Quantitative Evaluation of Extraction Socket Healing Following the Use of Autologous Platelet-Rich Fibrin Matrix in Humans

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Platelet-rich fibrin matrix (PRFM) is an autologous biologic material created by centrifugation of blood. This study quantified ridge changes associated with the healing of 21 extraction sites using PRFM alone as a graft. Standardized measurements of ridge width and height were recorded at extraction, after graft placement, and after 4 months of healing. Mean width resorption 3 and 5 mm apical to the crest was 0.32 mm (4.71% loss) and 0.57 mm (7.38% loss), respectively. Mean height resorption was 0.67 mm (7.13% loss). Sites grafted with PRFM alone displayed rapid clinical healing, minimal flap reopening, and excellent bone density. Advantages of PRFM alone include less surgical time, elimination of techniques and potential healing difficulties associated with membranes, and less resorption during healing, as compared to guided bone regeneration procedures. (Int J Periodontics Restorative Dent 2011;31:285–295.)

Guided bone regeneration (GBR) following tooth extraction is the most accepted technique for preservation and regeneration of host bone in localized defects of the alveolar ridge.1–4 Currently, GBR procedures employ membranes and nonviable commercially prepared or autogenous osseous graft materials. Healing following treatment with these membranes and graft materials often results in compromised outcomes because of the avascular and inert nature of the bone graft materials, movement and exposure of the membrane,5,6 mucoperiosteal flap design, and surgical and suturing techniques. With recent advances and increased knowledge of wound healing, new approaches are being developed that may play a more active role in healing while eliminating the potential downside of nonvital materials. One line of investigation that appears promising involves the use of platelet-rich plasma (PRP).

PRP is an autologous preparation of concentrated platelets and plasma created through centrifugation of a patient’s blood. There

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have been numerous publications on the use of PRP as an adjunct for periodontal and oral surgery applications.\(^7\)\textsuperscript{-12} Although many of these studies have reported excellent outcomes,\(^13\)\textsuperscript{-15} the results of other animal and human investigations have not demonstrated the beneficial effects of PRP.\(^9\)\textsuperscript{,16\textsuperscript{-18}} The contradictory evidence appears to be related to differences in study design, PRP preparation methods, methods for quantifying tissue healing, and graft materials used, to name a few variable factors.

The rationale, which supports the use of PRP, is that PRP is a reservoir of autologous growth factors.\(^11\)\textsuperscript{,19\textsuperscript{-20}} The platelets in PRP contain granules that release multiple growth and differentiation factors on activation (Table 1).\(^12\)\textsuperscript{,18\textsuperscript{-19}} These growth factors are normally released into the tissue during wound healing, and they regulate cellular events such as proliferation, differentiation, chemotaxis, extracellular

| Table 1: Growth factors found in platelets in PRFM |
|---------------------------------|---------------------------------|---------------------------------|
| Factor | Target cell/tissue | Function |
| PD-EGF | Blood vessel cells, outer skin cells | Cell growth, recruitment |
| Fibroblasts and many other cell types | | |
| PDGF, A+B | Fibroblasts, smooth muscle cells, chondrocytes, osteoblasts, mesenchymal stem cells | Potent cell growth, recruitment |
| | Blood vessel growth, granulation |
| | Growth factor secretion; matrix formation with BMPs (collagen and bone) |
| TGF-β1 | Blood vessel tissue, outer skin cells | Blood vessel (+/-), collagen synthesis |
| Fibroblasts, monocytes | Growth inhibition, apoptosis (cell death) |
| TGF gene family (includes BMPs) | Differentiation, activation |
| Osteoblasts (highest levels of TGF-β1) | |
| IGF-1,2 | Bone, blood vessel, skin, other tissues | Cell growth, differentiation, recruitment |
| Fibroblasts | Collagen synthesis with PDGF |
| VEGF/ECGF | Blood vessel cells | Cell growth, migration, new blood vessel growth |
| | Antiapoptosis (anti–cell death) |
| bFGF | Blood vessels, smooth muscle, skin | Cell growth |
| Fibroblasts, other cell types | Cell migration, blood vessel growth |

PRFM = platelet-rich fibrin matrix; PD-EGF = platelet-derived epidermal growth factor; PDGF = platelet-derived growth factor; TGF-β1 = transforming growth factor beta1; IGF = insulinlike growth factor; VEGF/ECGF = vascular endothelial growth factor/endothelial cell growth factor; bFGF = basic fibroblast growth factor; BMP = bone morphogenic protein.
matrix synthesis, and morphogenesis of tissues and organs. Therefore, if these growth factors can be released at higher concentrations at the onset of wound healing through the addition of PRP, enhanced and accelerated wound healing can be expected.

