Application of Platelet-Rich Plasma for Enhanced Bone Regeneration in Grafted Sinus

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Purpose: The present study was conducted to evaluate the effect of platelet-rich plasma (PRP) on new bone formation and remodeling after grafting of the maxillary sinus with an algae-derived hydroxyapatite AlgOss/C Graft/Algipore.

Materials and Methods: Fourteen consecutive patients with severely atrophic maxillae underwent uni- or bilateral grafting of the maxillary sinus with a mixture of collected bone, algae-derived hydroxyapatite AlgOss/C Graft/Algipore (ratio 1:10), and a combined addition of PRP and thrombin (Tissucol Kit; Baxter, Vienna, Austria) to allow for fast clotting. After an average healing period of 7.1 months bone samples were retrieved. Patients from a former consecutive series treated without PRP served as control group. Statistical analysis was done by Welch 2-sample t-test and mixed linear model testing.

Results: In the coronal specimen portions, mean values for newly formed bone area, biomaterial area and marrow space of 32.2% ± 10.4%, 20.1% ± 13.0%, and 47.7% ± 8.5% were found with PRP, respectively. In the control group the corresponding values were 27.6% ± 13.4%, 20.3% ± 12.9%, and 52.1% ± 9.3%. In the apical specimen portions in the PRP group, the newly formed bone area, biomaterial area, and marrow space was 25.7% ± 15.0%, 23.4% ± 14.9%, and 50.9% ± 12.5%, respectively. The corresponding values in the control group were 17.0% ± 8.6%, 34.5% ± 11.2%, and 48.5% ± 8.5%.

Conclusions: Statistical evaluation of the samples proved significantly better overall resorption of algae-derived hydroxyapatite AlgOss/C Graft/Algipore and increased new bone formation when PRP was used, especially in the apical region.

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A substantial, but often missing, prerequisite for the insertion of dental implants in the posterior maxilla is sufficient bone height. The sinus grafting procedure has brought significant progress in this field, and made implant treatment available for patients with initially insufficient bone in this region. Transplanted autogenous bone is considered the best material for sinus grafting, such as that taken from the iliac crest. However, this method has multiple disadvantages, including unpredictable resorption and postoperative donor site morbidity. Therefore, various bone substitutes, such as algae-derived hydroxyapatite (ACA), available under the brand names of AlgOss (Unexim, Moscow, Russia), C Graft (Clinician Preference, Golden, CO), and Algipore (Dentsply Friadent, Mannheim, Germany), were developed and have been used for sinus augmentation. ACA is manufactured from the calcifying marine algae Corallina officinalis and provides a large specific surface. In the manufacturing process, the fresh Corallina is collected, cleaned, dried, and pyrolized at a temperature of 750°C, which results in total burning of all the organic matrix. Details on this material have been previously described by Ewers et al. Clinical studies on the application of ACA for sinus grafting with histomorphometric analyses have been published by Schopper et al. In those investigations, the investigators could verify the formation of new bone after application of ACA, but they did not evaluate a potential effect of combining ACA with platelet-rich plasma (PRP) on the bone formation process. Several considerations suggested a potential positive effect of PRP in the course of bone formation. The first publication concerning centrifuged autogenous blood for the filling of large bone defects was presented in 1969. This method has since been refined and perfected. PRP is a volume of autologous plasma with a platelet concentration greater than baseline. The concentration, which is the working definition of PRP amounts to at least 1,000,000 platelets/µL. PRP is an important source of multiple growth factors. Platelet-derived growth factor, transforming growth factor-β1, transforming growth factor-β2, insulin-like growth factor, vascular endothelial growth factor, and epithelial growth factor use different mechanisms to increase the rate of bone formation in a graft. The most important specific activities of these factors include macrophage activation, chemotaxis, and mitogenesis of osteoblast precursors, inhibiting osteoclast formation or stimulating vascular ingrowth. In their report on autogenous bone grafts for mandibular continuity defects, Marx et al. showed that the bone density was significantly increased in the grafts when PRP was added.

