Platelet-rich plasma activity on maxillary sinus floor augmentation by autologous bone

Ugo Consolo
Davide Zaffe
Carlo Bertoldi
Giovanni Ceccherelli

Authors' affiliations:
Ugo Consolo, Carlo Bertoldi, Department of Neurosciences, Head-Neck, Rehabilitation, Section of Dentistry and Maxillofacial Surgery, University of Modena and Reggio Emilia, Modena, Italy
Davide Zaffe, Department of Anatomy and Histology, Section of Human Anatomy, University of Modena and Reggio Emilia, Modena, Italy
Giovanni Ceccherelli, Immuno-transfusion Center, General Hospital of Modena, Modena, Italy

Correspondence to:
Prof. Davide Zaffe
Dipartimento di Anatomia e Istologia
Sezione di Anatomia Umana Normale
Via del Pozzo 71, Modena 41100, Italy
Tel.: +39 0594224800
Fax: +39 0594224861
e-mail: zaffe@unimore.it

Key words: autologous bone, bone regeneration, maxillary sinus augmentation, platelet-rich plasma

Abstract

Objectives: This work aims to evaluate the regenerative potential of platelet-rich plasma (PRP) on an implant site of peculiar clinical impact, such as sinus augmentation.

Material and methods: Sixteen consenting patients (11 females and five males), with symmetrical maxillary sinus atrophy, underwent bilateral sinus floor augmentation, using autologous (iliac crest) bone on one side and PRP plus autologous bone contralaterally. Implants were inserted 4, 5, 6 and 7 months after surgery in the patients randomly split into four groups. Orthopantomographies, computed tomography with transverse image digital reconstructions and densitometries were used to monitor the treatment progress. A core biopsy was performed at the site of implant.

Results: Clinical performance across both sites showed no statistical significance ($P = 0.414$). Densitometric values were higher at PRP sites (mean Hounsfield units ~ +57%), even if densitometry converged in the two sites 8 months after surgery. Histology documents enhanced bone activities in sites treated with PRP, 4 months after surgery. Reduced bone activity was observed in both sites 5, 6 and 7 months after surgery. Bone amount, higher in sites treated with PRP (mean trabecular bone volume ~ +37%), decreased in both sites over time.

Conclusions: Our results seem to indicate a certain regenerative potential of PRP when used with autologous bone. The effect of this enhancement of bone regeneration appeared to be restricted to shorter treatment times. A progressive extinguishment of the PRP effect is recorded after an interval longer than 6–7 months.

The rate of achievable bone regeneration may be a decisive factor influencing the success of implant dentistry. Bone resorption with aging increases the cavity and reduces the thickness of the maxillary sinus floor, thus influencing implant insertion and stability in the posterior maxilla [Hutton et al. 1995; Jensen 1999; Misch 1999]. Several authors [Adell et al. 1981; Cox & Zarb 1987; van Steenberghe et al. 1987; Friberg et al. 1991; Jaffin & Berman 1991; Fürst et al. 2003] have reported that a higher rate of implant failures occurs in the edentulous upper jaw than other oral regions. Sinus floor augmentation, as first described by Boyne & James in 1980 and Tatum in 1986, constitutes one of the most important procedures for improving bone amount in pre-prosthetic surgery. Although various grafting materials are available, autogenous bone is considered the best material owing to its osteoinductive potential, histocompatibility and satisfactory clinical outcomes [Jensen et al. 1998]. Bone-healing processes have been analyzed in depth over the last...
few years and the biological fundamentals better understood, taking into account growth factor (GF) activities on bone formation processes. Bone growth and differentiation factors as well as the appropriate extracellular matrix and stem cells are needed for bone regeneration [Lynch et al. 1991; Bruder & Fox 1999; Ramoshebi & Ripamonti 2000; Fürst et al. 2003; Jakse et al. 2003]. These authors stated that the combined use of GFs and graft material might considerably improve bone healing, fostering great expectations for their clinical use.

