

Platelet-Rich Preparations to Improve Healing. Part I: Workable Options for Every Size Practice

Vicki L. Davis, PhD^{1*}

Alaeddin B. Abukabda, MS²

Nicholas M. Radio, PhD³

Paula A. Witt-Enderby, PhD⁴

William P. Clafshenkel, PhD⁴

J. Vito Cairone, BS²

James L. Rutkowski, DMD, PhD^{3,5}

Numerous studies have demonstrated that platelet-rich preparations applied to surgical sites, injuries, or wounds are a safe and effective way to promote soft tissue healing and bone growth. Various protocols have been developed for preparing platelet-rich preparations, with subtle but important differences between them. Unfortunately, only a minority of clinicians use platelet-rich preparations, such as platelet-rich plasma and platelet-rich fibrin, in their practice, possibly due to confusion about the different methods and their advantages and disadvantages. Therefore, the different types of preparations are described to help guide the selection of the best method for any size practice. Classic methods generally require large volumes of blood and can be expensive, complicated, and time-intensive. Simpler protocols have been developed recently, which require relatively inexpensive equipment and small blood volumes and, thus, may be more applicable for small clinical practices. Platelet-rich preparations accelerate healing at earlier time points to reduce discomfort and the potential for adverse outcomes, including infection, poor wound closure, and delays in forming strong bone for subsequent procedures (such as implants). However, platelet-rich preparations may also improve long-term outcomes in patients expected to have impaired healing, such as with lifestyle choices (eg, smoking), medications (eg, steroids), diseases (eg, diabetes, osteoporosis, atherosclerosis), and aging, by supplementing the deficient wound environment to restore proper healing. Therefore, both large and small clinical practices would benefit from utilizing platelet-rich preparations to enhance healing in their patients.

Key Words: *bone, growth factors, leukocytes, platelet activation, platelet-rich plasma, wound healing*

INTRODUCTION

Platelet-rich preparations are a safe, reliable, and cost-effective means to accelerate healing and to improve the probability of efficient repair following surgery or injury. Platelet-rich plasma

(PRP) is a preparation of plasma that contains an increased concentration of platelets compared to whole blood. An essential criterion for PRP is for it to be autologous: for the donor of the blood and the recipient of the PRP to be the same person. The use of PRP to facilitate both soft and hard tissue healing following surgical procedures is often used in clinical dentistry,^{1–8} because it is reported to accelerate bone formation and induce healing in nonhealing wounds.^{8–14}

Many medical and dental studies support the use of autologous PRP in clinical practice,^{15–24} including for soft tissue injuries; chronic diabetic ulcers; injuries to muscles, tendons, or ligaments;

¹ Center for Applied Research & Intellectual Property Development, Clarion University, Clarion, Pa.

² Biology Department, Clarion University, Clarion, Pa.

³ Clarion Research Group, Clarion, Pa.

⁴ Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, Pa.

⁵ Private practice, Clarion, Pa.

* Corresponding author, e-mail: dr.vicki.davis@gmail.com

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bone fractures; molar extractions; urologic, dental, ophthalmic, and plastic surgery procedures; and orthopedic, periodontal, sinus lift, and oral/maxillofacial surgeries. Since growth factors play crucial roles in soft and hard tissue regeneration,²⁵⁻²⁷ the proposed mechanism for the enhanced healing outcomes by PRP is through the release of critical growth factors by activated platelets.

PLATELET GROWTH FACTORS

Due to their essential role in hemostasis, platelets are deployed to sites of injury or infection to modulate inflammatory processes through the secretion of growth factors, chemokines, and other inflammatory mediators.²⁸⁻³¹ The majority of secreted substances found in platelets are localized within α -granules.^{32,33} Numerous growth factors with healing roles are released by activated platelets, including insulin-like growth factor (IGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), transforming growth factor- β 1 (TGF- β 1), and platelet-derived growth factors (PDGF).

PDGF was given its name after it was discovered within platelets. PDGF is composed of disulfide-linked A and/or B subunits.³⁴ Platelet α -granules contain a mixture of the PDGF-BB homodimer and PDGF-AB heterodimer. PDGF has crucial regulatory roles in the migration, proliferation, and survival of mesenchymal cells³⁵⁻³⁷ and in each stage of wound healing.²⁵ PDGF released from platelets in the wound chemo-attracts fibroblasts and increases collagen production for remodeling of the extracellular matrix during healing.³⁸ Another role of PDGF is to stimulate macrophages to synthesize and secrete TGF- β growth factors.²⁵ It also regulates mesenchymal cell lineages important for bone development and remodeling^{35,39} and for cicatrization (fibrous scar formation)^{35,40} and enhances proliferation of cells involved in bone formation.^{16,19} Topical delivery of recombinant PDGF-BB aids healing of chronic wounds and diabetic ulcers.^{25,41} In patients with severe periodontal disease, local delivery of PDGF-BB has increased biomarkers for bone metabolism and improved periodontal regeneration.^{41,42}

