

Platelet-Rich Plasma: Anatomical Application to Date: Does it Really Work?

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Platelet-rich plasma is increasingly used in the management of tendon injury in sports, supposedly accelerating the healing process, tissue regeneration, and return to play. However, the scientific clinical evidence to support its use is scanty, and more level I studies need to be conducted to justify its widespread use.

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Platelet-rich plasma (PRP) is increasingly used in sports medicine, supposedly accelerating the healing process, tissue regeneration, and return to play, particularly in elite and professional athletes. Introduced in the late 1980s as a topical adjuvant therapy to promote physiological wound healing of chronic leg ulcers,¹ PRP provides regenerative and healing effects in oral implantology.² Therefore, the use of PRP has spread to other clinical fields such as ophthalmology, orthopaedics, sports medicine, cardiology, dermatology, plastic surgery, and neurology. In orthopaedics, PRP has been administered to enhance the healing of meniscus defects and muscle injuries, stimulate chondrocytes to engineer cartilaginous tissue, reduce pain and produce better and more balanced synovial fluid in arthritic knees, improve outcomes after total-knee arthroplasty and subacromial decompression, accelerate bone formation, stimulate the healing of anterior cruciate ligament injury central defects, its primary repair or its reconstruction, improve the outcome of operated ruptured Achilles tendons, reduce pain in chronic tendinopathies, and prevent and reverse intervertebral disc degeneration.³

PRP Therapies and Healing Mechanisms

Activated platelets secrete multiple growth factors (GFs) involved in the healing of musculoskeletal tissues. These factors include transforming GF- β 1, platelet-derived GF (PDGF)-AB and PDGF-BB, vascular endothelial GF A, epithelial GF (EGF), hepatocyte GF, and insulin-like GFs I and II.⁴ When interacting with membrane receptors, different intracellular signaling pathways are activated. In this way, angiogenesis and extracellular matrix formation are induced, which are involved in the repair of tendons, muscles, ligaments, cartilage, and bone injuries. The rationale for PRP therapy lies in reversing the blood ratio by decreasing the amount of red blood cells, which are less useful in the healing process, to approximately 5% and increasing the platelet amount to 94% to stimulate recovery.⁵ These GFs and further released GFs supposedly improve the healing process in chronic injuries and accelerate repair in acute lesions.⁵

Preparation of PRP, Products, and Product Safety

The preparation of PRP is relatively rapid and straightforward and can be undertaken in the clinic or the operating room. Peripheral blood is drawn from the patient, with or without anticoagulants, and the plasma is centrifuged or filtrated. On the basis of the size and phase of injury, different volumes, varying from 10-100 mL, may be applied. Based on the composition and concentration of leukocytes, erythrocytes, and platelets in a given plasma volume, there are 3 methods of preparing PRP: (1) double-spinning methods using automated machines along with commercial kits, (2) single-spinning methods using conventional laboratory centrifuges followed

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by manual PRP separation, and (3) selective blood filtration using commercial available technology.⁶ When using single spinning, the platelet yield is 1-3-times the baseline levels, whereas a yield of 5-8-times the baseline levels is achieved by double spinning. Double spinning also concentrates leukocytes. Pure PRP (P-PRP) formulations do not contain leukocytes; leukocyte and PRP (L-PRP) preparations contain high concentrations of leukocytes.⁶ The role of leukocytes is still controversial,⁷ but P-PRP is more homogeneous and presents reduced donor-to donor variability. Double-spinning techniques provide a PRP concentrate of approximately 10% of the blood volume drawn (ie, 20 mL of whole blood would result in 2 mL of PRP), in contrast to 40%-50% of the blood volume obtained after single spinning. These differently obtained products present varying biological properties and potential uses, but the clinical relevance of this is unknown.⁷ The biological benefit is optimal when platelet concentrations are only moderately elevated. Suboptimal and inhibitory effects have been reported when platelet concentrations are lower or higher, respectively.⁸ The plasma should be prepared and immediately used at the point of care, not be stored, and before application, platelets can be slowly activated with the addition of calcium chloride, a cofactor necessary to convert prothrombin to thrombin. Once plasma has been activated, a fibrin scaffold can be formed *in vivo* or *ex vivo* to gradually release GFs.⁹