Several investigations demonstrated that a granular bone augmentation material produced from calcifying marine algae ACA is a convenient alternative to transplanted autogenous bone grafts for sinus grafting. The purpose of the present study was to evaluate the hypothesis that PRP would enhance new bone formation and bone remodeling after grafting of the maxillary sinus if added to a mixture of the biomaterial ACA and autogenous bone.

Materials and Methods

For the present study, the legal requirements and local ethical committee regulations were considered. As only products with all required approvals have been used and no experimental substances or interventions have been involved due to the study, an additional IRB decision was not necessary at the time the study has been conducted. A consecutive series of 14 patients with severely atrophic posterior maxillae were included in the present study. Of the 14 patients, 9 were women and 5 were men, with a mean age of 55.7 years (range 26 to 82 years). Only patients with a residual crestal bone height of less than 5 mm were enrolled in the present study. These inclusion criteria correspond to atrophy grades V and VI according to the Cawood and Howell classification system. The patients had undergone no previous implants or grafting surgery. No general diseases were known in the medical history of these patients. Old age or moderate smoking habits (ie, fewer than 10 cigarettes daily) did not represent an exclusion criteria. Smokers and nonsmokers were equally distributed within the study and control groups to avoid a bias in the results from this parameter. A total of 18 sinus grafting procedures (10 patients unilateral and 4 patients bilateral) were performed. The patients from the former consecutive series who were treated without PRP (before the introduction of PRP at our clinic) served as the control group.

Surgery using PRP and ACA is a procedure comprising several distinct steps, starting with the acquisition and centrifugation of blood to separate the PRP, taking bone from the bone collector, and mixing the components. After the healing phase of the graft, trephine samples were taken during implant surgery for the following histomorphometric investigations. The procedure in detail was as follows.

PREOPERATIVE PHASE

Clinical and radiographic examinations were completed before surgery. All patients underwent the full range of standard intra- and extraoral investigations, including the hard and soft tissue. The radiologic investigation included the residual crestal bone of the maxilla, which was analyzed using panoramic radiographs and
paraxial views from thin-slice spiral dental computed tomography.

Surgery

A full-thickness mucoperiosteal flap was raised at the surgical site, and a round bur was then used under sterile saline irrigation to outline a window in the buccal maxillary sinus wall. The obtained bone fragments were collected with a suction trap. After exposure and elevation of the Schneiderian membrane, the site was prepared for the placement of the graft. To ensure undisturbed bone regeneration and avoid soft tissue invagination, the osteotomy site was covered with a titanium shield (Bone Shield; Friadent, Mannheim, Germany) after placement of the graft. Primary closure of the flaps was obtained using nonabsorbable sutures.

In all patients, surgery was performed by the same surgeon (R.E.) with the patient under general anesthesia and under sterile conditions. All patients signed a surgical consent form of their agreement to sinus grafting with a combination of the biomaterial ACA and PRP.

Processing PRP

Preoperative blood tests were performed to determine the human immunodeficiency virus and hepatitis B and C status. PRP processing was performed the day of surgery under control of qualified personnel at the Department of Transfusion Medicine. The Platelet Concentrate Collection System (3i Implant Innovations, Palm Beach Gardens, FL) was used for PRP processing.

All PRP was acquired solely from the patients themselves. The mean platelet count of the patients before processing was 259,600 ± 58,000/µL. The mean platelet count in the produced PRP was 1,484,972 ± 1,198,865/µL. Therefore, the increase in concentration was about 575%.

Graft

The graft was a mixture of collected bone and biomaterial ACA (1:10 ratio, measured by volume) with an additional 5 mL PRP and 1 mL thrombin (Tissucol Kit; Baxter, Vienna, Austria) to allow for faster clotting to receive a moldable graft, as first described by Marx et al.12 After clotting of the PRP, the graft was inserted into the deficient site and compacted until the required volume was reached.

