Does platelet-rich plasma promote remodeling of autologous bone grafts used for augmentation of the maxillary sinus floor?

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Abstract: The aim of this study was to evaluate the effect of platelet-rich plasma (PRP) on remodeling of autologous bone grafts used for augmentation of the floor of the maxillary sinus. In five edentulous patients suffering from insufficient retention of their upper denture related to a severely resorbed maxilla, the floor of both maxillary sinuses was augmented with an autologous bone graft from the iliac crest. Randomly, PRP was added to the bone graft used to augment the floor of the left or right sinus (split-mouth design). Three months after the reconstruction, bone biopsies were taken with a trephine from the planned implant sites (N = 30). Subsequently, three implants were placed in the left and right posterior maxilla. Microradiograms were made of all biopsies (N = 30), whereafter the biopsies were processed for light microscopic examination. In addition, clinical parameters were scored. Wound healing was uneventful, clinically no difference was observed between the side treated with PRP or not. Also microradiographical and histomorphological examination of the biopsies revealed no statistical difference between the PRP- and non-PRP side. One implant placed in the PRP side of the graft was lost during the healing phase. Implant-retained overdentures were fabricated 6 months after implantation. All patients functioned well (follow-up 20.2 ± 4.3 months). In this study, no beneficial effect of PRP on wound healing and bone remodeling was observed. It is posed that PRP has no additional value in promoting healing of grafted non-critical size defects.

Implant dentistry is a dynamic field, both from a scientific and clinical point of view. Many clinicians are in search for rather simple pre-implant surgical procedures that are less inconvenient to the patient, but possess the ability to create optimal circumstances for implant placement. Various augmentation techniques are in use to create sufficient bone volume for reliable insertion of endosseous implants in the severely resorbed maxilla (Raghoebar et al. 2001). Today, reconstruction of the jaws requires transplantation of osteocompetent cells. These cells are expected to survive, produce bone, and finally mature into a functioning bony mandible or maxilla. Although elevation surgery of the maxillary sinus floor has proven to be a reliable method with good results, bone healing is not always predictable and occasionally the bone volume at the time of placement of the implants is not sufficient for predictable implant placement.

To improve soft-tissue healing and bone remodeling, platelet-rich plasma (PRP) has been introduced as a new and potentially useful adjunct in oral and maxillofacial bone reconstructive surgery. Platelets play an important role in wound healing. They
arrive quickly at the wound site and begin coagulation. They release multiple growth factors and cytokines involved in wound healing, including platelet-derived growth factor, transforming growth factors, vascular endothelial growth factor, platelet-derived endothelial cell growth factor, interleukin-1, basic fibroblast growth factor, and platelet activating factor-4 (Aghaloo et al. 2002; Weibrich et al. 2002). These growth factors are thought to contribute to bone regeneration and to increase vascularity, both of which are considered vital features of a healing bone graft.

Up to now, only one study has been reported in the literature evaluating the effect of PRP on bone density and healing time following reconstruction of mandibular continuity defects with autogenous bone. The authors claimed a 1.62–2.16-fold increase in bone density when PRP was added to an autogenous bone graft [Marx et al. 1998]. Although this beneficial effect has not been proven in other clinical studies yet, it already has been proposed to utilize PRP in combination with alloplasts, xenografts or other non-autogenous materials, even without combination with a bone graft (Fürst et al. 2003).

Preliminary case reports claim formation of some osteoid material or bone using such a technique, but the preliminary results are not equivocal and sometimes conflicting [Anitua et al. 1999; Kassolis et al. 2000; Rosenberg & Torosian 2000; Shanaman et al. 2001; Vanasse & De-françois 2001; Froum et al. 2002; Wiltfang et al. 2003]. No scientific conclusions can be drawn from these preliminary reports. Additional studies are needed. Therefore, the aim of this study was to evaluate the effect of PRP on remodeling of autologous bone grafts used for augmentation of the floor of the maxillary sinus.