Given the autologous nature of PRP, some diseases, such as human immunodeficiency virus infection, hepatitis, or Creutzfeldt-Jakob disease, or those of immunogenic reactions (a concern with allografts or xenografts), may be transmitted unless heterologous thrombin is used for activation.¹⁰ Therefore, human recombinant thrombin is now being advocated. Regarding the possible role of PRP in the development of neoplasms, these GFs act on receptors on the cell membranes and activate normal gene expression,² with no direct mutagenic effect having been reported.

PRP therapies are used in the management of tendon injuries. Tendinopathy is a failed healing response characterized by increased turnover and remodeling and gradual transformation of the extracellular matrix. Tenoblasts and tenocytes repair and maintain the extracellular matrix, which are influenced by external GFs and cytokines released from PRPs.¹¹ PRP is chemotactic,¹² stimulates cell proliferation, and synthesis of angiogenic factors^{13,14} and molecules of the extracellular matrix.¹⁴ In a study on 12 athletes undergoing surgical Achilles tendon repair, Sanchez et al¹⁵ applied P-PRP with a moderate concentration of platelets (2-3 times the concentration of platelets compared with whole blood) clotted *ex vivo*, whereas controls received an identical surgical procedure with no PRP administration. Enhanced range of motion and faster return to sporting activities were reported by the group of patients who had received PRP during surgery. Recently, buffered L-PRP injection did not improve pain or activity in patients with Achilles tendinopathy concurrently undergoing eccentric exercise regimen.¹⁶ Only 1 injection of L-PRP was performed, but most benefits in Achilles tendinopathy, and tendinopathies in general, have been reported after 23 injections. Different clinical effects of PRP have been observed, with favorable preliminary studies in

patients managed for wrist extensor and flexor tendinopathy. Significantly improved pain relief has been observed after 8 weeks from buffered L-platelet concentrate injections.¹⁷ In a randomized clinical trial¹⁸ reporting on patients with chronic tennis elbow tendinopathy, the group treated with corticosteroids recovered initially and declined successively, whereas the L-PRP group progressively improved. PRP application provided significant functional improvement at 12 and 24 months after arthroscopic rotator cuff repair and in athletes with chronic patellar tendinopathy (jumper's knee). In a prospective case-control study, activated L-PRP was effective in patellar tendinopathy at 6-month follow-up. Concerning the effects of PRP on rotator cuff pathology, Everts et al¹⁹ reported better functional recovery and less pain in patients undergoing L-PRP injections after open subacromial decompression, with no group differences at 2 years. Recently, Castricini et al²⁰ reported that PRP applied during rotator cuff surgery produced the same outcomes as no application.

Platelet-Rich Therapies and Tendinopathy

PRP therapies include all PRP, technologies, and readministration procedures. Activated at the site of tissue injury, platelets release intracellular stores, predominantly alpha granules, dense granules, and lysosomes.²¹ Even though the best effectors of PRP therapies are GFs such as PDGF, transforming GF, fibroblast GF, endothelial GF (EGF), hepatocyte GF, connective tissue GF, and vascular endothelial GF, this process seems to be more complex because the alpha granules contain more than 300 proteins.²¹ PRP therapies also contain structural proteins (eg, fibrin, fibronectin, and vitronectin), which facilitate cell adhesion by forming 3-dimensional scaffolds. The term tendinopathy refers to the pain and swelling of a tendon, associated with the histopathologic findings of intratendinous healing failure, occurring when tissue breakdown exceeds the rate of tissue healing or the capacity for tissue repair is impaired.²² Currently, cell apoptosis, deregulated angiogenesis or pain, and inflammation have been hypothesized to be involved, with no mutual exclusion, but simultaneously occurring at different temporal stages.²²

