

Growth Factor of Platelet-Rich Plasma and its Application in Trauma and Orthopedic Surgery

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Abstract

Platelets were considered as hemostatic cells. Platelets are small discoid blood cells produce in bone marrow. There are many intracellular structures inside the platelets containing glycogen, lysosomes, and three types of granules including: alpha-granules; dense-granules; and lysosomes. Alpha-granules, when platelets become degranulated, release hundreds of proteins those are composed of an array of chemotactic and mitogenic growth factors, adhesion molecules, hemostatic factors and different cytokines. Platelet growth factors including: PDGF, TGF- β , FGF, ILGF1, ILGF2, VEGF, EGF, I18, KGF and CTGF. Platelet-rich plasma is a volume of autogenous plasma which has a platelet concentration. PRP with 1,000,000 platelets/ μ l can provide by concentration of platelets in a 5-ml volume of plasma, is the working definition of PRP today. Thrombin act as platelet activator, induce immediate PGF release of the PRP. It is used in medicine for a "scarless repair", or regeneration in situ. Use of PRP as a regenerative medicine has affordability, availability, and minimally invasive properties. The applications of PRP in medicine are for wound healing bone healing, tendon healing, ligament healing, cartilage healing, joints healing, muscle healing and nerves healing. We conclude that the use of platelet-rich plasma in different tissues can result different outcome and more studies are needed. The main purpose of this paper is to evaluate and summarize the applications of PRP in the regenerative literature, with particular focus on advantage and disadvantage of PRP therapies.

Keywords: Platelet Rich Plasma, Healing, Repair

Introduction

There is considerable interest in the use of platelet-derived preparations to promote bone healing. Platelets have an important role in the complex local inflammatory response at sites of bone healing where, on activation, platelets promote angiogenesis, recruit mesenchymal cells and are a source of growth factors required in bone healing. PRP with some characteristics such as osteogenesis and osteoinduction provide a more rapid regeneration of bone defects [1]. It has the advantage of using the host as a donor source, which avoids bone graft donor site morbidity. In addition, PRP provides numerous growth factors necessary for bone healing, including those that are normally released at the fracture site by degranulating platelets. Animal studies have yielded variable results [2]. Several factors influence the healing of these tissues and increase the risk of nonunion, delayed union, osteomyelitis and other problems. PRP has been suggested to be effective in healing of the injured hard tissues, in different species of animals [3]. An easy and more physiological way of application these growth factors to bone defects are via the use of platelet-rich plasma (PRP), a thrombocyte concentrate made up of autogenous blood. Platelet gels are produced by mixing autologous platelet-rich plasma and bovine thrombin dissolved in calcium chloride solution. Bovine thrombin may be added to platelet-rich plasma to initiate fibrin polymerization and to release platelet factors and cytokines, depending on the surgical requirements [4]. Several factors in the preparation of platelet concentrates may differ, such as centrifuge acceleration,

coagulation initiator. Because saline solution does not contain fibrinogen, no adhesive effect can be expected in such a case. The platelets collected in PRP are activated by addition of thrombin or calcium chloride, which induce the release of these growth factors from alpha granules [5]. The use of PRP was proposed in order to provide a microenvironment for the orchestration of the sequential process of bone regeneration involving migration, proliferation and differentiation of osteogenic cells. Several investigations have previously demonstrated the positive effect of PRP on wound healing [6]. Several in vitro studies have shown the role of PRP on cell migration, proliferation and viability but some of them reported adverse effects of this reagent in different cells. Generally, at in vitro level, the reports showing the beneficial effect of PRP on cell migration, proliferation and viability are predominant [7]. PRP has been tested at in vitro and in vivo level in many studies. In the recent years several studies revealed a complex regulation of growth factors for the normal tissue structure and reaction to tissue damage and an important role and effectiveness in using growth factors for healing the damaged tissue [8]. In humans, PRP has been investigated and used as a clinical tool for several types of medical treatments, including nerve injuries, tendinitis, osteoarthritis, cardiac muscle injury, bone repair and regeneration, plastic surgery, and oral surgery. Our hypothesis is that the PRP with autograft can promote bone regeneration in radial bone defects in rat model. The main purpose of this paper is to evaluate and summarize the applications of PRP in the regenerative literature, with particular focus on advantage and disadvantage of PRP therapies [6].

