Effect of Platelet-Rich Plasma With Autogenous Bone Graft for Maxillary Sinus Augmentation in a Rabbit Model

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Purpose: To evaluate the effect of platelet-rich plasma (PRP) on autogenous bone graft remodeling during sinus augmentation in a rabbit model.

Materials: Twelve New Zealand White rabbits were divided randomly into 3 groups based on their time of sacrifice (2, 4, and 8 weeks). All animals underwent a general anesthetic and harvesting of an autogenous bone graft from the right iliac crest with subsequent bilateral maxillary sinus augmentation. PRP was prepared via standard approved technique by acquiring 21 cc of autogenous blood and performing differential centrifugation to obtain PRP. One cc of PRP was produced that was mixed with bovine topical thrombin and calcium chloride. The left maxillary sinus received only autogenous bone, while the right maxillary sinus received a mixture of PRP mixed with autogenous bone, thus each animal acted as its own control. Equal volumes of bone were inserted in each maxillary sinus. Animals were sacrificed at 2, 4, and 8 weeks and all specimens were harvested for peripheral quantitative computed tomography (pQ-CT), static, and dynamic and histomorphometric analysis.

Results: Student t tests were performed comparing bone density via pQ-CT analysis, histomorphometric parameters of total bone area, and bone apposition rate. PRP had no statistically significant effect on bone graft healing in maxillary sinus augmentation when compared using standard pQ-CT, static, and dynamic histologic criteria.

Conclusion: This study fails to find a direct stimulatory effect of PRP on healing of autogenous bone grafts using pQ-CT, static, and dynamic histomorphometric analyses.

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More than 25 million Americans are completely edentulous, with even more being partially edentulous. Osseointegrated implants are considered the ideal replacements for missing teeth; however, successful osseointegration is often compromised by bone loss at edentulous sites. In the maxilla, both alveolar resorption and expansion of the maxillary sinus reduce 3-dimensional volume of the alveolus. To restore bone volume and facilitate the placement of osseointegrated implants, a therapeutic procedure involving maxillary sinus floor augmentation is often performed.1

The maxillary sinus represents a unique environment in that it has decreased vascularity, decreased oxygen tension, and is subject to varying levels of intra-antral pressures that have been shown to influence bone graft healing.1,2 A graft to this region is further complicated by the integrity of the thin, delicate sinus membrane that is susceptible to perforation and sinusitis.

Strategies to accelerate autogenous bone graft healing have recently included the use of platelet-rich
plasma (PRP). PRP is a highly concentrated form of autogenous platelets, providing a rich and readily obtainable source of a diverse group of osteogenic growth factors. Its use with autogenous bone grafting was first reported by Whitman et al in 1997. A subsequent study by Marx et al compared PRP’s effect on autogenous bone grafts for mandibular reconstruction in humans and found its addition resulted in a statistically significant increase in radiographic graft maturation versus control over time. Unfortunately, a paucity of literature exists in which an objective histomorphometric analysis of PRP’s effect on skeletal healing is evaluated over time. Thus the present study was undertaken to objectively quantify the effect of PRP on autogenous bone grafts of the maxillary sinus in a rabbit model.

Materials and Methods

This study was approved by the Institutional Ethics Review Committee for Animal Research at the University of Connecticut (Farmington, CT). Twelve New Zealand male rabbits, each weighing between 3 to 3.5 kg, were randomly assigned to 3 groups of 4 animals each. All groups received the same surgical procedures and evaluation, but were sacrificed at different times: group 1 at 2 weeks; group 2 at 4 weeks; and group 3 at 8 weeks.

All animals received general anesthesia using a combination of ketamine 80 mg/kg intramuscular (Pfizer Parke-Davis, New York, NY) and xylazine hydrochloride 10 mg/kg intramuscular (Lloyd Pharmaceuticals, Shenandoah, Iowa) induction. Immediately before surgery, 21 cc of whole blood (approximately 10% circulating blood volume) was withdrawn via ear arterial aspiration and mixed with citrate phosphate dextrose at a ratio of 1 cc citrate phosphate dextrose to 5 cc whole blood, achieving anticoagulation through calcium binding. One cc was saved for determination of platelet concentration. This was followed by placement of a peripheral intravenous catheter with infusion with 0.45% normal saline. The preparation of PRP was performed simultaneously during the surgical procedures. A small sample of whole blood, platelet-poor plasma, and PRP was saved for determination of platelet concentration via standard Coulter Counter technique (Coulter Corporation, Miami, FL).