Discussion

In orthopaedic practice, PRP has been used for the management of bone defects, fractures, nonunions, after laminectomy, spinal fusion and joint arthroplasty to promote bone implant osteointegration, and in traumatic or degenerative conditions of tendons, ligaments, muscle, and cartilage.²³ Given the initial enthusiasm and popularity, PRP has been unreasonably used indiscriminately, relying on the fact that platelets are involved in healing process of the damaged tissue. Even though no deleterious effects have been reported after PRP application, it is known that GFs are potent, and consequently, even small changes in their concentrations may unbalance their actions. Some randomized controlled trials have been recently

published, but results are not univocal yet.^{20,24} Some trials report a great positive effect,¹⁸ but more recent well-executed and scientifically stricter ones report at best no effect,^{16,20} and possibly detrimental effects of PRP.²⁵ A meta-analysis has recently indicated predictably disappointing results of different studies, highlighting the limits of the current literature.²⁶ However, a meta-analysis is a statistical work that includes and examines the findings of independent studies considered to be “combinable,” but the variables in this specific topic are countless. To date, the concentration of PDGFs; the appropriate dose, timing, and number; and the length of applications have not been defined yet.^{7,11,27} In addition, many doubts arise about the cellularity of these products as they contain also leukocytes, monocytes, macrophages, and mast cells. The role of leukocytes in these preparations is controversial; even though leukocyte depletion may be advocated because they are reservoir of proteases and reactive oxygen species, which are deleterious, they also contain cytokines and enzymes that prevent infections. As the amount and speed of GF release are variable, these molecules work with different patterns of action and provide different results, it is difficult to ascertain the real influence of such products on the healing process of musculoskeletal injuries.²⁸ It is also mandatory to report and monitor complications. No systemic effects have been evidenced after local PRP injection, but infections, further injuries, and possible systemic effects related to autologous GFs administration should be monitored.²⁸ No scientific reports suggest potential cause-effect relationships between GFs present in PRP and carcinogenesis.²⁹ In 2010, PRP was specifically mentioned in the World Anti-Doping Agency prohibited list for the first time, but the different PRP formulations and treatment methodologies have not been found to increase muscle growth beyond return to a normal physiological state, and the use of PRP injections for only therapeutic purposes does not violate the spirit of sport. Even though PRP has been removed in the 2011 Prohibited List, World Anti-Doping Agency will continue to review PRP use as new medical and scientific information becomes available.²⁹

Conclusion

PRP is innovative and promising, but there is a need of biological evidence to assess the action mechanisms of GFs contained in PRP formulations. Basic and clinical researchers should spend more effort in answering the many questions still open on this field by documenting the clinical experience and performing more preclinical and clinical high-level studies to demonstrate the real potential of this biological approach.

