Platelet-rich Plasma: Is It Ready to Use?

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ABSTRACT

For over 20 years, autologous blood products, such as platelet-rich plasma (PRP) have been employed as a means to facilitate the healing process. Platelet-rich plasma has been advocated as a way to introduce increased concentrations of growth factors and other bioactive molecules to injured tissues in an attempt to optimize the local healing environment. This article reviews the basic principles involved in creating PRP and outlines the specific effects of these growth factors, both in vitro and in vivo, on periodontal wound healing.

Keywords: Healing, Platelet-rich plasma, Regeneration.


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Conflict of interest: None

HEALING OF TISSUES: DOWN THE LANE

The ultimate goal of periodontal therapy is not only to prevent periodontal disease progression but also to regenerate the lost dentition’s supporting structures, such as cementum, periodontal ligament, and bone to a diseased root surface where appropriate.1,2 But, at best, surgeons attempt to remove the known obstacles to healing, such as infection, instability, foreign bodies, etc.

A review of the literature related to wound healing shows that debridement and primary closure was the ‘hot topic’ of the 1950s. In the 1980s, the three seminal works by Knighton,3 Hunt,4 and Marx et al5 marked a paradigm shift by focusing attention on actively promoting healing rather than just removing the obstacles to it. First, introduced clinically by Knighton’s platelet-derived wound healing factor (PDWHF),6 and then through topical recombinant human platelet-derived growth factor bb (PDGFbb) (Regranex, Ortho McNeal) and today’s platelet-rich plasma (PRP), platelets have found to be the pivotal cells that initiate all human wound healing.

PLATELET- RICH PLASMA: WHAT IS IN A NAME?

Platelet-rich plasma has been classically (Table 1) described as ‘a volume of plasma that has a platelet count above baseline (of whole blood).’7 Although this definition would suggest a pure mixture of plasma (the acellular, liquid portion of blood that contains proteins involved in the clotting mechanism as well as other bioactive molecules that play a significant role in wound repair) and platelets (and their associated growth factors and cytokines), the generic term ‘PRP’ has recently expanded to include a variety of final products. These products can vary markedly not only in the final concentration of platelets they produce but also in the amount of red blood cells and/or white blood cells that are included in the final preparation. In addition, some techniques for creating PRP actively initiate the clotting cascade as part of the process, creating a fibrin scaffold. Because, the inclusion of these additional blood components may affect the indication(s), potency, and efficacy of the final PRP product, the generic classification ‘PRP’ does not allow distinction between the different systems and protocols.8 Therefore, to more precisely delineate these various products based on their leukocyte and fibrin content, categories, such as pure PRP, leukocyte rich PRP (L-PRP), pure platelet-rich fibrin, and leukocyte- and platelet-rich fibrin have been proposed.8

METHODS OF PREPARATION

At least 16 commercial PRP preparation systems are currently available (Table 2). A sample of peripheral venous blood is drawn and immediately spun in a centrifuge to separate the erythrocytes from the platelets and leukocytes. The increased density of the erythrocytes causes them to sink to the bottom of the centrifuge tube more rapidly than do the platelets and leukocytes. Further concentration then isolates PRP from platelet-poor plasma. Commercially available PRP kits concentrate platelets in the final injectate up to 9 times the normal concentration found in whole blood. The resultant volume of PRP and the final platelet and leukocyte concentrations differ among preparation systems.9

HOW MANY PLATELETS ARE ENOUGH?

This question has been elegantly answered by the work of Haynesworth et al,10 who showed that the proliferation
<table>
<thead>
<tr>
<th>System</th>
<th>Volume of blood (ml)</th>
<th>Final PRP volume (ml)</th>
<th>Final platelet concentration (compared with whole blood)</th>
<th>Available activator</th>
<th>Leukocyte concentration (10^3/l) (compared with whole blood)</th>
<th>Fibrinogen (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous conditioned plasma (Arthrex, Naples, FL)</td>
<td>9</td>
<td>3–5</td>
<td>2–3X</td>
<td>None</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Cascade (Musculoskeletal tissue foundation, Edison, NJ)</td>
<td>9 or 18</td>
<td>2 or 4</td>
<td>1.6X</td>
<td>Calcium</td>
<td>1.1 ± 0.2 (6-fold)</td>
<td>283.8 ± 34.2</td>
</tr>
<tr>
<td>GPS III (Biomet, Warsaw, IN)</td>
<td>27 or 54</td>
<td>3 or 6</td>
<td>2.1–9.3X</td>
<td>Calcium chloride/thrombin</td>
<td>34.4 ± 13.6 (5-fold)</td>
<td>286.0 ± 42.7</td>
</tr>
<tr>
<td>SmartPreP (Harvest Technologies, Plymouth, MA)</td>
<td>20 or 60</td>
<td>3 or 7</td>
<td>4.4–7.6X</td>
<td>Thrombin</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Magellan (Arteriocyte Inc, Cleveland, OH)</td>
<td>30,45 or 60</td>
<td>Variable</td>
<td>2.8–14X</td>
<td>NA</td>
<td>11.0 ± 8.2 (2-fold)</td>
<td>277.4 ± 30.5</td>
</tr>
</tbody>
</table>

