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A REVIEW ON PLATELET RICH PLASMA

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ABSTRACT

The utility of platelet-rich plasma (PRP) has spanned various fields of dermatology from chronic ulcer management to trichology and aesthetics, due to its role in wound healing. Though PRP is being used over a long time, there is still confusion over proper terminology to define, classify and describe the different variations of platelet concentrates. There is also a wide variation in the reported protocols for standardization and preparation of PRP, in addition to lack of accurate characterization of the tested products in most articles on the topic. Additionally, the high cost of commercially available PRP kits, precludes its use over a larger population. In this article, we review the principles and preparation methods of PRP based on available literature and place our perspective in standardizing a safe, simple protocol that can be followed to obtain an optimal consistent platelet yield.

KEYWORDS: Platelet-rich plasma, preparation, principles, standardization.

INTRODUCTION

Platelet-Rich plasma (PRP) is an evolving, versatile component of regenerative human medicine. Platelet-based therapy began in the early 1990's after multiple growth factors were identified in platelet alpha granules. (The component of PRP are growth factors, WBC and phagocytic cells, native fibrinogen concentration, vasoactive and chemotactic agents and high concentrations of platelets, PRP preparation involves a series of centrifugation and separation cycles to concentrate platelets without inducing their premature activation.[1]

Once the final product is prepared, platelet activation should be done. The PRP may or may be activated prior to use. To use PRP as a hemostatic or tissue sealing agent it is necessary to activation the platelets. This is generally done with a ration applicator and blending tipm which mixes the activating agent with the PRP just prior to tissue contact. Upon activation, platelets release their granular contents into the surrounding environment. The platelet granules are abundant and of particular interest because they contain many of the growth factors responsible for the initiation and maintenance of the healing response. TGF-B, PDGF, VEGF and FGF are a few of the growth factors released. Growth factors derived from platelets initiate connective tissue healing, bone regeneration and repair, promote development of new blood vesels and stimulate wound healing process.

The fibrin matrix formed following platelet activation also has a stimulatory effect on wound healing. The fibrin matrix form by polymerization of plasma fibrinogen following either external activation or internal activation. This matrix traps platelets allowing a slow release of a natural combination of growth factors. A number of variables exist in the preparation, form, and administration of platelet products.[2]

PRP can be percutaneously injected as a liquid, activated and applied as a gel, or inserted into a defect as a platelet-rich fibrin clot. The variety of PRP formulations and the ease of preparation provide a myriad of options based on the treatment goals and logistical limitations of a given case. Viewed in this way, PRP is especially unique in that it can be custom-crafted according to the goals. Any concerns of immunogenic reactions or disease transfer are eliminated because PRP is prepared from autologous blood. No studies have documented that PRP promotes hyperplasia, carcinogenesis, or tumor growth. Growth factors act on cell membranes rather than on the cell nucleus and activate normal gene expression. Growth factors are not mutagenic and naturally act through gene regulation and normal wound healing feed-back control.[3]

The versatility and biocompatibility of PRP technology has stimulated its therapeutic use in many different fields including orthopedics, sports medicine, ophthalmology, dentistry, and cosmetic, plastic and maxillofacial surgery

mechanisms. In bone repair, PRP is used to create bone substitution and develop bone healing. In arthroscopy PRP is used to treat joint injuries and diseases through small incision in the skin. The PRP can be applied in the intraarticular space to improve synovial cells, chondrocytes and subchondral ossteoblast. In sports medication PRP is used to treat soft tissue disorders, including muscle tendon, ligament and capsular injuries. PRPs are used in tendon pathology to relieve chronic pain in tendon injuries. In dentistry PRP benefits by the actions such as jump start osteogenesis, speed up mineralization, improvement of trabecular bone density.[4]