Patient and methods

Patients

Five consecutive patients referred to the Department of Oral and Maxillofacial Surgery of the University Hospital, Groningen because of insufficient retention of their upper denture related to a severely resorbed maxilla were selected on basis of the following inclusion criteria:

- severely resorbed maxilla [classes V–VI, Cawood & Howell 1991] with reduced stability and retention of the upper denture,
- comparable bone height between the maxillary sinus and top of the maxilla on both sides,
- class IV bone quality [Lekholm & Zarb 1985];
- edentulous period of at least 1 year;
- no history of radiotherapy in the head and neck region;
- no history of reconstructive, pre-prosthetic surgery or previous oral implantology.

In all patients, two superstructures were planned supported by three implants to obtain an optimal aesthetic and phonetic result (Fig. 1).

Informed written consent to participate in this study was obtained from all patients [three women, two men; mean age 58.4 ± 1.9 years at time of surgery, range 57–62 years]. The patients had been edentulous in the maxilla for 5–12 years. Orthopantomograms, lateral cephalograms, and postero-anterior oblique radiographs were made to assess the height of the maxillary alveolar bone, the dimensions of the maxillary sinus, and the antero-posterior relationship of the maxilla to the mandible. The radiographs were also screened for sinus pathology. The mean vertical height of the alveolar bone on the orthopantomogram between the most caudal part of the maxillary sinus and the oral cavity was in the premolar and molar 3 ± 2 mm [range 1–4 mm] and 2 ± 1 [range 1–3 mm], respectively.

Platelet-rich plasma

During the time needed for harvesting bone from the iliac crest, PRP was made using a commercially available Platelet Concentration Collection System kit (PCCS kit, 3i Implant Innovations Inc., Palm Beach Gardens, FL, USA). Before the surgical procedure, 6 ml of anticoagulant citrate dextrose-A was collected in a 60 ml syringe. From the venipuncture the syringe was filled with whole blood up to 60 ml. Immediately following the whole blood collection, the blood-filled syringe was inverted six times to ensure the anticoagulant has been evenly dispersed. Using the platelet concentration system, the 60 ml of whole blood mixed with coagulant was processed to a PRP solution. As a control for the effectiveness of the work-up of PRP, the transforming growth factor [TGF]-ß concentration of each PRP sample was measured according to the method described by Waarde et al. (1997).

To promote the release of growth factors from the platelets, 10% calcium chloride solution and the patient’s serum, as source of autologous thrombin, were added before actual reconstruction of the defects with the bone graft. The resulting gel was mixed with the bone graft and some gel was applied at the closure of the wound at the side reconstructed with the bone graft mixed with PRP.

To check whether applying a bone graft mixed with PRP does not result in a systemic rise of the concentration of growth factors released by platelets making a split-mouth design less applicable in patients locally treated with PRP, whole-blood samples were collected just before application of the bone graft mixed with PRP, immediately after placement of the bone grafts mixed with PRP and 24 h after surgery.

Surgical protocol

The maxilla of the patients was reconstructed with autologous anterior medial iliac crest bone grafts under general an-
Esthesia. In all cases, bilaterally a two-stage procedure (first stage, bone grafting; second stage, placement of implants) was performed because the height of the maxillary bone and/or the width of the alveolar crest were less than 5 mm. A bone height of 5 mm or more is a prerequisite for implant placement with sufficient primary stability (Raghoebar et al. 2001). In addition to elevation of the floor of the maxillary sinus, the width of the alveolar crest was reconstructed too. An osteotomy was prepared in the lateral wall of the maxillary sinus using the surgical procedure described by Raghoebar et al. (2001). After harvesting of the bone grafts from the iliac crest, the bone was split into two equal parts. Randomly, one side was reconstructed with autologous bone mixed with PRP gel and one side with autologous bone only. The floor of the maxillary sinus was augmented with bone blocks and the remaining space occupied by cancellous bone particles that were obtained by grounding the graft in a bone mill (Leibinger®, Freiburg, Germany). Subsequently, placing monocortico-cancellous bone blocks buccally of the cortex of the alveolar defect increased the width of the superior alveolar process. The cancellous side of the bone graft was in contact with the jawbone and again cancellous bone particles were used to fill the small gaps between the bone graft and the alveolar crest. The grafts were fixed with titanium screws to the alveolar bone. PRP gel was applied over the graft at the PRP side. No membranes were used to cover the lateral wall defect after the bone graft was placed. PRP gel was also applied over the wound after closure with sutures at the PRP side.

