoblasts, osteoclasts, osteocytes, and osteoprogenitor), capillary vessels, and inflammatory cells. The outcome variables were recorded 6 months postoperatively.

### 2.5 Histologic and histomorphometric analysis

Patients were called for implantation 6 months after sinus augmentation. Posterior superior alveolar nerve blockage and palatal infiltrative anesthesia were performed with 2 mL Ultracaine DS-Forte (4% articaine, adrenaline 1/100 000), and the mucoperiosteal flap was elevated. Bone biopsies were harvested by the use of trephine burs,
2 mm in diameter, prior to implant placement. The samples were placed in 4% formalin solution for 72 hours, and then in the 6% nitric acid solution for 12 hours. The samples were washed for 4 hours. They were dehydrated in an ascending series in alcohol, xylol, and paraffin. Bone cores were finally embedded in paraffin. Six cut sections with 5-μm thickness were sectioned from each specimen parallel to the long axis of the cylindrical core using a microtome (Leica RM2125 RT, Leica Biosystems Nussloch GmbH, Nussloch, Germany). Subsequent to deparaffinization of sections, they were exposed to a descending series of alcohol and xylol, then washed with water. Finally, the specimens were stained with Hematoxylin and Eosin (H&E).

Histologic and histomorphometric assessments were carried out by Stereo Investigator System for stereology, which, according to manufacturer, gives accurate, unbiased estimates of the number, length, area, and volume of cells or biological structures in a tissue specimen. The stereology workstation contains the Stereo Investigator software (Microbrightfield, Version 9.0, Microbrightfield, Inc, Colchester, VT) and a modified light microscope (Leica DM4000 B). Measurements were performed on the sections using a personal computer-based image analysis system (Microbrightfield, Version 9.0, Microbrightfield, Inc, Colchester, VT). The system was calibrated in micrometers using a slide, and the setting remained unchanged during the analysis of all samples.

Sections of 5-μm thickness were examined using a systematic random sampling method. Thirty frames were determined in the sections by using systematic random sampling method. The numerical densities of osteoblasts, osteoclasts, osteocytes, osteoprogenitor cells, inflammatory cells, and capillary vessels in the bone samples were evaluated via the fractionator frame method and counted using a Leica Plan Apo microscope in all groups.

The percentage of the newly formed bone, graft particle area and fibrous connective tissue areas relative to total measured area were recorded in all sections by using the area fraction fractionators method in all groups.

All histologic and histomorphometric evaluations and analyses were carried out by the same investigator, and the investigator was blinded to which group a specimen was assigned.

2.6 | Implant placement

Implants were placed with suitable diameter and size (TSI-Tidal Spiral Dental Implant System, Germany) in the drilled cavities. Drugs were prescribed and cold compress application was recommended for all patients. All patients were contacted for suture removal 10 days after implant surgery.

2.7 | Statistical analysis

All statistical analyses were carried out using the SPSS (Statistical Package of Social Sciences, Chicago, Illinois) for Windows software program version 17.0. A P value of less than .05 was considered statistically significant. The results were expressed as the mean ± standard deviation. Descriptive and bivariate statistics were computed. The Shapiro-Wilk test was performed to test normality. The Shapiro-Wilk test showed that all parameters in both groups showed normal distribution (P < .05). The data were analyzed by ANOVA and Tukey HSD tests.

3 | RESULTS

Schneiderian membrane perforation was observed in 5 of 26 patients (19.23%) (2 in the control group, 1 in the P-PRP group, and 2 in the PRF group) and the perforation area was covered with suitable sized collagen membrane. No postoperative maxillary sinus infection was observed in either group.

No significant differences at baseline were observed among the three groups for any study variables, including age, gender, and follow-up period. The mean age was 31.51 ± 8.52 years in the control group, 34.01 ± 9.59 years in the P-PRP group, and 35.48 ± 9.53 years in the PRF group (P > .05). The mean follow-up period was 6.14 ± 0.57 months in the control group, 6.08 ± 0.67 months in the P-PRP group, and 6.29 ± 0.53 months in the PRF group (P > .05) (Table 1).