Investigations employing PRP have reported accelerated fibroblast proliferation and bone repair, increased tissue vascularity and rate of collagen formation, and mitosis of mesenchymal stem cells as well as osteoblasts. If PRP releases growth factors, then the use of PRP in socket preservation procedures should lead to enhanced wound healing, as compared to those sites treated with nonbioactive graft materials. To test this hypothesis, a histologic and histometric study was performed in dogs comparing platelet-rich fibrin matrix (PRFM) (FIBRINET, Cascade Medical), which is an autologous concentrated platelet-rich thrombin-free fibrin gel, to conventional GBR techniques, which use demineralized freeze-dried bone allograft (DFDBA) and absorbable collagen membranes. PRFM is an autologous biologic material created by a two-step centrifugation of whole blood. The first spin separates the PRP and the platelet-poor plasma (PPP) from the red and white blood cells. The second spin of PRP and PPP results in viable platelets in a fibrin matrix. The investigation, on four mongrel dogs, evaluated the healing of sockets, treated with PRFM and GBR, at 10 days and 2, 3, 6, and 12 weeks postoperative.

By 2 weeks, PRFM-treated sites were filled with well-organized connective tissue and new bone was observed growing from the periphery of the socket toward the center. At 6 weeks, PRFM sites had complete osseous fill. The sites treated with DFDBA and a membrane exhibited much slower healing. At 3 weeks, GBR-treated sockets were still filled with nonvital DFDBA particles, a dense inflammatory infiltrate, and no new bone could be seen at the periphery of the socket. By 12 weeks, GBR-treated sites still contained osseous particles surrounded by an inflammatory infiltrate, a small quantity of connective tissue in the coronal two-thirds of the socket, and some new bone in the apical third of the site. It appeared that the 12-week sites treated with DFDBA displayed some resorption of thin crestal bone not noted in PRFM sites.

Clearly, these results would seem to suggest that extraction sites grafted with PRFM alone have more rapid healing and possibly less bone resorption than sites treated with DFDBA, at least in dogs. Consequently, a clinical study was designed to determine whether a PRFM graft alone would result in rapid healing and minimal bone resorption during ridge preservation healing in humans. The specific purpose of the investigation was to quantify the dimensional change of alveolar ridge changes that occur when using PRFM alone as a graft material in extraction sockets for ridge preservation procedures.

Method and materials

The study population consisted of 21 subjects (12 women, 9 men; age range, 24 to 63 years) who required tooth extraction and a GBR procedure, followed by implant therapy. The Institutional Review Board approved the protocol for this investigation. Patients were enrolled in the study after informed consent was obtained. Exclusion criteria consisted of individuals with known systemic risk factors for impaired wound healing such as diabetes or smoking, patients on medications that impair platelet function, pregnant women, and immunoincompetent subjects.

Each patient received a standardized diagnostic examination, which included periapical radiographs, study casts, clinical photographs, and a clinical evaluation of the patient’s dental and periodontal status. All necessary adjunctive treatment was performed before the approved tooth extraction and implant placement were initiated.

To standardize the measurements, a customized vacuum-form stent was fabricated to measure width at 3 and 5 mm apical to the crest, and an acrylic stent was fabricated to measure height. The stents ensured standardized measurements since they served as fixed reference guides for height and width measurements of the alveolar ridge. Stability of the stents was achieved by including the occlusal surfaces of the teeth adjacent to the extraction site. The stents had three demarcations that...
would allow for reproducible measurements to be made: (1) the midpoint of the extraction socket, (2) 3 mm distal to the midpoint demarcation, and (3) 3 mm mesial to the midpoint.

Measurements

One examiner performed all measurements. The collected data included measurements of alveolar height and width at three specific time intervals: (1) immediately following tooth extraction, (2) after ridge augmentation, and (3) after 4 months of healing, at the time of implant placement. Alveolar ridge height was measured by inserting a patient-dedicated UNC Williams periodontal probe (Hu-Friedy) perpendicularly through each of the three demarcation grooves located on the height stent. The probe extended to the most coronal aspect of the palatal aspect of the crest of the alveolar ridge. Measurements were recorded in millimeters at the coronal edge of the stent.

