Influence of PRP on autogenous sinus grafts
An experimental study on sheep

Author's affiliations:
Norbert Jakse, Antranik Eskici, Christof Pertl, Department of Oral Surgery and Radiology, School of Dentistry, University of Graz, Graz, Austria
Stefan Tangl, Robert Haas, Department of Oral Surgery, School of Dentistry, University of Vienna, Vienna, Austria
Renate Gilli, Department for Transfusion Medicine, University of Graz, Graz, Austria
Andrea Berghold, Department for Medical Informatics, Statistics and Documentation, Graz, Austria
Martin Lorenzoni, Department of Prosthodontics, School of Dentistry, University of Graz, Graz, Austria

Correspondence to:
Norbert Jakse
Department of Oral Surgery
School of Dentistry
Karl-Franzens University Graz
Auenbruggerplatz 12
A-8036 Graz, Austria
Tel: +43-316-385-2936
Fax: +43-316-385-6858
e-mail: norbert.jakse@uni-graz.at

Key words: PRP, bone regeneration

Abstract: Since platelet-rich plasma (PRP) has been introduced to the field of oral surgery, it has become a widely accepted additive for bone regeneration treatment. The aim of this study was to evaluate the regenerative capacity of PRP in a sinus graft study on sheep. Twelve adult sheep underwent a bilateral sinus floor elevation procedure with cancellous bone from the iliac crest. Unilaterally, PRP was administrated to the bone graft. After 4 (six sheep) and 12 weeks (six sheep), bone biopsies were obtained from each site. With histomorphometric analysis we evaluated both the percentage of newly formed bone within the grafted site and the percentage of the contact area between the grafted bone and the newly formed bone. After 4 weeks the mean proportion of newly formed bone on the control side was 26.1%, whereas it was 29.2% on the test side. After 12 weeks it was 46.9% on the control side and 51.1% on the test side. The area of contact between the graft and the newly formed bone was 73.0% on the control side and 78.5% on the test side after 4 weeks, and 87.2% on the control side and 90.1% on the test side after 12 weeks. A statistical analysis did not reveal significant differences between the control and the test side. The results of the present experimental study show a regenerative capacity of PRP of quite low potency. Further basic research is needed to investigate more profoundly the possibilities of PRP in bone regeneration.

Advanced implant dentistry is highly dependent on the successful regeneration of alveolar bone. The addition of growth factors to grafting material in healing sites has raised high expectations on its clinical potential. Platelets are a known source of growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor (TGF-β) [Ross et al. 1986; Pierce et al. 1989; Bonewald & Mundy 1990; Roberts & Spron 1993]. The use of platelet-rich plasma (PRP) for bone augmentation procedures in dental implantology has been introduced by Marx et al. [1998]. Their studies indicate that the supplementation of growth factors by adding PRP to bone grafts results both in a faster radiographic maturation rate and in a higher bone density than in the control group. Other authors have further described the method in more detail and presented their own clinical experiences. Anitua [1999] used PRP to enhance bone regeneration successfully and accelerate soft tissue healing in fresh extraction sites. Kassolis et al. [2000] present their experience in sinus elevation and alveolar ridge augmentation in combination with freeze-dried bone allografts, while Landesberg et al. [2000] have compared and evaluated two different methods of preparing PRP.
Although the use of PRP has been promoted by several respected clinicians (Marx et al. 1998; Anitua 1999; Kassolis et al. 2000; Landesberg et al. 2000), Schmitz and Hollinger point out the insufficient biologic understanding concerning the clinical therapy surrounding PRP. They highlight that the exact effects of PRP on bone grafts are still unknown and suggest further research (Schmitz & Hollinger 2001).

The aim of this study was to evaluate the regenerative potential of PRP in a sinus graft model on sheep using a standardized split-face protocol. In particular, we were interested in whether the effect of PRP is limited to the early stage of bone regeneration or there would be an influence over a longer period. Additionally, we determined both the platelet and growth factor concentration of PRP in each animal.

Material and methods

The animal study protocol was approved by the Austrian Federal Ministry of Education, Science and Culture.

General procedure

A total of 12 adult female sheep (mean weight of 53.4±6.2 kg) were used for this study. They all underwent a bilateral two-stage sinus floor elevation procedure with a cancellous bone graft from the iliac crest carried out by the same surgeon. PRP was added to the left sinus graft in each case. During the second-stage surgery after 4 (six sheep) and 12 weeks (six sheep) respectively, two biopsies were obtained from each grafted site and dental implants were inserted. According to the split-face study design the biopsies of the PRP site were compared to the control with the pure cancellous bone graft. The histological investigation of the osseointegration of the inserted dental implants is the subject of an additional study not discussed in this paper.

