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Critical review of the clinical use of PRP and BMAC

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INTRODUCTION
Platelet-rich plasma (PRP) has emerged as an economical method to provide a source and delivery method for autologous growth factors and cell-rich fractions. PRP has application in management of extensive skin wounds, and in orthopedic diseases, primarily for tendon and ligament repair, and cartilage resurfacing. In equine medicine, our experiences are primarily restricted to its orthopedic application. Platelet-enriched plasma products have also an increasingly broad application in human medicine.

The concept uses blood collected from the individual animal or patient in an acid-citrate-dextrose (ACD) anticoagulant followed by separation in a centrifuge. Various manufacturers produce centrifuge devices that use specific centrifugal force and either a barrier specific density shelf or a countercurrent elutriation technique to enrich platelet numbers approximately 4X, and in some devices up to 10X. Platelets are a rich source of growth factors, particularly platelet-derived growth factor (PDGF) and transforming growth factor β (TGF-β). Both growth factors play a role in musculoskeletal repair, and supplementing these recombinant growth factors has enhanced repair in many models. Autologous platelet gel was developed in the early 1990s as a byproduct of multicomponent pheresis (plasmaphoresis centrifugation). The fibrinogen content is typically 2-4 mg per ml, which is lower than the 100-200 mg per ml contained within commercial fibrinogen preparations or those harvested by cryoprecipitation from plasma, and has the tensile strength and adhesive capabilities to form a depot for growth factor release and also act as a vehicle for cell implantation.

Fibrin sealant (glue) has previously been used as the principal autologous biologic in equine orthopedic applications. Additionally, it has been used in hemostasis, sealing blood vessels on parenchymatous organs, re-attaching cartilage flaps, and as a vehicle for cell-based cartilage repair. However, fibrin use is limited by the preparation time required to produce autologous fibrinogen. Preparation by cryoprecipitation requires 3 days before use. Additionally, fibrinogen prepared by cryoprecipitation has a limited shelf life. Autologous PRP minimizes the time required to harvest a self-setting bioactive compound from blood products. The platelet-rich gel can be used by the surgeon to control bleeding in unsuturable regions, minimize oozing, improve healing in tendon and ligament tissues, and as a vehicle for cell implant into cartilage, bone, and tendon sites. The direct draw method of platelet isolation can provide product within 20 minutes of harvest of blood.

PLATELET-ENRICHED PLASMA PREPARATION
Platelet-enriched plasma can be effectively prepared from the patient within 20 minutes. Sterile preparation of the jugular vein for blood aspiration is routine. Either 30 or 60 ml of whole blood is then drawn into a syringe containing 3 or 6 ml of acid-citrate-dextrose. The blood is then injected into the separation device or centrifuge.

Several manufacturers provide specific disposable devices, packaged with syringes, needles, and anticoagulant. The separation of the buffy coat and plasma uses differential centrifugation and a specific density floating shelf (Figure 1), to separate the bulk of the red blood cell mass from the small white blood cells, platelets, and plasma.

Figure 1. (Left) Centrifuge developed for PRP preparation, using floating density shelf. (Right) PRP is collected in the side decant chamber of centrifugation device (Harvest Technology, M A, U S A). Courtesy Dr. Lisa Fortier.
The platelet-enriched plasma is concentrated by centrifugation immediately above the buffy coat layer of white blood cells (Figure 1). The upper layers of plasma represent platelet-poor plasma (PPP), and are generally drawn off and discarded. Sixty ml of whole blood generally results in generation of approximately 7 ml of PRP. Other devices use counter-current centrifugal elutriation to separate out the specific cell fractions in whole blood.

These units are generally more expensive, as are the disposable devices. Our experience is predominantly with the less expensive differential centrifugation technique (Harvest Technology Inc, MA, USA), using a floating specific density shelf to compress and retain the red cell mass during decant of the platelet-rich plasma and buffy coat. Other simple devices include the filtration system using retention filtration for platelets (Acelere™ PRP; Pal/VetCell Biosciences), and the ACP system (Autologous Conditioned Plasma) available through Arthrex. By using an autologous source of PRP, blood-borne disease transmission is avoided.

The two growth factors of particular interest in PRP are PDGF and TGF-β. Both have a major role in recruiting connective tissue progenitors, stimulating matrix formation, and improving the rate of healing. As a result, PRP is particularly useful in treating flexor tendinitis, suspensory desmitis, bursitis and synovitis, and focal cartilage erosion.