3.1 | Histologic analysis results

Histologic view of a biopsy material in the control, P-PRP, and PRF groups is shown in Figures 2–4. Biopsies of the P-PRP, PRF, and control groups showed very similar composition and distribution of histologic structures.

A large amount of new bone formation was observed in all groups. Dense fibrous tissue formation was observed in the P-PRP and PRF groups, whereas partly fibrous and cartilaginous tissue formation was observed in the control group. Ossification around the TCP particles was seen evidently in all groups. The exis-
6.34% in the PRF group, with no significant differences (P > .05). The mean percentages of residual graft particle area were 30.39 ± 10.29%, 28.98 ± 7.94%, and 32.66 ± 7.46% in the control, P-PRP, and PRF groups, respectively, with no significant differences (P > .05). The mean percentages of soft-tissue area were 36.21 ± 10.59%, 36.19 ± 13.94%, and 35.31 ± 10.81% in the control, P-PRP, and PRF groups, respectively, with no significant differences (P > .05) (Table 2).

One-way ANOVA showed insignificant differences between the groups in the parameters of mean densities of osteoblasts, osteoclasts, osteocytes, and capillary vessels (P > .05) (Table 3). Mean densities of osteoblasts, osteoclasts, osteocytes, and capillary vessels showed insignificant differences between groups (P > .05), but osteoprogenitor cells were found to be lower and inflammatory cells were found to be higher in the PRF group than those in the P-PRP and control groups (P < .01) (Table 3).

Studies have demonstrated that β-TCP is a highly biocompatible and osteoconductive material, creates a resorbable interlocking network within the grafted sinus room, and provides attachment, proliferation and differentiation of osteoblasts and mesenchymal cells to promote healing and bone growth. Khatiblou found increased

**FIGURE 2** Histologic view of a biopsy material in the control group (β-TCP). Thick black arrow: New ossification area around cartilaginous tissue. Green arrow: New ossification area around the β-TCP particles. Thin black arrow: Osteoblast. Thin orange arrow: osteoclast, hematoxylin and eosin staining (H&E) (100 μm)

**FIGURE 3** Histologic view of a biopsy material in P-PRP group. Thick black arrow: new ossification area around the β-TCP particles. Thin black arrow: bone cells. Thin orange arrow: osteoblast cell group releasing osteoid tissue and new ossification areas. Thin green arrow: angiogenesis. Thick blue arrow: loose fibrous tissue, hematoxylin and eosin staining (H&E) (100 μm)

**FIGURE 4** Histologic view of a biopsy material in PRF group. Thick black arrow: new ossification area composed of β-TCP particles. Thin black arrow: osteoblast cell group. Thick orange arrow: angiogenesis. Thin orange arrow: inflammatory cells, hematoxylin and eosin staining (H&E) (100 μm)
implant stabilization and bone formation after sinus-floor augmentation with β-TCP. However, β-TCP does not have osteoinductive properties and does not include growth factors. PRP or PRF contains several growth factors (in rich), and, therefore, adding PRP or PRF to β-TCP graft materials for maxillary sinus-floor elevation gained popularity among surgeons.

In this study, 33.40% of new bone formation was found in the β-TCP group after an average healing period of 6.14 ± 0.57 months. However, the percentages of residual graft particles and soft-tissue areas were similar to the percentage of new bone formation in this group. Results of a recent histomorphometric study supported our findings, which reported that newly formed bone, residual graft particles, and soft-tissue areas were approximately 30% in β-TCP bone biopsies obtained 6 months after sinus augmentation. Lezzi et al. reported 30.5% of new bone formation 6 months after sinus augmentation with β-TCP. However, the rate of new bone formation observed in this study was greater than the rate reported by Schulten et al. (19%) and Kürtçü et al. (21.09%) after 6.5 months of sinus augmentation with β-TCP.

In the P-PRP and PRF groups, 34.83% and 32.03% new bone formations, respectively, were found after an average healing period of approximately 6 months. Conversely, percentages of residual graft particle and soft-tissue area were also found similar to the percentages of new bone formation in each group.

Wiltfang et al. compared β-TCP plus PRP with β-TCP alone during sinus augmentation, and they found average bone formation of 38% in the β-TCP plus PRP group and 29% in the β-TCP alone group.