Histologic Investigation

After an average healing period of 7.1 months (range 6 to 9), the patients underwent implant placement. Before insertion, biopsies were harvested during the same surgical procedure using 11-mm-long trephine drills through the graft at the corresponding implant sites. A total of 28 bone samples were taken from 14 patients. The biopsies were immediately placed in thin pipet tips for later apicocoronal orientation and fixed in 4.5% buffered formalin. After dehydration in a graded series of ethanol and embedding in metacrylate, the hardened undecalcified samples were sliced (mean thickness 10 µm) using a modified technique according to Donath.15,16 After staining the bone slices with 1% thionine, they were histologically evaluated by transmission light microscopy (Eclipse 800, Nikon, Tokyo, Japan). For histomorphometric examination, image analyzing software (Lucia 32G, version 4.10; Laboratory Imaging, Prague, Czech Republic) was applied. The areas of the newly formed bone, the biomaterial ACA, and the marrow space were measured by 1 operator (F.Z.). The analysis was performed using a semiautomatic method within the software, accompanied by additional manual corrections, as necessary. To prevent a falsification of the data and to exclusively evaluate the bone regeneration in the graft, the residual crestal bone at the sinus floor was excluded from the measurement. In the remaining part of the sample, 2 regions of interest (ROIs) were determined. The apical and coronal ROIs had the same surface size in each measurement (Figs 1-3).

Control Group

Within the control group, 14 samples were taken from 11 patients. Before the introduction of PRP into clinical use for sinus floor augmentation in our department, exactly the same operation and postoperative treatment protocols were used. Therefore, we designated this consecutive series as our control group. The graft material placed in the 11 patients in the control group, with similar average age and conditions, was a combination of collected bone and ACA in the same ratio (1:10). The only difference from the patients in the study group was the absence of PRP. Instead of PRP, 2 mL of venous blood was applied to the graft for better clotting. Because this amount of venous blood is very small compared with the blood volume from which the growth factors were extracted, no bias could have resulted. The same histologic and histomorphometric procedures were used (Table 1).

Implants

After a mean interval of 6 months, the 28 Frialit-II implants (Friadent) were loaded.

Statistical Analysis

According to the study design and evaluation protocol, we did not choose a blinded procedure. For each variable, the Welch 2-sample t test was
applied to investigate whether statistically significant differences were present between the mean values of the distinct groups. Although for the presentation of the results, 3 digits are adequate and sufficient, we included more digits with respect to the following meta-analysis or Bonferroni’s correction.

Because some patients had more than 1 implant and therefore the assumption of independence was compromised, the data were reanalyzed using a mixed linear model. The histomorphometric bone percentage and biomaterial percentage was the primary and secondary outcome variable, respectively. The group (PRP vs no PRP) and position (coronal vs apical) were included as fixed effects, as was their interaction. The patient was modeled as a random effect. The model was fitted using the restricted maximal likelihood method. The Akaike information criterion and the Bayesian information criterion corresponding to the model are reported, as well as the t-table of the fixed effects. The correlation table of the fixed effects is listed. All statistical analyses were performed using the open source statistical package R (available from http://www.r-project.org).

Results

No acute inflammatory responses after combined application of ACA and PRP were observed postoperatively. All sites healed primarily without wound infection. Of the 28 placed implants, 1 (3.6%) was lost 2 months after loading because of peri-implantitis.

HISTOLOGIC RESULTS

The histologic investigation of the bone samples showed the adjacent hard tissue growing through most of the ACA particles (Fig 2). They were completely embedded in, or partially covered by, the newly formed bone. The ACA granules were degraded during physiologic bone remodeling, and the new bone gradually replaced them (Fig 3). Thus, newly formed bone was present in the pores of the bone graft. Nonosseointegrated particles were in contact with the soft tissue.

HISTOMORPHOMETRIC FINDINGS

To assess the bone regeneration in the grafted volume, the residual crestal bone of the sinus floor was...
excluded from the examination. Two fields of equal size in the coronal and apical part of the graft were designated as the coronal and apical ROI, respectively (Fig 1), and were measured histomorphometrically.

**Coronal ROI**

In the coronal part of the graft (Fig 4), the average proportion of newly formed bone was 32.2% ± 10.4% (range 15.2% to 54.1%). The relative amount of ACA in the bone graft was 20.1% ± 13.0% (range 0.0% to 50.1%). This resulted in a remaining ratio for the marrow space of 47.7% ± 8.5% (range 24.9% to 60.8%).