Alveolar width measurements were made using a ridge mapper, which had the specificity to measure the width at 3 and 5 mm apical to the alveolar crest. The width dimension was determined by the opening of the ridge mapper’s tips and measured using an electronic caliper that recorded to a tenth of a millimeter. To ensure the utmost reproducibility, the ridge mapper was inserted into the grooves, parallel to the Ala Tragus line, at the three measurement points.

Surgical procedure

A 9-cc venous blood draw was performed and collected in a tube, which contained trisodium citrate and a patented thixotropic separation gel. The tube was placed in a centrifuge and spun at 1,100 xg for 6 minutes. The patented gel separates the plasma and platelets in the supernate from the red blood cells at the bottom of the tube (Fig 1). This separation is stable and can remain in the tube for up to 1 hour.

Following administration of a local anesthetic (2% Xylocaine with 1:50,000 epinephrine, Cook-Waite) buccal and lingual full-thickness flaps were reflected (Fig 2). After atraumatic extraction and socket debridement (Fig 3), the extraction sockets were categorized into five different classes (Table 2).

Table 2 Extraction site classification*

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
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<tr>
<td>I</td>
<td>All four walls of the socket remaining</td>
</tr>
<tr>
<td>II</td>
<td>Three intact walls and the fourth wall having coronal loss up to one half</td>
</tr>
<tr>
<td>III</td>
<td>Three intact walls and complete loss or almost complete loss of the fourth</td>
</tr>
<tr>
<td>IV</td>
<td>One intact wall and the other walls either partially missing or completely lost</td>
</tr>
<tr>
<td>V</td>
<td>All extraction socket walls are partially missing and some are completely lost</td>
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</table>

*Classification based on the number of walls remaining after extraction.
Upon completion of the extraction, the supernate in the collection tube was transferred to a second test tube, which contained calcium chloride (Fig 4). The transferred supernate contains the PRP (which includes intact platelets) and plasma proteins (notably fibrinogen/fibrin). The second test tube was spun at 1,450 xg for 15 minutes. During the spin, the stents were placed and initial measurements of alveolar bone height and width were recorded. Following the second spin, PRFM grafts (Fig 5) were removed from the test tube and immediately placed into the extraction sites (Fig 6). For extraction sites that were not Class I, PRFM was placed to reconstitute the missing walls. The stents were then reapplied for the second measurements. Primary closure of the flaps was achieved with 4-0 Teflon sutures (W.L. Gore).
Prescriptions included systemic antibiotics for 10 days (amoxicillin, 500 mg tid), an oral rinse of 0.12% chlorhexidine gluconate (twice a day; Procter and Gamble), and ibuprofen (400 mg, as necessary for pain). Sutures were removed 14 days postoperative, and patients were followed for 4 to 6 weeks to monitor soft tissue healing and oral hygiene.

After 4 months of healing, the third set of measurements was performed using the measurement stents (Fig 7). Dental implants were placed according to the manufacturer’s protocol (Fig 8), and patients were again prescribed systemic antibiotics for 10 days (amoxicillin, 500 mg tid), a 0.12% chlorhexidine rinse (bid), and ibuprofen (400 mg PRN pain). The postoperative follow-up sequence for all patients was identical to that after the first surgical procedure.

Statistical analysis

The measurements taken at the time of extraction, after PRFM augmentation, and after 4 months of healing were compared using repeated analysis of variance and the Student paired t test. The difference between the measurements at extraction and PRFM augmentation is the amount the extraction site was augmented; the difference between measurements at PRFM augmentation and after 4 months of healing is the amount of alveolar height and width loss that took place over the 4 months of healing. Since there were only slight differences in measurements, the data from the mesial, midpoint, and distal were pooled at each of the measurement time intervals.
Results

The results of this study were derived from 21 experimental extraction sites in 21 patients who started and completed the investigation. There were 6 molar sites and 15 nonmolar sites. Five molar sites were classified as Class I extraction sockets and 1 as a Class II extraction socket. Of the remaining 15 nonmolar sites, 8 sites were categorized as Class I extraction sockets, 5 as Class II, and 2 as Class III extraction sockets. All sites exhibited rapid soft tissue healing. There were no signs of infection or other adverse tissue reactions at any time. Only 3 surgical areas had minute flap reopening during the first week of healing, and those areas were completely filled in and epithelialized by the second week postoperative. The remaining 18 sites (86%) maintained closure throughout the 4 months of healing until implant placement. Sites that exhibited areas of flap reopening had no measurable differences in net width and height compared to sites without flap reopening.