Animal management

Housing and feeding of the animals were performed according to standard animal care protocol at the Department of Biomedical Research of the University of Graz. Preoperatively, the animals had to starve for 24 h. One hour before surgery they were sedated with an intramuscular injection of 500 mg xylacinhdrochlorid (Rompun®; Bayer AG, Leverkusen, Germany). Additional premedication followed immediately before surgery: 0.2 mg of glycopyroniumbromid (Robinul®; Wyeth Laboratoires, Havant, Hampshire, UK), 25 mg of s-ketaminhydrochlorid (Ketanest S®; Pfizer Corporation Austria, Vienna) and 5 mg of midazolam (Dormicum®; Roche Austria, Vienna) intramuscularly. Anaesthesia was then induced with intravenous administration of 0.4 mg of Robinul®, 25 mg of Ketanest S® and 40 mg of triflupromazinhydrochlorid (Psyquil®; Bristol-Myers Squibb, Anagni, Italy). Anaesthesia was maintained with intravenous administration of Ketanest S® and Dormicum® according to effect. A standard monitoring of general anaesthesia was performed during the entire course of anaesthesia. All animals received oxygen (5 min) through a transnasal tube for the time of anaesthesia. The general anaesthesia was supplemented by local administration of 4% articain containing epinephrine (1:100,000) [Ultradacin Dental Forte®, Hoechst Marion Roussel GmbH, Vienna, Austria] in order to reduce haemorrhage in the surgical field. Intraoperatively, all animals received 500 mg of amoxicillin (Augmentin®, Beecham, Heppignies, Belgium) intravenously as a one-shot administration. Postoperatively, the animals received 75 mg of diclofenac [Voltaren®, Novartis Pharma, Basel, Switzerland]. PRP was collected and prepared during first-stage surgery.

Surgical procedure

First-stage surgery (sinus floor elevation):

A bloc-type bone graft was harvested from the iliac crest. The whole graft was ground with a bone-mill (R. Quetin Bone-Mill, Roswitha Quetin Dental Produkte, Leimen, Germany). The bone graft was then stored in physiologic saline solution. According to a split-face design, the sinus floor elevation procedure was performed identically on both sides of each sheep, except for adding the PRP on the test site. From an extraoral incision below the lower eye lid, the facial antral wall was exposed. A bone window of a 10 mm diameter was created with a burr. The sinus membrane was elevated from the lateral sinus wall using variably bent dissectors (Frios Sinus Set, Friatec, Friedrichsfeld, Germany), carefully preventing any perforation. On both maxillary sides, the created extrasinusoidal space was then packed with the harvested and ground bone graft [Fig. 1]. On the test site a volume of 3–4 ml of PRP was added extraorally in several mixes to the bone graft until fibrin formation bound the loose cancellous bone particles. The bony defect in the facial sinus wall was then covered by a resorbable collagen membrane [BioGide®, Geistlich, Wolhusen Switzerland]. Two titanium nails (Friatec, Friedrichsfeld, Germany) were used to mark the augmented area for the second-stage surgery.

Second-stage surgery (biopsies and insertion of implants):

The reentry surgery was performed 4 and 12 weeks after augmentation. The same extraoral incision was used to expose the lateral sinus wall. After removing the titanium nails, two bone biopsies were obtained exactly from the former location of these nails using a trephine burr of 3.1 mm diameter. The biopsies included original facial sinus wall and augmented bone.

Collection and preparation of PRP

Simultaneously with the surgical procedure of harvesting bone from the iliac crest, platelepheresis was performed with a blood cell separator (CS-3000Plus, Baxter Healthcare Corporation, Fenwal Division, Austria). The animal study protocol was approved by the local Ethics Committee of the University of Graz. The study was performed according to the guidelines of the Austrian Federal Ministry of Education, Science and Culture.
Deerfield, IL, USA) using the small-volume [30-ml] collection chamber (PLT-30). A total blood volume of 1000 ml was processed [50 ml/min] under anticoagulation with ACD-A. The extracted 30 ml of platelet concentrate was resuspended, and in a second step the platelet concentrate was concentrated by further centrifugation [centrifugation: 800 × g, 15 min] to adjust the final concentration of PRP. Platelet counts [Sysmex, TOA Medical Electronics Co., Ltd, Kobe, Japan] were determined in the whole blood before plateletpheresis and in the final product of PRP. Additionally, the concentration of PDGF and TGF-β was measured in the concentrated PRP (Quanti kin® R & D Systems, Inc., Minneapolis MN, USA).

