Research Article

Role of Platelet Rich Plasma in Orthopedic Surgery

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Abstract
Platelet-rich plasma (PRP) is a new technology focused on enhancing the healing response after injury of different tissue types. PRP is prepared by withdrawal of patients' peripheral blood and centrifugation to obtain a highly concentrated sample of platelets, which undergo degranulation to release growth factors with healing properties. It also contains plasma, cytokines, thrombin, and other growth factors that are implicated in wound healing and have inherent biological and adhesive properties. The prepared concentrate is then injected back into the patient at the site of morbidity. This may be intralesional, intra-articular, or surrounding the involved tissue bed. PRP preparations have been used therapeutically in various medical fields with a more recent evolution and promising results in the field of orthopaedic surgery. In cases of tendon, ligament, muscle,cartilage disorders, bone repair, Acl reconstruction, planter fasciitis, intervertebral disc degeneration and osteoarthritis that have failed conservative treatment measure.
Key words: Platelet-rich plasma, withdrawal**,** orthopaedic surgery

Introduction
According to the world health organization musculoskeletal injuries are the most common cause of severe long-term pain and physical disability, and affect hundreds of millions of people around the world. In fact, the years 2000–2010 have been termed ''the decade of bone and joint'' as a global initiative to promote further research on prevention, diagnosis, and treatment of musculoskeletal injuries. [(1)]

The rapid interest in platelet rich plasma and its case-based success has led to widespread use of the technique in the treatment of the musculoskeletal injuries.[(2)]

Platelet rich plasma is defined as a volume of the plasma fraction of autologous blood having a platelet concentration above baseline. [(3)]

Platelet rich plasma operates on a very simple principle: platelet concentrations, when increased in a specific area, stimulate rapid healing. The logic behind platelet rich plasma is that platelets are the first to arrive at the site of tissue injury and thus have the potential to release growth factors that play a critical role in mediating healing.[(4)]

More recently, Platelet rich plasma in an injectable form has been used for the management of common muscle, tendon and cartilage injuries. [(5)] As tennis elbow , chronic rotator cuff tendinopathy, jumper's knee, achilles tendon rupture, osteoarthritis, osteochondral defects, hamstrings tear.[(6)]

Biotechnology of platelet rich plasma
Platelet biology
Platelets are discoid cellular elements that are heterogeneous in size and have the smallest density of all blood cells, at 2 μm in diameter (a leukocyte is about 20 μm in diameter). They are anucleate and originate in the bone marrow as bulges along the length of pseudopodial extensions of megakaryocytes.[(7)]

Platelets are replete with secretory granules, which are critical to platelet function. Among the three types of granules, dense granules, alpha-granules and lysosomes, the alpha-granule is the most abundant.[(8)]

Platelet function

Not long ago, platelets were merely considered to function as haemostatic agents.Around 1980, platelets were recognized for their healing function. More than a decade later, the involvement of platelets in angiogenesis was discovered. Subsequently, Folkman showed that angiogenesis regulating proteins were selectively pumped into the budding pro-platelets from the mother megakaryocyte and that PF-4 is captured by platelets in tumor-bearing animals[7].

The rational for the use of PRPs involves replacing the blood clot with adhesive PRP, thus minimizing the presence of red blood cells (about 95% in volume) while increasing platelet concentration at the injury site. In doing so, we would achieve a supra-physiological concentration of platelet and plasma proteins that accelerates the repair process by direct or indirect mechanisms [7].

Platelet-rich plasma biotechnology: New tools for tissue repair

The biological properties of PRP

A concentration of 1- 407 640 / **µ**L (SD 320 100) of platelets in plasma has been suggested to be the working definition of PRP.[9] This is a platelet count five times higher than that of the blood, which is normally 150 000 **µ**L to 350 000/**µ**L, with an approximate mean of 200 000/**µ**L. PRP refers to autologous preparations.

The properties of PRP are based on the production and release of multiple growth and differentiation factors when the platelets are activated. Platelets begin actively secreting these proteins within ten minutes of clotting[11] , with more than 95% of the pre-synthesised growth factors secreted within one hour[12]. After the initial burst of growth factors, the platelets synthesise and secrete additional such factors for the remaining several days of their lifespan.[12]

Biological activity of PRP

Healing of both soft and hard tissue is mediated by a complex array of intra- and extracellular events that are regulated by signalling proteins. Disruption of the vascular structure as a result of injury leads to the formation of fibrin and platelet aggregation. A stable blood clot is then formed by coagulation of the blood. Subsequently, several growth factors are released into the injured tissue from the platelets and other cells that induce and support healing and tissue formation[10,11]. PRP is also activated by the addition of thrombin and calcium, resulting in the release of a cascade of growth factors from the α granules[12].