Width

When the width measurements were pooled for Class I, II, and III extraction sites, as summarized in Table 3, the mean width 3 mm apical to the crest measured at extraction was 6.79 mm. At the time of implant placement (after 4 months of healing), the mean width measurement 3 mm apical to the crest was 6.47 mm. This resulted in a 0.32-mm (4.71%) loss of alveolar width during healing. Although quite small, this difference was statistically significant ($P < .05$).

The mean pooled width measurement at 5 mm apical to the crest taken at extraction was 7.72 mm. At the time of implant placement, the mean width measurement 5 mm apical to crest was 7.15 mm. This meant that there was a 0.57-mm (7.38%) loss of alveolar width during the 4-month healing period (Table 3). This slight difference in width was also statistically significant ($P < .05$).
The mean pooled height measurement at extraction was compared to the measurements taken at implant placement. There was a net loss of 0.67 mm of alveolar height in comparison to the height of the original socket (Table 3). This loss resulted in a statistically significant net loss of 7.13% of the preoperative height ($P < .014$).

**Height**

The changes in alveolar width and height recorded in this study compare favorably to changes in alveolar dimensions noted in investigations using bioabsorbable membranes with and without graft materials. Lekovic et al.\(^2\) recorded a 1.31-mm (17.79%) mean net loss of alveolar width and a 0.38-mm (11.59%) net loss of height after 4 to 6 months of healing when a polygalactide/polyactide membrane was used for ridge preservation in intact extraction sockets (Class I sockets in this study). A more recent investigation by Iasella et al.\(^5\) found that extraction sites treated with DFDBA and a collagen membrane incurred a mean net loss of 1.2 mm (13.04%) of preoperative width. Other studies have shown an even greater net loss of width and height following GBR healing.\(^{28,29}\)

**Net gain/loss relative to extraction socket classification**

Table 4 compares the width and height measurements for Class I, II, and III extraction sockets. In this study, there were 13 Class I, 6 Class II, and 2 Class III sites. The small number of Class II and III extraction sites did not permit valid statistical evaluations. However, the data suggest that the more walls that remain following extraction, the less net loss of bone that occurs during healing. The only statistically significant comparison noted was the difference in width measurements at 5 mm apical to the crest when Class I sockets were compared to the pooled means of the study, which included Class II and III sockets. Class I extraction sockets had only a 0.16-mm (2.04%) net loss of width during healing 5 mm apical to the crest, whereas the pooled mean loss was 0.57 mm (7.38%).

**Discussion**

PRFM is a viable and biocompatible autologous biologic material that can be used alone to maintain ridge dimension during preservation procedures, while at the same time stimulating rapid osseous fill of the socket. In this investigation, it was found that after 4 months of healing, the sockets were filled with bone that appeared quite mature, and the sites did not exhibit discernable coronal invagination. The dimensions of the alveolar ridge were almost completely preserved during healing, exhibiting only a small but statistically significant net loss of width and height.

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Although all of the studies cited employed different methodologies and cannot be compared directly,
it would appear that the net loss of alveolar bone width and height measured when PRFM alone was used as the graft for ridge preservation was less than that noted when membranes alone or membranes and grafts were used.

The favorable bone and soft tissue responses undoubtedly exist because PRFM possesses the ability to accelerate tissue regeneration by stimulating the normal physiology. The viable platelets in PRFM contain intrinsic growth factors, described in Table 1, that affect every aspect of soft tissue and osseous healing. For these growth factors to accelerate wound healing, they need to be present over time to affect wound healing events that take place sequentially in soft tissue and bone regeneration. A study by Leitner et al30 that compared four preparations of PRP, including PRFM, suggested that the continued release of growth factors for up to 5 days is a common property of all tested preparations of PRP. However, only one growth factor, PDGF, was actually measured in the investigation. It is possible that the other intrinsic growth factors not measured may have diminished in concentration over the 5-day duration of the study.