Specimen preparation

The bone biopsies were obtained with a trephine burr and were immediately stored in 10% formalin solution. The preparations of the histological sections were performed according to the technique of Donath [1988]. After dehydration in ascending grades of alcohol, the specimens were embedded in light-curing resin [Technovit 7200 VLC + BPO, Kulzer & Co. Germany]. One section was produced from each biopsy with the help of saws and grinding machines [Exact Cutting and Grinding Equipment, Exakt Apparatebau, Norderstedt, Germany]. Each section was reduced to a thickness between 10 and 20 μm followed by a Levai–Lazcko staining.

Histomorphometric analysis

The investigator who performed histomorphometric analysis was blinded to animal treatment status. The undecalcified sections were photographed and digitized with a Kodak Professional DCS 420 digital camera [Eastman Kodak Company, Rochester, NY, USA] mounted on a Nikon Microphot-FXA microscope [Nikon Corporation, Tokyo, Japan], resulting in pictures where 1 mm measures 872 pixels [1 pixel = 1.13 μm]. As many digital photographs were taken and assembled in an overlapping manner with the Adobe Photoshop program as were necessary to depict the complete biopsy. For histomorphometric analysis, an interactive colouring of pristine bone, grafted bone and newly formed bone was obtained. The area of newly formed bone (m²) within the augmented region (e.g. the sinus area of the biopsy) and contact length (mm) between the graft and newly formed bone and the length of uncovered graft surface were determined with the morphometry program Lucia G 4.51 [Laboratory Imaging Ltd, Bmo, Czech Republic]. From these direct measurements, the percentage of newly formed bone and the percentage of the graft surface covered by newly formed bone were calculated.

Statistical analysis

The results of histomorphometric analysis were subjected to ANOVA procedures for a split-plot design using the General Linear Models procedure of SPSS. The model included time (i.e. 4 and 12 weeks after surgery), sheep (time), treatment (i.e. PRP and no PRP) and time × treatment interaction as possible sources of variation. A P-value of <0.05 was regarded as being statistically significant. Data are presented as mean and standard deviations. Additionally the Pearson correlation coefficient was used to calculate correlation between platelet count/concentration of PDGF and TGF-β in the final product of PRP and the results of the histomorphometric analysis.

Results

The animals tolerated the surgical procedures well and were healthy during the entire observation period.

Platelet count study

Platelet counts performed on each animal resulted in a mean platelet count value of 100 × 10⁶/ml, with a range of 6–268 × 10⁶/ml (SD 67 × 10⁶). After plateletpheresis the mean platelet count of the extracted platelet concentrate was 821 × 10⁶/ml, with a range of 218–1751 × 10⁶/ml (SD 785 × 10⁶). After a further centrifugation the mean final concentration of the administrated PRP was 3810 × 10⁶/ml, with a range of 1533–7121 × 10⁶/ml (SD 2468 × 10⁶).

Concentration of growth factors (PDGF and TGF-β) in the final product of PRP

The mean concentration of PDGF of the administrated PRP was 42.00 pg/ml with a range of 19–107 pg/ml (SD 27.43). The mean concentration of TGF-β was 997.58 pg/ml with a range of 184–4467 pg/ml [SD 1187.78].

Clinical and macroscopic findings

In each of the 12 cases it was possible to harvest a sufficient quantity of cancellous bone graft. All of the 24 sinus floor elevation procedures were performed without complications. In particular, no perforation of the sinus membrane occurred. The addition of PRP always caused a clotting of the otherwise loose cancellous grafting material, allowing easier packing of the material into the extrasinusoidal space. Soft tissue wound healing was uneventful in all cases. Four [six animals] and 12 weeks [six animals] after the first-stage surgery, two biopsies were taken from each grafted sinus, which finally resulted in 48 biopsies. When taking the biopsy with the trephine burr, an evident resorption of the augmented area led to sinus perforations in seven cases. Three of these [control group 1 and test group 2] occurred after 4 weeks and four perforations [control group 2 and test group 2] occurred after 12 weeks.

