

Platelet-Rich Plasma (PRP): What Is PRP and What Is Not PRP?

Robert E. Marx, DDS

Platelet-rich plasma (PRP) has been a breakthrough in the stimulation and acceleration of bone and soft tissue healing. It represents a relatively new biotechnology that is part of the growing interest in tissue engineering and cellular therapy today. Because of its newness, there is a potential for misunderstanding, misuse, and application of what the practitioner may incorrectly think is PRP. The purpose of this paper is to discuss the definition of PRP, its safety, its proper development, and its most efficacious means of application.

WHAT IS PRP?

Platelet-rich plasma is just that; it is a volume of autologous plasma that has a platelet concentration above baseline. Normal platelet counts in blood range between 150,000/ μ l and 350,000/ μ l and average about 200,000/ μ l. Because the scientific proof of bone and soft tissue healing enhancement has been shown using PRP with 1,000,000 platelets/ μ l, it is this concentration of platelets in a 5-ml volume of plasma which is the working definition of PRP today. Lesser concentrations cannot be relied upon to enhance wound healing, and greater concentrations have not yet been shown to further enhance wound healing (Fig. 1).

WHAT IS PRP IN RELATION TO RECOMBINANT GROWTH FACTORS?

Because PRP is developed from autologous blood, it is inherently safe and is free from transmissible diseases such as HIV and hepatitis. Within PRP, the increased number of platelets delivers an increased number of growth factors to the surgical area. The seven known growth factors in PRP are: platelet derived growth factor as (PDGF $\alpha\alpha$), PDGF $\beta\beta$, PDGF $\beta\alpha$, transforming growth factor beta-, (TGF- β_1), TGF- β_2 , vascular endothelial growth factor (VEGF), and epithelial growth factor (EGF). These are native growth factors in their biologically determined ratios. This is what distinguishes PRP from recombinant growth

factors. Recombinant growth factors are pure human growth factors, but they are not native growth factors. Human cells such as platelets do not synthesize them. Instead they are synthesized usually by a culture of Chinese hamster ovarian cells that have a human gene inserted into their nucleus through a bacterial plasmid vector. Recombinant growth factors are single growth factors and are delivered in high doses within either a synthetic carrier or a carrier derived from processed animal proteins. PRP is the combination of seven native growth factors within a normal clot as the carrier. The clot is composed of fibrin, fibronectin, and vitronectin, which are cell adhesion molecules required for cell migration such as is seen in osteoconduction, wound epithelialization, and osseointegration. PRP, however, contains only the same concentrations of these cell adhesion molecules as does a normal blood clot (200 μ g-400 μ g/ml). Therefore, PRP is not a fibrin glue. Platelet Rich Plasma is also not osteoinductive. It cannot induce new bone formation de novo. Only the bone morphogenetic proteins (BMPs) are known to induce bone de novo. However, the prolonged length of time required by recombinant BMP to produce de novo new bone formation and its immature osteoid nature suggest an opportunity for PRP to accelerate BMP activity in the future.

PRP acts on healing capable cells to increase their numbers (mitogenesis) and stimulate vascular ingrowth (angiogenesis). Therefore, it is unlikely to significantly promote bone substitutes and other non-cellular graft materials. However, because it has been shown to stimulate autogenous marrow grafts, it is likely to enhance the bone formation when applied to combinations of cellular autogenous bone and non-cellular bone substitutes.

TERMINOLOGY

There has already been some mistaken terminology related to PRP. Some have advanced the term "platelet concentrate." This is not correct because a platelet concentrate is a solid composition of platelets without plasma, which would therefore not clot. The clinically useful product is a concentration of platelets in a small volume of

Human Platelet Derived Growth Factor AB (PDGF-AB)

