Platelet-Rich Preparations to Improve Healing. Part II: Platelet Activation and Enrichment, Leukocyte Inclusion, and Other Selection Criteria

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Multiple platelet-rich preparations have been reported to improve wound and bone healing, such as platelet-rich plasma (PRP) and platelet rich fibrin (PRF). The different methods employed during their preparation are important, as they influence the quality of the product applied to a wound or surgical site. Besides the general protocol for preparing the platelet-rich product (discussed in Part 1 of this review), multiple choices need to be considered during its preparation. For example, activation of the platelets is required for the release and enmeshment of growth factors, but the method of activation may influence the resulting matrix, growth factor availability, and healing. Additionally, some methods enrich leukocytes as well as platelets, but others are designed to be leukocyte-poor. Leukocytes have many important roles in healing and their inclusion in PRP results in increased platelet concentrations. Platelet and growth factor enrichment reported for the different types of platelet-rich preparations are also compared. Generally, TGF-β1 and PDGF levels were higher in preparations that contain leukocytes compared to leukocyte-poor PRP. However, platelet concentration may be the most reliable criterion for comparing different preparations. These and other criteria are described to help guide dental and medical professionals, in large and small practices, in selecting the best procedures for their patients. The healing benefits of platelet-rich preparations along with the low risk and availability of simple preparation procedures should encourage more clinicians to incorporate platelet-rich products in their practice to accelerate healing, reduce adverse events, and improve patient outcomes.

Key Words: bovine thrombin, growth factors, leukocytes, platelet activation, platelet-rich plasma, wound healing

INTRODUCTION

Autologous platelet-rich plasma (PRP) was first developed in the early 1970s, but its use was rarely reported until after the 1980s. Historically, PRP was mixed with thrombin and excess calcium resulting in activated platelets trapped within the fibrin network; within the matrix, platelets secrete bioactive substances that slowly
diffuse into the surroundings tissues.\textsuperscript{1–9} PRP was introduced to the dental community by Whitman and colleagues, who hypothesized that the activation of platelets and the subsequent release of growth factors would enhance surgical healing.\textsuperscript{10} PRP is now commonly applied to surgical sites and injuries to promote wound healing.\textsuperscript{1,11} Types of platelet-rich preparations were discussed in Part I of this review,\textsuperscript{12} including cell separators, classic PRP, preparation rich in growth factors (PRGF), platelet-rich fibrin matrix (PRFM), simplified buffy coat (BC) method, and platelet-rich fibrin (PRF). To guide selection and preparation of PRP, information is discussed below on leukocyte inclusion, activation methods, platelet and growth factor enrichment, and other criteria.

**Leukocyte-Rich Versus Leukocyte-Poor Platelet Preparations**

Only some of the platelet-rich preparations will enrich leukocytes along with the platelets (reviewed in Part 1).\textsuperscript{12} For example, classic PRP, simplified BC-PRP, and PRF are leukocyte-rich—unlike PRGF, most cell separator methods, and PRFM. Therefore, when selecting the PRP method for clinical use, the potential advantages and disadvantages of leukocyte inclusion should be considered.

**Potential benefits of leukocytes in platelet concentrates**

Leukocytes have key roles in wound healing,\textsuperscript{13–18} so their presence in PRP may also enhance the repair process. The first white blood cells (WBC) that migrate to the wound site are the neutrophils. Their primary role is to phagocytize debris, microbes, and necrotic tissue to clean the wound and prevent infection. After completing these functions during the early inflammatory phase of healing, the neutrophils die by apoptosis. Monocytes are the second type of leukocytes recruited to the wound site, where they mature to become macrophages. Macrophages phagocytose the dead neutrophils and further debride the wound; debridement of the wound is critical for normal wound healing. Together with the neutrophils and platelets, macrophages also prevent infections by killing microbes.

