Literature review details and supports the application of platelet-rich plasma products in canine medicine, particularly as an orthobiologic agent for osteoarthritis

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ABSTRACT
Platelet-rich plasma (PRP) and other platelet-derived products represent a subset of regenerative medicine and have been researched in the veterinary community for the treatment of osteoarthritis, soft tissue wounds, tendinopathies, periodontitis, and fracture repairs. PRP is simple to produce, relatively affordable, safe, and can be delivered on site, making it an appealing therapeutic agent in veterinary medicine. As an orthobiologic for the treatment of osteoarthritis, it is one of few interventions with clinical study support that possess anabolic potential. Platelet product variability is wide ranging and often described in terms of cellular content or platelet enrichment. Growth factors associated with platelet activation and subsequent degranulation may mediate inflammation, modulate cellular immune response, and promote tissue repair. Product composition, dosage, and application likely influence treatment outcomes depending on the classification of the disease targeted. Sufficient canine data regarding the formulation and clinical application of canine PRP exist to warrant review. The aim of this narrative is to provide scientific background and clinical insight for veterinarians regarding platelet product content/formulation, mechanisms of action, considerations for use, and clinical application in dogs.

Keywords: platelet, platelet-rich plasma, PRP, dog, arthritis

Introduction
Regenerative medicine represents a broad field of therapies aimed at healing or replacing damaged tissues or organs. Platelet-rich plasma (PRP) is a biological therapy within the regenerative medicine field; it is believed to alter the host environment and modulate the immune system to promote healing and mediate inflammation and pain. Platelets are essential for both hemostasis and wound healing, aiding in these processes through the release of various growth factors and cytokines.1 PRP and other platelet-derived products are simple to produce, relatively affordable, and can be delivered on site, making them an appealing therapeutic in veterinary medicine. As such, platelet-derived biologics are one of the most researched regenerative therapies in veterinary medicine and are commonly used to treat osteoarthritis (OA), soft tissue wounds, tendinopathies, periodontitis, and fracture repairs.1,2

Various processing methods dictate both the leukocyte and fibrin content of platelet concentrates, helping to further define and characterize these products.1 PRP is a blood-derived plasma suspension with an increased concentration of platelets and variable quantities of leukocytes and RBCs.1 Likewise, autologous conditioned plasma (ACP) is a commonly used and researched platelet product in canine medicine. No specific definition of PRP or its distinction from ACP exists; however, as a generally accepted rule of thumb, PRP should enrich platelets about 3-fold or greater compared to whole blood, whereas ACP provides a 1- to 3-fold enrichment.1-3 Because ACP technically enriches platelets within plasma for the same general therapeutic purposes, it will be considered a type of PRP preparation within this article.

PRP Composition and Constituents
Introduction to formulation and product variability
Numerous formulations of PRP are commercially available for humans, equines, and canines. Various platelet products often differ in RBC and leukocyte
hepatocyte growth factor, and keratinocyte growth factor, insulin-like growth factor, which have been correlated to tissue healing include von Willebrand factor–rich alpha-granules. The associated growth factor concentration and desensitization of growth factor receptors may result in cellular apoptosis as well as down-regulation and desensitization of growth factor receptors. Theoretically, a ceiling effect may also limit benefit. Because platelet concentrations depend both on volume and cell number, a calculated product dose based on absolute platelet count should be considered.

**Cellular content: RBCs**

Reducing RBCs in PRP is recommended as RBCs have been shown to have a harmful inflammatory effect. Iron within RBCs may act as a catalyst to form reactive oxygen species, damaging cartilage, and synovial tissues. Strong inflammatory mediator (IL-1 and TNF-α) concentrations positively correlate with red cell increases. When leukocyte-rich PRP (LR-PRP), leukocyte-poor PRP (LP-PRP), RBC concentrate, and PBS were all applied to synovial sites ex vivo, RBC concentrate resulted in the most synovial cell death. Current evidence suggests minimizing RBCs in PRP, with most commercially available PRP systems reflecting this ideal.