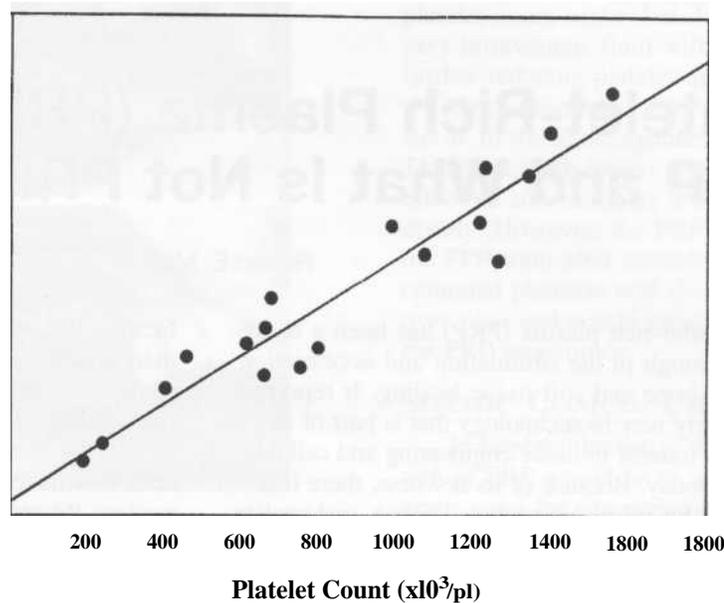


Fig. 1. Human platelet-derived growth factor AB (PDGF-AB). Graph of PDGF-ab versus platelet count indicates growth factors available to tissues as directly proportional to the concentration of platelets.

plasma and is therefore a "platelet-rich plasma." Some have advanced the term "platelet gel." This is also incorrect because PRP is nothing more than a human blood clot with increased platelet numbers. The clot by virtue of its cell adhesion molecules has additional biologic activity, whereas a gel does not. Still others have reversed the term platelet-rich plasma into plasma rich in platelets, plasma very rich in platelets, and even plasma very very rich in platelets. The ludicrousness of this terminology is obvious and is more reminiscent of a coffee house than a clinical science.

PROCESSING PRP AND PRP DEVICES

The professions have already seen numerous individuals and corporations promoting devices to process PRP for either cost savings or economic rewards. The practitioner should keep in mind that any PRP device should process a concentration of at least 1,000,000 platelets/ μ l in a 5-ml volume, process viable undamaged platelets, and process PRP in a sterile fashion and be pyrogen free. Liability, consent, and licensing must be discussed because both patient and auxiliary staff safety issues are pertinent. It should be noted that "sterile" and "pyrogen free" are not the same. Sterile means the absence of microorganism. Pyrogen free means the absence of any microorganism products or foreign body particle that might produce a fever. Therefore, the PRP device must use only certified pyrogen free disposable materials.

To truly concentrate platelets from autologous

blood, the device must use a double centrifugation technique. The first spin (called the hard spin) will separate the red blood cells from the plasma, which contains the platelets, the white blood cells, and the clotting factors. The second spin (called the soft spin) finely separates the platelets and white blood cells together with a few red blood cells from the plasma. This soft spin produces the PRP and separates it from the platelet poor plasma (PPP) free from the obstruction provided by a large number of red blood cells. To attempt PRP with a single spin would not produce a true PRP. Instead, it would produce a mixture of PRP and PPP and have disappointingly low platelet counts. Regardless of the rate of centrifugation or the time of centrifugation, a single spin cannot adequately concentrate platelets, because the red blood cells will interfere with the fine separation of the platelets. This is germane to those who may use a laboratory centrifuge to develop PRP or may purchase a device that is merely a modification of laboratory centrifuge. Such centrifuges are designed for diagnostic purposes-not PRP development. They may not produce a sufficient platelet yield, they may damage platelets, they may not use pyrogen free test tubes, and they are not FDA cleared. Therefore, they should not be used.

The FDA clearance is indeed important. Although the patient is protected from transmissible diseases because of the autologous nature of PRP, the practitioner and the auxiliary staff are not. Devices that leak blood or have the potential to mal-

function from centrifuge misbalance, or design characteristic intended for diagnostic blood work, are a real health, medical, and legal risk. Practitioners are recommended to look to devices that have the simple FDA clearance to process PRP from autologous whole blood. Further FDA clearances to mix PRP with autologous grafts and bone substitutes is an advanced security of some devices.

No dental practitioner or medical practitioner is licensed to infuse or re-infuse blood or blood products systemically in an office setting. However, it is within the licensure of each to apply blood products topically in the office as is done with PRP. Office devices that produce PRP use only 45 ml to 60 ml of blood, which is insignificant related to a normal 4- to 5-L blood volume. There is no reason to re-infuse the blood that is not used, and it would be risky to do so.