Each animal underwent harvesting of a corticocancellous autogenous bone graft from the right posterior iliac crest via standard surgical technique. A skin incision was then made in each maxillary region, 4 mm above the inferior junction of the incisive bone and maxilla. The subcutaneous tissue was divided to expose the maxillary periosteum, which was incised and elevated dorsally. A round diamond bur was used to outline the lateral antral wall of the maxilla, and the antral membrane was elevated from the sinus floor, with care being taken not to compromise its integrity. Each autogenous bone graft was morselized to uniform size and consistency using an MX Grafter (Maxilon Laboratories, Hollis, NH) into 2 equal volumes. The non-PRP side had 1 cc of autogenous morselized corticocancellous graft placed as per standard sinus augmentation technique. The contralateral side had 1 cc of autogenous bone graft mixed with 0.5 cc of PRP. The PRP was mixed with a combination of calcium chloride/topical bovine thrombin (10 cc of 10% calcium chloride mixed with 10,000 units of topical bovine thrombin (Gentrac, Middleton, WI)) (Fig 1), initiating the coagulation process. The graft mixture was placed per routine technique, and followed with usual periosteal/soft tissue closure using 3-0 polygalactin sutures (J & J Ethicon, Somerville, NJ).

Postoperative analgesic control was maintained as appropriate with buprenorphine (Reckitt and Colman Pharmaceuticals, Wayne, NJ). The subjects remained in a standard laboratory research housing facility, receiving water and food ad libidum, and medical care as needed.

The animals were injected with the fluorochromes calcine (10 mg/kg subcutaneous) and xylene orange (60 mg/kg subcutaneous) at 10 days and 2 days, respectively, prior to sacrifice to label de novo bone formation for dynamic histomorphometric analysis.

Each group of animals was sacrificed at their predetermined time (2, 4, or 8 weeks).
QUANTITATIVE COMPUTED TOMOGRAPHY

Quantitative computed tomography was used to image the maxillary sinus grafts at incremental steps of 0.5 mm (XCT Research M, Norland Medical Systems, White Plains, NY). Following phantom calibration of the instrument, bone mineral density (mg/cm³) was measured within the grafted region to 1% precision (± 3 mg/cm³).

HISTOMORPHOMETRY

All specimens were harvested and fixed in 70% ethanol at the time of sacrifice. Undecalcified specimens were dehydrated in increasing concentrations of ethanol, cleared in xylene, and embedded in methyl methacrylate. Five micron thick longitudinal serial sections were cut on a Reichert-Jung Poly-cut S microtome (Reichert-Jung, Germany) with a D profile knife (Delaware Diamond Knives Corp, Wilmington, DE). Sections were taken from the middle of the grafted region. Unstained sections were used for dynamic histomorphometric analysis, while static histomorphometric parameters were measured using alternate sections stained with Masson-Trichrome. All grafts were evaluated in a blinded, nonbiased manner using the Bioquant Computerized Image Analysis System (BIO-QUANT; R & M Biometrics, Nashville, TN) interfaced with a Nikon E400 microscope (Nikon Inc, Melville, NY). Measurements were confined to a total area of 1 × 10⁶ μm² of tissue.

Results

PLATELET COUNTS

Platelet counts confirmed that the PRP preparation technique used in this study produced a source of highly concentrated platelets. The average whole blood platelet count was 468,000 ± 182,000/mm³. The average PRP platelet count was 2,061,000 ± 854,000/mm³, while the platelet count in the platelet-poor plasma was 15,000 ± 5,200/mm³ (Fig 2).

QUANTITATIVE COMPUTED TOMOGRAPHY

Bone density measurements via quantitative computed tomography revealed no statistically significant differences between PRP and non-PRP groups at 2, 4, and 8 weeks postoperative (Table 1). Density in the PRP 350 ± 51 mg/cm³ at 2 weeks to 396 ± 47 mg/cm³ at 8 weeks, while in the non-PRP group density increased from 331 ± 55 mg/cm³ at 2 weeks to 370 ± 58 mg/cm³ at 8 weeks (Fig 3).