The interaction between these growth factors and surface receptors on the target cells activates the intracellular signalling pathways that induce the production of proteins needed for the regenerative processes such as cellular proliferation, matrix formation, osteoid production and collagen synthesis[13].

Growth factors in PRP

The levels of growth factors released from the platelets upon activation are commonly quantified by enzyme-linked immunosorbent assay (ELISA)[14]. PDGF was first found in platelets, especially in the α granules[15] . Three isoforms exist: αα, ββ and αβ isomers[16]. The reason for three distinct forms remains. unclear, but differential binding by various receptor cells such as endothelium, fibroblasts, macro-phages and marrow stem cells has been suggested[17].

The most important specific activities of PDGF include angiogenesis and macrophage activation[18], proliferative activity on fibroblasts[19], chemotaxis for fibroblasts and collagen synthesis[20]. It also enhances the proliferation of bone

3. PRP preparation

This can be prepared in a laboratory, an operating theatre or a clinic from blood collected in the immediate period before treatment. A small amount of autologous PRP can be obtained in minutes. There are three techniques for preparation: the gravitational platelet sequestration (GPS) technique, standard cell separators, and autologous selective filtration technology (plateletpheresis).

The GPS is a table-top centrifuge system. When anticoagulated blood is centrifuged, three layers become evident. The bottom layer is comprised of red blood cells (specific gravity = 1.09), the middle of platelets and white blood cells (buffy coat, specific gravity = 1.06), and the top of plasma (specific gravity = 1.03). This system used a flat-bottomed, 60-ml plastic centrifuge tube. The PRP volume of about 5 ml can be collected following a 12-minute spin at 3200 rpm. Standard cell separators and salvage devices generally operate on a full unit of blood.In general, they use a continuous-flow centrifuge bowl or a continuous-flow disk separation technique and both a hard (fast) and a soft (slow) spin, yielding platelet concentrations from two to four times baseline[21].

Selective filtration technology or plateletpheresis depends on a single-use disposable proprietary filter designed to concentrate platelets from whole blood. The platelets are captured on the filter and are then harvested to provide a platelet-rich concentrate (PRC) without the need for centrifugation. Compared to a commercial centrifuge based method, the filtration device produces a blood fraction similarly enriched in platelets and growth factors[22].

There are several choices of anticoagulant that can be used during preparation of PRP. Anticoagulant citrate dextrose- A (ACD-A) works well, as the citrate binds calcium and prevents coagulation, whereas the dextrose and other ingredients support platelet metabolism and viability. Citrate phosphate dextrose is similar to ACD-A but has fewer supportive ingredients and may therefore be less effective at maintaining platelet viability[23].The use of ethylenediaminetetra- acetic acid (EDTA) is potentially more harmful in the preparation of PRP, and a large number of damaged platelets have been observed. Trisodium citrate solution is an anticoagulant with no negative effects on PRP preparation[24], and consequently, ACD is the preferred anticoagulant.

Handling and application of PRP

Once the PRP is prepared it is stable in the anticoagulated state for eight hours or longer, permitting the blood to be drawn before operation and used as needed during lengthy procedures[25].

It must be activated for the platelets to release the contents of their α granules, with the clot that forms providing a vehicle to contain the secreted proteins and maintain their presence at the site of application. This is most commonly accomplished by adding a solution of 1000 units of topical bovine thrombin per millilitre of 10% calcium chloride to the PRP[24].

Marx[26] described a technique in which 6 ml of PRP, 1 ml of the calcium chloride/thrombin mix and 1 ml of air are introduced into a 10 ml syringe, with the air acting as a mixing bubble. The syringe is agitated for six to ten seconds to initiate clotting, and the clot then delivered.

