Sinus Floor Elevation Using Platelet-Rich Plasma and Beta-Tricalcium Phosphate

Case Report and Histological Evaluation

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INTRODUCTION

The successful placement of dental implants in the edentulous posterior maxilla can be compromised by the lack of adequate alveolar bone present between the alveolar crest and the floor of the maxillary sinus. A minimum of 10 mm of vertical bone height is usually required for predictable implant success. Maxillary sinus floor elevation surgery, initially described by Boyne and James, was developed to increase the height of bone available for implant placement in the posterior maxilla. The efficacy and predictability of this procedure have been demonstrated.

The basic surgical approach involves an osteotomy performed on the lateral maxillary wall, elevation of the sinus membrane, and placement of bone graft material. The graft material can be categorized into 4 groups: autograft, allograft, xenograft, or alloplast. These graft materials can be used alone or in combination with each other. Tong, et al compared success rates of implants placed in sinuses grafted with different materials and concluded that autogenous bone grafts should be considered as the gold standard for bone replacement. However, the harvesting procedure of autogenous bone graft leads to morbidity at the donor site and prolonged operation time. Therefore, research is ongoing to identify efficacious bone substitute materials. Nevertheless, there is a lack of clarity regarding the bone formation supporting properties of synthetic bone substitutes.

Among the currently used materials, betatricalcium phosphate (β-TCP) may have use in sinus floor augmentation procedures. β-TCP is a resorbable material that can be replaced by bone. For example, a histomorphometrical study showed no significant difference in bone density volume between test (human sinuses augmented with β-TCP) and control groups (augmented with autologous bone). In a similar study, Zerbo and co-workers reported that β-TCP is an acceptable bone substitute material for sinus augmentation, but the rate of bone formation is delayed in comparison to autologous bone. Consequently, the use of bone substitutes can become more predictable if they are used in combination with growth factors, which have the capacity to accelerate deposition of new bone in...
association with the graft material, thereby shortening the time to achieve adequate consolidation.

Platelet-rich plasma (PRP), which is a volume of plasma with a concentration of platelets, has been suggested to increase the rate of bone deposition and bone volume in combination with bone grafts when used during augmentation procedures. It has been demonstrated that the application of PRP in dentistry may be beneficial for bone healing. The positive impact of PRP on bone healing can be attributed to the angiogenetic, proliferative, and differentiating effect of the transforming growth factor-β and platelet-derived growth factor that are present in high concentrations in PRP. Therefore, the combination of PRP with β-TCP may result in an increased rate of bone formation in a sinus augmentation procedure.

As autologous PRP preparation requires blood to be drawn from the patient, the use of allogeneic platelet rich plasma (aPRP) may serve as an alternative option in the case of patients who refuse to be subjected to a venipuncture and blood-drawing procedure.

The purpose of this report is the presentation of a patient who was treated for sinus augmentation by means of a PRP and β-TCP. The report includes the histological evaluation of a biopsy harvested from the augmented area.

**CASE PRESENTATION**

A 37-year-old nonsmoking female was referred to the Department of Periodontology and Biomaterials of the Radboud University Nijmegen Medical Centre (The Netherlands) for periodontal treatment. The patient was in good general health and did not have any subjective dental complaints except bleeding during brushing. The dental history revealed that all 4 third molars had been surgically removed in childhood, and teeth Nos. 17 and 27 had been removed approximately 10 years ago. Also, multiple amalgam restorations were present.

During the oral examination and evaluation of the periodontal status, the oral hygiene appeared to be inadequate, and supragingival calculus was present in the anterior lingual area. The bleeding score upon gentle probing was moderate, and probing pocket depths up to 9 mm were recorded. Furcation involvement and localized recession were also noticed. The radiographic examination showed irregular bone loss up to 70%, especially in the area of the maxillary right second molar.