TGF- β 1 is a member of the TGF- β superfamily, which includes the bone morphogenic factors and the 3 isoforms of TGF- β , TGF- β 1, TGF- β 2, and TGF-

β 3. After the initial injury, active TGF- β 1 is released from platelets; latent TGF- β is also produced and stored in the fibrin clot and released by proteases to allow its sustained release.²⁷ TGF- β 1 has many important roles in wound healing, including in inflammation, angiogenesis, re-epithelialization and granulation tissue formation, connective tissue regeneration, chemo-attraction of additional immune cells, and augmentation of macrophage debridement.^{25,27} TGF- β 1 stimulates collagen production and synthesis of protease inhibitors to inhibit collagen breakdown.^{25,38} TGF- β 1 induces fibrous wound healing leading to scar formation.^{35,43} Wounds in TGF- β 1-deficient mice have impaired wound healing and reduced TGF- β levels are detected in aberrant wound healing from aging, steroids, and diabetes.²⁷ TGF- β 1 release by platelets at sites of bone injury is also important for the stimulation of bone repair and healing as it acts as a paracrine growth factor, and stimulates pre-osteoblast proliferation.¹⁶ Local delivery of TGF- β 1 also stimulates bone healing.⁴⁴

EGF influences two critical roles in tissue repair: cell proliferation, and cytoprotection.⁴⁵ EGF accelerates re-epithelialization and increases tensile strength in wounds.²⁵ In clinical trials with chronic wounds, topical EGF therapy increases epithelialization and shortens healing times.^{25,45}

IGF-1 is a positive regulator of proliferation and differentiation for most cell types. IGF-1 is released during platelet degranulation and is also ubiquitous in circulating blood.³⁵ IGF-1 influences proliferation and matrix secretion from osteoblasts and its decrease with age corresponds with lower bone mineral density.¹⁶ Systemic delivery of IGF-1 in rats^{44,46} and localized delivery in sheep⁴⁷ improved bone healing. Locally delivered IGF-1 also has potential efficacy for regeneration of periodontal tissue.¹⁶

VEGF promotes wound and bone healing by stimulating angiogenesis. After injury, VEGF is released from activated platelets^{25,48} and by macrophages in the wound.⁴⁹ Other growth factors enhance VEGF-A expression, such as TGF- β 1, PDGF-BB, bFGF, and EGF.²⁵ Its central role in wound healing is to stimulate angiogenesis by regulating endothelial cell proliferation and migration.^{25,48} VEGF also works in concert with fibroblast growth factor (FGF), which is also released from platelets, to stimulate angiogenesis. Inhibition of angiogenesis

impairs wound healing.⁴⁸ Exogenous VEGF therapy has been proposed to accelerate healing and for closure of nonhealing wounds.⁴⁸

Multiple studies have tested isolated growth factors to increase their local levels in wounds.* However, with PRP, growth factors are secreted by the platelets into the fibrin clot, and then are bound to this provisional matrix. The growth factors have a longer half-life, are slowly released into the wound over the several days of the 5–9 day platelet life, and have their activity controlled by the wound environment, such as by secreted activating and inactivating factors and cell types. In contrast, isolated growth factors are easily degraded, have a short half-life, and are generally applied in a bolus, so their release in the wound is not regulated or controlled. In addition, PRP provides multiple growth factors, which are expected to be more effective than the actions of a single growth factor. Therefore, PRP provides an ideal and safe means to supplement growth factor concentrations to enhance soft and hard tissue repair.

TYPES OF PLATELET-RICH PREPARATIONS

Only a minority of clinicians, both in the United States and internationally, use PRP preparations in dentistry and oral-maxillofacial surgery. One reason for its lack of general use may be related to many practitioners being confused by the different types of PRP. Not understanding the benefits and disadvantages, costs, and ease of use may also impede many from trying PRP in their practice. As a guide, the major types of PRP are reviewed below.

The major types of preparations can be summarized as cell separators, 1- and 2-spin methods, and platelet-rich fibrin (PRF). After collection, PRP may be further processed into the application biomaterial, such as platelet gel (PGEL). PRF is the only preparation that uses coagulated blood to form a fibrin clot (FC) and, therefore, is technically not classified as platelet-rich plasma.

There are multiple variations on the major techniques used to concentrate autologous platelets from whole blood in the medical or dental office setting. In general, almost all involve centrifuging whole blood to concentrate the platelets. Preparation variations involve the number and

speed of the centrifuge spins, the amount of blood required, the collection of platelets with or without the leukocytes, the means of platelet activation, the type of instrument required, the use of purchased kits, and cost or ease of use. The variations in PRP preparations are of interest because their differences may result in compounds with significantly dissimilar growth factor and platelet concentrations as well as biomechanical properties of the matrix. Different PRP preparations have been reported to have differing effects on platelet organelles, biochemical activity (eg, oxidative phosphorylation capacity), and surface marker expression.⁵¹ The variations in PRP preparations are relevant when examining scientific studies as the outcomes cited for 1 type of PRP may not hold true for others.