Platelets are a natural source of GFs, stored in α-granules. The β transforming growth factor (TGF-β) family, which includes bone morphogenetic proteins (BMPs), seems to be essential in tissue regeneration [Centrella et al. 1986; Bone- wald & Mundy 1990; Roberts & Spron 1993]. The efficacy of BMPs and of GFs in tissue regeneration has been documented in several studies [Urist 1965; Lynch et al. 1991; Boyne 1996; Cochran et al. 1999; Terheyden et al. 1999; Ramoshebi & Ripamonti 2000; Ruskin et al. 2000; Boyne 2001; Roldán et al. 2004]. When platelets are activated by some factors, such as thrombin or calcium, the α-granule content is released into the surrounding medium and GFs execute their regeneration potential [Mannaioni et al. 1997; Marx et al. 1998]. The local application of platelet-rich plasma (PRP) to achieve bone regeneration in oral and maxillofacial surgery was described by Marx et al. [1998]. This study indicated that the group treated with supplementation of GFs, obtained by mixing autologous PRP with bone graft, had better bone healing and higher bone density than the control group. Anitua [1999] later used PRP to enhance successfully bone regeneration and improve soft tissue healing in fresh extraction sites. Kassolis et al. [2000] used PRP with freeze-dried bone allografts in sinus floor elevation and alveolar ridge augmentation.

Although the clinical advantages of PRP use have been highlighted by several authors [Marx et al. 1998; Anitua 1999; Kassolis et al. 2000], the exact activity of PRP on bone processes is still unknown today [Schmitt & Hollinger 2001]. The aim of this study is to evaluate the regenerative potential of PRP on an implant site of peculiar clinical relevance, which allows clinical and histological comparative assessments in the same subject. Bilateral or unilateral sinus augmentations using PRP and graft were recently performed in experimental studies on animals [Jakse et al. 2003; Fürst et al. 2003; Roldán et al. 2004; Suba et al. 2004]. These studies did not always yield equivalent results, but they were almost always clinically suitable. Our study was performed on consenting patients, performing bilateral maxillary sinus floor augmentation, using autologous bone on one side and autologous bone plus PRP contralaterally. The work intends to analyze the PRP effect on bone regeneration over time.

**Material and methods**

**Preparation of PRP**

Our protocol, optimized according to the Immuno-transfusion Department of the Modena Medical Center, required that blood (about 450 ml) had to be drawn 24 h before surgery, using an ordinary blood container containing acid citrate dextrose solution (80 ml) as an anticoagulant. The drawn sample was centrifuged (RC3C, Sor- vall, Thermo Electron Corporation, Wall- tham, MA, USA) at 1200 g for 6 min at 20°C to obtain two moieties: FR1 [about 280 ml], plasma and platelets, and FR2 [about 250 ml], erythrocytes. FR2 was re- infused into anemic patients. Immediately before surgery, FR1 was centrifuged at 4400 g for 6 min at 14°C to obtain the two fractions: about 240 ml of platelet-poor plasma and 40 ± 5 ml of PRP. Part of the PRP was used to prepare the autologous thrombin by calcium chloride addition [recalcification]. PRP activation, i.e. α-granules release, and a quick PRP gelation was obtained by mixing PRP with autologous thrombin (3:1).

**Clinical protocol**

This study started in 1999 and patient grafting ended before March 2002. The preliminary results were communicated at the Ninth International Congress on Reconstruction and Preprosthetic Surgery, Kiel D, 2001, whereas the local ethical committee [Modena University, General Hospital and Healthy District] was established on December 2001. Moreover, we obtained informed consent from the patients, in particular explaining the objectives and protocol of the study, and possible side effects. Patients were guaranteed specific and continual health assistance and could opt out of the procedure at any phase of the study.

**Patient selection, criteria of inclusion and procedures**

All patients whose medical conditions might increase surgical risks of the research protocol were excluded from this study (Table 1). All patients judged as being unreliable for continuity of care and availability for follow-up were also excluded. Patients included in the study had a bilateral maxillary sinus pneumatization with corresponding alveolar atrophy [similar in both sinuses] of classes C and D [Jensen

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**Table 1. Clinical criteria for patient selection**