Platelets

Platelets were considered as hemostatic cells. They perform myriad diverse functions. Platelets are small discoid blood cells produce in bone marrow and have a 7 - 10 days lifespan. The average platelet count ranges in circulating blood is 1.5 to 3.0×10^5 /mL of circulating blood. The platelets life cycle intruding differentiation of hematopoietic stems cells, manufacturing megakaryocyte progenitors, formation of proplatelet extensions from megakaryocytes, and proplatelet maturation and producing platelets [9]. There are many intracellular structures inside the platelets containing glycogen, lysosomes, and three types of granules including: alpha-granules; dense-granules; and lysosomes. Alpha-granules occupy 10% of the platelet volume and when platelets become degranulated, release hundreds of proteins those are composed of an array of chemotactic and mitogenic growth factors, adhesion molecules, hemostatic factors and different cytokines [10]. Adhesive glycoproteins secreted from alpha-granules of the platelet, such as Vitronectin, Fibronectin, Von Willebrand factor, and Thrombospondin make platelet adhesion to endothelial cells. Dense granules of the platelets store and release ADP, ATP, calcium ions and serotonin [11]. The role of the ADP is promoting platelet aggregation while ATP can act on P2X1 and join in the platelet response to collagen contact under blood flow. Calcium is an essential cofactor in platelet aggregation and fibrinogen transformation to fibrin. It has a regulatory role in wound healing; calcium ions modulate keratinocytes proliferation and differentiation [6]. Serotonin causes vasoconstriction and increase in capillary permeability. Histamine can have anti-inflammatory and pro-inflammatory effects. Platelets contain Lysosomes, which can secrete cathepsin D, and E, elastases, acid hydrolases, lysozyme etc. whose have important role in healing process that should not be underestimated. The most famous form of platelets which have been used in the clinics is platelet-rich plasma [12].

Platelet growth factors

Platelet-rich plasma is a simple way of delivering autogenous growth factors. Transforming growth factor (TGF- β) consists of structurally and functionally related factors with regulate many biological processes, for example adhesion, differentiation, cell growth, migration and apoptosis. TGF- β has been associated with majority of the bone healing processes. TGF- β stimulates the proliferation of fibroblast and MSC; and has three isoforms TGF- β 1, TGF- β 2 and TGF- β 3 [13]. The platelets constitute a very big source of TGF- β , also it produce by macrophage, osteoblasts and chondroblasts; cause a significant deposit of TGF- β in bone matrix. TGF- β promotes chondrogenesis during endochondral bone formation. TGF- β has osteogenic potential make it to become the stimulator of chondrogenic and osteogenic MSC differentiation [14]. It also has a role in osteoclast apoptosis and inhibition in the endochondral bone healing. Platelet Derived Growth Factor (PDGF) can begin callus formation through the chemotaxis of mesenchymal stem cells, and the mitogenesis and chemotaxis of fibroblasts. PDGF involves in angiogenesis via the endothelial cell proliferation promotion, and the chemotaxis of neutrophil and macrophage that can supply a secondary stage of growth factor release, highlights PDGF as a crucial promoter of healing [15]. PDGF have three isoforms of

with the most understood roles in bone healing: PDGF-AA; -AB; -BB. The source of Insulin-like growth factor (IGF) is from the bone matrix, osteoblasts, chondrocytes, endothelial cells, and platelets, with presence of BMPs possibly stimulating the IGF secretion. In the endochondral pathway chondrocytes, Proliferation and maturation, is regulating by IGF. IGF have a role in bone maturation and remodeling [16]. Vascular endothelial growth factor (VEGF): Angiogenesis is essential for successful healing by providing O₂ and nutrients to the injured region via the newly formed capillary. VEGF is in has an important role in neovascularization as a strong endothelial chemokine and mitogen. VEGF have a role in matrix degradation by metalloproteinases (MMP) which digest the surrounding extracellular matrix [17].

