

PLATELET-RICH PLASMA: THE REST OF THE STORY

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Platelet-rich plasma (PRP) is an evolving, versatile component of regenerative human and veterinary medicine. Autologous concentrated platelets in plasma are used in such applications as articular resurfacing, tendon repair, and reconstruction, by providing a concentrated growth-factor cocktail and a provisional matrix to facilitate healing. New evidence suggests that PRP may have analgesic properties and can also slow the progression of osteoarthritis. Platelet-based therapy began in the early 1990's after multiple growth factors were identified in platelet alpha granules. PRP preparation involves a series of centrifugation and separation cycles to concentrate platelets without inducing their premature activation. Once the final product is prepared, platelet activation (exogenous or endogenous) may be important in order to maximize growth factor release. This can be achieved either on the bench top, i.e. in the form of a platelet-rich fibrin clot, or during the process of injection to create a gel in situ within the lesion. Whether to, and if so how to best activate PRP is an ongoing area of study, based on the desired growth factor release profile and the timing of subsequent clearance of the platelet clot from the lesion site. Since the optimal growth factor release profile to facilitate healing is unknown even in a generic wound model (i.e. burst vs. slow release/depot), and probably varies according to tissue type, determining the "ideal" PRP and growth factor strategy presents a multifactorial challenge. Until such time as specific evidence in support of one PRP methodology over another becomes available, the clinician can develop their own preferences from an array of PRP choices in order to augment healing in their patients.

A number of variables exist in the preparation, form, and administration of platelet products. PRP can be percutaneously injected as a liquid, activated and applied as a gel, or inserted into a defect as a platelet-rich fibrin clot. The variety of PRP formulations and the ease of preparation provide a myriad of options based on the treatment goals and logistical limitations of a given case. Viewed in this way, PRP is especially unique in that it can be custom-crafted according to the veterinary surgeon's goals.

Another variable in PRP therapy is the degree of platelet concentration within the product itself. Although in vitro studies indicate that anabolic effects in cell culture are directly proportional to platelet number, the ideal platelet concentration is not known. In humans, the arbitrarily cited minimum desired concentration is 106/ul, representing a roughly 5-fold (humans, dog) to 9-fold (horses) increase over the whole blood platelet count. Recently some human physicians have begun using extremely concentrated PRP (i.e. 20-fold concentration over whole blood), and report anecdotally that therapeutic benefit appears to be enhanced, although an attendant increased risk for acute inflammatory response may also exist. This is unsurprising because platelets contain many other soluble mediators of varying purpose (such as serotonin), in addition to their therapeutically-desired growth factor cargo.

As with many new therapies, the lexicon used to describe platelet-derived products is varied, sometimes confusing, and often redundant. However, the diverse terminology also indicates the aforementioned versatility of platelet products. A brief glossary is provided for the reader:

PRP (platelet-rich plasma): By the conventional human definition, PRP is plasma containing resting platelets at a concentration of at least 106/ul. The PRP may or may not be activated prior to use; the term “PRP” does not indicate that activation has occurred.

PRCR (platelet-rich clot releasate): Ex vivo (i.e. test-tube) activation of PRP is performed, using thrombin, calcium chloride, glass beads, or a combination. The liquid serum above that clot is the PRCR, which is drawn off and used as the treatment product. In this case, the treatment product does not contain platelets (or other cells) and the growth factor content is therefore fixed (i.e. no potential for “sustained release” since no cells or matrix are contained).

PRFM (platelet-rich fibrin matrix): Usually refers to a fibrin clot developed slowly (1 hour minimum) from PRP and calcium-chloride in a glass (i.e. red top) tube. The platelet-rich fibrin clot itself is then inserted into a surgical site. Purported advantages include ease of handling (i.e. a solid, physical substance that can be specifically positioned as intended) and the nature of a matrix itself, in theory providing a construct for tissue regeneration as well as a degradable (autologous) biologic from which growth factors are slowly released.

PC (platelet concentrate): Often used synonymously with “PRP”, but generally implies a higher platelet concentration than the term “PRP” might encompass. Does not indicate whether used in resting or activated form.

PRP Frequently Asked Questions:

Do I need to buy an automated preparation system, or can I make PRP myself?

PRP is nothing more than the name suggests: plasma with a concentration of platelets which is greater than whole blood. There are many commercial systems based on programmed, user friendly centrifuges and containers which partition the majority of the erythrocyte and leukocyte components from the plasma and platelets. The advantages of these systems are ease of use and maintenance of sterility; the “barrier” is generally only breached twice, by injecting the whole blood into it and then removing the PRP from it. In evaluating these systems, the platelet concentration and avoidance of premature platelet activation (which ultimately results in clots and therefore a lower platelet concentration in your treatment syringe) must be considered. The difficulty in doing so is that for most systems, all literature and data provided by the manufacturer is in reference to human PRP, and there may be differences with between those and the platelets of veterinary species. (Species differences exist in platelet size, optimal centrifugation protocol for their isolation, and platelet reactivity). In addition, common sense should be employed: for instance, if a system “doesn’t require” anticoagulant, how is it possible that platelets will be retained in the final liquid portion and not sequestered in a clot?