Histological findings

All histological sections, both of the PRP group and the control group, showed a similar structure. The condition of the pristine crestal bone hardly differed in the individual sections. In the 4-week group, and particularly in the 12-week group, extensive osteonal remodelling as a result of the surgical trauma of the first-stage surgery had taken place. In this respect there were no differences between the control and the test side. In the augmented area, the macroscopic impression was confirmed histologically. Most of the 48 biopsies presented signs of advanced resorption of the bone graft. Osteoclasts in resorptive lacunae [Howship lacunae] were found on the surface of the grafted bone particles. This was especially observed in the 12-week group. Again there was no obvious difference between the control and the test side. Near the pristine cortical bone and around the pieces of the cancellous bone graft, all different stages of new bone formation were observed. There were regions with osteoblasts, granular preosteoid depositions and osteoid formation. In the 4-week group the
The statistical analysis has revealed no significant treatment \( P = 0.39 \) or time \( \times \) treatment interaction \( P = 0.84 \) effects.

Both the percentage of newly formed bone and the percentage of area of contact between the graft and the newly formed bone after 4 and 12 weeks did not present a significant correlation with the platelet count in the final product of PRP. Also the growth factors PDGF and TGF-β showed no evident correlation with the results of the histomorphometric analysis.

**Discussion**

Our results showed no significant differences between the augmentation utilizing PRP in combination with a cancellous bone graft and the autogenous sinus graft alone during the observation period of 12 weeks in 12 sheep. Furthermore, no evident correlation was found between platelet count/concentration of growth factors of the added PRP and results of the histomorphometric analysis of the regenerated bone.

Marx et al. (1998) described a simplified model of bone healing that focused on the influence of two growth factors – PDGF and TGF-β. Both factors have been described to be physiologically secreted by platelets to initiate and promote wound healing processes, including bone regeneration and repair (Ross et al. 1986, Roberts & Spron 1993). The basic hypothesis of PRP’s addition to bone grafts is that a high concentration of platelets in a bony wound would increase the local concentration of secreted growth factors and subsequently would enhance initial bone regeneration. After a few days, the direct influence of the administrated PRP would fade (Pierce et al. 1989) and physiological mechanisms of bone repair continue the course of bone regeneration on an accelerated level. Marx et al. demonstrated the effect of PRP in a clinical study of 88 bone graft reconstructions of mandibular continuity defects. The radiographic data from Marx et al. indicate that PRP-supplemented bone grafts promote significant early new bone formation and a higher level of maturity. The results of the histomorphometric analysis indicate that bone grafts with growth factors from PRP demonstrate, after a 6-month healing period, a higher trabecular bone density than grafts without PRP (percentage of trabecular bone: 74.0±11% vs. 55.1±8%).
However, the conclusions from their results, that PRP enhances the rate and amount of newly regenerated bone were criticized by other authors [Schmitz & Hollinger 2001]. In fact, there is a lack of experimental data concerning the exact effects of PRP on bone grafts.

Our protocol was initiated to study PRP’s ability to promote bone healing in an animal model. PRP always caused a clotting of the otherwise loose cancellous graft material, which made it easier to pack the graft into the extrasinusoidal space. This higher degree of compression of the cancellous bone graft into the defect during augmentation seemed to be beneficial from a clinician standpoint as it helps to improve the immediate space maintaining effect. It might also result in a better density during early radiographic assessment. Independently of whether the cancellous graft was treated with growth factors or not, all augmented areas showed a clear tendency to resorption. Pronounced resorptions were particularly observed after 12 weeks of bone healing. The histological findings confirmed this macroscopic impression. There was evident resorption of the bone graft in many biopsies increasing from 4 to 12 weeks. This resorption has been described and it is a specific disadvantage of cancellous bone grafts [Haas et al. 1998]. The extent of resorption is certainly species dependent and might not have such a great relevance in humans. In this study we could not detect the ability of PRP to reduce this resorative process in cancellous grafts.

This experimental study shows a regenerative capacity of PRP of quite low potency. The results reflect the healing pattern in an animal model and might be different in humans. In order to provide the clinician with profound scientific back ground for the use of PRP in dental implantology, further research is urgently needed.

Acknowledgements: The authors thank the Department of Surgical Research (chairman: Prof. Dr Salman Uraňus), the Department of Anaesthesiology (chairman: Prof. Dr Werner List) and the Department of Biomedical Research (chairman: Prof. Dr Heinz Juan). The study was supported by Friatec (Friedrichsfeld, Germany) and Friadent Schütte (Linz, Austria).