Based on the history and the clinical and radiographic examinations, the diagnosis was generalized chronic periodontitis. The treatment plan included oral hygiene reinforcement, supragingival and subgingival debridement with hand and ultrasonic instruments, and reevaluation of the periodontal state at 3-month intervals. Further, extraction of the maxillary right first molar during initial periodontal therapy was scheduled due to excessive mobility (class 3), extensive loss of bone support (70% of the root length), and deep probing depths (8 to 9 mm). Surgical intervention for sinus floor elevation and implant replacement of the lost tooth in this area was proposed. As the patient did not wish any autologous graft harvesting or blood drawing, aPRP (obtained from a transfusion laboratory) in combination with alloplastic graft material (β-TCP) were selected for the sinus augmentation. A re-evaluation of the case after long-term follow-up was also planned. The patient was fully informed concerning the treatment to be performed and signed a written consent form.

**SURGICAL TREATMENT**

Three months after completion of the initial periodontal therapy and extraction of the first molar, a re-evaluation of the periodontal status was performed, indicating no need for periodontal surgical therapy. Therefore, the sinus floor augmentation procedure was performed.

Preoperatively, the residual bone height was assessed based on radiographic examination (Figure 1a). A 2-stage procedure (first stage, sinus elevation; second stage, placement of implants) was performed because the height of the alveolar crest was less than 5 mm. The patient received antibiotic prophylaxis prior to surgery. The sinus augmentation was then carried out according to Tatum. After careful elevation of the mucosal layer, the space created between the maxillary alveolar process and the new sinus floor was filled with 1.5 g β-TCP granules of 1 to 2 mm in diameter (Cerasorb [Curasan AC]). The β-TCP granules were mixed with the aPRP volume prior to delivery into the opened sinus cavity. Care was taken to distribute
the aPRP equally over the β-TCP granules. For the preparation of the aPRP, a blood volume of 250 cm$^3$ was obtained from a transfusion laboratory (Department of Blood Transfusion and Transplantation Immunology, Radboud University Nijmegen Medical Centre). The blood sample was compatible (group O-), avoiding any immunogenic reactions, and was checked for potentially transmissible diseases. The PRP fraction was prepared at a transfusion laboratory (SanQuin Bloed-bank, Nijmegen, The Netherlands) according to a standard protocol.\textsuperscript{17} Eleven milliliters of platelet-rich plasma suspension was obtained, and the platelet concentration was 1,000 x 10$^6$/mL. Normal platelet counts range between 150,000/µL and 350,000/µL, with an average of approximately 200,000/µL.

Finally, a bioresorbable collagen membrane of porcine origin (BioGide Perio [Geistlich]) was applied to cover the lateral wall defect after the bone graft was placed. Complete wound closure was achieved with nonabsorbable, ePTFE sutures (Gore-Tex [W.L. Gore]).

Postsurgical pain and edema were controlled with 600 mg ibuprofen, and the patient was instructed to rinse twice daily with 0.12% chlorhexidine for 2 weeks and to use modified oral hygiene procedures in the treated area for the first 4 postoperative weeks. The patient was examined one week later and the sutures were removed. Radiographic examination was also performed (Figure 1b). The patient was maintained in a supportive care program and received full-mouth professional prophylaxis and calculus removal every 4 months.

After a healing period of 6 months, implant placement was performed. A surgical template was used, and an implant 13 mm long and 5 mm in diameter (Replace Select [Nobel Biocare]) was inserted in the site of the maxillary right first molar. During this procedure, a bone biopsy from the augmented site was harvested using a trephine bur of 5 mm diameter. The biopsy was taken in the region of the maxillary right second molar next to the site where the implant was placed. Only 4 mm of the apical end of the bone biopsy cylinder was taken for histological examination, to guarantee that the augmented region of interest was examined. Six months after insertion, the implant was exposed and an abutment was connected. A prosthesis was fabricated 4 weeks later (Figure 1c).