Other critical roles of macrophages include the release of growth factors and cytokines, recruitment of other cell types to the wound, and stimulation of angiogenesis. Macrophages express transforming growth factor alpha (TGF\textsubscript{α}), transforming growth factor beta (TGF\textsubscript{β}), and platelet-derived growth factor (PDGF) only in wounds and wound sites depleted of macrophages that have reduced levels of TGF-β1 and other growth factors.\textsuperscript{17} Their other secretions also help the healing progress from the initial inflammatory phase to the proliferative phase. Inducing angiogenesis is a key role of macrophages. New blood vessels are essential for delivering nutrients, inflammatory cells, and oxygen; forming granulation tissue; and removing necrotic tissue. Accordingly, inadequate angiogenesis can result in impaired wound healing and ulcer formation.\textsuperscript{16} Adding activated macrophages to wounds in aging mice and humans accelerates healing time\textsuperscript{17}; thus, the principle concept of adding leukocytes with platelets to wounds may be similar. In the patient, before circulating monocytes can extravasate the blood vessels, applied PRP would supply monocytes to the wound. Since monocytes must attach to the extracellular matrix for differentiation,\textsuperscript{19} entrapment of the monocytes in the PRP fibrin clot would induce their maturation to macrophages. Thereby, perhaps the early and supplemented macrophage levels from leukocyte-rich PRP could shorten the inflammatory phase and wound healing.

Like platelets, macrophages and other leukocytes secrete many growth factors important in healing, including TGF-β1, PDGF, vascular endothelial growth factor, insulin-like growth factor (IGF), epidermal growth factor, TGF-α, and basic fibroblast growth factor (bFGF).\textsuperscript{14,20} One study compared PDGF-AB, PDGF-BB, and TGF-β1 levels in PRP samples that were either leukocyte-rich or leukocyte-poor. The presence of leukocytes resulted in significantly higher mean absolute levels of PDGF-AB and PDGF-BB compared to leukocyte-poor PRP.\textsuperscript{21} For TGF-β1, higher concentrations were detected for the leukocyte containing PRP when its levels were related to platelet concentration (per 10\textsuperscript{5} platelets).\textsuperscript{21} Statistics correlated platelet or monocyte concentrations as important variables for influencing these growth factor levels. These data suggest that leukocyte-rich PRP could augment growth factor availability to wounded tissues compared to leukocyte-poor methods.

In addition to growth factors, leukocytes express many proteinases, such as serine and metallopro-
teinases (MMP), which have critical roles in wound healing. Serine proteinases have multiple roles, including platelet and lymphocyte activation, antimicrobial activities, activation and inactivation of cytokines, and formation of the fibrin-platelet plug. Leukocyte proteinases contribute to migration of polymorphonuclear leukocytes and modulate inflammatory responses. Proteinases control the inflammatory response by deactivating inflammatory cells to limit injury to surrounding tissues during wound healing. Another important role of leukocyte-derived proteinases is to degrade the provisional matrix (fibrin-platelet clot) and remodel the extracellular matrix in normal wound healing. Degradation and remodeling of the matrix by proteases, especially MMPs, is important for many processes in wound healing, such as leukocyte migration, angiogenesis, re-epithelialization, and tissue remodeling. One crucial role of proteinases is to control the activity of growth factors, such as TGF-β, PDGF, and bFGF. TGF-β is released in an inactive (latent) form, and proteinases convert it to its active form. In addition, TGF-β and bFGF are stored bound to the extracellular matrix, and degradation of the matrix releases these growth factors so they can act on the wounded tissues. MMPs also have important roles in cartilage and bone remodeling and angiogenesis. Therefore, the inclusion of leukocytes in the PRP can directly provide these essential proteinases and their functions to the wound.

In preclinical studies in wild-type mice, some body sites failed to trigger the normal high-level inflammatory response with tissue damage. For example, damage to the oral mucosa results in a substantial reduction in neutrophils and macrophages migrating to the wound and a corresponding decline in several inflammatory cytokines and in TGF-β1. Therefore, the application of leukocyte-rich PRP may be especially important in enhancing the healing process with oral surgery. However, clinical and preclinical studies will be needed to demonstrate whether leukocyte-rich PRP is more advantageous for oral and periodontal surgery as compared to PRP without leukocytes.

