
PRP: Platelet Rich Plasma

Wes Sutter, DVM, ACVS
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I. Abstract/introduction

Slow or poor quality healing of bones, tendon & ligaments and other soft tissues are common clinical problems in horses. Injuries of the extremities appear to be the most susceptible to prolonged healing, exuberant granulation tissue, boney non-union and sequestration and infection. Minimal muscular protection and a resultant decreased blood supply likely contribute strongly to the poor healing response.

Wound healing involves a complex and incompletely understood array of cellular and molecular intracellular and extracellular events. However, it is known that platelets and the formation of a provisional matrix play a prominent and likely determinant role in the initiation and maintenance of wound healing. Platelets are naturally activated by exposure to damaged tissue. Primary hemostasis and initiation of the clotting cascade are just the beginning of the platelets role in healing.

Upon activation, platelets release their granular contents into the wound environment. The contents of the platelet α -granule are of particular interest to wound healing as they contain a host of anabolic growth factors responsible for the initiation, propagation and maintenance of wound healing. Growth factors such as platelet derived growth factor (PDGF), transforming growth factor β (TGF- β) and vascular endothelial growth factor (VEGF) are examples of the milieu of growth factors released upon activation. Individually and synergistically these growth factors stimulate progenitor cell localization to a wound, wound fibroblast expansion and subsequent wound matrix production. In concert with the provisional matrix or scaffolding, growth factors initiate and propagate wound healing.

Whole blood can be taken from the patient and used to concentrate platelets. Platelets have been used for nearly a decade in humans to treat non-healing wounds, augment bone grafts, provide hemostasis, and improve healing in invasive surgeries such as sternotomies and total joint replacements. Similar and novel therapies are applicable to the horse and require little or no modification to the devices used for humans.

PRP gel contains:

- growth factors
- fibrin matrix
- high concentration of platelets
- WBC and phagocytic cells

Benefits of PRP gel:

- “Jump” starts healing: increases growth factor levels at the injury site
- Provides a provisional matrix or scaffold for healing
- Works well in combination with stem cells or grafts
- Improves hemostasis

- Has anti-inflammatory properties
- Has antimicrobial properties
- Processing and application patient side is simple and rapid

II. What is autologous PRP?

Platelet rich plasma (PRP) is derived from anti-coagulated whole blood. Centrifugation separates whole blood into two major components based on their respective density: plasma and erythrocytes (red pack). At the erythrocyte cell-plasma interface is a small visible layer called the buffy coat. It is in this layer and in the immediate surrounding red pack and plasma that platelets are concentrated following centrifugation. Depending on the device and technique used, PRP can contain variable amounts of plasma, erythrocytes, white blood cells and platelets. The platelet concentration should be increased above baseline or whole blood concentration. It is generally agreed upon that PRP should have a minimum of 5 times the number of platelets compared to baseline values for whole blood to be considered “platelet rich.” This conclusion is supported by *in-vitro* work showing a positive dose-response relationship between platelet concentration and the proliferation of human mesenchymal stem cells, the proliferation of fibroblasts, and the production of type I collagen (Haynesworth et al, 2002; Liu Y et al, 2002). With current technology, achieving a 5-fold increase in platelets is relatively simple in a clinical setting.

PRP is used autologously (donor and patient are the same) avoiding graft vs. host complications. The term platelet concentrate (PC) is sometimes used interchangeably with PRP. There does not appear to be an official distinction between the terms, however it is possible to produce a platelet pellet that is mostly devoid of plasma. Platelet releasate is another term often used in platelet science. This term is generally used to describe the supernatant obtained following platelet activation and release of the proteins from the platelet granules into the plasma or more commonly serum. Platelet leukocyte rich plasma (PLRP) is a new term that has been proposed by Everts et al. in an effort to more accurately describe PRP used clinically (Everts et al, 2006). Leukocytes are concentrated in nearly every system that concentrates platelets at the suggested therapeutic concentration. PRP, or PLRP gel, are terms used simply to describe the coagulum formed after the addition of calcium, bovine thrombin, or autologous thrombin alone or in combinations. These products, commonly considered necessary for external applications, activate the platelets. It is somewhat controversial if activation is necessary for internal application as there is likely enough endogenous tissue thromboplastin for platelet activation. To use PRP/PLRP as a hemostatic or tissue-sealing agent it is necessary to activate the platelets. This is generally done with a ratio applicator and blending tip, which mixes the activating agent with the PRP just prior to tissue contact.