The importance of this knowledge is that our studies have indicated that only the aforementioned FDA cleared devices consistently achieve this therapeutic level of platelet concentration, and hence growth factor release.

PLASMA: THE OTHER ‘P’ IN PRP

Perhaps the most consistent component of many of the PRP products is the plasma component. Plasma, the fluid portion of blood, is a remarkable liquid containing numerous ions, inorganic and organic molecules, and many of the same proteins found in platelets. Plasma differs from serum in that plasma still contains fibrinogen as well as a number of clotting factors. Therefore, when plasma is exposed to thrombin (either by the addition of
exogenous thrombin or by coming in contact with tissue thromboplastin (also known as tissue factor), the clotting cascade is initiated and platelets are activated. The resulting formation of a fibrin clot provides a provisional scaffold for cell migration as well as a reservoir of growth factors.\textsuperscript{12} Although \textit{in vitro} studies have documented significant differences in cell proliferation between PRP and platelet-poor plasma preparations,\textsuperscript{13} it is possible that the plasma component of PRP actually plays a more significant role in creating a proper local environment for tissue repair.

**Exogenous Thrombin: To Activate or not to Activate**

Platelet activation can be initiated by a number of methods, such as shear forces caused by fluid flow, contact with a variety of material including fibrillar collagen and basement membranes of cells, and thrombin. Most current PRP protocols rely on bovine or autologous thrombin with or without calcium chloride to congeal the solution and activate the platelets. Gelation of the PRP solution is necessary to localize the product (and thereby its effects) onto the area of interest without loss into the surrounding tissues. To minimize the risk of immune reaction associated with thrombin, some have used calcium chloride as an activator by itself.\textsuperscript{14} An alternative activator recently reported is type I human collagen.\textsuperscript{15} As previously mentioned, when PRP is injected into connective tissues, it comes into contact with tissue thromboplastin (tissue factor), which can activate platelets and initiate the formation of a fibrin scaffold. Therefore, the need for platelet activation with exogenous thrombin before injection is not clear.

**Mechanism of PRP-related to Growth Factors**

The growth factors secreted by the platelets (i.e. PDGFaa, PDGFbb and PDGFab) usually have two active sites and are, therefore, called dimers. They attach only to cells that have receptors to accommodate them. These receptors are on the surface membrane of the target cell (Fig. 1). The growth factor never enters the target cell; instead, it activates the membrane receptor, which has an intracytoplasmic portion and, therefore, is often termed as ‘transmembrane receptor’. Two adjacent transmembrane receptors are then brought within a critical distance of each other to activate dormant intracytoplasmic signal transducer proteins. A signal transducer protein, then detaches from the transmembrane receptor and floats in the cytoplasm toward the nucleus. In the nucleus, the transducer protein unlocks a specific gene sequence for a regulated cellular function, such as mitosis, collagen synthesis, osteoid production, etc. The significance of this process is that it explains why an exogenous application of growth factors, even in the highest concentration possible, cannot produce a sustained overreaction, such as a hyperplasia, a benign tumor, or a malignant tumor. Growth factors are not mutagenic; they are natural proteins acting through normal gene regulation and normal wound healing feedback control mechanisms.\textsuperscript{16}

**Effects of PRP Growth Factors on Cells Involved in Periodontal Wound Healing**

Periodontal wound healing involves gingival fibroblasts, gingival epithelial cells, periodontal ligament fibroblasts and osteoblasts, all of which are important for tissue repair and hard-tissue regeneration. A series of well-orchestrated cell—cell interactions is initiated after injury. Disruption of the vasculature as a result of injury leads to fibrin formation and platelet aggregation. Several growth factors are then released into the tissue from the platelets and from the adjacent cells after injury, including platelet-derived growth factor (PDGF), transforming growth factor-alpha, transforming growth factor-beta (TGF-beta) and insulin-like growth factor I (IGF-I). Bone and cementum may also release growth factors during wound healing (Table 3).