Table 1. Q-CT EVALUATION OF BONE DENSITY: PRP VS NON-PRP

<table>
<thead>
<tr>
<th>Week</th>
<th>PRP Mean (mg/cm³)</th>
<th>PRP SD</th>
<th>Non-PRP Mean (mg/cm³)</th>
<th>Non-PRP SD</th>
<th>P &lt; .05</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>350</td>
<td>51</td>
<td>331</td>
<td>55</td>
<td>.08</td>
</tr>
<tr>
<td>4</td>
<td>381</td>
<td>77</td>
<td>349</td>
<td>67</td>
<td>.16</td>
</tr>
<tr>
<td>8</td>
<td>396</td>
<td>47</td>
<td>370</td>
<td>58</td>
<td>.12</td>
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</table>

FIGURE 2. Platelet counts.


FIGURE 3. Quantitative computed tomography: Coronal image of bilateral maxillary sinus grafts in rabbit at 4 weeks. Note the equal bone graft volume and density between the PRP-grafted side (right) and non-PRP side (left).

was no statistically significant difference ($P > .05$) between PRP and non-PRP groups at any of the times assessed (Fig 5). Within-group comparisons showed a statistically significant difference within the PRP group between 2 and 4 weeks, and 2 and 8 weeks, but not at any other time (Table 5).

**Discussion**

Maxillary sinus augmentation is a viable therapeutic intervention for increasing the height of the posterior maxilla when there is inadequate bone to allow for the placement of osseointegrated implants. Numerous materials have been used for sinus-floor augmentation with variable results. Of these materials, autogenous bone graft is still considered to be the gold standard for such procedures.\(^1\)

Watanabe et al were the first to use and validate the rabbit model for maxillary sinus augmentation with autogenous bone.\(^3,9,10\) The rabbit also serves as an appropriate model for PRP investigations because its hematologic status is similar to humans (ie, rabbit blood has equal or higher levels of coagulation factors compared with humans), with platelet counts that average 400,000/mm\(^3\) while also actively coagulating with bovine thrombin.\(^11,12\) Previous investigations have shown that most osteogenic remodeling of the autogenous bone graft in a rabbit model will occur within the first 6 to 8 weeks postsurgery.\(^10,13-16\) Albrektsson’s study\(^13\) of bone graft healing showed a revascularization rate of 0.2 to 0.4 mm/day in rabbits, with early vascularization equating to early bone remodeling. Cancellous bone healing showed vascular ingrowth at day 5, with full graft vascularization by day 20. Graft healing was found to be complete by day 40. In cortical bone healing, full graft vascularization was complete by day 30, with the osteogenic phase ending by day 35. Similar results were found by Roberts et al,\(^16\) who determined that the resorption and reversal phase is approximately 1 week in rabbits; while the duration of the bone formation phase was about 5 weeks. Thus, by evaluating the animals at day 14, 28, and 56, the entire resorption/deposition phase

### Table 2. Between groups comparison for percent bone area: PRP vs non-PRP

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Non-PRP Mean</th>
<th>Non-PRP SEM</th>
<th>PRP Mean</th>
<th>PRP SEM</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>25.4</td>
<td>4.0</td>
<td>26.4</td>
<td>5.7</td>
<td>0.9</td>
</tr>
<tr>
<td>4</td>
<td>20.6</td>
<td>4.3</td>
<td>17.8</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>8</td>
<td>20.7</td>
<td>6.7</td>
<td>21.1</td>
<td>5.9</td>
<td>0.97</td>
</tr>
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</table>

of corticocancellous bone graft healing would have been observed.

The PRP preparation technique used in this study provided clinically significant concentrates of platelets, with a mean PRP platelet count of 2,061,000/mm³, fully 4.4 times the mean of the whole blood platelet count. Little consensus exists in the hematology literature as to the exact definition of PRP, or its ideal preparation technique. The platelet-pelleting technique used in this study was similar to previously published techniques.4,5,17-27 Numerous studies provide evidence to support the use of a standard centrifuge for 2 separate spins to produce viable PRP.17,19-23,25,27 Some studies have questioned the effect of differential centrifugation on the integrity of the remaining platelets. Ledent et al28 showed that whole blood centrifuged at 1,500 g for 90 seconds resulted in a minimal release of growth factors from the platelets. However, PRP subjected to centrifugation forces of 2,500 g resulted in a significant release of growth factors from the platelet alpha-granules (17 ± 2% for platelet-derived growth factor, and 19 ± 3% for transforming growth factor-β). Thus, the PRP preparation technique in this study would have had minimal effect on platelet integrity and resultant concentrations of platelet growth factors.