Platelet Rich Plasma And It's Growth Factors

Blood contains plasma, red blood cells (RBC), white blood cells (WBC), and platelets. Plasma is the liquid component of blood, made mostly of water and acts as a transporter for cells. Plasma also contains fibrinogen, a protein that acts like a net and catches platelets at a wound site to form a clot. RBC helps pick up oxygen from the lungs and delivers it to other body cells, while removing carbon dioxide. WBC fights infection, kills germs and carries off dead blood cells. Platelets are responsible for hemostasis, construction of new connective tissue, and revascularization. Typically a blood specimen contains 93% RBC, 6% Platelets, and 1% WBC. The rationale for PRP benefit lies in reversing the blood ratio by decreasing RBC to 5%, which are less useful in the healing process, and increasing platelets to 94% to stimulate recovery.[4]

Platelets

Platelets are small discoid blood cells made in bone marrow with a lifespan of 7-10 days. Inside the platelets are many intracellular structures containing glycogen, lysosomes, and two types of granules. The alpha granules contain the clotting and growth factors that are eventually released in the healing process. Normally at the resting state, platelets require a trigger to activation and become a participant in wound healing and hemostasis. Upon activation by thrombin, the platelets morph into different shapes and develop branches, called pseudo-pods that spread over injured tissue. This process is termed aggregation. Eventually the granules contained within platelets release the growth factors, which stimulate the inflammatory cascade and healing.[4]

Platelet Rich Plasma (PRP)

Platelet Rich Plasma is defined a volume of the plasma fraction of autologous blood having a platelet concentration above baseline. Normal platelet concentration is 200,000 platelets/ul. Studies have shown that clinical efficacy can be expected with a minimum increase of 49 fold of this baseline (1 million platelets/ul). Slight variability exists in the ability to concentrate platelets, largely depending on the manufacturer's equipment. However, it has not been studied if too great an increased platelet concentration would have paradoxical effects. The use of autologous PRP was first used in 1987, following an open heart surgery, to avoid excessive trasnsfusion of homologous blood products. Since that time, the application of autologous PRP has been safely used and documented in many fields. An increased awareness of platelets and their role in the healing process has lead to the concept of therapeutic application.[5]

Growth Factors in PRP

Growth factors play a central role in the healing process and tissue regeneration therapy of platelet rich plasma. Alpha granules are storage units within platelets, which contain pre-packaged growth factors in an inactive form. The notable components of PRP include transforming growth factor (TGF)-ß, platelet derived growth factors (PDGF-AB and PDGF-BB), insuling-like growth factor (IGF0, vascular endothelial growth factors (VEGFs), epidermal growth factor (EGFs) and fibroblast growth factor (FGF)-2. The granules also contain vitronectin, a cell adhesion molecule which helps with osseointegration and osseoconduction. TGF-B1 and PDGF stimulate proliferation of mesenchymal cells. TGF-B1 also stimulates extracellular matrix production, including collagen. Principally, these factors stabilize the damaged tissue during initial stages of tissue repair, and direct the local mesenchymal and epithelial cells to migrate, divide and increase collagen and matrix synthesis, ultimately leading to fibrous connective tissue and scarformation. VEGF and FGF-2 are important for stimulating new blood vessel formation to brig nutrients and progenitor cells to the injury site; however, additional factors are also required for neo-vascularization. PRP is postulated to improve the early healing of tendon defects by over-expression of IGF-1. The 70 amino acid polypeptide hormone IGF is a normal component of the plasma and is transported by IGF-binding proteins. IGF-1 storage in platelets is unclear, with few proteomic studies reporting it to be absent and most literature detecting IGF-1 in platelets; however, most studies have detected IGF-1 PRP. For PRP's role in multiple healing pathways, it deserves due consideration as an adjunctive therapy for specific applications.[6]