In addition to their critical roles in soft tissue healing, leukocytes also contribute to hard tissue repair, such as bone fractures. For example, monocytes were demonstrated to be important in the early and late stages of fracture healing in mice. Reduced levels of macrophages at the fracture site were accompanied by reduced blood vessel density, delayed bone formation, and impaired callus remodeling. Leukocytes also release PDGF and TGF-β1, which have important roles in fracture repair. Therefore, increasing leukocyte concentrations with PRP at fracture sites may improve bone healing.

Consequently, leukocyte-rich PRP may increase platelet production by megakaryocytes, attract other leukocytes, accelerate wound and bone healing, prevent infection in nonsterile wounds as well as provide important cytokines, proteinases, and growth factors. Leukocyte-rich PRP has resulted in improved outcomes in joints, tendons, and bones, as well as after cardiac, oral, maxillofacial, plastic, and endoscopic surgeries.

Potential concerns with leukocytes in platelet concentrates

Using autologous PRP removes the possibility of severe complications from heterologous donor leukocytes, such as Creutzfeld–Jacob disease, cytomegalovirus transmission, or HLA alloimmunization. Still, some reports suggest that leukocyte inclusion in PRP may have deleterious effects due to excessive release of reactive oxygen species (ROS) by neutrophils damaging surrounding tissues. For example, leukocyte-poor PRP has been suggested for muscle injuries due to potential tissue damage from ROS released from neutrophils. However, this hypothesis needs to be tested, especially since nature provides controls to prevent (under normal circumstances) excessive actions of any specific cell type. In their role as the first invaders into the wound, the neutrophils release ROS and nitric oxide to kill foreign invaders and debride the wound of damaged tissue. However, macrophages induce neutrophil apoptosis to prevent potential detrimental effects on wound healing from too many neutrophils. Increasing monocyte concentration in the wound and fibrin clot with leukocyte-rich PRP (which are activated to macrophages) may control neutrophil activity and the time they must spend in the wound to reduce any potential adverse effects of these immune cells on neighboring tissues.

When selecting the type of PRP to use, the potential benefits of including leukocytes with the platelets may vary with the type of application, such
as oral surgery, tendon or muscle repair, orthopedic surgeries, and so on. For example, oral surgery may benefit more from the prevention of infection by using leukocyte-rich preparations due to the inability to keep the site sterile after closure, unlike skin wounds that can be covered and kept dry. Further, including leukocytes in PRP may result in higher platelet yields.

**Platelet Activation in Platelet-Rich Preparations**

The platelet activation method is another important criterion to consider for the platelet-rich product. Activation of platelets induces α granules to release their stored coagulation factors, platelet activating factors, adhesion molecules, cell-activating molecules, cytokines, integrins, inflammatory molecules, and growth factors. Activation follows platelet aggregation and occurs when platelets undergo several biochemical changes before secreting their active components. Factors that activate platelets include thrombin, collagen, fibronectin, laminin, thromboxane A2, epinephrine, adenosine diphosphate (ADP), or contact with a negatively charged surface (e.g., glass). Thus, when drawing blood for PRP preparation, an anticoagulant is used to control the time of activation (except with PRF). With platelet activation, growth factors begin to be released within 10 minutes; within 1 hour, 95% of the growth factors have been released. Due to this rapid release with activation, the timing between activation and administration to the wound site is important to ensure the maximum exposure of the short-lived growth factors to cell surfaces involved in the repair process. After this initial burst of secretory factors, the platelets continue to synthesize and secrete these cytokines for the remaining few days of their lifespan.