Periodontal and oral surgical techniques may involve use of these factors in both soft and mineralized tissues.
For example, local application of growth factors is used to promote healing, especially regeneration. Numerous studies, including some dental research, have shown that PDGF, TGF-beta and IGF-I are found in PRP and, because of their impact on wound healing, the use of these factors has led to promising results.12,14

Platelet-derived growth factor is a basic dimeric glycoprotein with two disulphide-bonded polypeptides, referred to as A and B chains. Three isoforms of PDGF are possible: AA, BB and the heterodimeric AB. All isoforms of PDGF are released after adhesion of platelets to an injured site. In vitro, all isoforms have proliferative activity on periodontal ligament fibroblasts.17 Platelet-derived growth factor is also chemotactic for these fibroblasts, and it promotes collagen and protein synthesis.

Insulin-like growth factor has two forms, I and II, each of which has two single chain peptides. Both forms of IGF are potent factors for survival of hematopoietic cells, fibroblasts and the nervous system.18 They are found in bone, and IGF-II is the most abundant growth factor in bone matrix. This form of IGF is chemotactic for periodontal ligament cells, and it has strong effects on periodontal ligament fibroblasts and protein synthesis.

Insulin-like growth factor-I stimulates bone formation by proliferation and differentiation,19 and it is synthesized and secreted by osteoblasts. It also has dose-dependent chemotactic effects on osteoblasts.20

Human patients treated with a combination of 150 mg/ml each of recombinant human platelet-derived growth factor-BB (rhPDGF-BB) and rhIGF-I in a methylcellulose vehicle experienced 43.2% osseous defect fill, whereas the control group (vehicle only) had 18.5% osseous fill.21

Transforming growth factor-beta is the name given to a group of homodimeric proteins involved in the formation and development of many tissues. Transforming growth factor-beta enhances collagen gel construction in vitro, and its effects are influenced by the combination of PDGF and IGF. In addition, TGF-beta stimulates biosynthesis of type I collagen and fibronectin and induces deposition of bone matrix.22

In a recent review, Yao and Eriksson23 reported that short shelf life and inefficient delivery to target cells are major concerns associated with local administration of recombinant human growth factors. The growth factors are expensive, and many doses may be required to achieve any therapeutic effect.23 In light of this PRP can be used as a pool of concentrated growth factors.

Clinical Effects on Osseointegration

Osseointegration of dental implants arises from cell migration, differentiation, bone formation, and bone remodeling along the implant surface; each of these processes is platelet and blood clot dependant.

During implant placement, the blood clot, or the PRP that is placed into the drill site, coats the implant surface as well as the microgap (about 25 μm wide) that lies between the actual bone and the metal surface.

<table>
<thead>
<tr>
<th>Growth factor</th>
<th>Effect</th>
<th>PRP concentration (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGF</td>
<td>Macrophage activation and angiogenesis</td>
<td>α: 117.5 ng/ml (63.4)</td>
</tr>
<tr>
<td></td>
<td>Fibroblast chemotaxis and proliferative activity</td>
<td>β1: 169.9 ng/ml (84.5)</td>
</tr>
<tr>
<td></td>
<td>Enhances collagen synthesis</td>
<td>β2: 0.4 ng/ml (0.3)</td>
</tr>
<tr>
<td></td>
<td>Enhances the proliferation of bone cells</td>
<td></td>
</tr>
<tr>
<td>TGF-β</td>
<td>Enhances the proliferative activity of fibroblasts</td>
<td>α1: 169.9 ng/ml (84.5)</td>
</tr>
<tr>
<td></td>
<td>Stimulated biosynthesis of type I collagen and fibronectin</td>
<td>α2: 0.4 ng/ml (0.3)</td>
</tr>
<tr>
<td></td>
<td>Induces deposition of bone matrix</td>
<td></td>
</tr>
<tr>
<td>PDEGF</td>
<td>Promotes wound healing by stimulating the proliferation of keratinocytes and dermal fibroblasts</td>
<td>470 pg/ml (320)</td>
</tr>
<tr>
<td>PDAF</td>
<td>Induces vascularization by stimulating vascular endothelial cells</td>
<td></td>
</tr>
<tr>
<td>PF-4</td>
<td>Stimulates the initial influx of neutrophils into wounds</td>
<td>0.189 nmol/ml (0.07)</td>
</tr>
<tr>
<td></td>
<td>A chemoattractant for fibroblasts</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A potent antihemarthropokinine agent</td>
<td></td>
</tr>
<tr>
<td>EGF</td>
<td>Cellular proliferation</td>
<td>51 pmol/l (5)</td>
</tr>
<tr>
<td></td>
<td>Differentiation of epithelial cells</td>
<td></td>
</tr>
</tbody>
</table>
| VEGF          | Angiogenesis | PDGF: Platelet-derived growth factor; TGF-β: Transforming growth factor beta; PDEGF: Platelet-derived endothelial growth factor; PDAF: Platelet-derived angiogenesis factor; PF-4: Platelet factor-4; EGF: Endothelial growth factor; VEGF: Vascular endothelial growth factor