**HISTOLOGICAL AND HISTOMORPHOMETRICAL EVALUATION**

The biopsy sample was fixed in 4% formaldehyde. Subsequently, it was dehydrated through a graded series of ethanol, embedded in methylmethacrylate (MMA), and finally polymerized. Longitudinal, thin, non-decalcified sections (10 mm) were made with a diamond blade sawing microtome. The sections were stained with methylene blue and basic fuchsin and examined for histological and histomorphometrical evaluation. The histological evaluation was performed with a light microscope (Leica MZ12 [Leica BV]) and included a description of the observed tissue response. Additionally, microscopic images were projected with a magnification of 25x on a color monitor using a CCD/RGB camera (Sony DXC151P), and digital image analysis software (Leica Qwin Pro-image analysis system).
was used for the histomorphometrical analysis. The new bone area ratio was defined as the area of new bone inside the examined image frame.

The histological examination (Figures 2a and 2b) revealed that the sections were composed of trabecular bone, marrow spaces with fat cells, and particles of the β-TCP bone substitute. β-TCP particles could clearly be observed, but no active degradation appeared to occur as characterized by the absence of osteoclast-like cells. The particles showed a wide variety of sizes, which may be due to a different degree of resorption or a different level of sectioning during the histological processing. The bone had a lamellar structure, and there was a varying degree of contact between the β-TCP particles and bone. The lamellae of the bone tissue contained osteocytes that were located in lacunae. The bone was clearly distinguishable from the β-TCP particles. While new bone lamellae appeared long and thin, the particles of the bone substitute material were short with a round shape and were a different shade after staining.

Occasionally, fibrous connective tissue was formed around and infiltrated into the β-TCP particles. Sporadically, inflammatory cells surrounded graft particles. Further, large bone marrow spaces were present between the formed bone and β-TCP particles, which contained fat cells, fibrous tissue, and blood vessels of various diameters. The percentage of new bone, the residual bone substitute, marrow space, and fibrous tissue varied among the sections. The percentage of bone area had a mean value of approximately 14% (±4%), whereas the bone marrow spaces and graft material particles occupied on average 52% (±7%) and 33% (±10%) of the total surface area, respectively.

ONE YEAR REEVALUATION

One year after prosthetic rehabilitation, a reevaluation of the case was performed. The general periodontal status was stable with shallow probing pocket depths and reduced bleeding scores. The patient had achieved a very good level of plaque control. No dental complaints were reported. Clinical examination of the implant placed in the augmented area revealed no mobility and healthy peri-implant mucosa.

The radiographic examination confirmed bone-implant contact without any sign of radiolucency around the implant. During the first year of functional loading, no additional vertical bone resorption was observed during radiographic examination.

DISCUSSION

Maxillary sinus floor elevation surgery has been developed to increase the height of bone available for implant placement in the posterior maxilla. This surgical approach involves the elevation of the sinus membrane and placement of bone graft material.

As donor site morbidity is often a problem in bone-harvesting techniques, there is great interest in the use of bone substitute material. Among various bone substitutes, β-TCP is a promising material for augmentation of the maxillary sinus. However, this material has only osteoconductive properties, resulting in a delayed rate of
bone formation in comparison to autologous bone. Therefore, an extended postoperative healing period is required before the placement of an implant in a second-stage surgery. On the other hand, use of PRP has been suggested to increase the rate of bone deposition and bone volume in combination with bone grafts during augmentation procedures.

Because autologous PRP preparation requires an additional procedure of blood drawing, the hypothesis that the use of aPRP may serve as an alternative option was evaluated in this case. Considering the limitations of the present report (a single case), the addition of aPRP did not apparently result in a remarkable increase in bone formation. In a histomorphometrical human study, Zerbo, et al reported that the average new bone volume formed in the augmented sinus with the use of β-TCP particles did not exceed 20% at a 6-month postoperative period.