In wounds, sites of tissue damage, and surgical sites, endogenous factors activate platelets spilling from damaged blood vessels. At the injured site, platelets aggregate to form a fibrin clot to stop bleeding from the cut vessels. The purpose of the fibrin clot is not only to prevent additional blood loss but also to provide a provisional extracellular matrix to support the participating cells, platelets, growth factors, and cytokines in the healing process. As the size of the clot is based on the size of the wound, the goal of PRP is to enhance this fibrin clot to enhance these healing factors. The composition of a natural clot is different compared to a clot formed with PRP. Composition of a natural clot is approximately 95% erythrocytes, with the remaining 5% being leukocytes and platelets. In contrast, the clot from PRP contains approximately 95% platelets; however, this percent will vary with the PRP procedure, as leukocyte-rich PRP will have a low percent of RBC and enriched leukocytes compared to leukocyte-poor methods. The platelet-enriched fibrin clot serves as a reservoir of platelets, growth factors, and leukocytes. The half-life of growth factors attached to this matrix is considerably longer than free growth factors in plasma; they remain localized to the wound environment, and their activity can be enhanced or inhibited as needed.

**Bovine thrombin and calcium chloride**

The most common means to activate platelets in PRP is by the addition of bovine thrombin and calcium, which creates a platelet-rich gel (PGEL). This combination is a potent coagulant that is able to overcome the anticoagulant added for collecting the blood. Once these reagents are added, gelling occurs quickly, so the components are often added to the wound site with a mixing syringe. The polymerized fibrinogen concentrated during PRP preparation by the thrombin and calcium results in a fibrin matrix with hemostatic and adhesive properties. Unfortunately, many studies indicate that the clinical effects of PGEL are very near those observed with conventional fibrin adhesives, which could be related to the type of matrix formed by the added gelling agent.

One problem with the final fibrin matrix is that with high thrombin concentrations, fibrin polymerizes via tetramolecular or bilateral junctions, resulting in a rigid network not favorable to cytokine enmeshment or cellular migration. Although PGEL should support cytokine action, cytokines may be released too quickly during the activation step to be completely incorporated into the fibrin. As growth factor activity and stability are enhanced and regulated by their sequestration and release from the matrix, their level and time of activity may be reduced without proper incorporation into the fibrin clot. In a study examining growth factor levels in PRP activated with a bovine thrombin and calcium gluconate solution, TGF-β1, PDGF-AB, and PDGF-BB levels were significantly decreased in
thrombin/calcium activated PRP (with or without leukocytes) compared to PRP prepared by freeze-thaw or Triton-X lysis. Therefore, the type of matrix and the consequences of its structure may explain the modest clinical effects observed with PGEL.

Another potential explanation for the limited positive reports with PGEL is that different studies have used varying concentrations of bovine thrombin to form the gel. Intini suggests that one reason for the lack of improved healing with PRP may be due to lower concentrations of thrombin or to adding only thrombin or CaCl$_2$, as these activation methods would not result in complete activation and release of the platelet growth factors. The optimal concentration for full activation is suggested to be 142.8 U/mL bovine thrombin and 14.3 mg/mL CaCl$_2$ for full degranulation of the α-granule contents.

Thrombin, also known as Factor IIa, is a serine protease that converts fibrinogen into insoluble fibrin stands. It is a naturally occurring enzyme that has been widely characterized for its roles in hemostasis, inflammation, and cell signaling. Unfortunately, bovine thrombin can induce a robust immune response in humans. There are several reports documenting adverse events following direct application of bovine thrombin in surgical patients, including the development of cross-reactive antibodies against autologous thrombin, prothrombin, factor V, and cardiolipin. In some cases, these antibodies cross-react with human factor V, resulting in hypocoagulopathies. Lawson and colleagues have hypothesized that applying immunologically dissimilar, yet biologically active, thrombin (such as bovine thrombin) to surgical sites—especially at physiologically extreme concentrations (1000U/mL)—may lead to immune recognition and result in deleterious autoimmune complications.