Before harvesting bone grafts, the patients received broad spectrum antibiotics, starting 1 h preoperatively [intravenously] and continued orally for 2 days after surgery. Postoperatively, the patients received a 0.2% chlorhexidine mouth rinse [1 min, five times daily] for 2 weeks. One month postoperatively, the edentulous patients were allowed to wear dentures if possible, after relining them in the operated areas with a soft liner.

After a healing period of at least 3 months, the implant placement procedure was performed. A surgical template was used. Using the template and a trephine bur (2 mm) biopsies were taken in the region of the canine, the first premolar and first molar on the same spot as the endosseous implants will be placed [Fig. 2a, c]. The length of the biopsy was the same as the length of the implant [at least 13 mm]. The implants were inserted at the biopsy locations after widening these holes to the required dimensions using the standard burs for the implant system chosen. In all cases, the bone volume was sufficient. Six months after insertion, the implants were uncovered, the oral mucosa was thinned where applicable and the abutments connected and the prosthetic construction was fabricated.

**Evaluation**

The investigators were blinded for both the clinical and laboratory investigations with regard to the PRP-treated side. Clinically, all patients were evaluated according to a standardized protocol 1, 3, 6 and 12 weeks after surgery. The clinical protocol included assessment of complications during surgery and postoperative healing (inflammation, wound dehiscence, sequestration, and loss of bone particles). In addition, TGF-β concentration of PRP and blood were measured and the bone biopsies were studied by microradiography and light microscopy.

**Microradiography**

An X-ray source [Philips PW 1730, Eindhoven, the Netherlands] was used that produced monochromatic radiation with a specific wavelength of 1.537 Å. The X-ray radiation used is Cuka radiation with a Cu [copper] X-ray tube and a nickel filter. The wavelength produced is especially sensitive to be absorbed by calcium. The biopsies were placed between the 35 mm film [Fuji B&W POS/71337, Rotterdam, the Netherlands] and the X-ray source and exposed for 25 s, with a tube charge of 35 kV and 12 mA.

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**Fig. 2.** Bone biopsies and microradiographs of the premolar region 3 months after grafting. (a) Bone biopsy of the platelet-rich plasma [PRP] side. (b) Microradiograph of the biopsy of the PRP side. (c) Bone biopsy of the non-PRP side. (d) Microradiograph of the biopsy of the non-PRP side.
Histological examination
Immediately after taking, the biopsies were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, at 4°C for at least 2 days. During this period, microradiography of the biopsies was performed. Subsequently, after rinsing in 0.1 M sodium cacodylate buffer, the samples were dehydrated in a graded concentration of ethanol and embedded in glycolmethacrylate. Two micrometer-thick serial sections were obtained using a Jung 1140 autocut microtome with a D-hard metal knife (Shandon, Life Sciences, Cheshire, UK). The sections were mounted on glass slide and stained with toluidine blue and alkaline fuchsin (Merck, Darmstadt, Germany).

All sections were studied lightmicroscopically on the following items: quality of bone biopsy, ratio bone–bone marrow, type of bone marrow (loose connective tissue, fibrous, fat), presence of hematopoietic tissue, presence of bone formation, osteoblastic rimming, and presence of bone resorption.

Histomorphometric analysis of the biopsies was performed using Leica Qwin® image analysis software (Leica Microsystems Image Solutions, Leica, Switzerland). Under a magnification of ×100 (Leica DM RA microscope, Leica, Switzerland), every image field of the histological section was digitized by a Leica DC 200 digital camera. Subsequently, the percentage of bone area was determined in each image field using the Leica Qwin® image analysis software and then averaged. The bone area in an image field was determined by setting a threshold to the color of the pixels of the bone, whose area then could be measured.