APPLICATIONS OF PRP

PRP may be mixed into a bone graft, layered in as the graft is placed, sprayed on a soft tissue surface, applied on top of a graft, or used as a biologic membrane. However, clotting of the PRP should be done only at the time of use. Clotting activates platelets, which begin secreting their growth factors immediately (Fig. 2). Within 10 minutes they secrete 70% of their stored growth factors and close to 100% within the first hour (Fig. 3). They then synthesize additional amounts of growth factors for about 8 days until they are depleted and die. Therefore, clinicians should only clot (activate) PRP when they are ready to use it and not in advance. Clinicians should also critically assess publications, which may claim to

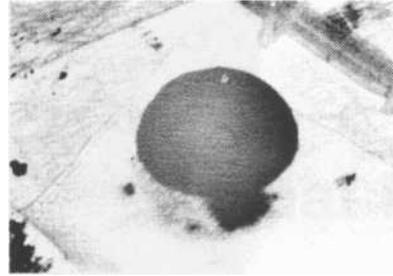


Fig. 2. Platelet-rich plasma (PRP) membrane is an activation of the clotting mechanism, which in turn activates platelets and stimulates the release of their growth factors.

study PRP but are actually studying growth factor depleted clots or supernatants. Complete PRP is both a fresh clot and the supernatant.

This knowledge is germane to those who have advanced the concept of developing PRP from clotted blood or to companies that have promoted "serum separator tubes." Serum is not plasma and contains almost no platelets. It is impossible to develop PRP from clotted whole blood. Because the two functional roles of platelets in nature are initiation of healing and hemostasis, platelets become part of the physical blood clot and, therefore, the serum is devoid of platelets. PRP can only be developed from anticoagulated blood.

WHICH ANTICOAGULANT TO USE?

There are several choices of anticoagulants the clinician can use. However, only two support the metabolic needs of platelets and the viable separation of platelets in an undamaged manner. Anticoagulant citrate dextrose-A (ACD-A) is preferred

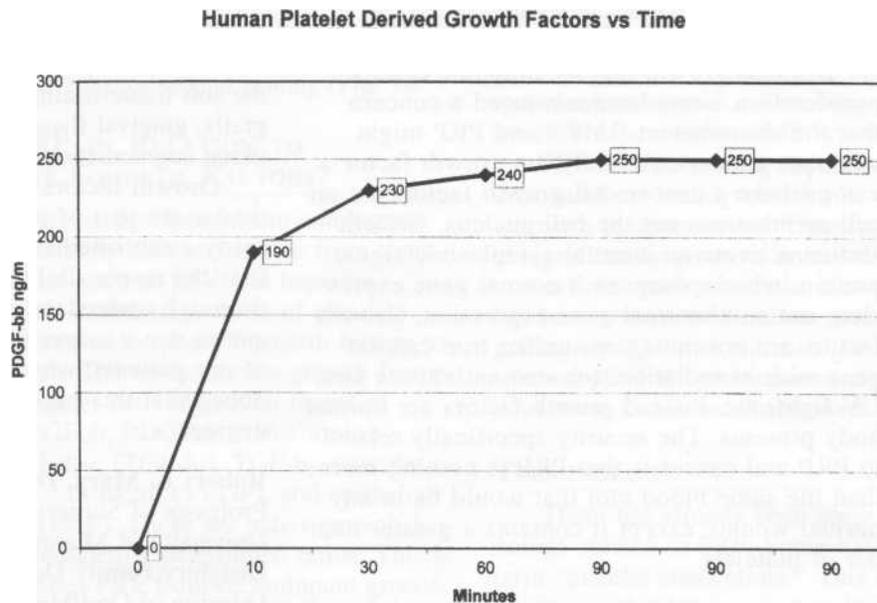


Fig. 3. Graph of PDGF-bb versus time indicating the release of 90% of the growth factors within the first 10 minutes of clot activation and the remainder within the first 1 to 1112 hours.

