

Autologous Platelet Concentrate for the Production of Platelet Gel

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Abstract

Platelets are attracted to a wound or injury site stimulating the clotting and healing cascades. Degranulated platelets release numerous substances including proteins known as growth factors. Growth factors signal undifferentiated stem cells to the site, promote cell mitosis, and

stimulate osteogenesis and angiogenesis. Cytokines, which attract neutrophils, are also released from platelet granules. Concentrating platelets 4x to 5x the baseline level accelerates the healing process. When platelet rich plasma is mixed with an activator, a platelet gel will form. Clinical applications of platelet gel are

numerous. Some benefits include a marked decrease in post-surgical swelling and bruising, reduction in surgical site pain, elimination of drains, and acceleration of bone growth and soft tissue healing. Scientific publications support that autologous biomaterial accelerates the healing process.

Platelets are known to perform multiple functions during injury and tissue repair. Platelets initiate the body's response to a normal sequence of events that provide clotting and healing of the damaged tissue. Following surgical incision, which is a "controlled tissue injury," the wound healing process immediately begins. There are 4 phases of wound healing: the hemostatic phase (clot formation), the inflammatory phase (clean-up and recruitment), the proliferative phase (regeneration), and the tissue remodeling phase. Although these phases appear independent from one another, they overlap significantly during the healing process. Naturally occurring materials present in the body provide the signals and structures for repair, regeneration, and healing. These may be considered as "autologous biomaterials" distinguishing them from recombinant or synthetic "biomaterials."¹ Increasing the concentration of autologous biomaterials to a wound site enhances healing.

Two important autologous biomaterials, platelets and fibrin, play a central role in the formation of the clot. The clotting cascade is initiated with the interaction of platelet membranes and receptors with damaged vascular endothelium. Underlying collagen is exposed to the blood, which acts as an agonist and triggers changes in the platelet membrane. The once disc-shape platelet changes to many long pseudopodia and binding sites with platelet membrane receptors GPIIb and GPIIIa becoming activated. Some of these binding sites have an affinity for fibrinogen and some for the von Willebrand factor (vWF) protein enabling activated platelets to remain at the site of injury. This stage is known as adhesion and is followed by platelet aggregation (a platelet plug of activated platelets). Fibrinogen is converted to fibrin strands forming a 3-dimensional mesh which entraps more platelets. These sticky platelet membranes bind to the fibrin strands adding further structural integrity to the clot. The clot is a hemostatic barrier that halts bleeding and prevents foreign organisms from entering the site. It is important to note that the red blood cells provide no function to the clot. The body will send macrophages to the clot to remove the red cells and the released hemoglobin.

The principal purpose of the inflammation phase of healing is for the infiltration of white cells to the wound and activation of platelets to release growth factors (GF).² Monocytes become phagocytic and are referred to as macrophages. Together with neutrophils, they are able to destroy bacteria, remove red cells and released hemoglobin, and to digest injured tissue and other foreign

materials. Cytokines, released from both white cells and platelets, will attract neutrophils and fibroblasts. A number of derived GF are released from the platelets. Growth factors are needed to start the proliferation phase. They are critical for any wound to heal and are involved in every phase of wound healing. They are contained in the alpha granules of platelets as well as other cells such as macrophages and endothelium. Platelet GF are responsible for the early migration of cells to the injury site and the triggering of mitosis of these cells once at the site. Specific platelet-isolated growth factors include platelet-derived growth factor (PDGF), transforming growth factors-beta (TGF-beta), vascular endothelial growth factor (VEGF), and epithelial growth factor (EGF). (Table 1)