Summary of Growth Factors Contained In Platelet-Rich Plasma

Growth factor	Functions
Transforming growth factor-B (TGF-B)	Stimulates undifferentiated mesenchymal cell proliferation Regulates endothelial, fibroblastic and osteoblastic mitogenesis Regulates collagen synthesis and collagenase secretion Stimulates endothelial chemotaxis and angiogenesis Inhibits macrophage and lymphocyte proliferation
Fibroblast growth factor (FGF)	Promotes growth and differentiation of chondrocytes Mitogenetic for mesenchymal cells, chondrocytes and osteoblasts
Platelet-derived growth factor a and b (PDGF)	Mitogenetic for mesenchymal cells and osteoblasts Stimulates chemotaxis and mitogenesis in smooth muscle cells Regulates collagenase secretion and collagen synthesis Stimulates macrophage and neutrophil chemotaxis
Epidermal growth factor (EGF)	Stimulates endothelial chemotaxis or angiogenesis Regulates collagenase secretion Stimulates epithelial or mesenchymal mitogenesis
Vascular endothelial growth factor(VEGF)	Increases angiogenesis and vessel permeability Stimulates mitogenesis for endothelial cells
Connective tissue growth factor (CTGF)	Promotes angiogenesis Cartilage regeneration Fibrosis and platelet adhesion
Insulin like growth factor (ILGF 1 and 2)	Chemotactic for fibroblasts and stimulates protein synthesis Enhances bone formation

Platelet Rich Plasma Preparation

Peripheral blood is the supply source for the preparation of PRPs; the mean number of circulating platelets is 200,000 plt/ul. For PRP preparation, peripheral blood is drawn from the patient under sterile conditions, with or without anticoagulants, and the plasma is prepared by centrifugation or filtration. The volume can be adapted to the clinical needs, ranging from 10 to 100 mL. Essentially, the methods of producing PRPs determine the composition and concentration of leukocytes, erythrocytes and platelets in a given plasma volume.

There are three methods:

1) The double spinning method using automated machines and commercial kits.

2) The single spinning method using conventional laboratory centrifuges followed by manual PRP separationselective blood filtration using commercially available technology[3] Single spinning yields a 1-3 fold change in platelet concentration over baseline levels, and double spinning yields a 4-8 fold change in platelet concentration over baseline levels. Double spinning also concentrates leukocytes. Accordingly, platelet concentrates have been categorized as pure platelet-rich plasma (P-PRP), in which leukocytes are purposely eliminated from the PRP, and leukocyte and platelet-rich plasma(L-PRP), which contains a high concentration of leukocytes.[7]

3) Platelets are enriched by 33.8% in the PRP preparation. PPP is the upper layer obtained after blood centrifugation in which platelet counts are negligible and is composed of a cellular plasma containing fibrinogen and plasmatic growth factors. PRP is the bottom layer and is a volume of autologous plasma that has a platelet concentration approximately 3-4 times higher than baseline levels (150000-450000/ul). Specific procedures have been established for the preparation of PRP including Curasan PRP kit, platelet concentration collection system and Smart PreP. A variety of factors influence the reliability of these systems including cell separator used, centrifugation steps, amount of blood collected preoperatively, baseline platelet concentration, amount of platelet concentrate obtained, final platelet concentration, type of blood anticoagulant and platelet activator used. Any of these factors may play a major role in PRP activation and affect the expected outcome in terms of biologic properties.[7]

Smart Preparation is an FDA approved system in which PRP, and autologous thrombin can be obtained. The unique property of the system is its autologous thrombin that is not present in any of the other systems which is an important step in the activation of release of PGFs from platelets. Since the activator of the system is autologous, there is no risk of disease transmission. When PRP is taken into account for use in clinical practice, clinicians may encounter with several issues, including the clinician's proficiency in drawing the necessary blood, the cost of the procedure, the efficacy of centrifugation machine in obtaining appropriate concentratios of platelets, extra time and steps to prepare the coagulated PRP for its actual use. The centrifugation process is considered to be critical since different platelet counts correspond to the differences in centrifuagation machines and techniques.[8]