In another human study, Wiltfang and co-workers evaluated the effect of autologous PRP in combination with β-TCP particles in sinus augmentation procedures, and they demonstrated that the formation of new bone was about 10% higher when autologous PRP was used. The volume of new bone reached a mean value of 29% in the non-PRP group (only β-TCP particles), and 38% in the PRP group (combination of PRP and β-TCP particles). While that study and this case cannot be compared directly, less bone formation was observed in the present case with the use of aPRP. Bone formation did not exceed a mean percentage of 14% of the total examined area. An explanation for the lack of beneficial effect might be that in this report aPRP was used. The aPRP may not have the same properties as the autologous material. Furthermore, in the current study PRP was applied without the use of thrombin. The platelet concentrate is activated by the addition of thrombin and calcium chloride, resulting in the release of a cascade of growth factors from the alpha granules. Some authors have questioned the necessity of using thrombin. Dugrillon, et al proposed that PRP gel formation can be performed with the addition of calcium, and no other activator is required. The mixture of PRP with the bone graft material may provide a sufficient viscosity, and induce coagulation and release of growth factors. Wiltfang and co-workers reported more bone formation in sinus elevation cases applying PRP compared to the control group (no application of PRP) despite the fact that no thrombin was used.

In addition, it must be emphasized that conflicting results have been reported in the literature regarding the beneficial effect of the autologous PRP on bone formation in augmentation procedures. Raghoebar, et al did not observe any additional benefit on bone formation when they applied PRP in combination with autologous bone grafts in sinus floor elevation. Preparation time and centrifugation forces may play a role in the effectiveness of PRP. The active secretion of these growth factors is initiated by the clotting process and begins within 10 minutes after clotting. More than 95% of the presynthesized growth factors are secreted within one hour, therefore PRP must be developed in an anticoagulated state and should be used within 10 minutes after clot initiation. However, the concentrated platelets can remain viable for up to 8 hours in the anticoagulated state. In addition, the centrifugation forces may be a critical step in PRP preparation, and the mechanical forces may activate platelets, with the consequence of releasing the granular load that contains the growth factors into the concentrated plasma volume.

Furthermore, this histological study confirmed that β-TCP is an acceptable bone substitute material for sinus augmentation, but the rate of bone formation was delayed at 6 months. The synthetic material occupied one third of the histological section, and some particles did not reveal any sign of degradation. This has been reported by other authors.8 In summary, the present case report shows that the use of aPRP in a sinus augmentation procedure neither provoked any adverse tissue reaction nor caused any discomfort to the patient. On the other hand, the beneficial contribution of aPRP to bone formation after mixture with a synthetic bone substitute in sinus augmentation procedures is not defined. Within the limitations of this report, allogeneic PRP did not appear to have any additional effect on bone formation in combination with alloplastic graft material in sinus elevation surgery. However, the treatment outcome was successful, resulting in adequate bone volume that could host the oral implant, with proper tooth replacement and sufficient masticatory function.

Acknowledgement

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REFERENCES


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1. The objective of sinus floor elevation procedures is to ____.
   a. treat chronic sinusitis  
   b. increase implant success  
   c. increase bone height  
   d. all the above

2. The graft material that has been considered as the gold standard for bone replacement in sinus elevation is ____.
   a. autograft  
   b. allograft  
   c. xenograft  
   d. alloplast

3. A 2-stage procedure (first stage sinus elevation; second stage: placement of implants) seems necessary when the height of the alveolar crest is ____.
   a. less than 3 mm  
   b. less than 5 mm  
   c. less than 8 mm  
   d. none of the above

4. PRP can be best defined as a volume of ____.
   a. plasma with a high concentration of platelets  
   b. blood including a high concentration of platelets  
   c. plasma without erythrocytes  
   d. plasma without leucocytes and erythrocytes

5. In sinus augmentation procedures, PRP has been suggested to ____.
   a. delay the resorption of the bone graft  
   b. enhance the resorption of the bone graft  
   c. increase the bone volume in combination with the bone graft  
   d. improve bone-implant contact after implant placement

6. Platelets contain growth factors located in the ____.
   a. alpha granules  
   b. beta granules  
   c. cytoplasm  
   d. all the above

7. The positive effect of PRP on bone healing can be attributed to the effect of ____.
   a. interleukin 1  
   b. erythropoietin  
   c. transforming growth factor  
   d. none of the above

8. The addition of thrombin and calcium in PRP results in the ____.
   a. increase of the biomaterial viscosity  
   b. activation of release of growth factors  
   c. induction of the coagulation process  
   d. all the above
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