Platelet-rich Plasma (PRP)

Platelet-rich plasma is a volume of autogenous plasma which has a platelet concentration. PRP with 1,000,000 platelets/ μ l can provide by concentration of platelets in a 5-ml volume of plasma, is the working definition of PRP today (Normal platelet counts in blood flow average is about 200,000/ μ l). Lesser concentrations of platelets cannot be relied upon to enhance healing [18]; and greater concentrations of it have no further enhance healing. Platelet-Rich Plasma is a concentrate of autogenous blood platelet growth factors, obtained simply, minimally invasive and low cost method, has been proposed into clinical therapies to improve healing [19].

Terminology

The concentration of platelets in a small volume of plasma is called “platelet-rich plasma”. Platelet rich plasma is a generic term but many terms have appeared to differentiate PRP components and state of activation. The variety of names does little to help standardize the PRP derived products [20]. There has been some mistaken terminology about PRP. Sometimes the term “platelet concentrate” has advanced. This is wrong because a platelet concentrate is a solid constitution of platelets without plasma that would not clot. The term of “platelet gel” is incorrect because PRP is not more than a human blood clot with enhanced platelet numbers. The clot has biologic activity; but a gel doesn't [21].

History

In 1974, Ross., et al. determined that the addition of platelets and calcium resulted in significant improvements in the mitogenic capacity of the serum derived from whole blood. They concluded that platelets can be the big source of the proliferative effect provided by serum. At 1978, the term “platelet derived growth factor (PDGF)” was invented. Initially the identification of platelet growth factors and use of PRP, reported in 1998 by Marx as beneficial for use in mandibular bone healing [22]. This cause more interest of PRP use in the oral surgery. Then PRP was used in variety of surgical fields, including soft tissue healing, cosmetic surgery, nervous tissue, chronic skin ulcers, and burns; but conclusive indication for the PRP use in bone healing it is not established [23].

PRP Activation

Platelets must be activated to initiate of growth factors release. Thrombin act as platelet activator, induce immediate PGF release of the PRP. Thrombin is derived from bovine plasma; it is used as a gold standard; bovine thrombin usually is associated with antibodies and it had occasionally led to life-threatening coagulopathies. In addition PRP can be activated by autogenous thrombin, which produced with commercially available thrombin production kits [24]. Mixing PRP with calcium chloride and thrombin for antagonize the citrate present in the blood bag would cause the activation of the platelet concentrate. Add of thrombin and calcium chloride to PRP activates the alpha granules to release the following biological growth factors. PG can be applied with a syringe or as a solid jelly applied to soft tissues, bone, or synthetic bone. An ideal PG producing procedure is defined as a procedure within 10 seconds. However, formation of coagulum is a function of the activated fibrinogen concentration, rather than the amount of platelets [25].

Advantages of PRP use

Platelet suspensions in plasma is prepared for the therapeutic proposes and experimental study. It use in the fields of growth factor, stem cell, extracellular matrix, tissue engineering, Regenerative and Medicine research. It is used in medicine for a “scarless repair”, or regeneration in situ. In the regenerative medicine new tissue takes place within the lesion zoon [26]. The propos of this is optimization and enhancing the natural healing process. Use of PRP as a regenerative medicine has affordability, availability, and minimally invasive properties. Since it's produced from the patient's blood the risk of diseases transmission is Minimum. Furthermore, because it's autogenous, PRP doesn't provoke immune response. The preparation process of PRP is rapid and doesn't need special instruments; so it can be applied to a patient in short time. These features cause PRP attractive for clinical use in hospitals [27].