The PRP processed by means of Smart PreP system, ensures high percentages of platelet concentration with less blood drawn from the patient and with less complex procedures. In theis system, for the PRP preparation, one

hour before surgery, 20 ml of blood was drawn from the patient through a venipuncture in the antecubital vein. The drawn blood was mixed with 2ml of of anticoagulant solution and was then processed through a centrifuge to obtain 3ml of the PRP according to the manufacturer's instruction. Immediately before application, this PRP was mixed with autologous thrombin. For the preparation of autologous thrombin, 10 ml of blood was also drawn from antecubital vein. The drawn blood was mixed with 1 ml of anticoagulant solution and following 5 minutes of incubation period, was centrifuged to obtain 1 ml of autologous thrombin. A delivery syringe was used to mix PRP with autologous thrombin.[8]

Activation and Mechanism of Platelet Rich Plasma
Activation
The plasma should be prepared and immediately used at the point of care, and the plasma should not be stored. Prior to application, platelets ca be slowly activated by setting in motion the coagulation cascade with the addition of calcium chloride, a necessary cofactor for prothrombin conversion to thrombin. Alternatively, coagulation and platelets can be instantly activated by adding a standard solution of bovine or human thrombin with 10% calcium chloride to the PRP. After plasma activation, the fibrin scaffold can be formed in vivo or ex vivo: the latter is suitable for implantation in surgery or in ulcer care and provides a gradual release of growth factors in the area where it has been applied. Depending upon the activation mechanism, induced by $CaCI_2$, collagen or thrombin can achieve a sudden burst of GFs or a gradual release. Indeed, a central question in biology and cell signaling is how extracellular factors elicit a complex set of signaling events to achieve specific cellular functions.[9]

Fibrin is a natural biopolymer involved in the coagulation cascade formed upon fibrinogen cleavage by thrombin. It acts as reservoir for growth factors, cells and enzymes during wound healing and provides a scaffold for the synthesis of the extracellular matrix. Fibrin scaffolds provide nature's cues for tissue regeneration. Fibrin is a key scaffold material for the delivery of biomolecules, and it mimics natural processes and provides adequate exposure time to maximize biological interactions.

The kinetics of signaling may be influenced not only by distinct cell surface receptors but also by the method that their cognate ligands and secreted or delivered. A receptor may be acutely activated by an immediate increase in ligand concentration, a process mimicked in most pharmacological studies. In many cellular processes in vivo, however, cells encounter a gradual increase in the concentration of extracellular factors, i.e., constitutively secreted factors need to accumulate over time to reach a threshold set by the affinity of the receptor.[10]

Mechanism
Upon activation, platelets release their granular contents into the surrounding environment. The platelet a-granules are abundant and of particular interest because they contain many of the growth factors responsible for the initiation and maintenance of the healing response. TGF-ß, PDGF, VEGF, and fibroblast growth factor (FGF) are a few of the growth factors released. These growth factors have been shown to play an important role in all phases of healing. The active secretion of these proteins by platelets begins within 10 minutes after clotting, with more than 95% of the pre-synthesized growth factors secreted within 1 hours. After this initial burst, the platelets synthesize and secrete additional proteins for the balance of their life (5-10 days). The fibrin matrix formed following platelet activation also has a stimulatory effect on wound healing. The fibrin matrix forms by polymerization of plasma fibrinogen following either external activation with calcium or thrombin or internal activation with endogenous tissue thromboplastin. This matrix traps platelets allowing a slow release of a natural combination of growth factors while providing a provisional matrix that provides a physical framework for wound fibroblast migration and presentation of other biological mediators such as addesive glycoproteins.[11]