The comparative data that Leitner et al30 presented appears to be somewhat inaccurate because of important methodology issues. First, the authors did not follow the manufacturer's instructions relative to the speed and time of the first centrifugation for the FIBRINET system (1,000 xg for 15 minutes vs the manufacturer's directions of 1,100 xg for only 6 minutes). The increased time and slightly slower revolutions/minute potentially adversely influenced the platelet yield measurements. Second, measurements were performed after the first spin of all systems, but PRFM requires a second spin to fully concentrate the platelets (2× concentration after the first spin and 4× concentration of platelets after the second spin). This changed the platelet concentration per unit volume that was recorded. Finally, the investigators did not activate the PRP preparations with exogenous activation after the initial centrifugation. All PRP preparations tested, except PRFM, require exogenous activation to form the gelatinous material used in oral surgical procedures. Therefore, the measurements of PDGF-AB that Leitner et al30 recorded were not on the activated PRP of the three systems, nor on the second spin of PRFM.

The issue of exogenous activation of most PRP systems may be important with respect to the length of time that the platelet growth factors will be present in the wound during healing. According to the conclusions of a flow cytometric study by O’Connell and Carroll, exogenous activation of platelets using bovine thrombin at the time of PRP preparation results in an almost immediate degranulation and disintegration of platelet granules. Consequently, virtually complete growth factor release will take place at the time of PRP

<table>
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<th>Table 4</th>
<th>Net difference (mm) and % change of width and height in healed extraction sockets</th>
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<tr>
<td>Pooled</td>
<td>Class I</td>
</tr>
<tr>
<td>Net difference</td>
<td>% change</td>
</tr>
<tr>
<td>Height</td>
<td>-0.67</td>
</tr>
<tr>
<td>Width (3 mm)</td>
<td>-0.32</td>
</tr>
<tr>
<td>Width (5 mm)</td>
<td>-0.57</td>
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preparation and placement. Since wound healing requires a sequential appearance of vessels and cells over time, the immediate and almost complete release of platelet granules may have very little effect on the later stages of healing.

In contrast, PRFM has been shown to have a sustained release of growth factors. Carroll et al documented a sustained release of platelet growth factors in a 7-day in vitro investigation. The concentration of six growth factors released from PRFM at the end of the 7-day study was almost identical to the concentration of the growth factors initially quantified. It would appear logical to assume that the decrease in growth factor concentration beyond 7 days would take place incrementally, and, therefore, bioactive concentrations could be present in the wound for extended periods of time.

Most PRP preparations in their initial centrifugation separate the patient’s blood into three fractions: the blood cell (white and red) layer, the platelet-rich layer (PRP), and the platelet-poor layer (PPP). The platelet-rich layer, after activation by an exogenous activator or by adding the patient’s whole blood, is transformed into a gelatinous material. In other systems, the PPP is added back to the PRP with calcium chloride and thrombin to obtain a more substantial clot. In the preparation of PRFM, the blood cell layer is separated from the platelets and plasma, and it is the PRP and PPP layers that are centrifuged, after recalcification, in the second faster and longer spin. During the second spin, a cross-linking of fibrin takes place, resulting in the formation of a dense fibrin matrix, within which a concentration of viable platelets can be found. The cross-linking also acts to stabilize the clot, prevent retraction, creates a consistency that resists displacement, and maintains space; the dense fibrin cross-linking inhibits soft tissue invasion. In addition, the fibrin acts as scaffolding for migrating endothelial cells, osteoblasts, and other cells during tissue repair. Having an organized fibrin matrix at the start of healing increases the speed of vascular ingress into the wound compared to nonaccelerated healing, which requires a longer time for fibrin formation and the development of vascularity. The earlier vascularity is established, the faster bone-forming cells will be able to enter the wound and bone formation can begin.

An additional advantage of using PRFM alone for alveolar ridge preservation is that the procedure does not require the manipulation and placement of a membrane or the inclusion of other nonvital graft materials. The benefits of not using a membrane include a faster and less complicated surgical procedure and elimination of the issues associated with infection, membrane movement, and membrane exposure. The increase in healing time associated with using nonvital graft materials has been documented in many studies.

In this investigation, augmentation with PRFM beyond the confines of the existing socket walls was unpredictable. Although the consistency resisted displacement, it was still subjected to compression associated with flap closure. Studies using PRFM for the treatment of chronic lower extremity ulcers have indicated that further increasing the second spin speed and duration will lead to a much denser membranelike fibrin matrix that may have the texture and consistency required for augmentation beyond the confines of the extraction socket. An additional possibility is augmentation of sites using PRFM in combination with a biocompatible, osteoconductive scaffold material, such as resorbable tricalcium phosphate, calcium sulphate, or resorbable hydroxyapatite.

References