There are now a few non-centrifugation systems available, and again, each must be assessed based on whether the end product actually concentrates platelets. The advantages of these systems, if they do achieve that goal, are the true “patient-side” nature of the PRP preparation, labor- and cost-effectiveness that they provide.

However, PRP can be prepared manually by any operator with an appropriate centrifuge, protocol, and knowledge of sterile technique. Whole blood is collected in anticoagulant (i.e. 1 part ACD-A to 9 parts whole blood), and should be kept warm until centrifugation. For equine blood, for example, whole blood is spun at 200g for 15

minutes, the plasma is drawn off sterilely and transferred to a new tube, and that plasma is then spun at 400g for 15 minutes. Platelets will pellet at the bottom of the tube; excess plasma is drawn off and the platelets are gently resuspended with a pipet in whatever residual volume of plasma is desirable for use. Less volume=higher platelet concentration of course!

Should I activate the PRP? If so should I do so with thrombin or calcium chloride alone? If I use thrombin, should it be bovine or autologous?

The jury is still out on this topic. Although the standard human protocol has provided PRP activation just prior to injection or use, by way of bovine thrombin and 10% calcium chloride, some human and many equine practitioners skip activation and inject “resting” PRP only. Activation results in immediate and definitive growth factor release, but it’s uncertain as to whether this is required, or whether spontaneous platelet activation occurs in situ after injection, maybe even in “waves”, resulting in more of a sustained-release growth factor profile. Furthermore, we don’t know which of those is preferable, or whether it depends on the tissue type we are trying to regenerate. Although bovine thrombin has been widely used in people with very few side effects, autologous thrombin certainly seems more attractive, in order to minimize any chance for an immune response to the PRP injection. Autologous thrombin preparation kits are widely available, but the resultant thrombin is certainly of a lower concentration than the conventionally used bovine formulations. However, relative to actual physiologic events, these bovine thrombin concentrations are extremely high and likely beyond that required for maximal effect. Some groups use 23mM calcium chloride alone in their PRP, allow it to clot over an hour at room temperature, and then use the clot itself in a surgical site. This can be accomplished easily without specialized tubes or equipment, but the product is not injectable, it is now a solid and therefore only appropriate for “open” applications.

Does PRP enhance bone formation?

PRP use began with human oral surgeons, who are commonly faced with the challenge of trying to recreate bone in either the entire mandible or the alveolar socket. In these scenarios, they use bovine thrombin-activated PRP in conjunction with bone graft. There are many clinical studies indicating that bone formation is enhanced by local PRP application, although in vitro and lab animal results have been mixed. Interestingly, in a recent paper (Han et al, J Bone and Joint Surg 2009; 91:1459-70), bovine thrombin-activation of human PRP resulted in less cellular proliferation and less cartilage and bone formation than controls, while resting (non-activated) resulted in significantly more than controls. In addition, more inflammation was observed in association with the thrombin-activated PRP than with resting PRP, when injected into athymic rats. The majority of the literature suggests that PRP, resting or activated, does promote bone formation.

What evidence do we have supporting PRP use? Against its use?

The in vitro, in vivo and clinical evidence for PRP use in experimental animals and humans is substantial. At the time of this writing however, scientific reports relating to veterinary use of PRP included only about 12 papers specific to horses, less than five on dogs, and none on PRP use in cats. There are many studies employing dogs as an experimental model, most often for human periodontal applications. A brief cross-section of the literature is nonetheless provided here. Reported effects of PRP include:

- Increased proliferation in cell culture: human tenocytes (Anitua '05), osteoblasts (Ogino '06, Slater '95)
- Improved outcomes in tendon healing: equine (Bosch '09), rat (Aspenberg '04, Virchenko '06), human (Sanchez '07)
- Improved histological quality in experimental cranial cruciate ligament injury: dog (Murray '07)
- Improved post-operative pain scores and range of motion in total joint arthroplasty: human knee (Gardner '07), human shoulder (Zavadil '07)
- Improved healing in complicated wounds: diabetic foot ulcers (Giacco '06), deep sternal wound infection, decubitus (Mazzucco '04)
- Improved osteogenesis and vascularization of engineered bone grafts in conjunction with stem cells: dog (Li '09)

Detrimental effects of PRP are essentially not found in the literature, with one notable exception: a distal limb wound study in horses indicated that PRP treatment induced more undesirable fibroplasia (exuberant granulation tissue) than occurred in control wounds (Monteiro '09). Most negative reports on PRP use simply report a lack of therapeutic effect:

Does not enhance bone formation: craniectomy, rat (Roussy '07), dog (Por '08); ulnar or humeral ostectomy, dog (Rabillard '09), (Jensen '04)

Thrombin-activated PRP reduced bone and cartilage formation in vitro and in vivo (whereas non-activated PRP resulted in increased formation of both) (Han '09)

What are the advantages of PRP use over other regenerative therapies?

The main advantages of PRP are its autologous nature, non-invasive collection process, and rapid preparation. PRP is generally more cost-effective and time-saving than stem cell processing and treatment and can be prepared without specialized equipment, using a standard centrifuge. It can be modified into various forms according to whether an “open” (i.e. surgical) or “closed” (i.e. percutaneous) application is desired. PRP can provide a matrix/scaffold and growth factor concentrate to enhance stem cell treatment of a lesion.