The proliferative phase is responsible for restoring vascular integrity, replacing lost or damaged tissue, and the resurfacing the wound.² Growth factors released from platelets and contained within the clot send out signals to trigger cell division. Platelet-derived growth factors initiate connective tissue healing, bone generation and repair, increases mitogenesis of fibroblasts, stimulates angiogenesis in the wound bed, and activates macrophages.^{3,4} The newly created blood vessels and blood flow bring necessary nutrients and oxygen for optimal healing. Transforming growth factors-beta promotes cell mitosis and differentiation for connective tissue and bone. This growth factor acts on stem cells, osteoblast precursors, and fibroblasts. Vascular endothelial growth factors stimulates angiogenesis and related vascular permeability enhancing activities specific for endothelial cells. This GF is chemoattractive for osteoblasts. Epithelial growth factors induce (EGF) epithelial development and promotes angiogenesis.³

In the remodeling phase, collagen is continually produced and broken down. Inflammatory cells regress and the maturation of the scar may last up to 2 years. Several growth factors regulate

Table 1 Platelet-Isolated Growth Factors

- Platelet-derived growth factor (PDGF)
- Transforming growth factors-beta (TGF-beta)
- Vascular endothelial growth factor (VEGF)
- Epithelial growth factor (EGF)

the remodeling process. Restoration of the wound site to a mature scar depends on the perfect balance of collagen degradation and synthesis.²

With the overview of the clotting and healing cascades in mind, consider the autologous platelet gel clot. Research indicates that acceleration of the wound healing process requires proper preparation of the specimen to yield a product with minimal red blood cell (RBC) and with platelet concentration that has a 4x to 5x above baseline.^{4,6} Since most individuals have a platelet count near the range of 250,000 per cubic milliliter, a platelet-rich plasma (PRP) platelet count of 1,000,000 per cubic milliliter has become the therapeutic level. Increased numbers of degranulating platelets will produce increased concentration of GF or signal proteins.^{1,7,8} Undifferentiated stem cells migrate to the concentration of GF (chemotaxis) moving into the scaffold or matrix of the clot seeking fibrin strands of which to bind and proliferate.¹ Growth factors trigger proliferation of these cells once they are at the site. By increasing the bioactivity of the clot, the recruitment of undifferentiated stem cells to the site of injury or wound healing is accelerated.

Preparation of PRP

Platelet-rich plasma was first identified in the early 1990s through the use of plasmapheresis and PRP sequestration.⁹⁻¹¹ In these procedures, the intraoperative blood salvage devices, also known as autotransfusion machines, were used to process 1 to 2 units of anticoagulated whole blood. Blood was often drawn in cardiac surgery from the cardiac bypass patient prior to surgery for the purpose of platelet sequestration. This would spare the platelets from the mechanical trauma of the oxygenator and other heart-lung machine associated equipment.¹² After processing by differential centrifugation, RBC were immediately returned to the patient. Assessed by platelet aggregometry, platelet function was improved in these patients when plateletpheresis was carried out and PRP retransfused after termination from bypass.¹³ The large autotransfusion machines are still used to produce PRP; most often, these large machines are used in the operating rooms. Various companies are now marketing clinical APC machines that are compact table top centrifuges used at the point-of-care. They use differential centrifugation producing RBC, PRP, and platelet poor plasma (PPP). These clinical systems yield a high concentration of platelets from as little as 20cc of whole blood.

Prior to surgical incision, wound debridement, or other tissue injury, an anticoagulated whole blood specimen must be obtained. There are 2 anticoagulants that support the metabolic needs of platelets and the viable separation of platelets. They are anticoagulant citrate dextrose-A (ACD-A) and citrate phosphate dextrose (CPD). Anticoagulant citrate dextrose-A is preferred as it is the anticoagulant used to store viable platelets for platelet transfusions from donor blood.¹⁴ The procedure and amount of platelet gel (PG) required will dictate how much whole blood will be drawn. For example, if a procedure requires 10 cc of PG, 60 cc of anticoagulated blood must be drawn and processed. Per manufacturer's instructions for use, a volume of anticoagulant is drawn into a 60cc syringe. The skin is prepared in a sterile manner much like that of a blood donor. The blood is gently drawn into the anticoagulated syringe. Care is taken to not activate the platelets or rupture the RBCs. The specimen is transferred to a sterile, single-use processing device. A 2-stage centrifugation process is used. The first spin separates the red cells from the PRP where the RBCs are discarded. The second spin concentrates platelets and white cells with the supernatant being the PPP. The PPP is removed and

properly labeled. The remaining 7 to 10 cc of plasma is used to resuspend the concentration of platelets and white cells. This syringe is labeled PRP. The processing time may be as little as 12 minutes.