In the control group, the following values for these 3 areas were found. Newly formed bone had a ratio of 27.6% ± 13.4% (range 10.5% to 56.1%). The proportion of ACA (without PRP) was 20.3% ± 12.9% (range 1.7% to 47.9%), and the average amount of marrow space was 52.1% ± 9.3% (range 35.9% to 66.1%).

**Apical ROI**

For the apical ROI, the average ratio of newly formed bone (Fig 4) was 25.7% ± 15.0% (range 2.5% to 61.0%). The relative amount of ACA in the bone graft was 23.4% ± 14.9% (range 2.0% to 61.5%). The average proportion of the marrow space was 50.9% ± 12.5% (range 30.1% to 81.3%).

In the control group, in the apical part of the graft, the average proportion of newly formed bone was 17.0% ± 8.6% (range 3.5% to 32.1%) was measured. The proportion of ACA was 34.5% ± 11.2% (range 7.7% to 51.9%). The relative amount of the marrow space averaged 48.5% ± 8.5% (range 37.7% to 67.2%).

**Overall Sample**

The average ratio of newly formed bone (Fig 5) for the whole overall sample (ie, containing the coronal and apical ROIs) was 29.0% ± 13.2% (range 2.5% to 61.0%) in the study group. The average proportion of the ACA in the bone graft was 21.8% ± 14.1% (range 0% to 61.5%). This resulted in a remaining ratio for the
marrow space of 49.2% ± 10.7% (range 24.9% to 81.3%).

In the control group, the data for the overall sample were as follows. The newly formed bone had a ratio of 22.3% ± 12.3% (range 3.5% to 56.1%). The proportion of ACA (without PRP) was 27.4% ± 13.9% (range 1.7% to 51.9%), and the average marrow space ratio was 50.3% ± 8.9% (range 35.9% to 67.2%; Fig 5).

**STATISTICAL ANALYSIS**

The statistical analysis revealed first that the bone within the complete sample without PRP resulted in significantly less newly formed bone than did the bone with PRP (Welch 2-sample t test, \( P = .01348 \); mixed linear model, \( P = .0319 \)). Second, the apical bone without PRP resulted in significantly less newly formed bone than the apical bone with PRP (Welch 2-sample t test, \( P = .01074 \)). Third, the addition of ACA within the complete sample without PRP led to significantly less resorption than did ACA plus PRP (Welch 2-sample t test, \( P = .01074 \)). Finally, the apical ACA without PRP showed significantly less resorption than did apical ACA plus PRP (Welch 2-sample t test, \( P = .0054 \)). At the other regions, the differences were not statistically significant.

**Discussion**

Cricchio and Lundgren\(^{17}\) described in their report that 26% of the patients with iliac crest grafts had a prolonged period of pain that lasted from a few weeks to several months, and 11% of the patients still had some pain or discomfort 2 years after surgery. Of the 8 patients who still had some pain after 2 years, which was considered permanent, 3 also had gait disturbance. A total of 3 major complications (4%), 1 iliac wing fracture and 2 nerve injuries, occurred. With respect to such complications, it is difficult to consider autogenous pelvic bone for sinus grafting as the “reference standard.”

Several reports have been published concerning the histologic and histomorphometric characteristics of autogenous bone grafts and various bone substitutes, or a combination of both, as grafting materials. Schenk\(^{18}\) found that the original bone content of the human iliac crest was 20% to 25%, depending on age. Wheeler et al.,\(^{19}\) in 7 augmentations using bovine hydroxyapatite alone, showed an average bone area of 16.4%; however, augmentations using primarily iliac crest bone produced a mean bone content of 19.3%. Yildirim et al\(^{20}\) used a mixture of bovine hydroxyapatite (Bio-Oss, Osteohealth, Shirley, NY) and venous blood as the grafting material. In their histomorphometric examination, they found 14.7% new bone, 29.7% biomaterial, and 55.6% marrow space. Comparing the results of Schenk,\(^{18}\) Wheeler et al.,\(^{19}\) and Yildirim et al\(^{20}\) with the findings from our study showed a greater proportion of bone area and a greater resorption of the biomaterial after combined application of ACA and PRP.