<table>
<thead>
<tr>
<th>Systemic contraindications</th>
<th>Local contraindications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis of severe medical conditions, or disorders causing metabolic bone deterioration</td>
<td>Oral disease, diffuse acute gingivitis, complex periodontitis inadequately treated</td>
</tr>
<tr>
<td>Ongoing therapies unrelated to the present study</td>
<td>Aggressive periodontitis, diagnosis or history, also medical history</td>
</tr>
<tr>
<td>Ongoing radiotherapy or antiblastic therapy, or therapy completed within 36 months</td>
<td>Diagnosis of inadequately treated sinus disease, and poor outcome of sinus disease</td>
</tr>
<tr>
<td>Neurotic and psychotic disorders or probably poor compliance</td>
<td>Dental and/or rehabilitative therapies unrelated to the present study</td>
</tr>
<tr>
<td>Pregnancy, puerperium or lactation</td>
<td>Extreme alveolar atrophy of posterior maxilla with absence of continuous bony sinus</td>
</tr>
<tr>
<td>Alcoholism and/or habitual smoker</td>
<td>floor</td>
</tr>
<tr>
<td>Tetracycline or non-steroidal anti-inflammatory drugs (NSAID)</td>
<td></td>
</tr>
<tr>
<td>Intolerance</td>
<td></td>
</tr>
<tr>
<td>Underage or above 60 years</td>
<td>Parafunctions</td>
</tr>
</tbody>
</table>

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plants were never inserted concurrent to the sinus graft. In each patient, the side treated with bone graft and PRP was randomly chosen. The Langer & Langer (1990) overlap flap, a modification of the original technique [Boyne & James 1980; Tatum 1986], was performed to gain access to the maxillary sinus and to prepare it for the floor augmentation. The modification for the most part consisted in an incision effected distant from the sinus access, which guarantees a wide cover of both the grafting site and alveolar ridge. Moreover, surgery was planned to avoid ostium obstruction. Iliac crest was used as a donor site. Autologous bone was harvested from the anterior iliac crest. Conserving the inguinal ligament and femoral cutaneous nerve, a linear incision of soft tissue, along the outer lip of the iliac crest (1 cm behind the anterior superior iliac spine), was performed. The periosteum was dissected in the middle of the iliac crest and bone harvested from a surgical opeculrum performed by a chisel and a sharp spoon. One sinus was grafted with bone and the contralateral with bone mixed with PRP.

Patients were then randomized into four groups (four patients each) to distribute the patients according to the planned implant insertion. Clinical and roentgenographic evaluation of grafted sites were performed to monitor treatment progress. Densitometries were performed on patients about 15 days before implant insertion, in the same sites as the pre-surgical phase. Implant insertion, singly carried out in each of the four groups 4, 5, 6 or 7 months after surgery, concurred with the site biopsy. Cylindrical core biopsies were obtained through the surgical access of the grafted site with a hollow mill at 600 r.p.m. under saline jet for histology. Patients were treated with tetracycline [Bassado (doxycycline) – 100 mg twice a day, for 3 days starting 5 days before biopsy, Pharmacia Upjohn, Nerviano MI, Italy] to gain additional information on bone formation. The side of biopsy facing the oral mucosa was labeled with methylene blue after saline and heparin wash to remove clots.

**Histology**

Biopsies were treated and methacrylate embedded according to Consolo et al. [2006]. Microradiographs and sections obtained and treated as reported in Consolo et al. [2006] were analyzed and photographed using an Axioptophot microscope (Carl Zeiss AG, Oberkochen, Germany) under ordinary light. Thick sections were analyzed under fluorescent light (mercury lamp, fluorescent isothiocyanate filter setup) to detect newly formed bone. Trabecular bone volume (TBV) of all biopsies, an index of the amount of bone tissue [Parfitt et al. 1987], was evaluated on the micro-radiograph of the thick section of each biopsy using a suitable image analyzer and software (VIDAS, Carl Zeiss AG).

**Statistics**

Evaluations of clinical success, as previously reported, were performed using the χ²-statistic (Glantz 2003). Numerical data were evaluated by usual estimators (mean (m), standard deviation (SD), standard error (SE), median) to define the sample distribution. Comparisons were performed by means of nonparametric Mann–Whitney U-test, Friedman test and Kruskal–Wallis multiple comparison test (Glantz 2003). Simple linear regression and correlation was applied to densitometric data to define the trend with time, and the overall test of coincidence of regression lines was used to compare the two treatments (Glantz 2003). The null hypothesis H₀ was rejected for a critical significance level of P < 0.05.

**Results**

The 16 patients (11 female and five male) of this study ranged in age from 37 to 57 years (F + M = 47 ± 5.84; F = 47.09 ± 5.9; M = 46.8 ± 6.37 – m ± SD). All patients had the same degree of sinus atrophy [class D – Jensen 1999], and they showed an almost uniform atrophy of both the right and left sinus.

