Key Points:

- The clinical acceptance of intra-articular PRP has preceded needed scientific evidence.
- The relative levels of platelets, WBCs and RBCs in PRP are device and technique dependent. The optimum dose of platelets and/or WBCs as well as the necessity for activation is unknown.
- A high margin of safety and anti-inflammatory effects appear consistent in human clinical studies.
- Indications for PRP may include joint conditions refractory to or not amenable to traditional therapy.

While it is difficult to determine the exact number: the number of patients both human and animals treated with platelet rich plasma (PRP) has grown significantly over the last several years to likely hundreds of thousands. Initially, PRP was coveted as a treatment for slow or non-healing cutaneous wounds and dental applications. Over the past 10 years PRP evolved into a tendon and ligament therapy and more recently (last 5 years) an intra-articular therapy. To date there have been several in vitro and a few in vivo studies in human and laboratory animals outlining the potential mechanisms of PRP therapy. By definition, PRP is plasma that has a higher platelet count than whole blood. Depending on the device being used PRP contains a variable amount of RBCs and WBCs. Most PRP methods increase the concentration of WBCs compared to whole blood and the name Platelet-leukocyte rich plasma has been proposed but not universally adopted. Plasma Rich in Growth Factors is another term used to describe a proprietary method in which the plasma is drawn off the tubes manually at a predetermined volume. Platelet rich fibrin (PRF) is term used to describe PRP that has been clotted with an activator such as thrombin or whole blood that has been centrifuged during the clotting process producing a fibrin clot.

There are several proprietary devices used to make PRP. Most have been developed for humans and the protocols are used without modification in horses. The majority of the devices use a two-stage centrifugation. First a “soft” spin which separates the red cells from the plasma and second a “hard” spin, which separated the platelets from the plasma. Generally the majority of the red pack is removed or separated into another chamber prior to the “hard” spin. This technique maximizes platelet recovery from large volumes of blood. More recently single spin techniques have been marketed for small volume separation. These devices simply separate the RBCs from the plasma. The entire volume of plasma is then used. The result is a smaller dose of platelets in a given volume.

PRP devices can be classified as automated using optical sensors, automated using a density shelf or manual. The difference between automated and manual is that in manual systems the user determines the buffy-coat RBC interface. In the automated systems the machine determines the interface. Automated systems therefore produce a more consistent concentration of platelets in PRP. Because different devices produce different volumes, concentration is not a reliable measure for total dose; therefore when comparing systems the total dose of platelets should be considered. The volume should be multiplied by the concentration to
get the total dose. This number can be used to compare devices. Importantly, the optimum dose of platelets and role WBCs in PRP is not well understood at this time.

PRP systems that are closed have a distinct advantage for the practitioner over systems that require multiple needle aspirations. Needle aspirations can be done aseptically in the laboratory but are difficult to achieve in the field. Care should be taken to avoid bacterial contamination during the collection, processing and delivery. The reported antimicrobial activity of PRP does not obviate the need for good aseptic technique. While PRP has been shown to antimicrobial properties against *Staphylococcus aureus*, it appears to promote growth of other bacteria such as *Pseudomonas*.

**Intra-articular administration of PRP: potential mechanisms**

PRP contains hundreds of different proteins many of which are growth factors that may have beneficial affects in joints with synovitis and or arthritis. Because of the heterogeneity of PRP it is has been difficult ascertain a definitive or single mechanism of action. Potential beneficial affects of PRP on cartilage, synovium and inflammation have been demonstrated *in vitro* and *in vivo*. PRP and the releasate of PRP have an anabolic effect on chondrocytes. Akeda et al. (2006) showed in culture PRP stimulates greater production of proteoglycan and collagen when compared to platelet poor plasma and fetal bovine serum in porcine chondrocyte cultures. Similarly Mishra et al. (2009) showed PRP enhances mesenchymal stem cell proliferation and chondrogenic differentiation. In a clinical model of osteochondral defects in sheep (Sun et al., 2010) showed improved macroscopic, computed tomographic and histologic scores of newly formed cartilage and bone in the defect.

In the joint, the synovium regulates much of the nutrition and metabolism of the cartilage. A study using a proprietary platelet enriched plasma product showed an increase in synovial fibroblast secretion of hyaluronic acid (HA) and the angiogenic growth factors VEGF and HGF (Anitua et al., 2009). The increased levels of the proteins in the joint following PRP administration may be beneficial in healing and inflammatory conditions.

Inflammation is a key element in the pain and progression of arthritis and synovitis. Several studies have attempted to elucidate the anti-inflammatory pathways involved in PRP therapy. Following activation platelets release HGF, TNF-α, and IL-4. These anti-inflammatory cytokines have been shown to reduce NF-κB transactivation in chondrocytes. NF-κB is a principle transcription factor regulating the inflammatory process (Bendinelli et al., 2010). Another possible mechanism is increased lipoxin release. Lipoxins are endogenous lipid molecules that are increased in PRP compared to whole blood (2.2 fold with Biomet ® GPS device). Specifically LXA4 has been shown to decrease inflammation by increasing TGF-β in resolving exudates, promoting non-inflammatory infiltration of monocytes which appear necessary for wound healing and stimulating macrophages to ingest and clear neutrophils (El-Sharkawy et al., 2007).

Accumulation of osteoclasts in synovial tissues is thought play a role in the progression and pain involved in arthritis. Targeting the osteoclast in arthritic conditions involving subchondral bone loss is another approach to modifying the progression and pain in arthritis. Cenni et al. (2010) showed that PRP impairs osteoclast generation from human precursors in the peripheral blood *in vitro*. 
Clinical Studies demonstrating safety and suggesting efficacy.

With the exception of one small case series (Carmona et al., 2007) there are no published studies evaluating intra-articular use of PRP in horses. There are a few human studies. The first study was a cohort study comparing 60 patients with osteoarthritis (OA) in the knee treated either with a proprietary PRP (2.0X increase in platelets, no WBCs) product or HA. Three injections with at 1 week intervals with either PRP or HA were done. The patients were evaluated using the objective WOMAC scoring system at 5 weeks post injection. PRGF group showed 33.4% improvement and the HA group showed 10% improvement. A more recent prospective study evaluated 100 patients with chronic knee pain and evidence of OA on radiographs and/or MRI (Kon et al., 2010). Fifty-eight patients had identified chondral lesions and 27 had prior surgery. A standard two-stage centrifugation was used to yield PRP with 6X baseline platelets. Three 5cc doses were administered every 21 days for 3 total treatments. The PRP was activated with 10% CaCl. No major adverse events were reported. At baseline, end of therapy, 6 months and 12 months, IDKC objective and subjective scores were recorded. The IDKC objective score (% of normal) increased significantly from 46.1% to 78.3% at the end of therapy, remained at 73% at 6 months but dropped significantly to 66.9% at 12 months. There was no difference in scores in patients with previous surgery. A second study looking at the same group of patients in a 2 year follow up showed the IDKC continued to fall from 67% at 12 months to 59% at 2 years. (Filardo et al., 2010) The median duration of clinical improvement was 9 months with this protocol. Better results were achieved in younger patients with lower degrees of arthritis. In another cohort study reported by the same group (Kon et al, 2010), 150 patients with affect by chondropathy, early OA and severe OA were compared. Three groups of 50 patients each receiving either PRP as stated above, low molecular weight hyaluronan or high molecular weight hyaluronan were compared. Using the IKDC and EQ-VAS scores for clinical evaluation a comparison of outcomes was made. There was a statistically significant superiority of PRP at all times of follow up.