Disadvantages of PRP use

Clinical prospective randomized trials are needed to evaluate PRP use in medicine. The preparations of the injections do not underlay the rules of the European Medicines Agency so they are not a “medicament” in the pure sense. They’re often sold as a private offer to the patients and they not become refunded by the governmental institutions. Until now we are unaware of major health situations that have arisen through the therapeutic use of autogenous PRP-clots [28]. Low platelet counts or high haematocrits may be a limiting factor and more research is needed to establish the optimum number of platelets for use. Platelets release large numbers of microparticles that carry proteins which are prothrombotic. So, we must be careful in using this procedure in around large blood vessels, especially in patient with thrombotic risk factors. Anti-platelet drug may be needed to use [29].

Wound healing

Several investigations have been demonstrated the effect of PRP on wound healing. Hard-to-heal wounds cutaneous wounds and chronic wounds represent significant impact psychologically. The hard-to-heal wounds are those which don’t heal in any case of proper treatment of wound. A wound is said chronic wound when it doesn’t heal for period of 12 weeks [30]. In chronic pressure wounds there is a decreased growth factor concentration in comparing an acute wound. Wound healing is an organized and complex series of events (cell-cell and cell-matrix interactions) with growth factors serving as messengers to regulate the various processes involved. The action of PDGFs in wound healing has established previously. In wound healing repair begins with platelet clot formation, coagulation cascade activation, and platelet degranulation with release of its growth factors [31]. After tissue damaging platelets start to be degranulated by release of growth factors such as PDGF and TGF which are important growth factors and they start wound healing process. PGF is also chemotactic and mitogenic for inflammatory cells. PDGF amplifies the inflammatory response. PGF again plays a predominant role during the phase of matrix synthesis and matrix deposition. PDGF increased the volume of tissue granulation. Collagen production is initiated by the chemotactic and mitogenic actions of fibroblasts by FGF [32].

Bone healing

There is very much interest in the use of platelet and its derived preparations to promote bone healing. The clinical and experimental studies regarding the osteogenic potential of PRP are controversial. PRP may be has a role in bone healing, it usually is used when autogenous bone is not sufficient or possible for treatment for example in large defect. Recently one study examined the osteoinductivity of PRP on these cells, in comparison to treatment with autologous serum [33]. PRP enhance Gene expression for Type I collagen, osteopontin and osteocalcin. Platelet concentration is important for bone formation, but there is no bone produced at very high platelet concentrations or at very low intermediate concentrations. Osteoinductivity property of the PRP is because of the presence of different growth factors and it make the addition of it improves new bone formation. In a recent study PRP was shown to decrease osteoinductivity of demineralized bone matrixin (DBM). Another study has been shown osteogenicity of activated PRP with thrombin and calcium which provide a scaffold for soft extracellular matrix formation in bone defects [34]. Another study used high concentration PRP in with cancellous bone graft for treatment of critical unicortical defects in the tibia of pigs. The PRP group was compared to bone graft alone. The zoon of bone formation in the bone defect was significantly greater in PRP treated animals. Autogenous PRP has been used in bone surgery to stimulate bone growth and maturation [21]. Impregnated bone grafts with autogenous PRP are more effective in bone healing process and make a greater trabecular bone density than bone graft alone. The growth factor of Autogenous PRP produced quantifiably enhanced bone density and maturation, as well as an improved ultimate result. Addition of PRP influenced the tissue microenvironment by providing key hidden factors for regeneration, by means of increasing the progenitor cell recruitment, collagen production, bone matrix deposition and creating a bridging interface between the scaffold and bone [30].

Tendon healing

Tendinopathy is the degenerative condition of tendons which characterized by loss of collagen, tissue stability, integrity and strength. It is not an inflammatory condition with inflammatory cells. Tendinopathy is happened because of natural aging, repetitive stress, and injury and vascular, neural and hormonal inputs. The injured tendons which healed with scar tissue adversely affect its function. In addition tendons that heal slowly will have poor vascularization [35]. Different studies are supportive for PRP use in tendinopathy. Some studies have shown improved tenocyte proliferation and collagen and they have good results. Tendon was among the first investigators of the use of PRP on tenocytes in culture. Some studies have examined the effects of PRP on injured tendon and ligament. Collagen synthesis was greatest in platelet-poor plasma [6]. A study found increased collagen expression after treatment with PRP lysed but they saw there were no significant differences between PRP and plasma or whole blood controls. Another study on tendon explants reported a remarkable increased breaking strength and stiffness in explants treated with a collagen graft in combination of PRP [36].