The PRP produced by the patient’s whole blood [starting concentration of 300,000 ± 50,000 platelets/μl] contained 1000,000 ± 250,000 platelets/μl; therefore, platelet concentration increased more than threefold. Surgery generally produced positive results: a considerable resorption of grafted bone of both sinuses was only observed in one patient. However, the densitometric values of this patient were substantially coherent with those of the other patients. Patients had a lower level of local inflammation [swelling and red-
ness) and fewer additional side effects on
the facial side corresponding to the sinus
treated with PRP. Roentgenographic eva-
luations (Figs 1 and 2) confirmed the over-
all positive outcome of bone graft surgery
both in the immediate post-operative and
follow-up period. At 4 months (Fig. 2), we
observed a substantial conservation of the
initial volume of the graft, without remark-
able differences between the site treated
with bone and bone plus PRP, both of
which showed an almost uniform radi-
ographic aspect. Conversely, skeletal mass
had a smaller size, in particular in the
sinuses treated with autologous bone alone,
mainly 7 months after surgery. Moreover,
the uneven radiographic aspect of grafted
bone was often observed in sites treated
with autologous bone alone.

Roentgenographic analyses and clinical
evaluations of operative and postoperative
outcome allowed us to define temporally
the general clinical result. The contingency
tables (Table 2), calculated using the defini-
tion stated in Material and Methods, point
out that failures increased slightly with
time. Excluding the comparison at 4
months, where data were equal, \( \chi^2 \) was
always statistically insignificant (\( P > 0.4 \)).
The various clinical pictures are considered
not statistically different, as the \( P \) value was
higher than the critical level of significance.

### Densitometry

The basal bone densitometry of the max-
illary sinus of the 16 patients ranged from
33 to 104 Hounsfield units (HU) (Table 3).
The average densitometric results of grafted
sinuses obtained 15 days before implant
insertion (Table 4) highlighted the consist-
tently higher densitometric values of sites
treated with PRP. Densitometric evalu-
a tions were compared with nonparametric
tests. Despite the limited number of data
per group, data distribution (Table 5)
showed median values very similar to
mean values and low variance, which
constantly decreased over time. The
Mann–Whitney \( U \)-test showed no statisti-
cal significance between right and left den-
sitometric values of sinuses before
treatment (\( P > 0.57 \)). A significant statisti-
cal difference between the autologous bone
group and bone plus PRP was shown 4, 5, 6
and 7 months after sinus floor augmenta-
tion (\( P < 0.05 \): Kruskal–Wallis test, fol-
lowed by the Student–Newman–Keuls
test). Conversely, longitudinal analysis
showed statistical significance only among
7 or 6 and 4 months, but not the other
comparisons in the autologous bone group.
Instead, excluding 4 and 5 months com-
parison, longitudinal analysis showed sig-
ificant statistical differences among months
4–7 for the bone plus PRP group (\( P < 0.05 \) –
Kruskal–Wallis test, followed by the Stu-

Although the bone plus PRP group had
higher values than the autologous bone
group (+71% at 4, +81% at 5, +48% at
6 and +29% at 7 months), the general
performance of densitometric values de-
creased over time in both groups (Fig. 3).
The linear regression of densitometry from
4 to 7 months after sinus floor augmenta-
tion produced two equations with different
slopes (Fig. 4), both highly significant. The
overall test of coincidence of regression
lines for the two groups (\( HUB = 685.9–
42.63t; HUBP = 1460–136.6t; B, autolo-
gous bone; BP, bone plus PRP; t = months) pro-
duced a value of \( F = 111.97 \) correspond-
ing to a probability of \( P \leq 0.0001 \), meaning
that the two regression lines were statisti-
cally different. The theoretical point of
intersection (i.e., the concurrence of
densitometric values of sites of the two
groups) corresponds to a densitometric va-
 lue of 335.2 HU at 8.24 months after sinus
augmentation, independent of the sinus
graft.