The PRP is drawn into a 10 ml syringe and the activating solution is drawn into a 1 ml syringe. Both syringe plungers are connected to move in concert with both output ports connected to a dual spray applicator tip which allows both solutions to be mixed as they are applied to the surgical bed. Because the α granules quickly release their contents on activation. In the case of other mixing techniques, it is important to transfer the clot to the surgical site before retraction, otherwise the clot that is transferred may be deficient in the secretory proteins. Most commercially available preparation systems have a PRP delivery technique similar to that of Man et al.[27]

Contraindications to the Use of PRP

Absolute Contraindications include:[26]

- Platelet dysfunction syndrome
- Critical thrombocytopenia below 25,000/μL
- Hypofibrinogenemia
- Hemodynamic instability
- Septicemia
- Sensitivity to bovine thrombin (if using bovine thrombin with calcium to make platelet gel)

Relative Contraindications include:[26]

- Consistent use (anti-inflammatory use) of NSAID's within 48 hours of procedure
- Corticosteroid injection at treatment site or

systemic use of corticosteroids within 2 weeks of graft procedure
- Recent fever or illness
- Rash at graft donor site or at receptor site
- Cancer — especially hematopoetic or of bone
- Active history or history of Pseudomonas, Enterococcus or Klebsiella infection, as PRP has been shown in one study to potentially stimulate these pathogens
- HGB <10 g/dl
- Platelet count less than 105/μL

Contents, type and formulations of platelet-rich plasma

Content of PRP Preparations

The cellular response to platelet-rich plasma (PRP) is influenced by the composition of the PRP, including the relative concentrations of platelets, white blood cells (WBCs), fibrinogen and fibrin, and growth factors.

1- Platelets

Currently, PRP is consistently defined only by the absolute quantity of platelets, and not by other components. Normal platelet counts in blood range from approximately 150,000 to 350,000/μL, whereas PRP is often defined as at least 1,000,000 platelet/μL suspended in plasma[28].

2- Leukocytes

High leukocyte count has been associated with higher release of VEGF.[29] Dragoo et al have also demonstrated a large acute inflammatory response with increased cellularity and vascularity in rabbit tendons 5 days after treatment with leukocyte-rich (LR-PRP) compared with leukocyte-poor PRP (LP-PRP)[30].

3- Fibrin

Liquid PRP formulations contain soluble fibrinogen, which is the precursor molecule to fibrin monomers. Fibrinogen modulates the activity of monocytes and macrophages and therefore mediates the transition between inflammatory and regenerative stages of injury response.[31]

4- Growth Factors and Cytokines

PRP contains numerous growth factors, whose properties vary significantly. PRP typically contains a 3- to 5-fold increase in growth factor concentrations. VEGF, PDGF, and TGF-1 are key growth factors derived from platelets.

Platelet-Rich Formulations

1- Leukocyte-Rich PRP

LR-PRP includes leukocytes in the autologous blood fraction. Because a separation system is required to exclude leukocytes. Several recent studies have characterized the leukocyte concentration produced by commercially available PRP preparation systems.[32]

Tendons injected with LR-PRP showed a heightened acute inflammatory response at 5 days compared with other treatment groups.[10]

2- Leukocyte-Poor PRP

LP-PRP specifically excludes leukocytes in the autologous blood fraction through the use of cell separator systems.[33]

3- Platelet-Poor Plasma

PPP is a by-product of the PRP preparation process and is the blood fraction devoid of platelets that is produced after centrifugation to separate RBCs from the plasma. Some protocols recommend double-spin centrifugation to ensure that platelets are pelleted and contained within the PRP layer. PPP does not have the same therapeutic advantages because it lacks the PDGFs and cytokines. However, it still contains the full complement of plasma proteins responsible for the coagulation cascade and can be used clinically to aid hemostasis.

4- PRP Gel

PRP gel is a broader category encompassing platelet-rich fibrin matrix and platelet-rich fibrin membrane applications. These preparations include fibrin and are designed to provide some structural support for the tissue repair process.

5- PPP Gel

PPP gel, also known as fibrin glue or fibrin sealant, is produced by adding calcium chloride to the PPP blood fraction. Because PPP still contains all of the clotting proteins, it forms a fibrin matrix when activated with calcium chloride.

Application of platelet rich plasma in orthopaedic surgery ACL Reconstruction
By acceleration of the biological integration of the graft by use of PRP, patients could potentially advance through more intensive rehabilitation programs and return to sports more rapidly than patients treated with traditional surgical protocols.