III. How is Autologous PRP made?

Procuring PRP is straightforward and simple using the Vet-Stem GenesisCS device. The system is closed, minimizing any opportunity for contamination, and the single use sterile disposables are used only once on the selected patient. The amount of blood needed for horses is physiologically insignificant. Generally, whole blood is drawn aseptically from the donor into a syringe with either ACD (acid-citrate-dextrose) or CPD

(citrate-phosphate-dextrose) anticoagulant. Eight mL of either of the mentioned anticoagulants is mixed with 52mL of whole blood in a 60mL syringe. The blood should be collected as atraumatically as possible using an 18 gauge or larger needle to avoid activating the platelets prematurely by exposing them to excessive shear forces. The blood is mixed with anticoagulant by inverting the syringe gently a couple of times. Using the Vet-Stem GenesisCS kit the blood is then transferred into the centrifugation tubes and subsequently processed patient-side following simple instructions. Within minutes whole blood can be drawn, processed and PRP is ready for application. Simply processing more blood or adding more plasma to the final mixture can adjust the desired volume of PRP. Adding more plasma will decrease the platelet concentration by diluting the PRP.

IV. How does PRP work?

Upon activation, platelets release their granular contents into the surrounding environment. The platelet α -granules are abundant and of particular interest because they contain many of the growth factors responsible for the initiation and maintenance of the healing response. TGF- β , PDGF, VEGF, and fibroblast growth factor (FGF) are a few of the growth factors released. These growth factors have been shown to play an important role in all phases of healing. The active secretion of these proteins by platelets begins within 10 minutes after clotting, with more than 95% of the pre-synthesized growth factors secreted within 1 hour. After this initial burst, the platelets synthesize and secrete additional proteins for the balance of their life (5–10 days). The fibrin matrix formed following platelet activation also has a stimulatory effect on wound healing. The fibrin matrix forms by polymerization of plasma fibrinogen following either external activation with calcium or thrombin or internal activation with endogenous tissue thromboplastin. This matrix traps platelets allowing a slow release of a natural combination of growth factors while providing a provisional matrix that provides a physical framework for wound fibroblast migration and presentation of other biological mediators such as adhesive glycoproteins.

V. PRP for Cutaneous and Bone Healing.

Several human clinical studies have been published evaluating PRP gel topically as a treatment of chronic or non-healing wounds. Many of the studies focus on wounds in patients with diabetes since these patients often present a formidable challenge in terms of healing. The results have been encouraging (Everts PA et al, 2006). Platelet growth factors and many other growth factors are known to be important in the healing cascade. Unfortunately, several studies have shown that a single growth factor applied onto a wound is not as effective as multiple growth factors (Schilepake H, 2002). This highlights the complexity of the molecular events in wound healing where growth factors can have inhibitory and stimulatory functions during the different wound healing phases. A single study evaluated PRP treatment of wounds in horses (Carter CA et al, 2003). This study used allogenic platelets and a proprietary activator. Nevertheless, they were able to show significant histological differences in a 2.5cm² wound model on the forelimbs of horses. The PRP treatment group demonstrated accelerated epithelial differentiation and improved collagen organization (Carter CA et al, 2003).

Similarly, bone healing can be a formidable challenge in horses. Maintenance of a balance between bone absorption, formation, and modeling is critical for fracture repair or boney fusion. Platelets act as an exogenous source of growth factors stimulating bone healing. To date, no studies evaluating the effects of PRP on bone healing in horses have been published. However, there have been numerous clinical studies published evaluating PRP alone or in combination with synthetic or natural bone graft in humans. The majority of studies show enhanced early bone healing when PRP is used in combination with bone substitute or autogenous bone (Everts PA et al, 2006). In the future PRP may play an important role in augmenting bone healing particularly in combination with bone graft or stem cells.

VI. PRP for Tendon and Ligament Healing.

One of the first PRP tendon studies looked at the effects of PRP in a rat Achilles tendon gap model. A 3mm segment was removed from the Achilles tendon and PRP treatment was compared to controls. In one week, PRP-treated animals demonstrated an 18% increase in cross-sectional area and a 30% increase in force to failure. These variables were significant compared to those of the control group out to 4 weeks or until the termination of the study. Interestingly, there was no observable difference histopathologically at 11 days. At 21 days, the PRP treated group showed improved organization and fiber pattern alignment over controls (Aspenberg P et al, 2004). In a similar subsequent study, the PRP treatment group was divided into PRP controls and rats additionally treated with botox to prevent hind limb loading. Treatment with botox eliminated the PRP effects on the biomechanical characteristics demonstrating that the tendon needs mechanical loading for proper regeneration (Virchenko O et al, 2006).