Résumé
Puisse le plasma riche en plaquettes [PRP] a été introduit dans le champ de la chirurgie buccale, il s’est avéré être une amélioration du traitement de la régénération osseuse. Le but de cette étude a été d’évaluer la capacité de régénération du PRP dans une étude de greffe spongieuse chez le mouton. Douze moutons adultes ont subi un processus d’épaisissement du plancher sinusal avec de l’os spongieux provenant de la crête illiaca. Unilatéralement, le PRP a été administré au greffon osseux. Après quatre [six moutons] et douze semaines [six moutons] des biopsies osseuses ont été obtenues de chaque site. Le pourcentage d’os néoformé à l’intérieur du site greffé et le pourcentage de contact entre l’os greffé et l’os néoformé ont été évalués par histomorphométrie. Après quatre semaines la proportion moyenne d’os néoformé au niveau du site contrôle était de 26.1% et de 29.3% au niveau du test. Après douze semaines, il était de 46.0% au niveau contrôle et de 51.1% au niveau test. L’aire de contact entre le greffon et l’os néoformé était de 73.3% au niveau contrôle et de 78.5% au niveau test après quatre semaines, et de 87.2% au niveau contrôle et de 90.1% au niveau test après douze semaines. Une analyse statistique n’a révélé aucune différence entre les tests et contrôles. Les résultats de l’étude présente montrent que la capacité régénérative du PRP n’a qu’un potentiel très faible. Davantage de recherche s’avère nécessaire pour mettre en évidence les possibilités du PRP dans la régénération osseuse.

Zusammenfassung
Der Einfluss von PRP auf ein autologes Transplantat bei der Sinusbodenelevation. Eine experimentelle Studie am Schaf. Seit das mit Plättchen angereicherte Plasma (PRP) in der Disziplin der oralen Chirurgie Einzug hielt, hat es sich zu einem allgemein akzeptierten Hilfsmittel bei der Knochenregeneration entwickelt. Das Ziel dieser Studie war, die regenerative Fähigkeit des PRP bei der Sinusbodenelevation am Schaf zu untersuchen.


Nach 4 Wochen betrug der mittlere Anteil an neu gebildetem Knochen auf der Kontrollseite 26.1%, währenddessen der Anteil auf der Testseite 29.2% betrug. Nach 12 Wochen waren es auf der Kontrollseite 46.9% und auf der Testseite 51.1%. Die Kontaktfläche zwischen Transplantat und neu gebildetem Knochen betrug nach 4 Wochen auf der Kontrollseite 73.0% und auf der Testseite 78.5%, nach 12 Wochen waren es auf der Kontrollseite 87.2% und auf der Testseite 90.1%. Die statistische

Resumen
Desde que se introdujo el plasma rico en plaquetas [PRP] en el campo de la cirugía oral, este ha sido aceptado ampliamente como un aditivo para el tratamiento de regeneración ósea. La intención de este estudio fue evaluar la capacidad regenerativa del PRP en un estudio de injerto sinusal en ovejas. Se sometió a 12 ovejas adultos a un procedimiento de elevación del hueso del seno bilateralmente con hueso de la cresta ósea. Se administró PRP unilateralmente al injerto óseo. Tras cuatro [6 ovejas] y doce semanas [6 ovejas] se obtuvieron biopsias del hueso de cada lugar. Evaluamos con análisis histomorfológico el porcentaje de hueso neofijado dentro del lugar injertado y el porcentaje del área de contacto entre el hueso injertado y el hueso neofijado.

Tras 4 semanas la proporción media de hueso neofijado en el lado de control fue del 26.1%, mientras que en el lado de prueba fue del 29.2%. Tras 12 semanas fue del 46.0% en el lado de control y del 51.1% en el lado de prueba. El área de contacto entre el injerto y el hueso neofijado fue del 73.0% en el lado de control y del 78.5% en el lado de prueba tras 4 semanas y del 87.2% en el lado de control y del 90.1% en el lado de prueba tras 12 semanas. El análisis estadístico no reveló diferencias significativas entre el lado de prueba y de control.

Los resultados del presente estudio experimental mostraron una capacidad regenerativa del PRP de muy bajo potencial. Se necesitan ulteriores investigaciones para investigar más profundamente las posibilidades del PRP en la regeneración ósea.
References