**Cellular content: neutrophils**

Ongoing debate surrounds the inclusion, effects, and potential therapeutic applications of neutrophils (polymorphonuclear cells) in PRP. Neutrophils inherently release potent inflammatory mediators such as IL-1β, TNF-α, IL-6, IL-8, and matrix metalloproteinase-9. Such cell signaling effects may be desirable within some applications but not others. Because LR-PRP significantly induces more synoviocyte death compared with LP-PRP and PBS, it is likely advisable to minimize neutrophils within intra-articular PRP. On the other hand, certain polymorphonuclear interactions with platelets may result in anti-inflammatory signaling, lending further insight into the complexity surrounding the consideration of platelet product composition. In degenerative soft tissue injuries like tendinopathies, part of the treatment goals may be to reinitiate the inflammatory phase of healing and then progress toward remodeling; therefore, support exists for the application of LR-PRP. Such logic may be oversimplified when applied too broadly. For example, although both LR-PRP and LP-PRP may influence progenitor cell differentiation into tenocytes for healing, LR-PRP has been shown to counterproductively delay tendon healing due to increased catabolic and inflammatory effects.

**Cellular content: mononuclear cells**

Recent studies have shown that mononuclear cells (such as monocytes and lymphocytes) may have a beneficial effect. Monocytes are thought to increase cellular metabolism and collagen production in fibroblasts and decrease antiangiogenic cytokines interferon-γ and IL-12. Likewise lymphocytes exhibit collagen production potential when activated by platelets through increased production of IL-6. Macrophage subtypes have been categorized broadly as M1 polarized (proinflammatory) and M2 (anti-inflammatory and involved in tissue repair). M1-polarized macrophages have been

**Growth factor content**

Growth factor concentrations have been correlated with platelet concentrations in dogs and people; however, ideal concentrations for specific applications are unknown and platelets contain other bioactive molecules that may promote tissue healing. Activation of the platelet triggers the release of its granules, including growth factor–rich alpha-granules. The associated growth factors and cytokines present in alpha-granules, which have been correlated to tissue healing include PDGF, TGF-α, TGF-β, VEGF, basic fibroblastic growth factor, epidermal growth factor, connective tissue growth factor, insulin-like growth factor, hepatocyte growth factor, and keratinocyte growth factor. These growth factors and cytokines work alone or in concert, encouraging cell recruitment, cell migration, cell proliferation, angiogenesis, and osteogenesis. Additionally, some platelet products work synergistically with stem cells by providing a scaffold and stimulating their regenerative and trophic potential.

**Cellular content: platelets**

In principle, PRP should contain a sufficient platelet concentration to affect cell proliferation, cell migration, and immunomodulatory activities for the specific treatment application; however, no medical consensus has been established. Proposed platelet concentrations typically range from 2- to 6-fold. Moderate (2- to 3-fold) and high (4- to 6-fold) platelet concentrations may prove advantageous for the healing of soft tissue and bone injuries. Moreover, recent studies have shown that cells respond to PRP in a dose-dependent manner. On the other hand, platelet concentration greater than 6-fold (or > 1,800 X 10³ platelets/μL) may result in cellular apoptosis as well as down-regulation and desensitization of growth factor

counts but are typically autologous in nature; however, allogeneic preparations exist as well. Studies have examined different commercial canine kit preparations of platelet products, showing wide variability in cellular recoveries between kits as well as a large SD within some kits. In other words, the number of cells (leukocytes, RBCs, or platelets) recovered from the same animal can widely vary between different types of kits or, sometimes, even within the same kit using the same technique. Therefore, variability in cell recovery can be both processing and patient dependent.

The ideal formulation of PRP remains unknown and is likely contingent on numerous factors including the disease (process, location, severity, duration, etc) and the host (age, gender, health status, species, body condition, activity, nutrition, etc). Studies have shown that PRP constituents, such as RBCs, neutrophils, and mononuclear cells, contribute to the inflammatory response following PRP administration. PRP may also be affected by its activation status (granule release) before therapeutic application or whether it has been frozen and stored.

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identified in the synovium of joints affected by OA. Interestingly, monocyte-derived macrophages isolated from peripheral blood have been shown to respond to PRP by shifting from M1 toward a more M2 phenotype. PRP may influence monocyte-derived macrophages within the product or macrophages at the site of application. A transition toward a more dominant M2 synovial macrophage phenotype could explain the longer duration of effect seen by intra-articular PRP in joints affected by OA.

**Platelet activation**

Activation of PRP samples has been shown to affect the concentration of growth factors by inducing degranulation. Platelet degranulation triggers can include exogenous or endogenous parts of the clotting cascade as well as direct stimulation of exposed collagen or other physical stimuli. Platelets release most of their contents within 1 hour of activation; however, viable platelets may continue to release growth factors for up to 7 days after activation. Exogenous activation methods include thermal, thrombin solution, and calcium and are thought to increase the release of growth factors; however, this is not always true. The type of activation agent and platelet product composition influences the type and quantity of growth factor release. Furthermore, activation agents could directly affect changes in tissues, regardless of their platelet effects. For example, even when activation of PRP by calcium gluconate 10% failed to alter growth factor concentrations compared to an equivalent nonactivated product, it still improves the induction of osteoblastic and fibroblastic proliferation. Higher platelet concentrations have been documented with thermal activation compared to induction with calcium gluconate 10% or thrombin; however, freezing and thawing PRP have been shown to also negatively affect platelet morphology, function, and growth factor release.