No statistical differences were observed
for gender when comparing the initial den-
sitometric condition with time (4–7
months), even when elaborating the densi-
tometric data in relation to the two treat-
ments.
Histology

Four months after surgery, microradiographs of biopsies of grafted sites (Fig. 5) showed an indented surface (Howship’s lacunae, due to osteoclastic erosive activity) of almost all autologous-bone trabeculae. Microradiographs documented an appreciable amount of newly formed bone (Fig. 5) in sites grafted with bone plus PRP. In sites treated with only autologous bone, histology highlighted a substantial quiescence in both osteogenesis processes, low fluorescence (Fig. 6), very low expression of alkaline phosphatase (ALP; Fig. 7) and bone remodeling, few osteoclasts positive to tartrate resistant acid phosphatase (TRAP; Fig. 7). Fragments spared from osteoclastic erosion (Fig. 5) had a typical lamellar structure with ellipsoidal-shaped lacunae, empty at optical microscopy (Fig. 7) in sites treated with autologous bone alone. In contrast, live osteocytes inside some grafted bone fragments (Fig. 7) were detected in sites grafted with bone plus PRP. Osteogenic activity, positive to tetracycline labeling (Fig. 6) and ALP histochemical reaction (Fig. 7), was high in these biopsies. ALP expression was considerable not only along bone surfaces but also in the soft tissue of medullar cavities (Fig. 7). Osteoclastic activity was scanty or absent (Fig. 7). Five and six months after surgery, the histological differences between autologous bone grafts and bone plus PRP were not only less noticeable but sometimes even reversed. Bone formation

Table 2. Clinical outcome of sinus floor augmentation, with or without PRP

<table>
<thead>
<tr>
<th></th>
<th>S</th>
<th>F</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 months</td>
<td>AB</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>AB + PRP</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>5 months</td>
<td>AB</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>AB + PRP</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>6 months</td>
<td>AB</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>AB + PRP</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>7 months</td>
<td>AB</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>AB + PRP</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

S, clinical success; F, clinical failure; AB, autologous bone; PRP, platelet-rich plasma; T, total.
or erosion activities were not necessarily found in sites treated with PRP, particularly 6 months after surgery, but sometimes appeared on the contralateral side. A continued increasing deprivation of bone graft fragments was found on both sides. ALP expression gradually decreased, whereas osteoclastic erosion sometimes became relevant, without relation to the graft type. Seven months after surgery, no differences were detectable in the sites, grafted with only bone or with bone plus PRP. Histology showed the prevalence of newly formed woven bone containing typical irregular-shaped osteocytes and few residues of grafted bone on both sides. Histochemical results were similar: osteogenic activity appeared to be rather low (Fig. 8) and sometimes a feeble osteoclast activity was observed. Little formation of lamellar bone was recorded in sites treated with PRP [Fig. 8].

Four months after surgery, TBV evaluations [Fig. 9] revealed a significant difference \( (P = 0.021, \text{Mann–Whitney } U\text{-test}) \) between the site grafted with autologous bone and the contralateral \( (\text{bone plus PRP, } \text{TBV } = 66\%) \). The Friedman test was statistically significant \( (P = 0.048) \) for comparison of the TBV values [Fig. 9] between autologous bone \( (m \pm SD = 52 \pm 5.2) \) and bone plus PRP \( (m \pm SD = 43.3 \pm 9.1) \). Five months after surgery, the Mann–Whitney \( U\text{-test} \) highlighted a statistical significance \( (P = 0.043) \) between the two sites, and the Friedman test showed statistical significance \( (P = 0.046) \) comparing the TBV values [Fig. 9] between autologous bone \( (m \pm SD = 29.2 \pm 4) \) and bone plus PRP \( (m \pm SD = 39.3 \pm 5.7) \).

Six months after surgery, the Mann–Whitney \( U\text{-test} \) did not reveal statistical significance \( (P = 0.061) \) between the two sites, whereas the Friedman test showed statistical significance \( (P = 0.046) \) comparing the TBV values [Fig. 9] between autologous bone and bone plus PRP \( (\text{TBV of PRP sites } = 49\% \text{ and } 46\%) \). Seven months after surgery, the Mann–Whitney \( U\text{-test} \) gave \( P = 0.15 \) and the Friedman test \( P = 0.317 \), both statistically insignificant, between the two sites \( (\text{TBV of PRP sites } = 29\% \text{ and } 28\%) \).