Zhang et al. compared PRF plus deproteinized bovine bone (Bio-Oss) (PRF group) and Bio-Oss only (control group) for bone regeneration in bone specimens harvested after a healing period of 6 months of sinus-floor augmentation. The authors reported the percentage of new bone formation in the PRF group was approximately 1.4 times greater than that in the control group (18.35% ± 5.62% vs 12.95% ± 5.33%), whereas the percentage of residual bone substitute in the control group was approximately 1.5 times higher than that in the P-PRF group (28.54% ± 12.01% vs 19.16% ± 6.89%), with no significant differences between the groups.

Tanaka et al. evaluated additional effects of PRF on bone regeneration in sinus augmentation with deproteinized bovine bone mineral (DBBM), and reported that mean percentages of newly formed bone, residual DBBM, and fibrous connective tissue were 34.5%, 33.4%, and 32.1%, respectively. These rates are comparable with the rates observed in our study. The mean percentages of residual graft particle and soft-tissue area in β-TCP bone graft biopsies reported in the literature are extremely variable; approximately 20%-40% for residual graft particle and approximately 20%-60% for soft-tissue area.

Our results clearly revealed that similar capabilities of inducing new bone formation were observed in the three groups, and this finding indicates that there was no benefit to adding P-PRP or PRF to β-TCP in bone cell levels, capillary vessels, and new bone regeneration.

Conflicting clinical results have been reported in the literature regarding the beneficial effect of the autologous PRP or PRF on bone regeneration and formation in maxillary sinus-floor elevation procedures. Limited clinical studies were conducted to evaluate the efficacy of PRP plus β-TCP as graft materials for sinus augmentation in literature. Furthermore, no clinical study was conducted previously to evaluate the efficacy of PRF plus β-TCP during sinus-floor augmentation. Nikolidakis et al. observed that adding PRP to β-TCP graft substitute did not provide an additional contribution to new bone formation.

Contrary findings have been reported in studies evaluating adjunctive use of PRP to other bone graft substitutes during sinus-floor augmentation. Among these studies, Anitua et al. and others found additional benefits of P-PRP on bone formation, implant osteointegration, tissue healing, and regeneration potential during sinus-floor augmentations. Console et al. found a certain regenerate potential of RPP when it is used with autologous bone during sinus-floor augmentation. However, another group of other researchers reported minimal or no benefits of PRP on bone regeneration and formation or wound healing during sinus-floor augmentation. Thor et al. stated that PRP has a rather low regenerative capacity, and thus, beneficial effects of PRP in autologous bone grafts in the maxillary sinus-floor elevation is questionable.

PRP contains several concentrated growth factors. Torres et al. evaluated the concentration of the platelets in the P-PRP obtained with the one-step centrifugation procedure described by Anitua et al. that was used in our study. Torres et al. found 2.97 ± 0.7-fold platelet counts over peripheral blood after the one-step centrifugation procedure.

Optimal concentrated platelets and growth factors in the P-PRP or PRF can be expected to cause a profound effect on wound-healing enhancement and bone regeneration. The release of growth factors will provide a stimulus for nearby cells both in terms of chemotaxis, proliferation, and maturation. In this respect, living human bone cells are important requirements for bone regeneration and production.

However, β-TCP does not contain any living bone cells. It is well documented that the graft material must contain living bone cells responsive to these growth factors for bone regeneration. In other words, living

### Table 2: Means percentages of new bone, graft particle, soft tissue areas in the groups, and their intergroup comparisons

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group (β-TCP)</th>
<th>Study group A (P-PRP)</th>
<th>Study group B (PRF)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>New Bone, %</td>
<td>33.40</td>
<td>10.43</td>
<td>34.83</td>
<td>10.12</td>
</tr>
<tr>
<td>Graft particle, %</td>
<td>30.39</td>
<td>10.29</td>
<td>28.98</td>
<td>7.94</td>
</tr>
<tr>
<td>Soft (fibrous) tissue, %</td>
<td>36.21</td>
<td>10.59</td>
<td>36.19</td>
<td>13.94</td>
</tr>
</tbody>
</table>
bone cells are required for P-PRP or PRF to have an effect on bone regeneration.\textsuperscript{30} This essential deficiency of \(\beta\)-TCP bone graft material can explain why P-PRP or PRF added to the bone graft mixture revealed no significant superiority over the sole graft material used in this study. The slow degradation property of the \(\beta\)-TCP may have also retarded the actual bone-regeneration capacity of the PRF or P-PRP because scientific data suggested that the slow resorption property retards the replacement of new bone formation.\textsuperscript{16}