PRP is a source of a myriad of growth factors found within the alpha-granules of platelets, including29-33 platelet-derived growth factor, transforming growth factor-β1 and -β2, vascular endothelial growth factor, platelet-derived endothelial growth factor, interleukin-1, basic fibroblast growth factor, and platelet activating factor-4. Among the growth factors found in PRP, platelet-derived growth factor, basic fibroblast growth factor, and transforming growth factor-β1 and -β2 are the most osteogenically active growth factors found within PRP. PRP growth factors do not induce osteoprogenitor cell differentiation, as is seen with the bone morphogenetic proteins, but instead act via stimulation of chemotaxis, mitogenesis, and angiogenesis of surrounding cells, acting as a catalyst in the very early phases of bone remodeling.34 As was found

| Table 3. WITHIN GROUP COMPARISONS: PERCENT BONE AREA |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Non-PRP 2 vs 4 | 25.4 4.0 | 20.6 4.3 |
| Non-PRP 4 vs 8 | 20.6 4.3 | 20.7 6.7 |
| PRP 2 vs 4    | 25.4 4.0 | 20.7 6.7 |
| PRP 4 vs 8    | 17.8 0.7 | 21.1 5.9 |
| PRP 2 vs 8    | 26.4 5.7 | 21.1 5.9 |


<p>| Table 4. BONE FORMATION RATE BETWEEN GROUPS COMPARISON: PRP VS NON-PRP |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Weeks</th>
<th>Non-PRP Mean* SEM*</th>
<th>PRP Mean* SEM*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.23 0.13</td>
<td>0.09 0.06</td>
<td>.38</td>
</tr>
<tr>
<td>4</td>
<td>0.48 0.21</td>
<td>0.55 0.17</td>
<td>.80</td>
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<tr>
<td>8</td>
<td>0.80 0.05</td>
<td>0.85 0.02</td>
<td>.44</td>
</tr>
</tbody>
</table>

*(μm²/day/μm).*


FIGURE 5. Calcein/xylene orange staining of PRP/autogenous bone graft (A) and of non-PRP/autogenous bone graft (B) at 4 weeks. (Magnification ×10.) The interlabel distance between the fluorescent labels reveal a similar amount of new bone formed.

in earlier studies, neovascularization is evident at day 14 in the rabbit maxillary augmentation model without PRP. In the present study, a moderate number of new blood vessels entering the PRP-enhanced graft were present in the 14 day specimen, but this was not subjectively different from the non-PRP side.

Despite the theoretical benefits of osteogenic growth factors within PRP, the results of the present study did not show a significant increase in histologic total bone area, bone formation rate, or bone density when bone and PRP-enhanced bone grafts were compared. Comparisons within groups did show a statistically significant difference in total bone area and bone formation rate in the PRP group between 2 and 4 weeks. This is not unexpected, as the most influential time in which PRP would exert its effect is the very early healing period, as the lifespan of a platelet in a wound and the period of the direct influence of its growth factors is less than 5 days. These results are consistent with previous studies investigating the effect of PRP in rabbit bone healing, in which Aghaloo et al compared the healing of autogenous bone alone, and autogenous bone with PRP in noncritical size defects in the rabbit cranium at 1, 2, and 4 months. In that study, there was no statistically significant effect of PRP on bone healing when assessed for bone density and static histomorphometric changes. Marx et al evaluated the reconstruction of 88 mandibular continuity defects, with one half of subjects receiving PRP. The graft maturity was subjectively evaluated radiographically and the PRP grafts were assessed either at or slightly more than twice their actual maturity. Unfortunately, both the subjective nature of quantifying graft maturity and the lack of correlation between radiographic density and histomorphometric graft maturity recently reported by Schultz-Mosgau et al questions the authors’ conclusions that PRP has a direct stimulatory effect on bone graft healing. In contrast, the present study has shown through objective analysis that PRP failed to provide a statistically significant direct stimulatory effect on healing of autogenous bone grafts in maxil-