The activator is prepared by reconstituting 5,000 units of bovine thrombin with 5 cc of 10% calcium chloride. This mixture is drawn into a 1 cc syringe. The PRP syringe and the activator syringe are placed into a hand-held application system. The syringes are attached to a metering clip and a clip-on handle. This applicator system mixes components of the syringes on application and will control the PRP and thrombin/calcium mixture in a 10:1 ratio. Applied as either a liquid (bead or caulking) or spray, the dual mixture gels in 3 to 5 seconds. The liquid mixture can be placed directly into a wound, such as endoscopic browlifts, rhinoplasty, blepharoplasty, or incision lines for example, or mixed with bone graft material and then placed into a wound bed. The spray application is used when wide surface areas exist such as CO₂ laser resurfacing or undersurface of flaps in facelifts or other soft tissue surgeries. For additional hemostasis, the PPP and thrombin/calcium activator may be applied directly to a wound.

Evaluation of Methods

In making a decision for use of the commercial PRP-producing systems available for the preparation of PG, several factors must be taken into consideration. These factors include the type of setting for production and use of the product, the personnel obtaining and processing the specimen, the type of anticoagulant to be used, the type of collection system (donor bag, tubes, processing devices, etc), the (*g*)-forces during centrifugation, and the processing time. The various methods of preparation must be fully explored as they can directly affect the characterization of the product.^{6,15-18} First, the system must be able to concentrate platelets to 1,000,000 per cubic milliliter, which is considered the therapeutic level. Second, viable platelets must be obtained and preserved such that they have similar characteristics to platelets in circulation. Third, platelets must release GF for 7 days after they are activated.¹⁸

It has been well documented that increased levels of growth factors in PRP (PDGF, TGF- β , EGF) are in direct proportion with increased platelet concentration.^{8,18} The types of collection and processing systems, excessive (*g*)-forces during centrifugation, and types of anticoagulant can have an effect on the quality of the platelets causing fragmentation. These platelets result in reduced function, reduced activity, and increased spillage of GF. Depending upon the method of collection and device used for processing, the intact and viable platelets result in GF ready for activation.

Platelet viability is measured according to the requirements of the American Association of Blood Banks (AABB) and the Food and Drug Administration (FDA) for transfusable platelets.^{18,19} These tests include pH, hypotonic stress, P-Selectin, and platelet aggregation levels. To determine alpha granule release and activation during processing, P-Selectin levels are measured, and the percent of platelets that have undergone change during processing are determined. Better platelet quality is reflected with a lower P-Selectin level and higher level of platelet aggregation. Release time of the growth factors PDGF, TGF- β 1, and EGF in the platelet gel clot has been documented. From the time of clot formation, 95% of GF are released within the first hour. There is a variable release of GF as a function of time and temperature.¹⁸

When assessing the literature for PRP devices, it is important for the clinician to note which devices are cleared by the FDA. These devices will produce the greatest platelet concentrate and yield therapeutic levels of bioactive GF.

Clinical Applications of Autologous Platelet Concentrate

The clinical applications for APC are numerous. Currently, large randomized studies are taking place and being reported. A review of some clinical applications follow.