In general, the positive correlation between platelet and anabolic growth factor concentrations in PRP seems intuitive. In 1 canine study, both platelet concentration and ex vivo platelet activation correlated to growth factor concentration; however, ex vivo activation yielded the largest concentration of measured growth factors with thrombin yielding more platelet degranulation than calcium chloride. A stronger growth factor yield in activated versus quiescent PRP products is not surprising. Quiescent PRP has the potential for activation and granule release upon clinical application that may be equivalent to ex vivo activation but is challenging to measure.

Photoactivation of PRP with low-level laser therapy has been shown to be safe and favorably shift the balance between pro- and anti-inflammatory cell signaling. Photoactivation of PRP has been shown to prolong and improve growth factor release in 1 study when compared to PRP activated with calcium chloride. Another canine study demonstrated an additive clinical effect when PRP was augmented by photobiomodulation for hip OA when compared to PRP or photobiomodulation alone; however, this study lacked a true control group and did not directly measure the effects of light therapy on PRP. In vivo studies to measure for direct effects of laser therapy on injected PRP products would be complex, and there are none to date.

Platelet activation can also be affected by the site of venipuncture. A recent canine study demonstrated less preprocessing activation upon collecting blood from the cephalic versus jugular vein. Furthermore, this study demonstrated that delaying the processing of PRP from whole blood resulted in further unwanted preprocessing activation. The minority of PRP products are activated exogenously before administration because platelets will naturally degranulate endogenously when contacting collagen fibers or other in vivo activation agents. Therefore, most commercial systems have moved away from exogenous activation before administration. More evidence is warranted before making strong recommendations regarding platelet product activation.

**PRP anticoagulation**—Anticoagulants such as EDTA, anticoagulant citrate dextrose-A (ACD-A), and sodium citrate have been used for PRP processing. No anticoagulant is thought to be superior for minimizing effects on platelet function, morphology, and growth factor concentration. Since EDTA suppresses platelet degranulation, it is not commonly recommended for PRP. ACD-A maintains the optimal pH (7.2) while also preventing the coagulation cascade. ACD-A has been shown to preserve platelet morphology and not affect growth factor concentration. Sodium citrate and ACD-A have been shown to support greater proliferation of mesenchymal cells than EDTA. One recent study found that when compared to ACD-A, sodium citrate yielded a higher number of platelets and leukocytes while maintaining similar growth factor concentration. For all these reasons, ACD-A is the most common and well-supported choice.

**PRP cryopreservation**

While it is well documented that platelets are no longer viable once frozen, PRP is sometimes cryopreserved for future application, reducing repeated patient collection procedures. Although freezing PRP alters concentrations of growth factors and inflammatory mediators, freeze-thawed PRP still possesses anabolic potential. Currently, no studies have directly compared fresh versus frozen PRP in dogs. At this point in time, conclusions cannot be made regarding the benefit, detriment, or optimal procedure for freezing and thawing PRP.

**Clinical Applications**

**General application, safety considerations, and follow-up**

PRP is typically injected into target tissue using an aseptic technique with or without imaging guidance (ultrasound, fluoroscopy, etc) to optimize its effect (Figure 1). The administration of NSAIDs is...
common in patients suffering from musculoskeletal disease; however, NSAIDs inherently possess antiplatelet effects. Controversy, therefore, surrounds NSAID administration in patients receiving orthobiologic PRP. Interestingly, a recent canine study showed that dogs taking carprofen did not demonstrate PRP changes in platelet activation or growth factor release. There are insufficient data to support the general recommendation to discontinue antiplatelet therapies before harvesting blood or during PRP application. Despite ongoing debate, NSAIDs may be included in a patient’s treatment plan at the clinician’s discretion.

Autologous PRP products have proven to be relatively safe; however, there are circumstances (systemic or at the site of application) in which harvesting blood or applying PRP is contraindicated and may include but is not limited to thrombocytopenia, anemia, antiplatelet therapy, septic arthritis, sepsis, and neoplasia. Overall, PRP is considered safe with multiple meta-analyses finding no statistically significant increase in adverse events following PRP injection compared with other injected products. Nonetheless, even infrequent (< 1%) complications warrant disclosure with clients before procedures and should then be subsequently monitored. For example, a recent retrospective canine study demonstrated a high safety margin for IA PRP injections; however, major complications such as septic arthritis are possible. Risks can be reduced by sound candidate selection and sterile technique.