Specific protocols and automated systems for preparing PRP have been developed and commercialized, including Ace, PRGF, PRP-Landesber, Curasan, PCCS, Harvest SmartPREP, Vivostat, Friadent-Schütze, Regen, Fibrinet, and Plateltex. Dohan Ehrenfest and colleagues offer an excellent comparison between these traditional systems.¹⁵ While these widely used methods tend to be comparatively expensive and complex and often use 50 mL or more of blood, simpler and less expensive methods that require less than 10 mL of blood have also been developed in the last few years. These newer methods include Anitua's PRGF,⁵² Choukroun's PRF,⁵³ Fibrinet's PRFM,⁵⁴ and Rutkowski's simplified BC method.⁵⁵ Such new methods have been demonstrated to be similarly effective as the classic methods and are more readily available for small outpatient clinics performing multiple procedures. The main types of preparations are discussed below and summarized in Table 1.

Cell separators

Originally, apheresis methods were performed by blood banks or hospitals to separate platelets for PRP. As a unit of blood was collected, the other blood components were retransfused to the patient. More compact instruments have since been developed to avoid the need for a hematologist, to use less blood, and to prevent the need for retransfusion. Vivostat PRF Preparation kit (Vivolution, Denmark) uses 120 mL of uncoagulated blood to result in a platelet-rich, leukocyte-poor preparation.⁵⁴ With the Electa Cell Separator™ (E-CS; Sorin, Italy), a leukocyte- and platelet-rich preparation is

*References 16, 25, 27, 41, 42, 44, 45, 48, 50

TABLE 1
Types of platelet-rich preparationst

Names	PRP					PRF
	PC, PRC, cPRP; With Platelet Activation: PGEL or PG, PRP gel, PRG, PLG					
	P-PRP			BC-PRP‡		PRF, L-PRF, FC
	Cell Separators	PRGF	PRFM	Classic PRP	Simplified BC-PRP	
WBC rich or poor	Leukocyte poor§	Leukocyte poor	Leukocyte poor	Leukocyte rich	Leukocyte rich	Leukocyte rich
Centrifuge spins	Automated spins	1 spin	2 spins	2 spins	1 spin	1 spin
Type of centrifuge or instrument	Automated instruments	PRGF system or variable speed benchtop or floor centrifuge	Variable speed benchtop or floor centrifuge	Variable speed centrifuge (manual kits); automated or special instruments	Single speed clinical centrifuge	Variable speed benchtop or floor centrifuge
Examples of procedure	Vivostat PRF ⁵⁴ E-CS Electa Cell Separator ⁵⁶ (other blood products not re-transfused)	Anitua ⁵² Nahita ⁵⁸	Fibrinet PRFM ⁵⁴	PCCS, ⁵⁴ Regen, SmartPREP, ⁵⁴ Ace, ⁵⁸ Curasan, ⁵⁷ Friadent-Schutze, ⁷⁶ Plateltex, ⁷⁷ GPS ⁷⁸ (only 1 spin)	Rutkowski ⁵⁵	Choukroun ⁵³
Blood volume	> 100 mL	< 10 mL	< 10 mL	Varies; most ≥20 mL	< 10 mL	< 10 mL
Anticoagulant	Yes	Yes	Yes	Yes	Yes	No
Cost¶	\$\$\$	\$\$\$	\$\$\$	\$\$\$	\$	\$
Ease#	***	**	**	***	*	*
Platelet activator††	Bovine thrombin and CaCl ₂ (can use autologous or human thrombin) Other activators: CaCl ₂ , ADP, other kits, such as ITA or batroxobin (can be used without activation)				Endogenous in surgical/wound site (activation optional)	Blood collection without anticoagulant
Time issues	Before activation: Can draw blood before clinical procedures and let set before activation, length of setting time without loss of GF has not been determined After activation: Must use within < 10 minutes for optimal GF release in site				Can draw blood before procedures and set up to 2 hours	Must draw blood just prior to use with immediate spin and application
Disadvantages	Platelets may be damaged in procedure	Lower platelet yields; may be difficult to consistently obtain proper fraction	Lower platelet yields	More complicated methods	Results in a loose PRP preparation (without activation)	Must draw blood during surgical procedure; timing is critical for forming proper matrix

†PRP indicates platelet-rich plasma; PRF, platelet-rich fibrin; PC, platelet concentrate; PRC, platelet-rich concentrate; cPRP, concentrated platelet-rich plasma; PGEL or PG, platelet gel; PRG, platelet-rich gel; PLG, platelet-leukocyte gel; BC-PRP, buffy coat platelet-rich plasma; P-PRP, pure platelet-rich plasma; L-PRP, leukocyte- and platelet-rich plasma; PRGF, plasma rich in growth factors or preparation rich in growth factors; PRFM, platelet-rich fibrin matrix; WBC, white blood cells.