Therapeutical Applications of Platelet Rich Plasma
1) PRP In Bone Formation And Bone Disorders
(1a) Bone Formation
PRP and its soluble fraction stimulate the osteoblastic differentiation of myoblasts and osteoblastic cells in threedimensional cultures in the presence of bone morphogenetic protein (BMP)-2 BMP-4, BMP-6 or BMP-7. Moreover, heparin-binding fractions obtained from serum also stimulated osteoblastic differentiation in the presence of BMP-4. These results suggested that platelets contain not only growth factors for proliferation but also novel potent iator(s) for BMP-dependent osteoblastic differentiation.[12]

(1b) Osteoarthritis
Platelet released growth factors (PRGFs) regulate endogenous hyaluronic acid (HA) synythesis, thereby protecting the cartilage and lubricating the joint. PRGF enhances the secretion of HA and induces hepatocyte growth factor (HGF) production by synovial fi broblasts isolated from arthritic patients. Platelets preincubated in an acidic environment (pH 5.0) induced the highest degree of fibroblast proliferation and the concentration of PDGF in the different treated lysates. Intra-articular administration of PDGF might be beneficial in resorting HA concentration and switching angiogenesis to a more balanced status but does not halt the effects of IL-Iß on synovial cells.[13]

(1c) Arthroplasty
A retrospective case control study has described the effect of autologous platelet gel applied to exposed tissues, synovium and the lining of the wound at closure

following total knee arthroplasty. Interestingly, the patients receiving platelet gel during surgery had less postoperative blood loss as measured by differences in preoperative and postoperative hemoglobin on day 3, lesser narcotic requirement and a higher range of motion prior to discharge than their control counterparts. Researchers concluded that the application of autologous platelet gel might lead to improved hemostasis, better pain control and a shortened hospital stay. Autologous platelet gel and fabrin sealant application during unilateral total knee arthroplasty was found to help reduce the peri-operative incidence of blood transfusions.

(1d) Technique for Intra-Osseous Sites

- Alcohol or Betadyne preparation.
- +/- Ethyl Chloride spray.
- Local anesthetic either mixed with the PRP graft or to sites of tenderness to 'road test' the area prior to using the graft. This ensures that the PRP matrix graft is placed in the proper areas.
- Aspirate degenerative joint fluid prior to PRP matrix graft placement.
- Gel the PRP or utilize other stabilizing matrix for intra-articular sites. Ligaments, tendons, and inherent matrix sites do not require gel in the authors' experience.
- 8-10cc PRP matrix graft is the typical amount used for a knee or shoulder joint in out clinic.
- "Treat regionally, not locally" (treat all of the capsule that is tender along with tendinous and ligamentous sites of tenderness in addition to the intraarticular capsule.

2) PRP In Sports Medicine

(2a) Sport Medicine

Sports related soft tissue injuries cause athletes to lose a significant amount of time from their spot and represent a significant burden to society in terms of health care resources, personal disability and activity restriction. In 2002, an estimated 15.8 billion dollars in total health care expenditures was used for the medical management of these injuries. Soft tissue disorders, including muscle, tendon, ligament and joint capsular injuries, represent more than 50% of all the musculoskeletal injuries reported each year. Primary care studies have shown that 16% of the general population suffers from shoulder pain, whereas elbow tendinopathy affects 1-2% of the population. The importance of this problem is substantial because the field of sports medicine influences millions of people from athletes to those who participate in recreational sports or simply exercise to stay healthy and active.[14]

(2b) Muscle injuries

Muscle injuries resulting from extrinsic or intrinsic mechanisms are extremely common in sports, accounting for about 35-45% of all injuries. Contact sports and sports that require the generation of large eccentric forces present the highest risk. The vulnerability of soccer players to strains and confusions is a substantial problem for professional players and their clubs; such injuries involve significant time lost from raining and completion. Due to the increasing demands of the competitive soccer season, muscle treatments able to accelerate the recovery time without adversely affecting the recurrence rate (i.e.., those that can minimize the scarring response) are of paramount importance.[14]

At present, no drugs have been developed that hasten the restoration of muscle function after injury. Therefore, in the absence of any available evidence-based treatments, injection therapies may be an important option to help professional athletes. At the 2nd World Congress of Regenerative Medicine, Sanchez(2005) 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 Soccer League, resumed normal training activities in half the time needed by matched historical controls. Using the same leukocyte-free PRP preparation it was reported good outcomes (1 week to return to pre-injury activities) after three weekly PRP infections to treat an adductor longus strain in a professional body builder.