References

- Margolis DJ, Kantor J, Santanna J, et al: Effectiveness of platelet releasate for the treatment of diabetic neuropathic foot ulcers. *Diabetes Care* 24:483-488, 2001
- Marx RE: Platelet-rich plasma (PRP): What is PRP and what is not PRP? *Implant Dent* 10:225-228, 2001
- Mei-Dan O, Lippi G, Sanchez M, et al: Autologous platelet-rich plasma: A revolution in soft tissue sports injury management? *Phys Sportsmed* 38:127-135, 2010
- Nurden AT, Nurden P, Sanchez M, et al: Platelets and wound healing. *Front Biosci* 13:3532-3548, 2008
- Mei-Dan O, Mann G, Maffulli N: Platelet-rich plasma: Any substance into it? *Br J Sports Med* 44:618-619, 2010
- Dohan Ehrenfest DM, Rasmuson L, Albrektsson T: Classification of platelet concentrates: From pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol* 27:158-167, 2009
- Tidball JG: Inflammatory processes in muscle injury and repair. *Am J Physiol Regul Integr Comp Physiol* 288:R345-R353, 2005
- Graziani F, Ivanovski S, Cei S, et al: The in vitro effect of different PRP concentrations on osteoblasts and fibroblasts. *Clin Oral Implants Res* 17:212-219, 2006
- Vavken P, Saad FA, Murray MM: Age dependence of expression of growth factor receptors in porcine ACL fibroblasts. *J Orthop Res* 28:1107-1112, 2010
- Landesberg R, Moses M, Karparkin M: Risks of using platelet rich plasma gel. *J Oral Maxillofac Surg* 56:1116-1117, 1998
- Andia I, Sanchez M, Maffulli N: Platelet rich plasma therapies for sports muscle injuries: Any evidence behind clinical practice? *Expert Opin Biol Ther* 11:509-518, 2011
- Kajikawa Y, Morihara T, Sakamoto H, et al: Platelet-rich plasma enhances the initial mobilization of circulation-derived cells for tendon healing. *J Cell Physiol* 215:837-845, 2008
- Anitua E, Andia I, Sanchez M, et al: Autologous preparations rich in growth factors promote proliferation and induce VEGF and HGF production by human tendon cells in culture. *J Orthop Res* 23:281-286, 2005
- Anitua E, Sanchez M, Zalduendo MM, et al: Fibroblastic response to treatment with different preparations rich in growth factors. *Cell Prolif* 42:162-170, 2009
- Sanchez M, Anitua E, Azofra J, et al: Comparison of surgically repaired Achilles tendon tears using platelet-rich fibrin matrices. *Am J Sports Med* 35:245-251, 2007
- de Vos RJ, Weir A, van Schie HT, et al: Platelet-rich plasma injection for chronic Achilles tendinopathy: A randomized controlled trial. *J Am Med Assoc* 303:144-149, 2010
- Mishra A, Pavelko T: Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. *Am J Sports Med* 34:1774-1778, 2006
- Peerbooms JC, Sluimer J, Bruijn DJ, et al: Positive effect of an autologous platelet concentrate in lateral epicondylitis in a double-blind randomized controlled trial: Platelet-rich plasma versus corticosteroid injection with a 1-year follow-up. *Am J Sports Med* 38:255-262, 2010
- Everts PA, Devilee RJ, Oosterbos CJ, et al: Autologous platelet gel and fibrin sealant enhance the efficacy of total knee arthroplasty: Improved range of motion, decreased length of stay and a reduced incidence of arthrofibrosis. *Knee Surg Sports Traumatol Arthrosc* 15:888-894, 2007
- Castricini R, Longo UG, De Benedetto M, et al: Platelet-rich plasma augmentation for arthroscopic rotator cuff repair: A randomized controlled trial. *Am J Sports Med* 39:258-265, 2011
- Blair P, Flaumenhaft R: Platelet alpha-granules: Basic biology and clinical correlates. *Blood Rev* 23:177-189, 2009
- Longo UG, Ronga M, Maffulli N: Achilles tendinopathy. *Sports Med Arthrosc* 17:112-126, 2009
- Del Buono A, Papalia R, Denaro V, et al: Platelet rich plasma and tendinopathy: State of the art. *Int J Immunopathol Pharmacol* 24:79-83, 2011
- Randelli P, Arrigoni P, Ragone V, et al: Platelet rich plasma in arthroscopic rotator cuff repair: A prospective RCT study, 2-year follow-up. *J Shoulder Elbow Surg* 20:518-528, 2011
- Schepull T, Kvist J, Normman H, et al: Autologous platelets have no effect on the healing of human Achilles tendon ruptures: A randomized single-blind study. *Am J Sports Med* 39:38-47, 2011
- Sheth U, Simunovic N, Klein G, et al: Efficacy of autologous platelet-rich plasma use for orthopaedic indications: A meta-analysis. *J Bone Joint Surg Am* 94:298-307, 2012
- Toomey DP, Murphy JF, Conlon KC: COX-2, VEGF and tumour angiogenesis. *Surgeon* 7:174-180, 2009
- Maffulli N, Del Buono A: Platelet plasma rich products in musculoskeletal medicine: Any evidence? *Surgeon* 10:148-150, 2012
- Engebretsen L, Steffen K, Alsousou J, et al: IOC consensus paper on the use of platelet-rich plasma in sports medicine. *Br J Sports Med* 44:1072-1081, 2010