Follow-up after administration of PRP is advised and depends on the disease treated and expected outcomes. For orthobiological treatments, we often recommend strict rest with appropriate low-impact rehabilitation exercise until further assessment at a 2-week recheck for IA therapy and a 3-week recheck for intratendinous therapies. Exercise therapy has been shown to benefit patient outcomes as measured by objective gait analysis following plasma rich in growth factors treatment in dogs with OA. Photobiomodulation is a commonly used rehabilitation modality with some evidence that it may augment the effects of PRP. Finally, the frequency in which to repeat PRP remains unknown; however, multiple PRP injections at a single site have demonstrated safety 7 to 30 days apart.

Wound healing
Upon injury to the skin, platelets assist in clotting, provide a scaffold for cell migration, and deliver a robust cell signaling milieu integral to the healing process. PRP has numerous positive effects ranging from its ability to encourage wound epithelialization while reducing scar formation to demonstrating antimicrobial activity and stimulating vascular ingrowth. A study in dogs with decubital ulcerations demonstrated clear benefits from autologous platelet gel in mean percent reduction of wound area from day 5 to 25 compared to paraffin-impregnated gauze. Furthermore, PRP has helped assist in second-intention healing in acute wounds. Conversely, autologous PRP injected at the margins of surgically induced canine wounds failed to accelerate healing but improved tissue perfusion and collagen bundle formation. Such differences in findings may be due to the wound chronicity, sterility, patient health status, or type and application method of PRP.

Oral, dental, and maxillofacial disorders
The prevalence of oral, dental, and maxillofacial disorders in dogs is high, with most being characterized by bone loss or fractures and soft tissue damage including periodontitis, congenital palatal defects, and traumatic injuries, among others. The outcomes of standard surgical interventions are variable, and none are likely to result in bone regeneration. The use of PRP has been proposed for these and similar oral, dental, and maxillofacial applications to enhance tissue healing and promote bone regeneration. However, there are no clearly
defined clinical guidelines and the evidence base is considered weak in people and lacking in dogs with spontaneous disease.

Osteoarthritis

Osteoarthritis likely affects over 60% of the canine population. Platelet-enriched products have been shown to reduce pain and lameness associated with naturally occurring OA of various joints with intra-articular injections in prospective controlled clinical trials. Furthermore, PRP targets secondary stifle OA as a result of cranial cruciate ligament (CCL) disease with evidence supporting its use in dogs whether surgical stabilization has been conducted. In one such study, Yun et al. performed a randomized controlled trial in which PRP, mesenchymal stem cells, or a combination were compared to controls. PRP alone or in combination with mesenchymal stem cells showed a positive effect on lameness and preservation of tissue mechanics and histology compared to controls. Ideal timing of PRP application for the stage of OA remains debatable with some evidence supporting better clinical outcomes with earlier intervention. On the other hand, multiple studies in different species still demonstrate some positive effect of PRP on joints affected by severe OA. Despite uncertainty regarding optimal timing, evidence to support IA PRP use in a variety of OA stages exists. Although these studies appear promising, it should be noted that the product compositions differed, the total study populations were small, and some of the dogs had naturally occurring OA whereas others were surgically induced OA models. Interestingly, when contrasting these studies with anecdotal evidence of efficacy in clinical practice, it appears that both PRP and ACP may provide noticeable and measurable relief from OA in dogs from a single injection for up to 3 months in duration.

Soft tissue injury

Several studies have shown PRP has a beneficial effect on soft tissue healing. Histologic specimens confirm that tendinosis is not an acute inflammatory condition, but one dominated by degeneration. Intradendinous PRP application, particularly when leukocyte rich, can induce transient inflammation that may subsequently stimulate cell recruitment and proliferation to promote tissue regeneration. Additionally, PRP stimulates the secretion of angiogenic proteins, which has a beneficial immunomodulatory effect on tenocytes. Therefore, the paracrine effects of PRP may then help guide and support the local tissues through the appropriate phases of healing. The use of PRP for tendon healing and tendinopathy may be dependent on the site of injury, product used, and stage of disease, adding to the complexity and uncertainty of optimal patient selection. PRP applied during canine surgical tendon repair has been shown to increase neovascularization and fibrocyte proliferation, supporting its use in tendon healing.

References


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Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org.