‡Only some classic PRP methods are BC-PRP.

§E-CS is leukocyte-rich.

||Volume can vary with procedure, number of sites for application, site of application, and practitioner.

¶Costs based on requirements for specialized instruments or centrifuges, reagents, or kits compared to standard blood drawing supplies.

#Ease based on number of steps, specialized timing, or complexity of procedures.

††Blotting the resulting clot prior to application may remove many growth factors prior to application; different activators result in different types of matrix.

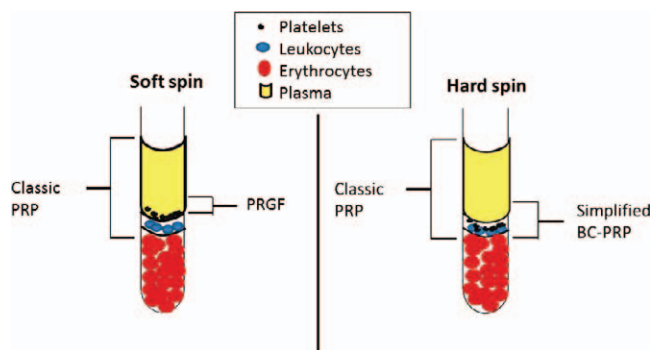


FIGURE 1. The speed of the first or only spin affects platelet location during platelet-rich plasma (PRP) preparation with anticoagulated blood. Whole blood plus anticoagulant is spun at low speed (soft spin) or high speed to separate the components. The blood separates into 3 layers: the top layer of plasma or platelet poor plasma (PPP), the middle layer or buffy coat, and the lower layer of red blood cells (RBC). The buffy coat contains leukocytes after the soft spin and the leukocytes and platelets after the hard spin. The platelets are located at the plasma/buffy coat interface after the soft spin and at the buffy coat/RBC interface after the hard spin. The collected fractions are noted by the brackets for the designated PRP preparations; the fractions outside the brackets are discarded. For the preparation rich in growth factors (PRGF) and simplified buffy coat PRP (BC-PRP), the PRP is composed of the collected fractions. For the classic PRP with either speed for the first spin, the collected fraction is spun in a separate tube to concentrate the platelets and leukocytes by removing most of the PPP after centrifugation.

concentrated from 375 mL of anticoagulated blood.⁵⁶ However, these methods are used infrequently as the instruments are expensive and require high volumes of blood, and the resulting platelets can be damaged.⁵⁴

Classic platelet-rich plasma

The classic PRP technique can be performed with automated instruments, such as Harvest SmartPReP, 3I PCCS (platelet concentrate collection system), or manual kits, such as Curasan, Friadent-Schütze, Ace, and Plateletex. Most procedures use 2 spins; the first spin collects the plasma and platelets, and the second spin concentrates the platelets in a small volume of plasma. With most preparations, the blood is first subjected to a low g-force or soft spin to produce 3 layers: a top layer of plasma, a middle layer (buffy coat) containing the leukocytes, and a bottom layer of red blood cells (RBC). An initial slow speed spin locates the platelets at the plasma and buffy coat interface (Figure 1). The plasma, buffy coat, and a small amount of the RBC layer is

collected and transferred to a second tube and spun at a higher speed to concentrate the platelets and leukocytes at the bottom of the tube in the buffy coat layer. After the second spin, most of the platelet-poor plasma (PPP) is removed and the platelets are gently mixed with the low volume of remaining plasma to resuspend them and leukocytes. This traditional PRP has a rosy color due to the residual RBCs. These procedures can be complex with multiple steps and handling, which could result in inconsistent quality or compromised sterility with an inexperienced preparer.

There are many variations of the classic PRP with different speed spins; some preparations use a higher speed for the first spin and are labeled buffy coat-PRP (BC-PRP), such as the Curasan PRP kit,⁵⁷ which concentrates both the platelets and leukocytes in the middle buffy coat layer (Figure 1). Another variation is the Gravitational Platelet Sequestration System (GPS), which employs only one hard (higher g force) spin to concentrate the platelets and leukocytes in the buffy coat and a specialized, disposable centrifuge tube to aid collection of the PRP. The amount of blood required may also vary as some methods use less than 10 mL, such as Curasan and Friadent-Schütze, while others require 50 mL, such as SmartPReP, PCCS, and GPS. Despite variations in the techniques, all of these methods result in leukocyte-and platelet-rich preparations.