In North America there is an increasing interest in PRP technology. Fanelli and colleagues[34] have reported their results using the Cascade PRFM system (Musculo-skeletal Transplant Foundation, Edison, NJ) in ACL reconstructions with allografts. They found less tunnel expansion and osteolysis in patients who underwent supplementation with PRFM.

Bone repair
The use of platelet rich preparations may help to fulfill an aid to bone regeneration. In fact, in vitro studies have clearly demonstrated that platelet derived growth factors stimulate the proliferation of human trabecular bone cells and the differentiation of human osteoblast-like cells.[35] Studies have confirmed that the local application of PRPs is especially important in pathological conditions in which bone healing is weakened due to an inadequate blood supply, such as that observed in atrophic nonunion fractures.

Muscle injuries
Muscle injuries resulting from extrinsic or intrinsic mechanisms are extremely common in sports, accounting for about 35-45% of all injuries.

At the 2nd World Congress of Regenerative Medicine,[36] reported for the first time the application of leukocyte-free PRP to 21 muscle injuries of different severities and different anatomical locations. Small tears progressed well with a single application, whereas more severe tears required 2-3 ultrasound-guided injections. The injected volume depended on tear severity. These athletes, who played in first division teams of the Spanish SoccerLeague, resumed normal training activities in half the time needed by matched historical controls.

Tendon pathology
The prevalence of degenerative rotator cuff tears is increasing as the population ages.[37] Failed healing and retearing of the rotator cuff are frequent complications following rotator cuff repair. Autologous PRP is an attractive biologic strategy to augment tissue healing.[38]

Evidence on surgical or nonsurgical use of PRP in treatment of tendon pathologies are available in the literature.[36] An early recovery after local application of platelet rich fibrin has been demonstrated during open Achilles tendon repair in athletes. These subjects were able to return to their previous sport activity at an average of 14 weeks, compared with an average of 22 weeks in the untreated group.[37]

Epicondylitis showed more reduction in pain and Disabilities of the Arm, Shoulder, and Hand (DASH) questionnaire scores at 52 weeks.[39]

Plantar fasciitis
The injection of platelet-rich-plasma (PRP) into the affected tissue addresses the healing stages necessary to reverse the degenerative process which are going on in the base of the plantar fascia. The individual cytokines present in the platelet α-granules have been shown to enhance fibroblast migration and proliferation, up-regulate vascularization, and increases collagen deposition in a variety of in vitro and in vivo settings The cytokines present in platelet α-granules have been shown to affect the healing stages necessary to reverse a chronic plantar fasciitis condition .[34]

Osteoarthritis and Articular Cartilage Defect
Van Buul et al.[40] suggested that PRP influences conversion of human osteoarthritic chondrocytes by inhibiting the action of inflammatory cytokines such as IL-1 and NF-kB. However, despite the possibilities based upon the result of some in vitro studies, there is still a lack of evidence to support the effect of PRP in the treatment of osteoarthritis in humans.

Intra-articular injection of autologous PRP has been increasingly implemented on patients with osteoarthritis and currently seems to be considered as one of the treatment options for osteoarthritis. Most of the studies on autologous PRP injection have been focused on the reduction of pain and improvement of function over time. Sampson et al.[41] reported the results of the PRP injection on primary or secondary knee arthritis, showing improvement in pain and symptoms without adverse effect on the scales of Knee Injury and Osteoarthritis Outcome Scores (KOOS).

Intervertebral Disc Regeneration

Lower back pain is currently a serious problem in industrial countries, with enormous financial and health care costs, and intervertebral disc (IVD) degeneration is one of the major causes of the condition.[42] Growth factor therapy is one of the promising modalities to regenerate IVD, because several growth factors have been reported to have therapeutic effects on IVD cells in vitro.Transforming growth factor-b1 (TGF-b1) has been reported to have strong effects on IVD cell proliferation and proteoglycan synthesis.[43]

Conclusions

PRP has demonstrated numerous clinical benefits to patients. PRP remains apotentially powerful autologous therapy for surgeons that want to enhance bone formation. The addition of growth factors such as PDGF, TGF-β1 and VEGF will promote cellular bioactivity by increasing cell proliferation, chemotaxis and angiogenesis. The benefits seen to date with PRP's use will continue to encourage researchers to actively investigate the use of PRP in orthopedics and in other fields of medicine.

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