APPLICATION

For injection into tendinitis and desmitis lesions, the PRP is injected directly and relies on local coagulation factors to induce clotting to form a platelet gel. When used as a surface spray on wounds or in cartilage repair, the platelet-rich plasma is activated to form a platelet gel by the addition of thrombin and calcium. The resulting coagulum or “platelet gel” has a significant adherent stickiness, largely due to its concentrated fibrinogen levels.

Tendon and Suspensory Ligament Repair

Recent in vitro research using equine tendon explants indicates PRP actively supports flexor tendon repair. PRP is used as the sole injectable treatment for flexor tendinitis within the first 8 weeks of injury. Ultrasonographic evidence of a core lesion is required to allow injection of the platelet-rich product. Depending on lesion size, 4-7 ml of platelet-rich plasma is harvested. Shorter and smaller cross-sectional area tendinitis lesions require only 1-2 ml of PRP.

More extensive lesions can accommodate 4-6 ml during the injection. The platelet-rich plasma can also be used as a vehicle for more aggressive treatment of tendinitis cases, to deliver enriched cultured bone-marrow derived stem cells (Figure 2).

Repeat injection of PRP can be done 2-4 weeks after the initial injection. Combination with check desmotomy of the accessory ligament of the superficial digital flexor tendon can be done, depending on surgeon preference. There are few published followup studies documenting the results of equine flexor tendinitis treatment with PRP.
Cartilage Repair

PRP makes a very effective vehicle for delivery of cultured chondrocytes or MSCs. PRP can be isolated patient-side, and when used in combination with calcium-activated thrombin, provides a malleable self-adherent platelet gel that combines enriched growth factors with chondrocytes that contribute to cartilage repair. An in vitro study of the metabolic function of chondrocytes in PRP compared to platelet-poor plasma or fibrin showed the positive impact of PRP on chondrocyte function (Figure 3). PRP enhanced chondrocyte cluster formation, collagen type II in deposition and toluidine staining proteoglycan accumulation (Figure 4).

Given its simplicity at harvest, compared to fibrinogen preparation from cryoprecipitation of plasma, PRP makes an effective substitute. The only drawback to the use of PRP is the considerable shrinkage that develops following platelet degranulation during the formation of the platelet gel. Clinical experience suggests that refilling cartilage defects with PRP is required at surgery. Additionally gas arthroscopy is required for application. No published data are available yet concerning cartilage resurfacing using PRP as a vehicle.

Bone Marrow Aspirate Concentrate (BMAC) alone for Cartilage Repair

The development of patient-side centrifugation techniques for intraoperative stem cell isolation and purification for immediate grafting have significant advantages in time savings and immediate application of an autogenous cell for cartilage repair. When considered together, cartilage studies reveal that three components are required for cartilage regeneration; cells, scaffold, and growth factor/s. Recent work has generated a stem cell concentrate from sternal bone marrow aspirate which can be centrifuged to concentrate the cellular population and platelets in bone marrow aspirate. Using flow cytometry, current data indicate that the final total nucleated cell population contains approximately 15% stem cells (Radcliffe & Fortier, unpublished data, 2008). The concentrate also contains a large number of platelets which are the body’s natural reservoir of several growth factors such as IGF-I, TGF-B, and FGF, which are known to enhance cartilage matrix synthesis. The concentrate can be mixed with thrombin to cleave the fibrinogen into a fibrin scaffold to hold the milieu of MSCs and growth factors. This method has the advantages of being a point-of-care technique (no laboratory culture period is necessary) that is completely autogenous, arthroscopically applicable, and delivers all three components believed to be important for cartilage regeneration; cells, growth factors, and a scaffold. In vivo data, using 10 research horses in which 15mm full thickness defects were made on the lateral trochlear ridge of the femur, revealed no post-operative synovitis or other detectable adverse reaction. In the research cases, the grafted limb had a significantly better score at 3-month recheck arthroscopy and the control limb. At 8 months (euthanasia) 3T MRI indices, gross score, and histologic scores were all significantly better in the grafted limb compared to the control limb. Subsequently 28 clinical cases have used BMAC as a sole graft product. BMAC is slow to clot in place, and careful drying is required to get solid adherence of the BMAC, with or without added MSC.

REFERENCE LIST