Preparation rich in growth factors

Preparation rich in growth factors (PRGF), also called plasma rich in growth factors, is a simplified method to collect only the platelets. Developed by Anitua and colleagues, the PRGF protocol uses 5–40 mL of uncoagulated blood.⁵² After blood collection, the tubes are immediately centrifuged at 270g for 7 minutes. The bottom 0.5 to 1 mL of the plasma fraction, immediately above and not including the buffy coat, are harvested as PRGF (Figure 1). Due to the low speed spin and absence of the buffy coat, this preparation is leukocyte-poor. However, by avoiding the buffy coat, this preparation results in lower platelet yields compared to other methods that collect it. Although the single spin makes this procedure easy, the collection of the platelets resting on the PPP and buffy coat interface without drawing up the buffy coat is difficult and may result in preparations with inconsistent quality. The Nahita

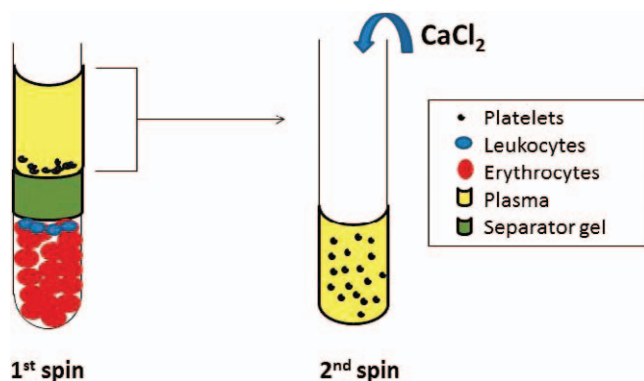


FIGURE 2. Following are the centrifugation steps for preparing platelet-rich fibrin matrix with uncoagulated blood. To prepare platelet-rich fibrin matrix (PRFM), whole blood is mixed with anticoagulant in the disposal PRFM tubes containing the separator gel and centrifuged to separate the platelets and plasma above the gel and the red blood cells (RBC) and leukocytes below the gel. The plasma and platelets are mixed and transferred to a second tube and mixed with CaCl_2 . The fibrin matrix forms during the second spin.

PRP preparation method is similar to the Anitua protocol and also results in a leukocyte-poor PRP.⁵⁸

Platelet rich fibrin matrix

The platelet-rich fibrin matrix (PRFM) is prepared using the Fibronet Autologous Fibrin and Platelet System (Cascade Medical Enterprises LLC, Plymouth, UK). This method collects uncoagulated blood into a tube with a thixotropic separator gel to isolate the plasma and platelets without the leukocytes and RBC.^{15,59} After the first spin, the platelets resting on the separator gel and the plasma are mixed and transferred to a second tube containing CaCl_2 . This tube is spun (second spin) to form the fibrin matrix (Figure 2). The fibrinogen in the plasma during the clotting step aids the matrix formation, making it stronger than methods that add calcium to minimal volumes of plasma.¹⁵ Some procedures use a second spin of over 10 minutes,^{59,60} which could result in most of the growth factors being prereleased from the platelets prior to application in the wound or surgical site.

Simplified buffy coat method

A simplified BC method using 1 spin of the uncoagulated blood was developed by Rutkowski and colleagues.⁵⁵ The hard spin results in the buffy coat containing platelets and leukocytes. In a single-speed, clinical benchtop centrifuge, longer spins (10

minutes, 1350g) consistently result in high platelet yields concentrated more than 6-fold over whole blood.⁵⁵ To overcome the concerns about “eyeballing” for collecting PRP,¹⁵ clear labels are affixed to the spun tube with a dotted line placed at the RBC and BC interface. Marks above (3 mm) and below (2 mm) the dotted line guide the clinician to consistently collect the proper amount of high quality PRP.⁵⁵ The PPP is removed to the first mark (3 mm above the BC/RBC interface) and discarded. Then, the PRP is collected between the 3 mm and 2 mm lines above and below the interface. Using clear sticky address labels preprinted with the correctly spaced markings, a consistent PRP preparation of 375 μL can be obtained with 4.5 mL blood collection tubes.

Platelet rich fibrin

Platelet rich fibrin (PRF) is a new member of platelet concentrates developed by Choukroun and colleagues. PRF is a cross between autologous fibrin glue and classic platelet concentrates.⁵³ This new biomaterial is similar to an autologous cicatricial matrix formed in natural wound healing. First, blood is drawn into 10 mL, anticoagulant-free tubes and then immediately spun in a tabletop centrifuge at 3000 rpm (approximately 400g) for 10 minutes. Due to the lack of anticoagulant, platelet activation occurs once the blood contacts the glass tube walls and subsequently initiates the coagulation cascade. By centrifugation, fibrinogen is concentrated in the top and middle of the tube, and then circulating thrombin transforms it into fibrin. This action leads to the development of a fibrin clot in the middle of the tube, between the RBCs at the bottom and serum at the top (Figure 3a). Platelets and growth factors are theoretically trapped in the fibrin clot as platelets are not measured and levels of growth factors in the exudate are well below other PRP preparations.⁵³ The contents are removed from the centrifuge tube and blotted to remove serum and RBC to result in a dense matrix (Figures 3b–d). However, blotting may also decrease the level of growth factors applied to the wound that were released into the serum during centrifugation. Timing is also critical to the success of the PRF technique. PRF must be prepared immediately before application to the surgical site. Unlike with PRP preparations using anticoagulants, the time required to collect blood and begin centrifugation