Statistical analysis
The data were analyzed using t-test. A significance level of 0.05 was chosen.

Results
Clinical assessments
One sinus membrane was perforated during surgery. Healing was uneventful and loss of bone particles through the nose was not observed. A small incision breakdown occurred in the first week in one patient in the non-PRP side. This patient was put on a regimen of rinsing with a chlorhexidine mouth rinse four times daily. The dehiscence healed within 2 weeks.

A total of 30 implants (length 13 or 15 mm, Bränemark®, Nobel Biocare, Göteborg, Sweden) was placed in augmented maxillae [Fig. 1]. Before the prosthetic phase, one implant was mobile on the PRP side and was removed. No other implants were lost during the follow-up (20.2 ± 4.3 months).

Comparison of the clinical features at the PRP and non-PRP sides revealed no differences in areas occupied with bone with osteoblastic rimming was seen in both the PRP and the non-PRP group [Figs 3 and 4]. Histomorphometric analysis revealed no differences in areas occupied with bone between PRP- and non-PRP-treated sides. Again because of the fact that biopsies derived from the canine region largely consisted of pre-existing bone no histomorphometric data are given for this region. The average area occupied by bone in the augmented (pre)molar region was 41.1 ± 8.3% at the non-PRP treated and 38.4 ± 11.3% at the PRP-treated side [Table 1].

Discussion
Researchers in oral and maxillofacial surgery continuously strive to improve bonegrafting techniques and to provide means to obtain a faster and denser bony regenerate. This study did not show a significant increase in bone formation by adding PRP to an autogenous bone transplant used to augment the floor of a maxillary sinus and to increase the width of the alveolar process. Although the sample size is small and...
biopsies were taken at insertion of the implants only [all placed in a grafted area], some conclusions may be drawn from this study. The small sample size might have contributed to the fact that no differences in remodeling of the grafts between the PRP- and non-PRP-treated sides were observed, but even no tendency of improved healing could be shown. An explanation for the lack of difference between the PRP- and non-PRP sides might be that the majority of the bony regeneration takes place within the first month of healing. This would be in line with the mode of action of platelet growth factors. Degranulation and release of growth factors occurs within 3–5 days and the growth factor activity may end in as soon as 7–10 days (Marx 2001; Froum et al. 2002). Consistent with the outcome of our human study, also in animal experiments no significant differences in wound healing between autogenous bone and autogenous bone mixed with PRP were observed 1, 2, and 4 months after grafting (Aghaloo et al. 2002). The grafting material used in latter study was cortical membranous bone from the rabbit cranium, which differs from the bone used in this study, the latter being cancellous endochondral bone from the iliac crest. More recently, Jakse et al. (2003) published a study in which they augmented the floor of the maxillary sinus with autologous bone in sheep, either with or without PRP. These authors also concluded that the regenerative capacity of PRP is of quite low potency. Finally Fürst et al. (2004) failed to show an effect of platelet-released growth factors on bone regeneration of cortical mandibular defects in rats. Based on their and our results, we like to pose that addition of PRP to a bone transplant used for sinus floor elevation surgery has apparently no adjuvant clinical value.

In contrast to the above-mentioned reports, other authors proposed that adding PRP to bone grafts will result in an increase the bone density of the reconstructed defect (Marx et al. 1998; Vanassche & Defrancq 2001). Furthermore, it has been claimed that platelets can act as local regulators of fracture repair and bone regeneration (Gru¨ ber et al. 2002). Vanassche & Defrancq included no controls in their study, however, Marx et al. (1998) showed the beneficial effect of adding PRP in mandibular continuity defects of 5 cm or greater. Our study as well as the studies by Aghaloo et al. (2002) and Jakse et al. (2003) did not confirm the observation of Marx et al. (1998) in defects of a lower magnitude. Therefore, we like to pose that PRP, because of its effect as local regulators of bone regeneration, may have a beneficial effect on bone healing in critical size defects and defects with compromised vascularization, while it has no adjuvant value in smaller defects like in sinus floor elevation surgery.