Although bovine thrombin has the potential for serious adverse events, other reports suggest that its addition to PRP has low risk of such events. The complications with bovine thrombin result from the development of antibodies to bovine factor Va, which cross-react with human factor Va. Bovine factor Va was a contaminant in some commercial thrombin preparations prior to 1997; this has since been eliminated during manufacturing. In addition, a lower concentration of thrombin is added to PRP and incorporated into the fibrin gel for PGEL application vs high concentrations applied directly to open surgical sites, where it easily reaches the systemic circulation. It is also proposed that as the PGEL fibrin clot is broken down and phagocytosed by macrophages, so is the incorporated bovine thrombin; therefore, it should never reach the systemic circulation to induce the antibodies against factor Va. As a result, bovine thrombin used to gel PRP may not pose the same serious risks previously reported for direct application to surgical incisions, especially with post-1997 formulations. However, with the lower growth factor levels, less favorable matrix formed during gelling, and previous reports of serious risks with bovine thrombin, other options may be preferable for activating PRP.

Because of the important role of TGF-β1 and PDGF in bone regeneration, their decreased levels in bovine thrombin/calcium-activated PRP may not result in improved bone repair. In a rat study comparing PRP with and without thrombin activation, PRP without thrombin promoted bone growth, whereas the thrombin-activated PRP did not.

Several randomized, controlled clinical trials for periodontal intraosseous defects using bovine thrombin/calcium-activated PRP did not result in significant improvement of clinical attachment levels compared to the control group without PRP treatment. Since some studies with thrombin activation resulted in improved outcomes, the lack of favorable results could be due to lower quality or inappropriate amounts of thrombin added. Additionally, in the rat diabetic fracture model discussed above, improved bone regeneration was detected with thrombin-activated PRP; however, the mechanical strength of the resulting bone was significantly lower than the repaired bone in nondiabetic rats. To determine if the activation method influences bone regeneration, clinical and preclinical studies are needed to compare bovine thrombin vs endogenous or other means of platelet activation to determine the best activator for healing efficacy.

Other methods of platelet activation

Due to potential risks with bovine thrombin, many preparations (including PRGF) use only CaCl$_2$ to activate the platelets. For PRGF, 50 μL of 10% CaCl$_2$ may be added per 1 mL of PRGF to overcome the
anticoagulant. Clotting time ranges from 5–8 minutes at room temperature or approximately 3 minutes at 37°C. However, since the main source of fibrinogen in PRGF is from the platelets, the lower fibrin concentration results in a weaker mesh. In contrast, the CaCl₂-induced matrix in PRFM is stronger due to more plasma being present during the gelling process. Since plasma contains fibrinogen and other clotting factors to reinforce the platelet source, their presence with the calcium aids in forming the more complex matrix.

PRF is different from PGEL in that it polymerizes naturally and comparatively more slowly during centrifugation, relying on native autologous thrombin rather than added thrombin. Thrombin in PRF acts on fibrinogen to produce a fibrin mesh that has trimolecular or equilateral fibrin junctions. These junctions establish a fine, flexible fibrin network capable of supporting cytokine enmeshment and cellular migration. The trimolecular fibrin organization gives greater elasticity to the matrix as compared to the tetramolecular or bilateral architecture seen in PGEL. The PRF protocol results in a flexible, plastic, and strong fibrin membrane.

Other gelling agents include ADP and ITA gelling agent (Natrex Technologies, Greenville, NC). Autologous thrombin can also be prepared in over 30 minutes with a separate aliquot of PRP. Plateletex (Bratislava, Slovakia) uses calcium gluconate and lyophilized purified batroxobin in place of bovine thrombin. Batroxobin induces clotting; however, unlike thrombin, it does not activate platelets. However, relatively little research exists regarding the potential complications or efficacy of such novel agents.

**Endogenous activation of PRP**

Most preclinical and clinical studies use an exogenously activated PRP since it is often presumed that the platelets must be pre-activated for the growth factors to be released and for the healing benefits to occur. However, endogenous factors can spontaneously induce their activation in the wound. For example, the calcium levels in the wound from blood exiting the damaged vessels could activate the platelets in PRP. Many other activating agonists are also found at the injured and surgical site, such as collagen, thrombin, thromboxane A₂, ADP, serotonin, vasopressin, thrombospordin, fibrinogen, platelet-activating factor, von Willebrand factor, immune complexes, and plasmin. However, without prior activation, the prepared PRP will be loose, making it harder to apply to the surgical site; the PRP can be applied with a syringe or by mixing with bone-grafting material to overcome this issue. Endogenous activation can result in effective clinical outcomes as evidenced in the split-mouth clinical trial using the simplified BC-PRP that resulted in accelerated bone regeneration.