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**Table 3. Hounsfield units (HU) densitometric values of maxillary sinuses before augmentation surgery**

<table>
<thead>
<tr>
<th>N</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>40.2</td>
<td>45.1</td>
<td>68.5</td>
<td>104</td>
<td>38.6</td>
<td>70</td>
<td>80</td>
<td>60.5</td>
<td>39</td>
<td>55</td>
<td>69.5</td>
<td>85.4</td>
<td>48.6</td>
<td>54.8</td>
<td>55.4</td>
<td>51.5</td>
</tr>
<tr>
<td>L</td>
<td>42.4</td>
<td>43.2</td>
<td>70</td>
<td>102.4</td>
<td>33.8</td>
<td>63.1</td>
<td>75.2</td>
<td>69.2</td>
<td>37</td>
<td>48</td>
<td>63</td>
<td>91.1</td>
<td>43.5</td>
<td>50.7</td>
<td>48.3</td>
<td>47</td>
</tr>
</tbody>
</table>

\(N\), patient number; R, right sinus; L, left sinus.

**Table 4. Densitometric values of maxillary sinuses at 105, 135, 165 and 195 days after surgery (15 days before implant insertion)**

<table>
<thead>
<tr>
<th>N</th>
<th>Bone</th>
<th>Bone + PRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>105 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>503.5</td>
<td>802</td>
</tr>
<tr>
<td>5</td>
<td>608</td>
<td>979</td>
</tr>
<tr>
<td>10</td>
<td>450.1</td>
<td>911.8</td>
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<tr>
<td>11</td>
<td>530</td>
<td>870</td>
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<tr>
<td>135 days</td>
<td></td>
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</tr>
<tr>
<td>2</td>
<td>550</td>
<td>900</td>
</tr>
<tr>
<td>8</td>
<td>450.5</td>
<td>780</td>
</tr>
<tr>
<td>9</td>
<td>450</td>
<td>750</td>
</tr>
<tr>
<td>14</td>
<td>400</td>
<td>850</td>
</tr>
<tr>
<td>165 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>705</td>
</tr>
<tr>
<td>7</td>
<td>360</td>
<td>700</td>
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<tr>
<td>12</td>
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<tr>
<td>15</td>
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<td>600</td>
</tr>
<tr>
<td>195 days</td>
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</tr>
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<td>1</td>
<td>470</td>
<td>500</td>
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<tr>
<td>6</td>
<td>380</td>
<td>450</td>
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<tr>
<td>13</td>
<td>400</td>
<td>550</td>
</tr>
<tr>
<td>16</td>
<td>320</td>
<td>500</td>
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</tbody>
</table>

PRP, platelet-rich plasma.

**Table 5. Descriptive statistics of Hounsfield units (HU) densitometric values of sinuses before and after augmentation**

<table>
<thead>
<tr>
<th>Time</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>b.s.</td>
<td>60.3</td>
<td>55.2</td>
<td>18.31</td>
</tr>
<tr>
<td>L</td>
<td>49.5</td>
<td>19.58</td>
<td>4.89</td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>4 m.a.s.</td>
<td>522.9</td>
<td>516.8</td>
<td>65.73</td>
</tr>
<tr>
<td>Bone + PRP</td>
<td>4 m.a.s.</td>
<td>890.7</td>
<td>890.9</td>
<td>74.25</td>
</tr>
<tr>
<td>Bone</td>
<td>5 m.a.s.</td>
<td>462.6</td>
<td>450.2</td>
<td>62.88</td>
</tr>
<tr>
<td>Bone + PRP</td>
<td>5 m.a.s.</td>
<td>820</td>
<td>815</td>
<td>67.82</td>
</tr>
<tr>
<td>Bone</td>
<td>6 m.a.s.</td>
<td>427.5</td>
<td>425</td>
<td>60.76</td>
</tr>
<tr>
<td>Bone + PRP</td>
<td>6 m.a.s.</td>
<td>626.2</td>
<td>650</td>
<td>97.07</td>
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<tr>
<td>Bone</td>
<td>7 m.a.s.</td>
<td>392.5</td>
<td>390</td>
<td>61.85</td>
</tr>
<tr>
<td>Bone + PRP</td>
<td>7 m.a.s.</td>
<td>500</td>
<td>500</td>
<td>40.82</td>
</tr>
</tbody>
</table>

b.s., before surgery; m.a.s., months after surgery; PRP, platelet-rich plasma.
Discussion