Since the publication of the data from Marx et al.,\(^{12}\) several groups have investigated the effect of addition of PRP to a variety of bone substitute materials on bone formation. The targets of PRP are vital preosteoblasts and stem cells; therefore, no positive clinical benefits can be expected with the use of PRP in the absence of vital bone cells.\(^{11}\) Increased bone regeneration by application of PRP can only be expected when used with mixtures of bone substitutes and vital bone cells.\(^{11}\) However, as presented in the present survey, neither pelvic nor autogenous bone

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**Table 2. CORONAL ROI: COMPARISON OF MEAN VALUES OF STUDY AND CONTROL GROUPS**

<table>
<thead>
<tr>
<th>Group</th>
<th>Newly Formed Bone (%)</th>
<th>Biomaterial (%)</th>
<th>Marrow Space (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No PRP (control)</td>
<td>27.6</td>
<td>20.3</td>
<td>52.1</td>
</tr>
<tr>
<td>PRP (change compared with control group)</td>
<td>32.2 (±16.7)</td>
<td>20.1 (−1.0)</td>
<td>47.7 (−8.4)</td>
</tr>
</tbody>
</table>

Abbreviations: ROI, region of interest; PRP, platelet-rich plasma.

grants from other sites are necessary. Collected bone from a suction trap is a sufficient and an equivalent alternative.

A number of research groups have evaluated the effect of PRP on bone formation by combining it with other bone substitutes. Their results suggest no significantly enhanced or improved bone regeneration using PRP. Nevertheless, the enhancement of new bone formation in the patients included in the present study could be attributed to the proliferative effects of PRP, the addition of collected bone, and the specific properties of the bone graft with ACA, allowing physiologic bone remodeling with appropriate resorption kinetics owing to its similarity to highly porous bone surface and chemistry. The addition of thrombin itself does not contribute to increased bone formation, as shown by Betoni-Júnior et al.

The results of the present study showed an increase in the newly formed bone area compared with that in the control group (Fig 5), which had the same study protocol, biomaterial, and methods, but without the addition of PRP to the grafts (Tables 2 and 3). These findings are supported by a report by Aimetti et al., investigating the effect of PRP in sinus floor augmentation. After the use of PRP, an increase in new bone formation in the whole sample and enhanced resorption of the biomaterial ACA could be detected, especially apically. The most likely reason for this observation was that the bone at the coronal locations at the sinus floor itself facilitates chemotaxis and mitogenesis of osteoblast precursors owing to already existing vessels. Therefore, no significant difference from the control group was found in the coronal ROI. In contrast, the increased formation of new bone and the enhancement of bone graft resorption in the apical regions (ie, regions with no vessels) in the study group were clearly induced by the properties of PRP. Consequently, the control group without PRP showed less new bone formation.

In the present study, no patient was excluded because of old age or smoking habits. Although increased age does not appear to affect the clinical potential for osseointegration, older patients, theoretically, have potentially longer healing times. Kan et al. showed that smoking was disadvantageous to the success of implants placed into the grafted maxillary sinus, regardless of the amount of nicotine smoked. Because of the known effects of smoking and the aim of our study was not to detect the histologic differences between smokers and nonsmokers and the influence of age, we ensured that the smokers and older patients were equally distributed within the study groups and the control group to avoid any bias of the results from these parameters. Furthermore, we did not detect any wound healing problems related to smoking in our group.

From the results reported in the present study, the need for any additional surgery to harvest the bone grafts for sinus floor elevations must be carefully considered.

It can be concluded that the application of PRP results in enhanced new bone formation when used in combination with the algae-derived hydroxyapatite ACA and collected bone. This method predictably leads to a newly produced bone volume that sufficiently supports dental implants in function and might help to supersede additional bone harvesting.

References

mentation of the maxillary sinus with Aligpore. J Long Term Eff Med Implants 1:203, 1999