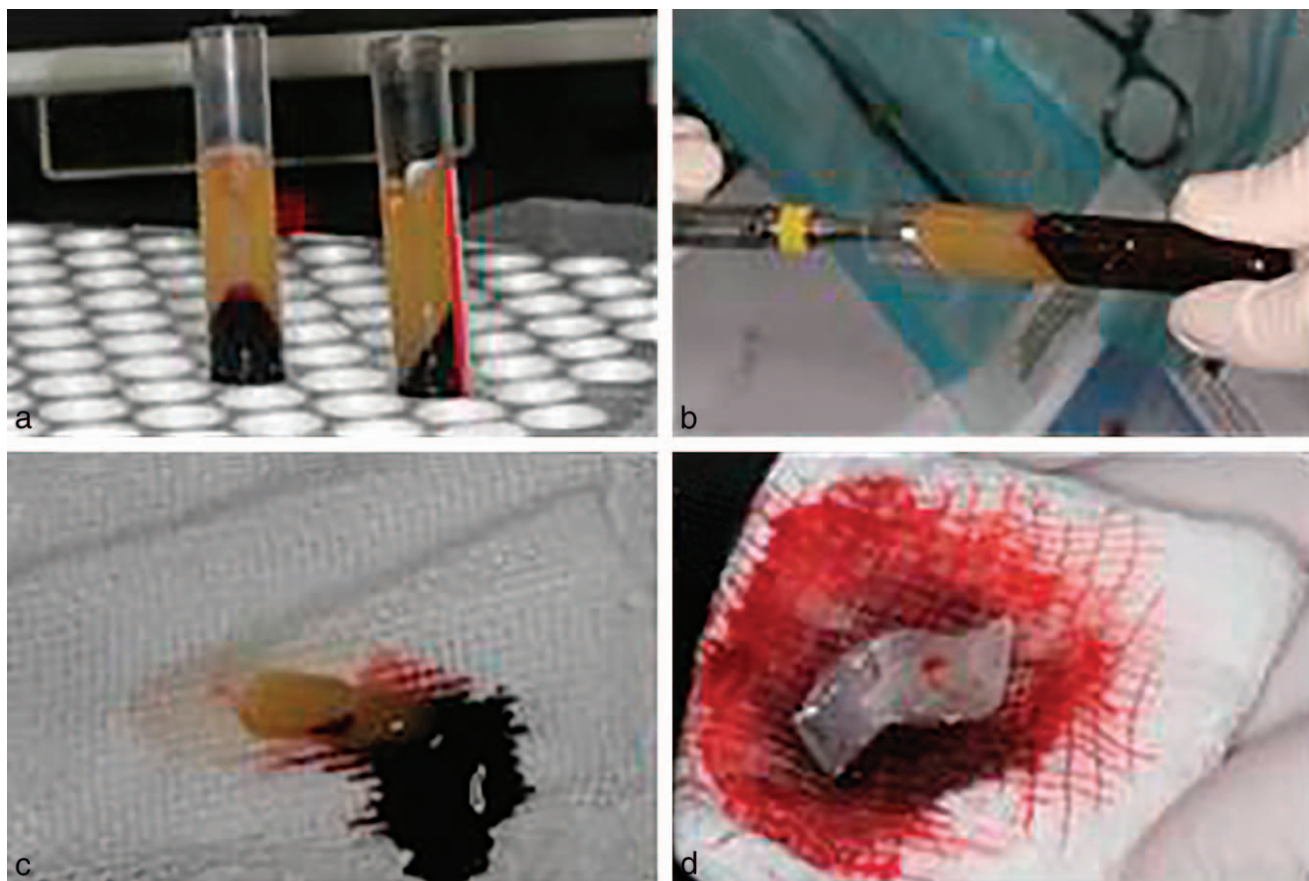


FIGURE 3. The following describes platelet-rich fibrin preparation with coagulated blood. The products obtained during platelet-rich fibrin (PRF) preparation⁵³ are shown. (a) Results of centrifugation of whole blood show the lower layer with compacted erythrocytes, the middle layer with the fibrin clot or PRF, and upper clear layer of serum. (b) The fibrin clot is removed from the tube with forceps. (c) The contents of the tube are placed on gauze to absorb the serum and the red blood cells. The red blood cell layer can also be cut away from the fibrin clot before blotting on the gauze. (d) The final PRF product for application in the wound is the white, autologous fibrin membrane after blotting.

cannot be prolonged or the fibrin will polymerize in a dispersed manner and only a small clot without consistency will be obtained.