The results indicate a role of PRP in stimulating bone formation in human graft sites. This agrees with some studies (Antuña 1999; Marx 1999) that pointed to PRP as a potential source of GFs for bone regeneration. Although several authors (Jakse et al. 2003; Suba et al. 2004) report good outcomes, some studies carried out in experimental animal did not achieve satisfactory results (Fu¨rst et al. 2003; Roldán et al. 2004). In humans, PRP has been used with various graft materials. When applied with autologous bone (Philippart et al. 2003), with the results analyzed without suitable quantitative measures and on decalcified specimens, PRP did not appear to be a constituent capable of activating cells. On the whole, the overall indications gleaned from the literature are varied, as some authors believe that results from studies on sinus augmentation cannot be suitably compared owing to the absence of an adequate standardization (Wallace & Froum 2003; Graziani et al. 2004). This is the main reason that drove us to plan accurately all the procedures of our study, so as to standardize all predictable variables before evaluating the results from PRP plus autologous bone grafts.

The method that we adopted for the preparation of PRP is commonly used in many studies on PRP behavior in tissue regeneration [Marx et al. 1998; Camargo et al. 2002; Fürst et al. 2003; Roldán et al. 2004]. Institutional health facilities for processing blood and its derivates can guarantee a standardized preparation and high a quality of PRP, due to their high standard of competence in this specific field.

The use of specific and restricted criteria for inclusions of patients in this research undoubtedly limited the number of patients enrolled, but also favored better results. In agreement with some studies (Danesh-Meyer & Filstein 2001; Mazor et al. 2004), we included a broad range of age, characterized by skeletal system aging (Dao et al. 1993), to study a large quota lifespan. Although a wide span, patient age ranged between 37 and 57 years, with a mean age of about 50 years both in men and women. This rather high mean was mainly due to the indication of treatment of severe bone atrophy taken unlikely to be found in younger healthy patients. Statistical analyses highlighted an age distribution matched for gender. All patients were classified as class D (Jensen 1999), corresponding to a thin sinus floor (1–3 mm) and, consequently, to a more problematic possibility of bone regeneration. The high homogeneity between male and female patients and also between the two sinuses of each patient was essential to realize reliable results.

No roentgenographic differences were detected between the two sinuses in the short term. The reduction of grafted bone mass was observed clinically in both sinuses, but a slightly greater subsidence was recorded in sinuses grafted with only autologous bone. Note also in the latter how osteoclasts have broken the grafted bone into small fragments. Field width a = b = 6 mm; c = d = 800 µm.

Fig. 5. Microradiographs of biopsies of the same patient performed in the sinus treated with only autologous bone (group A - a and c) or with autologous bone plus platelet-rich plasma (group B - b and d) 4 months after surgery. Note the newly formed (woven) bone (●) showing the high density of irregular-shaped osteocytes. Note how the grafted bone (●) shows an indented outer surface, particularly pronounced in (c) the site treated with only autologous bone. Note also in the latter how osteoclasts have broken the grafted bone into small fragments. Field width a = b = 6 mm, c = d = 800 µm.

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Our definition of full surgical success attempted to classify the progress of treatment without automatically jeopardizing the implant insertion, in particular, after longer times. Success was defined by indicators [complications, recovery, morphologic and dimensional features of bone graft] expressing good viability and optimal
capability of graft for supporting implant-based prosthetic rehabilitation (Branemark 1983; Nystrom et al. 1993; Cawood et al. 1994). The \( \chi^2 \)-test showed no statistical significance between the clinical outcomes of the two treatments. The poor outcome bilaterally observed in one patient was probably due to chronic sinusitis, later acknowledged by the patient, which, moreover, relapsed during the 7 months after surgery.

Our densitometric results agree with those obtained by Rodriguez et al. (2003), who achieved a mean densitometric value increase of 35% in the site treated with PRP and Bio-Oss \( ^{\circ} \), 4 months after surgery. Our follow-up indicated a marked densitometric difference between sinuses: the PRP-treated sides always showed a higher mean densitometric value and a comparative densitometric increase of 70% at 4 months. No densitometric differences were instead recorded between males and females, both of initial densitometric values and their progress. The autologous bone group showed a statistically significant uniform decrease of densitometric values over time, whereas the PRP group showed similar values up to 4–5 months (plateau-like increase), followed by a decline. Regression lines of data converged at about 8 months after surgery. Agreeing with Marx et al. (1998), our results suggest that PRP might be more effective in humans for the interval of time up to 6–7 months after surgery, whereas no differences could be found between the two grafted sites in the long term.