(2c) Tendon Healing

Chronic pain in tendons is very common and studies show that overuse, underloading, all contribute to tendon injuries and painl More than 30-50% of the injuries among professional and recreational athletes are overuse tendon injuries resulting in the onset of pain and discomfort. Thus, the development of innovative strategies to treat tendon injuries is an essential task,, but it requires a more thorough understanding of the underlying cellular and molecular mechanisms, The use of platelet rich preparations in this context may be focused on restoring the normal tissue composition while avoiding further degeneration. When we evaluated the effects of the pool of growth factors released from PRP on tendon cells, the results showed that human tendon cells increased their proliferation rate and were stimulated to release VEGF and HGF. The former promotes angiogenesis, which is directly related with tendon healing capability; the latter is a potent antifibrotic agent that can reduce scar formation around tendon tissues. Other studies have reported that injections of platelet rich plasma one week postoperatively increased tendon regenerate strength. The clinical translation of this approach was assayed in a pioneer study involving professional and recreational athletes. PRP was injected into the tendon fibers after the tendon was sutured. After closing the paratenon and before closing the overlying skin, the affected area was covered with the fibrin scaffold. The results showed that those receiving the PRP-therapy experienced a significant acceleration in functional recovery compared

with a matched group that underwent conventional surgery.

(2d) Anterior Cruciate Ligament Reconstruction

A great deal of effort has been paid to the development of novel medical tools for the repair of injured anterior cruciate ligaments (ACL). The ACL is one of the four major ligaments connecting the bones of the human knee. A torn ACL is a common injury and is typical among the active younger population. The injury requires surgical intervention to stabilize the knee and to prevent cartilage and meniscal injuries, which lead to degenerative joint disease. ACL reconstruction, namely ACL tissue engineering, involves the manipulation of cells and tissues to replace the injured ligament; this processs is a complex undertaking and involves many mechanical and biological challenges. It requires both the application of mechanical knowledge and an understanding of how cells are maintained and grow into functioning tissues to replace defective or injured ligaments. At present, the most common options in ACL replacement are allograts or autografts. A novel approach using PRP technologies seeks to facilitate ACL healing by mimicking the native tissue and improving tissue function with the appropriate clues ultimately leading to better p[a]tient care.[15]

Cell cultures and animal research, in addition to human clinical studies, drive the main hypotheses for the application of PRP grafts biotechnology in ACL reconstruction. These applications involve first promoting bone-bone and bone-tendon healing, and second, influencing the pattern of change within the autograft body (ligamentization). Finally, the application of PRP-therapies will help in donor site healing. One exciting option to enhance ligamentization is to simultaneously transfer multiple cytokines and growth factors (including PDGF, TGF-1 and VEGF, among others) to the graft by applying an endogenous PRP. Autografts could be loaded in situ with a balanced pool of signaling molecules. These molecules would have the potential to not only activate the graft tenocytes but also to attract cells, such as endothelial or stem cells, from adjacent niches (such as the synovium and/or the intrapatellar pad) to the graft structures using the synovial fluid for passage. The corroboration and clinical translation of this notion may be enhanced healing and intrasynovial adaptation of the tendon graft to the synovial milieu. When we compared the gross appearance and microscopic qualities of the PRP-treated and untreated grafts during the remodeling period(6-24 months). Using second-look arthroscopy focusing on graft thickness, apparent tension and synovium coverage. The overall arthroscopic evaluation provided evidence that a higher percentage of the grafts rated as excellent in the PRP group (57% versus 33%). No grafts were scored as poor in the PRP group, but 20% of the controls showed poor morphology. At the same time, PRP treatment influenced the histological characteristics of the tendon graft, which resulted in tissue that was more mature than in the controls. Histology displayed newly formed connective tissue enveloping the graft in 77.3% of the PRP-treated grafts and 40% of the controls. Other authors have used a compressed gelatin sponge soaked with leukocyte and platelet-rich concentrate sutured to the intra-articular part of the graft, which confirmed the acceleration of the maturation of the grafts treated with PRP as assessed by magnetic resonance imaging.[15]