HOLDING TIME FOR BLOOD OR PRP

When possible, blood is collected immediately prior to commencing the surgical procedure. The time until final use of the prepared PRP depends on the length of the procedure, which may vary from minutes to several hours. A recent study by the authors demonstrated that short-term storage of BC-PRP (2 hours or less) at room temperature does not result in aggregated platelets or in significant decreases in growth factor levels.⁵⁵ Other studies with classic PRP suggest that platelet viability and sterility can be preserved for PRP held for up to 8

hours prior to use.²⁴ Recently, holding time was tested on calcium and thrombin activated PRP and preparations refrigerated or frozen for 2–10 days had lower TGF- β 1 and PDGF-AB concentrations.⁶¹ When stored at -20°C and -70°C , the levels declined, but significantly less than when stored at 4°C . Therefore, if PRP needs to be stored, lower temperatures ($<-20^{\circ}\text{C}$) are preferred. However, any storage of activated platelets is less desirable to immediate use since high levels of growth factors would be pre-released, but not in the proximity of the damaged tissue.

If the PRP is not going to be used immediately, the preferred method would be to not activate the platelets before storage or, possibly, to not even prepare the PRP. For example, blood banks hold whole blood at room temperature for up to 24 hours prior to centrifugation for collecting platelets

with a hard spin (BC method). Additional studies are needed to compare the quality of PRP prepared immediately versus after specific holding times. However, for PRF, storage could only occur after activation since the coagulating blood must be spun immediately after collection.

PRP IN SOFT AND HARD TISSUE HEALING

PRP and wound healing

In acute wounds, TGF- β 1, PDGF, VEGF, bFGF, and EGF are detected at increased levels for inducing wound healing.²⁵ All of these factors are released by platelets and leukocytes in PRP, so its application would augment their levels to accelerate healing. In contrast, in chronic wounds, the levels of these same growth factors are decreased.²⁵ Therefore, PRP use would supplement these suboptimal levels to stimulate and accelerate the healing process. Indeed, improved healing has been demonstrated with PRP in chronic and diabetic wounds.^{23,62–65}

Many studies report PRP is effective for treating chronic wounds. In a randomized controlled pilot study with chronic vascular ulcers, PRGF resulted in a higher percentage of surface healed compared to the control group.²³ In another pilot study with chronic leg ulcers, PRFM increased the number of patients with complete healing.⁶⁴ These studies suggest that very different platelet-rich preparations can improve healing in wounds that fail to heal by conventional methods. In diabetic patients, chronic wounds occur frequently due to increased inflammation, altered angiogenesis, and an imbalance in protease activities that impairs healing.⁶⁶ The ability of PRP to influence all of these actions is the likely reason that multiple studies on diabetic ulcers report PRP shortened healing time and increased the number of patients with complete wound closure.^{62,63,65} Aging also suppresses the underlying mechanisms of healing, such as angiogenesis, local immunity, growth factor levels, and number of stem cells; accordingly, a higher prevalence of nonhealing wounds occurs after age 65.⁶⁶ Therefore, PRP use in the elderly may also improve surgical and injury outcomes with delayed- or nonhealing wounds. These studies suggest that patients with impaired healing are ideal candidates for PRP therapy.

PRP and bone repair

Bone is a continually remodeled structure with the osteoblasts producing and secreting the matrix and the osteoclasts breaking down the matrix in order to allow new bone formation. Osteoclasts are large, multinucleated cells that are of hematopoietic origin, differentiating from the monocyte-macrophage lineage,⁶⁷ which are responsible for bone resorption at sites of microdamage. The maturation and differentiation of osteoclasts is directly linked with that of osteoblasts, as a balance between bone resorption and matrix production must be maintained. Osteoblasts develop from mesenchymal stem cells (MSC) and are responsible for secreting the calcium phosphate matrix in bones.⁶⁸ One of the predicted roles of PRP is to stimulate the differentiation of MSC to osteoblasts to generate more cells that form new bone. Osteogenic growth factors in PRP, including TGF- β 1 and BMP2,⁶⁹ help regulate the commitment of the progenitor cells to the osteoblast lineage.⁷⁰ In addition, PRP also contains factors that stimulate MSC migration to the wound site²¹ to bring additional progenitor cells into the site of bone damage, which have the potential to differentiate and form new bone.