Histology revealed differences in biopsies only for a short time. A greater osteogenic activity was recorded in PRP-treated sites at 4 months. In these sites, newly formed bone surrounded several autologous bone fragments showing viable osteocytes inside. This evidence may stem from the greater cell viability maintained by PRP. Instead, no marked difference in ALP activity and fluorescence was observed in the medium and long term between the two group biopsies. A TBV decrease was recorded in sinuses from 4 to 7 months, in particular in PRP-treated sites. In sites grafted with only autologous bone, the initial bony mass roentgenographically decreased over time, but the biopsies showed an almost constant amount of bone (TBV). In the PRP-treated sites, the bony mass reduction was lower, although we recorded a decreasing TBV and a steeper regression line of densitometries. Although we do not have data before 4 months after surgery, we can speculate that the bony mass produced by PRP stimulation during the first 4–5 months was abundant, causing a subsequent marked decrease, probably due to the absence of mechanical loading.

Owing to the biologic features of maxillary sinus, the clinician does not target a bony mass greater than the graft in the maxillary sinus. The clinician aims to preserve an adequate and viable bony mass (architecture and volume) to meet implant–prosthetic requirements. The bone results obtained are in agreement with studies where PRP was used to heal intrabony defects, such as periodontal pockets and sockets in animal models or humans (Camargo et al. 2002; Suba et al. 2004). In those sites, defined in a multiparietal bone condition, PRP appeared capable of inducing relevant bone regeneration and stabilizing the bone mass, also with bone substitutes.

In conclusion, our results do not point out statistical clinical differences between sites treated or not treated with PRP, whereas densitometric and histologic ana-

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**Fig. 6.** Morphology (a and b) and fluorescences (c and d) of group A (a and c) and B (b and d) biopsies of the same patient, 4 months after surgery. Note in c the only labeled (fluorescent) bone found in the whole biopsy. More newly formed (labeled) bone can be observed in the group B biopsy (d). Field width a-d = 375 μm.
yses indicate better short-term results for PRP. Several studies (Marx et al. 1998; Marx 1999; Schmitz & Hollinger 2001; Rodriguez et al. 2003; Roldán et al. 2004) describe a decrease of the grafted bone mass using clinical observations and statistical regression models. The same authors state that no additional differences in a bone graft outcome were achieved 1 year after surgery, whether or not PRP was used. Larger studies are warranted to clarify this ostensible contrast.

Fig. 7. Morphology (a, b: toluidine blue stain; c, d: under polarized light, trypan blue stain) and histochemistry (e, f: TRAP, tartrate-resistant acid phosphatase; g, h: ALP, alkaline phosphatase) of group A (a, c, e and g) and B (b, d, f and h) biopsies of the same patient, 4 months after surgery. The red arrows in a point to the empty lacunae of the grafted bone (which appeared lamellar in c), surrounded by newly formed woven bone. Note how no osteoclast activity (e) and ALP expression (g) was found in the group A biopsies. The yellow arrows in b point to living osteocytes (inside lacunae) of grafted bone (lamellar in d). Note in f how a few osteoclasts resorb grafted bone fragments and in h the good ALP expression of soft tissues of the group B biopsy. Field width a–h = 405 μm.

Fig. 8. Morphology (a: toluidine blue stain; b: under polarized light, trypan blue stain) and histochemistry (c: alkaline phosphatase [ALP]) of a group B (autologous bone plus platelet-rich plasma) biopsy of the same patient, 7 months after surgery. The yellow arrows in a point to the lamellar bone (whose structure is displayed in b), containing the typical ellipsoid-shaped osteocytes, newly formed in apposition to the previously formed woven bone. Note in c the low ALP expression of the soft tissues of the group B biopsy. Field width a–c = 405 μm.

Fig. 9. Graph showing the trabecular bone volume (TBV) amount (express as bone percent of the whole biopsy) of all group A (autologous bone – ○ ) and group B (autologous bone plus platelet-rich plasma – □ ) biopsies, 4, 5, 6 and 7 months [m] after surgery. Bars correspond to the mean TBV value. Note how TBV remains almost constant in group A biopsies and decreases over time in group B biopsies, although they always have higher mean values.
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References