**Platelet and Growth Factor Enrichment**

The concentration of platelets and growth factors from different types of platelet-rich preparations are shown in Table. The lowest platelet concentrations and enrichment compared to whole blood occur with leukocyte-poor preparations PRGF and PRFM. Since the purpose of PRP is to increase platelet concentration, their levels may be a valid criterion for comparing different preparations. However, as the quality of the PRP varies with the preparer, the chosen method may initially need confirmation by each practice. Conversely, for PRF, the inability to measure platelets and growth factors (except for plasma IGF-1) with this method makes it difficult to assess its quality; accordingly, other methods are needed to compare it to other platelet-rich preparations.

Growth factor concentrations for TGF-β1 and PDGF-AB or -BB vary among the preparations (see Table), which is likely partially related to the methods for extracting the proteins (freeze-thaw, lysis, or activation), as these different methods can affect the cytokine levels. In addition, the means to release the growth factors from the platelets for the assays may not represent the capacity of each preparation to release these factors in vivo. These differences may be the reason that growth factor levels in some studies do not correlate with platelet concentrations. Therefore, in selecting a method, the platelet concentration may be more relevant than growth factor levels. This is especially true for IGF-1, as its concentrations in PRP are not increased over plasma levels due to plasma providing higher levels from liver production compared to the small amounts made by the platelets. Although, platelet-secreted IGF-1 would contribute to soft and hard tissue repair in the microenvironment of the wound, its levels are not representative of platelet enrichment and, as such,
are not a good criterion on which to base selection of the PRP method.

Another option to consider is selecting the number of centrifuge spins in preparing the PRP, since this may affect platelet enrichment. Marx suggested that single-spin methods for preparing PRP would be ineffective with low platelet yields.87 Despite requiring only one spin and having an easy method for collection, the Rutkowski method results in a platelet concentration (over sixfold higher than whole blood) similar to the classic 2-spin methods with automated instruments or manual kits. This platelet concentration is within the desired range of 1 million platelets per microliter and the range reported to improve bone repair in rabbits. In addition, this method accelerated bone formation after tooth extraction in a split-mouth clinical trial.27 In contrast, the single-spin, leukocyte-poor PRGF preparation does not concentrate the platelets as effectively as the leukocyte-rich centrifugation methods, with the fold increase over whole blood slightly below the range with positive effects (two- to sixfold), and its platelet concentration per microliter (see Table) is at the low end of the range observed to have significant effects on rabbit bone regeneration (503 000 to 1 729 000 platelets/lL).52 Anitua and colleagues suggest that the minimum effective concentration is only 300 000 platelets per microliter. For the single-spin PRF, the platelet concentration is unknown and, thus, cannot be correlated to the suggested range for platelet-rich preparations.

### OTHER SELECTION CRITERIA

The selection of PRP may be based on multiple criteria, including the size of the practice, number of staff, budget, and type of use. For example, large practices, hospital settings, and surgical centers are more likely to have the funds to purchase the automated or specialized instruments for PRP.
preparation. Commercial kits may be too costly for small clinical practices. Consequently, simpler methods with lower costs may be more advantageous for private practices. In addition, methods with lower blood volume requirements prevent stress to the patient and will not decrease their hematocrit. However, higher volumes may be required for PRP applications in multiple or large sites. Blood volume requirements, relative costs, and ease of preparation are reviewed in Part 1.\textsuperscript{12}