In the current study, using a very sensitive microradiography method assessing the density of bone at the implant side, no differences between the PRP- and non-PRP sides were observed. Quantitative microradiography is a commonly used technique to measure mineral distributions (calcium, phosphate) and mineral amounts of carious lesions in enamel and dentin (Ruben & Arends 1993; de Josselin de

### Table 1. Histomorphometrical analysis of the specimen

<table>
<thead>
<tr>
<th>Patient</th>
<th>Non-PRP side</th>
<th>PRP side</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>47.8 ± 14.5</td>
<td>53.9 ± 12.9</td>
</tr>
<tr>
<td>M1</td>
<td>45.9 ± 20.6</td>
<td>52.2 ± 5.5</td>
</tr>
<tr>
<td>#2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>57.3 ± 11.8</td>
<td>34.7 ± 8.8</td>
</tr>
<tr>
<td>M1</td>
<td>29.6 ± 14.4</td>
<td>49.3 ± 11.1</td>
</tr>
<tr>
<td>#3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>42.2 ± 17.6</td>
<td>33.8 ± 22.7</td>
</tr>
<tr>
<td>M1</td>
<td>36.2 ± 13.7</td>
<td>32.3 ± 12.6</td>
</tr>
<tr>
<td>#4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>33.1 ± 7.5</td>
<td>29.6 ± 11.4</td>
</tr>
<tr>
<td>M1</td>
<td>37.5 ± 7.7</td>
<td>46.6 ± 15.9</td>
</tr>
<tr>
<td>#5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>47.7 ± 21.5</td>
<td>23.8 ± 11.4</td>
</tr>
<tr>
<td>M1</td>
<td>36.7 ± 10.5</td>
<td>25.1 ± 6</td>
</tr>
</tbody>
</table>

The relative area occupied with bone in the first premolar (P1) and first molar (M1) region (%; mean ± SD) is given. PRP, platelet-rich plasma.
Jong et al. 1987). The technique has also been used to measure mineral distributions in bone [Hobson & Beynon 1997; Hobson 1998, Bovin & Meunier 2002]. Microradiography has the advantage over histology that an image is obtained from the entire specimen, making it possible to both locate and measure areas of bone growth (Schorthinghuis et al. 2003). Although the study by Marx et al. [1998] reported a 1.62–2.16 times greater radiographic maturation rate in PRP-treated reconstruction sites as measured on panoramic radiographs, in the current study, using a much more sensitive method no difference in bone density was observed between the PRP- and non-PRP sides. This again supports our hypothesis that PRP has no adjuvant value in sinus floor elevation surgery, while it still may have an adjuvant value in major reconstructive surgery.

With regard to grafting with bone substitutes, Kassolis et al. [2000] reported some beneficial effects of the use of PRP. They used PRP in combination with freeze-dried bone allografts in maxillary sinus floor elevation surgery. Histologic sections revealed numerous areas of osteoid and bone formation around freeze-dried bone allograft particles. Histomorphometry was not performed and there were no controls. This makes their results questionable as in our study, using autogenous bone, however, we also see proper ostoid and bone formation in the non-PRP-treated areas grafted with iliac crest bone. Kassolis et al. [2000] recognized this flaw in their experimental setup, as they did not draw firm conclusions from their study. They mentioned a need for studies to determine whether PRP enhances new bone formation or maturation with allogenic grafts or not. A recent study indeed indicated that addition of PRP to grafts of anorganic bovine bone used for sinus floor elevation surgery that contained minimal or no autologous bone did not make a significant difference either in vital bone or in interfacial bone contact on the test implants [Froum et al. 2002]. Also, combination of PRP with bovine hydroxyapatite was not demonstrably superior to hydroxyapatite alone [Fürst et al. 2003]. These are not surprising observations as PRP acts on healing capable cells to increase their numbers [mitogenesis] and stimulate vascular ingrowth [angiogenesis] [Marx 2001] that are not present in acellular bone substitutes. PRP is not osteo-inductive and must be used in combination with living bone cells. Therefore, it is unlikely to significantly promote bone substitutes and other non-cellular graft materials.