Oral and Maxillofacial Surgery. Platelet gel is applied to mandibular bone graft to accelerate bone growth for dental implants. Clinical observations have shown radiographic density and maturity of bone approximately twice that of grafts without PRP.⁴ "PRP accelerates the rate of bone formation and allows earlier return of function and earlier implant placement."² Mixing allograft or synthetic graft material with PG will make a more bioactive bone graft material. Other areas of use include surgical repair of alveolar clefts, oral-nasal fistulas, and procedures involving endosseous dental implants.²⁰ Platelet-rich plasma has been used for PG in patients undergoing elective impacted mandibular third molar extractions. The overall rate of alveolar osteitis (dry socket) in PRP treated site was 3.4% vs. 12.8% in the untreated site.²²

Neurosurgery. Platelet gel can be used as a biologic sealant in an effort to create a watertight dural closure. This is to prevent a CSF leak or to repair a documented leak in surgeries such as pituitary tumor removal, skull base tumor resection, acoustic neuroma excision, and intradural procedures involving tumor or release of tethered cords.²¹ In a Stanford University study, PG was used as an alternative to fibrin glue in dural wound repair with 39 of 40 patients having successful repair.²³ At Oakland Naval Hospital, 5 patients (3 young children, 2 young adults) were able to avoid prolonged hospital stay due to the use of PG for tethered cord dural repair.²⁴

Facial Plastic and Reconstructive Surgery. Platelet poor plasma and PRP are widely used in these surgeries. Both products are activated with thrombin/calcium activator solution. Once activated, the PPP forms a fibrin glue. It can be sprayed on exposed surfaces for hemostasis as well as in the surgical bed to decrease bleeding. Platelet poor plasma is applied to the undersurface of facial flaps serving as an adhesive. Gauze is rolled along the flap to spread the gel and remove any excess fluid.^{2,25} Some applications of PG include facelifts, endoscopic browlifts, blepharoplasty (eyelids), rhinoplasty, incision lines, skin grafts, bony reconstruction, and bone graft donor sites. Platelet gel was injected into 23 iliac crests bone harvest sites in place of the traditional hemostatic agents such as microfibrillar collagen. No hematoma or seroma formation occurred.²¹ Decrease in postoperative swelling, hematoma formation, seroma formation, and healing time have been reported.²⁶ The need for drains in some surgeries has been eliminated.²⁷ Spraying PG as a biologic dressing after CO₂ laser resurfacing significantly reduces bleeding, bruising, decreases post-operation pain, and speeds healing and recovery in patients.^{25,28} Due to the strong hemostatic properties of the gels, the use of cautery is minimized therefore reducing the risk of nerve injury. A study of split-thickness skin grafts (skin graft donor sites) of 20 patients was conducted. Each patient had 2 side-by-side split-thickness graft harvests. One site was treated with topical bovine thrombin, and 1 site was treated with PRP. Both sites were covered with an occlusive Opsite dressing. Early wound healing with quick epithelialization was noted in the PRP wounds. Less pain was also indicated in the PRP wound group.⁵

Otolaryngology-Head and Neck Surgery. During radical neck dissections and pectoralis mayor myocutaneous flaps, PG is used as a hemostatic agent and lymphatic sealant. Less post operative drainage allows for early removal of the drains. Platelet gel has been used in endoscopic paranasal sinus surgery as a packing material with excellent results.^{29,30}

Orthopedic Surgery. Total hips, total knees, and iliac crest harvest sites all benefit from the use of PG. When used in total knee arthroplasty (TKA), benefits include earlier functional range of motion, decreased IV and oral narcotic requirements, and lower drop in hemoglobin.³¹ Enhancement of fusion has been observed in total knees and lumbar fusions.^{32,33}

Chronic Wounds. An impressive office-based study of chronic wounds was performed. The wounds included diabetic ulcers, decubitus ulcers, venous stasis ulcers, and complicated surgical wound dehiscence. Included in the study were patients with a wound that failed to have any reduction in size after 4 weeks of standard treatment. Sixteen patients with 17 wounds were enrolled in the study. The number of autologous platelet concentrates applications varied. There was successful wound closure in 16 of 17 wounds. There was a 94% success rate with criteria for success being complete epithelialization.³⁴ In a randomized control-designed study of 20 decubitus ulcers, PG was found to promote wound healing in the treatment group compared to those wounds that did receive conventional therapy.³⁵ Chronic non-healing wounds including treated dehiscent sternal wounds and necrotic skin ulcers showed substantial improvement when treated with PG lesion dressings.³⁶

Burns, Snake Bites, and Spider Bites. Many other wounds have shown benefit from PG applications. If a wound is culture negative, it may have PG applied to it following thorough cleaning and complete debridement.