Multiple dental and oral surgery studies report that PRP administration improves bone repair. In a review of randomized, controlled clinical trials comparing the effects of the presence, or lack thereof, of PRP on bone regeneration in dentistry, PRP improved bone repair in 3 studies on periodontal defects and in most of the studies on sinus augmentation, oral-maxillofacial reconstructions, and tooth extractions.²² In a split-mouth study using simplified BC-PRP, a significant increase in radiographic bone density was detected in the first 2 weeks after extraction of mandibular third molars in the sites receiving PRP versus the control sites without PRP.⁷¹ In a preclinical study with PRP plus β -tricalcium phosphate, the PRP-treated extraction site of mandibular molars had significantly higher bone regeneration at only 6 and 12 weeks after the procedure, but not after 24 weeks, compared to the control side with β -tricalcium phosphate alone.⁷² These findings suggest that due to accelerated healing, early timepoints may be essential for assessing PRP effects on bone repair. Besides supporting that the function of PRP is to accelerate healing, these studies also indicate that split-mouth studies may provide a better chance for obtaining

statistical significance with PRP by avoiding the inherent variations in platelet concentrations and immune responses between individuals.

PRP stimulates bone repair in nonunion and diabetic fractures¹⁹; thus, similar to wounds, improved bone healing with PRP may be most evident in patients or animals with impaired healing. The early through late healing process is compromised with diabetic fractures, including reduced cell proliferation, delayed chondrogenesis, and decreased biomechanical properties.³ In a rat diabetic fracture model, several critical growth factors measured in the fracture callus were significantly reduced, including PDGF, TGF- β 1, IGF-1, and VEGF, which are all secreted by platelets.³ The expectation that PRP would supplement their deficient levels was verified as PRP delivered to the fracture site in diabetic rats rebounded early cell proliferation (2 and 4 days after fracture) to levels similar to PPP-treated, non-diabetic rats and significantly higher than PPP-treated diabetic rats.³ Seven days into the healing process, chondrogenesis recovered in PRP-treated rats to the levels of non-diabetic fracture callus treated with PPP. Additionally, at 6 weeks postfracture, mechanical testing demonstrated that PRP partially improved the strength of the repaired bone compared to nondiabetic rats.³ Therefore, early benefits observed with PRP result in improved bone regeneration in diabetic fractures.

For patients without impaired healing, the main effect may be the reduction of adverse events, which should occur with low prevalence. To see the advantages of PRP use, large studies may be needed to demonstrate significance. For example, a retrospective study from a dental practice with a single dentist, the incidence of alveolar osteitis (AO) with 904 tooth extractions in 506 patients treated with and without BC-PRP gelled with calcium chloride was examined.⁸ Since the incidence of AO was only 9.6% in extraction sites not treated with PRP, the large number of patients and extractions examined allowed the detection of a highly significant reduction in AO for sites treated with PRP (3.6%, $P = 0.0004$). PRP also reduced the incidence of AO in tobacco smokers to 0% (0/37) compared to 17% (10/59) without PRP. Since smoking negatively impacts wound healing in periodontal surgeries,^{73,74} these findings further demonstrate that PRP can improve wound and bone repair in dental patients at higher risk for

healing problems, similar to those observed in diabetic fracture studies.

CONCLUSIONS

The delivery of PRP versus isolated growth factors has many advantages, including controlled, sustained delivery; the potential for additive and synergistic interactions between the released growth factors; and the therapeutic benefits of the fibrin matrix. There are multiple procedures available for generating platelet-rich preparations. In addition to methods that use larger volumes of blood, have higher costs, and are more complicated; there are simpler, less expensive protocols that can be easily employed by a small clinical practice, including PGRF, PRF, PRFM, and simplified BC-PRP.^{52,53,55,60} Additional criteria to help guide selection of a plasma-rich preparation and activation methods are discussed in Part II.⁷⁵ Earlier soft and hard tissue healing following bone-manipulation oral surgery are important to reduce adverse effects, such as preventing infection and loss of blood clot and/or AO⁸, and to improve patient recovery and quality of life with lower treatment time and costs.²³ PRP may benefit patients with normal healing, with impaired healing, and with slower or incomplete healing by accelerating recovery and improving outcomes. Due to the many beneficial components of PRP, its reported advantages on wound and bone repair, its low risk, and the availability of easy and low cost methods for its preparation, small offices may find it worthy to test PRP in their own practice to determine its benefits for their patients, especially for those at higher risk or with potential for impaired healing.

ABBREVIATIONS

AO: alveolar osteitis
 BC-PRP: buffy coat platelet-rich plasma
 EGF: epidermal growth factor
 FC: fibrin clot
 FGF: fibroblast growth factor
 IGF: insulin-like growth factor
 MSC: mesenchymal stem cells
 PDGF: platelet-derived growth factor
 PGEL: platelet gel
 PGRF: platelet rich in growth factor
 PPP: platelet-poor plasma

PRF: platelet-rich fibrin
 PRFM: platelet-rich fibrin matrix
 PRP: platelet-rich plasma
 RBC: red blood cell
 TFG- β 1: transforming growth factor- β 1
 VEGF: vascular endothelial growth factor

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