An effective way to evaluate the effects of PRP on the formation of bone is to study the effect in bilateral sinus grafts, with the addition of PRP being the only controlled variable. This is the first study to be performed in this manner with autologous bone. In our study a beneficial effect of PRP on bone healing and remodeling could not be shown. In critical size defects, compromised bone after radiotherapy and in large bony defects, PRP still might have some value, as has been observed by some authors in the treatment of such a category of patients [Marx et al. 1998; Fennis et al. 2002]. Furthermore, in the current study the non-PRP-supported bone graft and PRP-supported bone graft were left unfunctioned. Implants were placed 3 months after reconstruction. At that time the grafts showed comparable signs of remodeling. This does not exclude a possible beneficial effect of PRP on early bone healing making earlier placement of implants after grafting possible. In such a case, the possible early enhancing effects of PRP on bone healing might play a role.

Controlled clinical trials are necessary to determine whether addition of PRP significantly enhances bone formation and maturation or not. Up to now, we like to pose that PRP has no additional value in promoting healing of grafted non-critical size defects. As there is a strong clinical potential associated with supplementing growth factors in healing wounds, there is a great need for well-designed studies.

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Résumé

Le but de cette étude a été d’évaluer l’effet du plasma riche en plaquettes sur le remodelage de greffons osseux autogènes utilisés pour l’épaississement du plancher sinusal. Chez cinq patients édentés souffrant d’une rétention insuffisante de leur prothèse supérieure en relation avec un maxillaire sévèrement résorbé, les planchers sinusoïaux des deux maxillaires ont été épaisseis avec un greffon d’os autogène provenant de la crête iliaque. Au hasard, du plasma riche en plaquettes (PRP) a été ajouté au greffon osseux utilisé pour épaissir le plancher du sinus gauche ou droit [modèle de bouche divisée]. Trois mois après la reconstruction, des biopsies osseuses ont été obtenues avec un trépan des sites planifiés pour placer des implants [N = 10]. Ensuite, trois implants ont été placés dans les parties maxillaires gauches et droites. Des microradiographies des 30 biopsies ont été effectuées, ces dernières ont ensuite été utilisées pour l’examen au microscope optique. De plus, des paramètres cliniques ont été enregistrés. La guérison a été parfaite, cliniquement aucune différence n’a été observée entre les sites traités avec PRP ou sans. L’examen microradiographique et histomorphologique des biopsies n’a révélé aucune différence significative entre les sites PRP et non-PRP. Un implant placé dans le site PRP du greffon a été perdu durant la phase de guérison. Des prothèses retenues sur implants ont été fabriquées six mois après l’insertion des implants. Tous les patients ont une mise en fonction excellente après un suivi de 20 ± 4,3 mois. Dans cette étude, aucun effet bénéfique additionnel du PRP sur la guérison et le remodelage osseux n’a été observé. Le PRP n’aurait aucune valeur supplémentaire à promouvoir la guérison dans ce type d’opération.

Zusammenfassung

Fondert plättchenreiches Plasma die Remodellierung von autologen Knochentransplantaten, welche für die Augmentation des Sinusbodens vom Sinus maxillaris verwendet werden?

La intención de este estudio fue evaluar el efecto del plasma rico en plaquetas [PRP] sobre el tiempo de cicatrización y la formación ósea de las biopsias revelaron diferencias estadísticamente significativas entre los lados con o sin PRP. Un implante colocado en el lado del PRP se perdió durante la cicatrización. Las dentaduras implanteorrenuevas se fabricaron a los seis meses de la implantación. Todos los pacientes funcionaron bien (seguimiento de 20.2 ± 4.3 meses).

En este estudio no se observó ningún efecto beneficioso del PRP sobre la cicatrización y sobre el remodelado óseo. Se plantea que el PRP no tiene ningún valor adicional en promover la cicatrización de defectos no críticos injeridos.