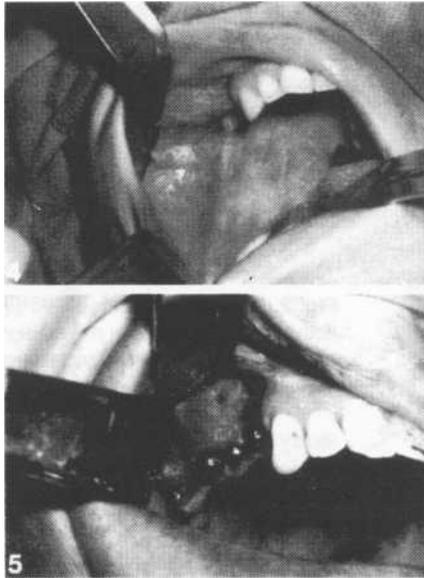


Fig. 4. Severe vertical maxillary ridge defect before bone grafting with PRP enhancement of bone formation.
 Fig. 5. Three months after bone graft reconstruction enhanced with PRP, a mature graft is capable of receiving dental implants with excellent primary stability.

and will best support platelet viability. The citrate binds calcium to create the anticoagulation. The dextrose, buffers, and other ingredients support platelet metabolism. ACD-A is the anticoagulant used to store viable platelets for platelet transfusions from blood banks. Citrate Phosphate Dextrose (CPD) is also useful for PRP development. It is similar to ACD-A but has fewer supportive ingredients and, therefore, is 10% less effective in maintaining platelet viability.

GROWTH FACTORS, PRP, AND CANCER

Because growth factors stimulate cellular proliferation, some have advanced a concern that the recombinant BMP's and PRP might stimulate cancers. Actually, no growth factor can provoke a cancer. All growth factors act on cell membranes, not the cell nucleus. Growth factors activate an internal cytoplasmic signal protein, which promotes a normal gene expression, not an abnormal gene expression. Growth factors are not mutagens, unlike true carcinogens such as radiation, tobacco anthracene tars, UV light, etc. Instead growth factors are normal body proteins. The security specifically related to PRP and cancer is that PRP is nothing more than the same blood clot that would be in any normal wound, except it contains a greater number of platelets.

CLINICAL DEVELOPMENT AND USE OF PRP

PRP is best developed from autogenous whole blood shortly before or at the very beginning of

the surgical procedure. This is because platelets will collect at the surgical site to initiate clotting and healing. This will reduce the whole blood platelet count somewhat. In addition, during surgery intravenous fluid will dilute whole blood, further reducing platelet numbers.

Once developed, PRP is stable and remains sterile in the anticoagulated state for 8 hours. Therefore, with longer surgeries PRP is just as effective and sterile as it would be if used immediately. However, the PRP must be separated from the PPP soon after centrifugation because the concentrated platelets will slowly diffuse into the PPP over time and would reduce the platelet count of the PRP preparation.

SPECIFIC CLINICAL USES

In implant dentistry, the most obvious application of PRP would be to accelerate autogenous grafts used for site preparations, sinus lifts, osseointegrations, ridge augmentations, etc. (Figs. 4 and 5). To date, no positive clinical benefits have been documented, nor can be expected, with the use of PRP with non-vital bone substitutes. The target of PRP remains viable osteoprogenitor cells and stem cells. However, an enhanced bone regeneration can be expected when PRP is used with mixtures of autogenous bone and bone substitutes and with recombinant human growth factors such as recombinant BMP.

In addition, early results are promising that PRP placed in the preparation site of a dental implant will promote and accelerate osseointegration. This may be of specific benefit in the maxilla, in areas of previous failures, in type IV bone, in the osteoporotic woman, etc.

Soft tissue healing enhancement and rapid epithelialization of skin with PRP has already been documented. The extrapolation is apparent to the soft tissue-healing enhancement to palatal grafts, gingival flaps, and cosmetic dentistry soft tissue augmentations.

Growth factors in general and PRP in particular are part of a new biotechnology with already established efficacy and future potential. It is the responsibility of the clinician to gain a thorough understanding of this biotechnology and to use it correctly and wisely for the benefit of our patients, who trust our judgment. It is hoped that this paper served that end to some degree.

Robert E. Marx, DDS

Professor of Surgery and Chief
 University of Miami, School of Medicine
 Daughtry Family Department of Surgery
 Division of Oral/Maxillofacial Surgery
 Deering Medical Plaza
 9380 SW 150 Street, #190
 Miami, FL 33157