In another study, the effects of PRP on normal tendon were evaluated in a model that compared saline, platelet poor gel (PP) and platelet rich gels (PR) injected into sheep Achilles tendons. Two mLs of each substance were injected into normal Achilles tendons weekly for four weeks. Histopathology of tissue at the injection sites was performed at five weeks. In the PR and PP injection groups, intense neovascularization with absence of inflammatory cells was observed, as well as a marked increase in cellularity that appeared to be aligned with the tendon fibers and tension. In contrast, the saline injected sites showed disorganized and disordered cells at the injection sites (Anitua E et al, 2006).

In a prospective cohort study, 12 human athletes with sutured Achilles tendons treated with PRP were compared to 6 without PRP treatment. Those treated with PRP showed improvements in several measured outcomes, including greater range of motion, earlier recovery (7 weeks versus 11 weeks) and improved functional recovery, earlier time to gentle exercise (11 weeks versus 18 weeks), and earlier time to training (14 weeks versus 21 weeks). Surgical complications were not observed in the PRP treated group; however, in the non-treated group there was surgical site infection in one patient and keloid formation in two patients. Ultrasound exams performed between 32 and 50 months demonstrated the treatment group to have adequate tendon remodeling as evidenced by a smaller increase in cross-sectional area as compared to controls (Sanchez M et al, 2007).

In-vitro evidence supports the clinical findings and gives some clues to the potential mechanisms of PRP improving healing characteristics of tendon injuries. When PRP is

added to cultured human tenocytes, proliferation is increased and cells produce higher levels of angiogenic growth factors VEGF and hepatocyte growth factor (HGF) as compared to controls. This stimulatory effect is not abolished by hirudin, a thrombin inhibitor (Anitua E et al, 2005). Collagen type I is produced in culture within 6 days by tenocytes incubated with either platelet poor or platelet rich fibrin matrices. However, a study looking at gap healing in the Achilles tendon of the rat found a significant difference in outcomes of rats treated with PRP or PRP & thrombin. Rats treated with thrombin (0.5 I.U./ml PRP) had significantly higher force to failure, energy and ultimate stress at 14 days. The clinical significance of this difference, particularly in relation to tendonitis and desmitis in the horse, is unknown.

Schnabel LV et al evaluated some of the in-vitro biological effects of PRP on equine tendon explants. When compared to other blood products, PRP-treated tendon explants demonstrated enhanced expression of collagen matrix molecules and cartilage oligomeric matrix protein (COMP) with no associated increase in the catabolic molecules MMP-3 and MMP-13 (Schnabel LV et al, 2006).

PRP may also have benefits in chronic painful degenerative tendon and ligament injuries. A recent cohort study from Stanford University evaluated the response of elbow epicondylar tendinosis patients, refractory to physical therapy and considering surgery, treated with regional injection of buffered platelet rich plasma (Mishra A et al, 2006). Between 2-3mLs of PRP were injected into the tendon with a 22-g needle using five tendon penetrations. Long term follow up showed 93% of patients had reduction of pain. Just 16% of the control group had reduction in visual analog pain scores at eight weeks. Clinical studies are necessary to determine if PRP may be useful in similar injuries in the horse.

VII. Clinical Application Instructions.

A. Platelet gel for external wounds.

PRP used for external application should be activated with thrombin and/or calcium chloride (.0425mL of 10% calcium chloride per 1mL of PRP). The volume of PRP needed is dependent on the wound surface area and/or volume. In general the wound surface should be completely covered with the gel. Five to ten mLs of PRP is sufficient for most wounds. The PRP is processed and collected in a 12mL syringe according to device instructions. A sterile plastic (a Petri dish works well) or stainless steel flat-bottomed container is coated with the PRP. A ratio applicator can be used to mix the PRP or the activator can be added to the PRP after placing it in the dish. If a ratio applicator is available, the PRP can be sprayed directly on the wound or beneath an adhesive bandage; otherwise it should be in gel form prior to application. Once the PRP gels in the dish, a 4X4 gauze moistened with sterile physiologic saline can be used to lift the gel off. The gel is then placed over the wound and bandaged in routine fashion. Prior to application, it is important that the wound undergo any necessary debridement and necrotic or unhealthy tissue be removed to minimize drainage and maximize the gel contact with the wound bed. The bandage is then left on for three to five days. The treatment can be repeated as many times as necessary.