Ligament healing

Tendon and ligament was the first investigation of PRP use in culture. Most of ligament studies have been in combination with surgical anterior cruciate ligament reconstruction. The researches suggest improved healing and graft quality with using PRP. In some orthopedic surgeries regard to non-surgical care is absent at this point. In sports studies the evidence show improved time of healing, reduced time to return to sport and reduced pain [10].

Cartilage healing

When PRP added to the culture medium, it induced a high mitogenic response in the chondrocytes. PRP promotes proliferation, adhesion, and migration of muscle-derived MSCs. PRP tended enhance the number of collagen-producing cells and increase cell apoptosis. Researchers showed high expression and synthesis of collagen by chondrocytes. In addition they inspected the combination with hydrogel allowed the retention of PRP at the defect site of cartilage and filling up irregularities at the cartilage surface [37].

Joints healing

Osteoarthritis is a chronic degenerative disease of articular cartilage. Improve the clinical condition of an osteoarthrotic joint with degenerative process and symptom is hard. Because of the lack of healing response to osteoarthritis, injection of PRP which has growth factors is sensible. Previous studies for using PRP in osteoarthritis have favorable results [38]. However it is unknown that PRP acts in healing process by new hyaline or fibrocartilage formation. Animal studies describe enhance healing in glenohumeral labrum, meniscus and OCD with induced defects. Researchers showed the usefulness of PRP because of its proliferation effect. PRP regulated the viability of meniscal cells as well as the expression of biglycan and decorin [7]. The studies of the PRP effects on articular tissues have reported positive effects of PRP on chondrocytes or their precursors, in terms of proliferation and increased matrix synthesis. PRP induced a dramatic chemotactic effect on cortico-spongious progenitor cells as well as significant increases in collagen. The effects of PRP use on synovial fibroblasts and meniscal chondrocytes of the joints have been examined: it was Showed normal synovial fibroblasts produced significantly more Hyaluronic Acid after exposure to PRP [39].

Muscle healing

Muscles have rich capillary stroma and usually heal with 8 times faster than ligaments. PRP treatment is used when a chronic or sub-acute condition developed. The distribution an acute injury in a trying to promote function is considered, but there is inadequate evidence to endorse this presently [40]. A study has shown faster recovery of acute muscle injury with PRP usage. The applicable of PRP to larger scale in human cases, it is unknown that is relevant functionally, clinically or for return to sport. Treatment of myositis ossificans with PRP injection is a usual procedure [41].

Nerves healing

Enmeshment neuropathies that have unsuccessful “conservative management” have been customarily treated with surgical neurolysis or release/decompression. There is cultivate experience in execute percutaneous release of nerves using different solutions. There is insufficient information to approve PRP treatment for neuropathies treatment, however, in ischemic damage cases to a nerve due to scar tissue banding, there is a role for PRP and we encourage further investigation [42].

Conclusion

In general, PRP is an effective protective treatment without complications and surgical cost, and has been successful in the repair of various musculoskeletal conditions, but should not be treated in the first line of treatment. PRP plays an important role in wound healing, especially chronic ulcers, and although most studies believe that PRP has the characteristics of Osteoinductivity. But its role in bone healing in various studies is controversial. PRP increases the proliferation and invocation of the stem cells and causing increase collagen deposition and thus extracellular matrix production. As a result, in most tissues, it accelerates the repair process. PRP in most clinical studies on joint repair has yielded acceptable results. But its mechanism of action is unclear. The role of PRP on muscle repair is related to experimental studies, and there are no clinical studies in humans, and more studies are needed on muscle and nerve repair.

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