Table 3: Effect of growth factors produced by platelets and their average concentration in platelet-rich plasma (PRP)24

For example, local application of growth factors is used to promote healing, especially regeneration. Numerous studies, including some dental research, have shown that PDGF, TGF-beta and IGF-I are found in PRP and, because of their impact on wound healing, the use of these factors has led to promising results.12,14
Table 4: Studies showing clinical effects of PRP with various bone substitutes

<table>
<thead>
<tr>
<th>PRP therapy</th>
<th>Researcher and year of study</th>
<th>Study protocol</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRP with autogenous bone</td>
<td>Marx et al (1998)</td>
<td>Forty-four continuity bone grafts to the mandible, placed without PRP, were assessed against 44 grafts placed with PRP at 2, 4, and 6 month maturity intervals with panoramic plain-film radiographs</td>
<td>Investigators assessed PRP grafts to be 2.16 times more mature at 2 months, 1.88 times more mature at 4 months, and 1.62 times more mature at 6 months. These differences were statistically significant (p = 0.001). The authors report 8% or 10% greater new bone formation in PRP group.</td>
</tr>
<tr>
<td>PRP with anorganic bone mineral</td>
<td>Wiltfang et al (2003)</td>
<td>A total of 45 sinus lifts were performed, with half receiving PRP and tricalcium phosphate</td>
<td>The authors report 8% or 10% greater new bone formation in PRP group.</td>
</tr>
<tr>
<td>PRP with organic bone matrix</td>
<td>Shanaman et al (2001)</td>
<td>Performed alveolar ridge augmentation on 3 patients, primarily using freeze-dried demineralized bone. The grafts were mixed with PRP and were protected with barrier.</td>
<td>The authors concluded that the addition of PRP did not appear to enhance the quality over that reported in comparable guided bone regeneration studies without PRP.</td>
</tr>
<tr>
<td>PRP when used alone</td>
<td>Papli and Chen (2007)</td>
<td>Compared the treatment of infrabony defects by an intralossional graft of PRP to guided periodontal regeneration (GPR) using a bioabsorbable barrier membrane (MEM) over a 52 week period.</td>
<td>Their case series suggested that an PRP graft achieves a similar CAL gain and PD reduction to GPR using an MEM over a 52 week period.</td>
</tr>
</tbody>
</table>

Within this microgap are found the usual components: platelets, red blood cells, white blood cells, and the cell adhesion molecules of fibrin, fibronectin, and vitronectin. In this situation, the cell adhesion molecules perform the important roles of coating the implant surface and bridging the microgap between the implant surface and the bone.

The model for osseointegration demonstrates that platelets degranulate and secrete their seven growth factors. As a result, the osteoclasts and marrow stem cells along the bony walls of the drill site proliferate and migrate along the strands of fibrin and other cell adhesion molecules spanning the microgap. As they migrate along the surface of the fibrin strands, the marrow cells can pull the fibrin strands off the implant surface. As the marrow cells migrate along the fibrin strands, they undergo differentiation and produce osteoid.

Clinical Applications of PRP

Different applications of PRP include sinus lift procedures, ridge augmentations, socket preservation, alveolar cleft palate repair, oral/nasal fistula repair, intrabony defects, furcation defects, jaw reconstruction surgeries, structural defects of the mandible and specially in medically compromised patients who undergo surgery have been suggested.25 Some clinicians also use PRP applications in soft-tissue procedures, like gingival grafts, subepithelial connective tissue grafts, etc., because of its property of increasing soft tissue healing.26 Platelet-rich plasma has also found its application in medicine as a treatment of skin ulcers, macular lesions, and corneal epithelial defects. Recent studies have shown that combining PRP with bone or bone substitutes present significant faster radiographic maturation and denser bone regeneration histologically. The results of few studies have been summarized in the Table 4.

CONCLUSION

Today’s understanding of bone science recognizes the pivotal role of growth factors in clinical bone grafting success. Platelet-rich plasma is seen as an available and practical tool for enhancing the rate of bone formation and the final quality of bone formed. Undoubtedly, all clinicians involved with bone grafting have high hopes that PRP will eventually prove to be of great benefit in bone graft healing. The conflicting results in today’s literature make it overwhelmingly evident that more research is needed before surgeons can feel confident in recommending this procedure to their patients.

REFERENCES