Another option to consider is the anticoagulant for blood collection (except with PRF, which is prepared with coagulated blood). Citrate-based anticoagulants are the most frequently used for most PRP preparations. The citrate binds to the calcium in the plasma to prevent platelet aggregation. The anticoagulant citrate dextrose solution A (ACD-A) has been suggested to be the preferred choice for supporting platelet viability and metabolism.\textsuperscript{74,87} This anticoagulant is used in blood banks to store platelets due to the presence of dextrose and other ingredients that protect and maintain the platelets. Citrate phosphate dextrose is a good alternative due to its similarity to ACD-A; however, it has fewer ingredients to support the platelets and may be less protective. No negative effects have been observed with trisodium citrate solution—unlike ethylene-diaminetetra-acetic acid, which can result in many damaged platelets,\textsuperscript{74} and was suggested as a possible reason for the lack of positive outcomes in a PRP preclinical study.\textsuperscript{23}

The matrix formed by the clot should also be considered when choosing the PRP method. The ideal matrix would have an elastic, flexible nature with equilateral junctions.\textsuperscript{24} The method of matrix formation can affect the resulting properties, cytokine enmeshment, cellular migration, and durability of the clot in the healing wounds.\textsuperscript{24} A weaker matrix may be removed from the wound earlier by the neutrophils and macrophages and potentially could have reduced cell adhesion and migration needed for the healing process. Activation that is too rapid and harsh due to high thrombin concentrations may result in the fibrin network being too dense with bilateral junctions that are less conducive for cytokine storage and cell migration.\textsuperscript{24} Thus, inferior matrix characteristics may affect proper wound healing.

The desired application of the PRP may also need to be considered when selecting the PRP method, such as for dental procedures, surgery, fractures, or muscle or tendon injuries. Compared to a muscle, a fracture hematoma has fewer leukocytes and more necrotic tissue.\textsuperscript{22} Therefore, bone fractures or oral surgeries may benefit more from using leukocyte-rich PRP than muscle injuries. In addition, the higher growth factor levels reported for leukocyte-rich preparations may aid bone repair.\textsuperscript{21}

\textbf{Conclusions}

Selecting the methods for preparing platelet-rich products involves considering the types of preparations and their ease of use, cost, and blood volume required (reviewed in Part 1).\textsuperscript{12} Other considerations include platelet activation methods, leukocyte inclusion, anticoagulant, and level of platelet and growth factor enrichment. Although bovine thrombin and calcium are the most commonly used activators, other methods—including endogenous platelet activation in injured and surgical sites—may produce a better matrix\textsuperscript{24} and improve bone healing outcomes.\textsuperscript{91} Leukocyte- and platelet-rich preparations may also enhance healing due to the many beneficial actions of leukocytes in wound healing and infection prevention.\textsuperscript{13–18} Leukocyte-rich preparations will also have higher platelet and growth factor concentrations to aid in the repair of soft and hard tissues (see Table). Optimal procedures still need to be identified by well-designed preclinical and clinical trials to determine the best activation and preparation methods for medical and dental applications, thereby accelerating healing and reducing adverse outcomes, such as inclusion or exclusion of leukocytes, minimum platelet and growth factor concentrations, and combinations with other biomaterials or treatments. However, numerous studies report improved healing with different platelet-rich preparations, suggesting that with conscientious consideration of the available methods and careful preparation of the platelet-rich product, most clinicians will see the benefits of its use. Consequently, the benefits of platelet-rich products in wound and bone healing, the low risks with its use, and the availability of easy and low-cost preparation methods should encourage more small offices to incorporate platelet-rich preparations in their own practice.
**ABBREVIATIONS**

ACD-A: anticoagulant citrate dextrose solution A  
ADP: adenosine diphosphate  
BC: blood cells  
bFGF: basic fibroblast growth factor  
MMP: metalloproteinase  
PGDF: platelet-derived growth factor  
PRP: platelet-rich plasma  
PRF: platelet-rich fibrin  
PGEL: platelet-rich gel  
PRFM: platelet-rich fibrin matrix  
ROS: reactive oxygen species  
TGF-α: transforming growth factor α  
TGF-β: transforming growth factor β  
TGF-β1: transforming growth factor-β1  
WBC: white blood cells

**REFERENCES**


Options for Preparing Platelet-Rich Products


71. Ortel TL, Moore KD, Quinn-Allen MA, et al. Inhibitory anti-factor V antibodies bind to the factor V C2 domain and are