B. PRP for tendon and ligament lesions.

The goal of intra-lesional PRP therapy is to minimize damage to surrounding and injured tissue thus requiring accurate placement of the PRP within the lesion. Ultrasound can be used reliably to accurately place a needle within the lesion in most locations. Radiographs are generally used for accurate needle placement where ultrasound is not possible such as lesions within or around the hoof capsule. A transverse image with the ultrasound transducer and needle perpendicular to each other provides the best image for placement. This may be difficult to achieve in some locations necessitating the transducer and needle be in the same plane. In this case the needle must be angled toward the transducer once it enters the skin.

The volume to be injected is subjective, dependent on the lesion size and available volume of the cell suspension. Desired volumes can be adjusted during processing of platelet rich plasma. The volume of the lesion can be roughly calculated from the ultrasound images if the distance between transverse images is known. A simpler method is to inject approximately 1mL per 3% lesion calculated on transverse images. The goal in most acute lesions is to fill the defect as best as possible without separating fibers and creating additional damage. Chronic scarred lesions are more difficult and often are injected under some pressure. Most tendon and ligament injuries will easily take between 3 and 6mLs of PRP.

A small gauge needle should be used because often relatively healthy tendon tissue will need to be penetrated to access the lesion. A 22 gauge needle is used in most locations. Smaller needles can make injection of PRP quite difficult. In the proximal suspensory and other locations that require deep injections, an 18 or 20 gauge needle can be used to avoid potential needle breakage. Pre-placing needles intra-lesionally every 2-3cm as needed, and then confirming their placement ultrasonographically prior to injection is an efficient method. Inserting the needle longitudinally with respect to the tendon fibers may result in less injection related tendon damage and facilitate filling of the injured area between tendon fascicles. This is also an advantageous needle orientation for injection of injuries at the enthesis e.g. suspensory origin or insertion.

C. PRP in combination with stem and regenerative cell therapy.

PRP can be combined with cell-based therapies such as adipose-derived stem and regenerative cell therapy. While this is a relatively new concept, the strategy is appealing as the regenerative matrix graft delivers a potent trilogy of regenerative cells, fibrin matrix, and growth factors. The applications are similar to those for PRP alone with the added benefit of regenerative cell enrichment. As more clinical data becomes available, this will likely be a more common therapy for bone and soft tissue regeneration.

Appendix:

Supplies Needed for Wound Treatment with PRP

- Surgical Pack for wound debridement
- ACD or CPD anti-coagulant (8mLs/52mLs whole blood)
- 60mL Syringe and 18-g needles
- Disposables and centrifuge for PRP procurement
- Activator: Bovine Topical Thrombin-JMI (NDC 52604-7102-1) and/or 10% calcium chloride solution
- Sterile saline and 4X4 gauze
- ± Sterile plastic or stainless steel dish (Petri dish works well)
- ± Ratio applicator (10:1) with blending tip (www.micromedics.com)
- ± Tegaderm® adhesive dressing available from 3M (can be placed over the wound and PRP injected between it and wound bed)
- Bandaging Materials

Supplies Needed for Intra-Lesional Tendon or Ligament Injection

- ACD or CPD anti-coagulant (8mLs/52mLs whole blood)
- 60mL Syringe and 18-g needles
- Disposables and centrifuge for PRP procurement
- Ultrasound suitable for tendon and ligament work with sterile probe cover
- 22-g X 1.5in needles
- ± Supplies for regional or field local anesthesia
- Surgical scrub
- Sterile gloves
- Bandaging Material

Supplies Needed for Regenerative Matrix Graft

- ACD or CPD anti-coagulant (8mLs/52mLs whole blood)
- 60ml Syringe and 18-g needles
- Disposables and centrifuge for PRP procurement
- Stem and regenerative cells
- Items from either list for wounds or intra-lesional injection

W. Wesley Sutter, DVM MS, Diplomate ACVS, is a referral surgeon at Ocala Equine Hospital, located in Ocala, Florida. Dr. Sutter completed his graduate research on the uses of Platelet Rich Plasma in horses during his residency program at The Ohio State University.

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