In an in-vivo study, Tajima et al.\textsuperscript{31} found that PRP had a negative effect on bone formation and osteogenic differentiation in a porous \(\beta\)-TCP scaffold. According to these authors, the effectiveness of PRP may depend on growth-factor concentrations and the stage of bone formation.

Some researchers suggested that PRF used as sole grafting material for maxillary sinus promotes new bone regeneration,\textsuperscript{32} but other researchers\textsuperscript{33} reported contrary findings.

In vitro studies showed that PRF significantly improved the proliferation of human osteoblasts, but this effect is in a dose-dependent manner.\textsuperscript{4,34} According to Dohan Ehrenfest et al.,\textsuperscript{4,34} the growth factors and fibrin matrix structure of the PRF might be responsible for biological events for bone regeneration. It has been revealed that the sinus-floor plays an important role in bone regeneration as the source of precursor cells.\textsuperscript{35} In a recent histological and histomorphometric study on sinus augmentation, Zhang et al.\textsuperscript{16} claimed that "PRF mixed with Bio-Oss might stimulate migration of precursor cells to situ by a lesser extent than PRF mixed with FDBA. The absence of precursor cells could be a reason of the lacking effect of PRF mixed with Bio-Oss on the bone formation." Accordingly, the lesser precursor cells could be a reason for the lacking effect on bone formation of PRF mixed with \(\beta\)-TCP, because the mean density of osteoprogenitor cells is significantly less in PRF group than those in other two groups.

We found insignificant differences between the groups with regard to the mean densities of osteoblasts, osteoclasts, osteocytes, and capillary vessels, but the mean densities of these parameters seemed considerably enough for bone regeneration. This indicated that graft materials in all groups were still replacing to new bone with simultaneously occurring active bone-graft resorption and bone formation after 6 months of sinus-floor augmentation. Miyamoto et al.\textsuperscript{35} reported that \(\beta\)-TCP granules attract osteoprogenitor cells that migrate into the interconnecting micropores of the bone-substitute material after 6 months of sinus-floor augmentation. Szabo et al.\textsuperscript{26} observed intense proliferation of mesenchymal cells and new capillary vessel formation composed in the pores of soluble \(\beta\)-TCP granules.

A surprising finding of this study is that osteoprogenitor cells were lesser and inflammatory cells were higher in the PRF group than those in the P-PRP and control groups. In contrast to our findings, some researchers found that PRP may suppress cytokine release and limit inflammation.\textsuperscript{37} Simunek et al.\textsuperscript{10} reported the presence of chronic inflammatory cells in several graft materials (ie, \(\beta\)-TCP, deproteinized bovine bone, autologous bone, and different mixtures with autologous bone harvested 9 months after sinus augmentation).

This study was not free of limitations. First, the sample size was limited with respect to the generalization of the findings. Second, the concentration of platelets in the P-PRP or PRF group was not determined in this study. The one-step centrifugation procedure used in this study was tested in other studies,\textsuperscript{28} and moderate elevated platelet concentration (two to threefold increase) were found, indicating sufficient biological benefits of P-PRP. Third, a 6-month follow-up period was considered to evaluate possible changes in the outcomes of this study. However, longer follow-up periods may be beneficial for better evaluation of bone regeneration.

### 5 | CONCLUSIONS

These findings suggested that adding P-PRP or PRF to \(\beta\)-TCP graft substitute was not beneficial on new bone formation and regeneration, and P-PRP plus \(\beta\)-TCP or PRF plus \(\beta\)-TCP is not superior to \(\beta\)-TCP alone.

### ACKNOWLEDGMENTS

This study was financially supported by scientific research project fund of Ataturk University (Funding Number: 2011/33).

### CONFLICT OF INTEREST STATEMENT

All authors have *no conflict of interest.*

### REFERENCES