Veterinary Medicine. Equine legs that have been treated with PG have shown wound repair in wound areas previously said to be untreatable.

Major Vascular Surgery and General Surgery. Vascular access grafts, abdominal aortic aneurysm, carotid endarterectomy are vascular surgeries that benefit from the use of PG. Laproscopic cholecystectomy, splenectomy, gastrectomy, pancreatic, liver resection, hernia repair, mastectomy, limb amputation, and numerous other general surgeries have also shown benefit from PG application.

Cardio-Thoracic. Platelet gel is widely used in the areas of coronary artery bypass grafts surgeries for leg and arm wounds. It is also used in valve repair/replacement, sternal repair, aneurysm repair, and cardiothoracic redos surgeries. In one study, the incidence of sternal infection was lower in the PG group than the control group.³⁷

Ophthalmology. Visual acuity had improved with the use of PG in macular hole surgeries.³⁸

Urology. Platelet gel may be used during radical retropubic prostatectomy and retroperitoneal lymph node dissections. Decreased drain outputs and postoperative fluid requirements have been observed. Also noted was the trend toward early discharge from ICU, less post operative blood transfusions, and early drain removals.²¹

Plantar fasciitis, sometimes called "heel pain," is the most commonly treated condition by podiatrist and foot specialist. In a small study, only APC (not gel) was injected into the feet using high-resolution diagnostic ultrasound for guidance. Both pre- and post-ultrasound measurements of medial, central, and lateral bands were obtained. Decrease in thickness of the symptomatic medial band and resolution of pain was noted for all patients.³⁹

Safety

Platelet-rich plasma is a safe, cost effective, autologous product. It is readily available at the point-of-care, provides antibacterial protection, and eliminates the concern of disease transmission and immunogenic reactions. Rare reactions to bovine thrombin have lead to lower dose use of thrombin and to better purification

processing. Although bovine thrombin is routinely used today as a safe initiator of clotting with PRP, human thrombin processing equipment has been developed.

Conclusion

Platelet-rich plasma represents an emerging biotechnology in current tissue engineering and cellular therapy. Acceleration of bone growth and soft tissue healing has been well documented. Autologous blood yields a product that is safe and free from transmissible diseases. With proper preparation, large numbers of platelets in the PRP are activated producing high concentrations of GF which stimulate soft tissue growth, osteogenesis, and angiogenesis. Many manufactures have presented equipment to process anticoagulated blood to PRP. When researching these clinical devices used to prepare PRP, one must consider a clinical system that results in platelets comparable to those which are produced for transfusion therapy. If minimal platelet activation and functional viability is maintained, maximum GF will be released within 1 hour of activation and continue to be released for 1 week. Benefits of PG include decreased pain and pain medication, decreased bleeding, bruising and swelling, reduction or elimination of drains, increased bone graft regeneration, and soft tissue healing. Clinical applications of PPR include oral and maxillofacial, neurosurgery, facial plastic, reconstructive surgery, otolaryngology, orthopedic, chronic wounds, major vascular, general surgery, cardiothoracic, ophthalmology, urology, and veterinary hospitals. Burns, snake bites, and spider bites that have been properly debrided show excellent results. Reimbursement issues are being addressed. Greater understanding of the effects of PRP will come from the large, randomized studies that are underway and being reported. 1M

The author welcomes comments. Please send to cjameson@plateletgeltech.com.

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