(2e) Technique for Myotendinous or Teno Osseous Sites administration of PRP

- Alcohol or Betadyn VGHHG e preparation
- +/- Ethyl Chloride spray
- Inject PRP with approximately I cc PRP peer cm^3 of tissue / interface
- Important to touch bone and ‘pepper’ the area of teno-osseous junction to stimulate the greatest number of fibroblast colonies.
- For myotendinous sites use ultrasound to ensure lay cred treatment throughout the tendon
- Sterile band-aid applied post injection
- Kinesiotape to protect motion if needed treatment.

3. PRP In The Treatment of Cutneous Ulcers

The Cutaneous ulcers re divided into acute and chronic wounds. Clinical differences between acute and chronic wounds are in part explained by alterations in the local biochemical environment. For example, acute wounds are associated with a greater mitogenic activity than chronic wounds. Chronic wounds are associated with a higher level of pro-inflammatory cytokines than acute wounds. As chronic wounds begin to heal, they progress to a less inflammatory state. Elevated protease activities in chronic wounds may directly contribute to poor healing by degrading the proteins necessary for normal wound healing. Chronic wounds can be defined as those failing to proceed through an orderly and timely process to produce anatomic and functional integrity. Practically, a chronic wound is one that has failed to heal within 3 months. The cellular, biochemical and molecular events that characterize chronic wounds have been well defined, including a prolonged inflammatory phase, cellular sensecense, deficiency of growth factors and their receptors, deficient fibrin productions and high levels of proteases. In chronic wounds, the orderliness of the healing process is disrupted Repeated trauma, foreign bodies, pressure necrosis, infection, ischemia and tissue hypoxia also amplify the chronic inflammatory state, which is characterized by excess neutrophils, macrophages and lymphocytes. Fragments of dead tissue, bacterial products and foreign bodies are powerful chemoattractants that sustain a continuous influx of inflammatory cells, which in turn produce a variety of growth factors, cytokines, and matrix-degrading enzymes. Among the most potent of these enzymes are elastase and MMPs, which are present in large quantities in chronic wounds. Therefore, any effective intervention must include a strategy for disrupting this cycle and setting the wound on permanent path towards healing. Historically, the first clinical application of platelet

derived preparations was conducted in chronic leg ulcers in which wounds were filled with collagen embedded in platelet secreted proteins. This initial product, known as PDWHF (Platelet-derived wound healing factors) stimulated the formation of the vascularized connective tissue found in healing wounds. Thereafter, various other types of platelet products have been assayed in several pilot studies, case series and clinical trails.

Growth factors are crucial for timely wound healing; inadequate levels of growth factors may be an important factor contributing to the chronicity of the wound, which may be degraded in excess by cellular or bacterial proteases. The platelet releasates were more effective than standard therapy. Subsequently, PRP formulations were refined and primarily applied as fibrin membranes for the treatment of non-healing ulcers. More recently, the use of PRP in the management of chronic diabetic foot ulcers has been successful. Moreover, PRP provides advantages I skin grafting for recalcitrant ulcers. Allogenic platelet preparations have been used recently to treat recalcitrant ulcers in very elderly hypomobile patients for whom autologous blood processing may be difficult. Finally, the use of PRP gel resulted I an improved quality of life and a lower cost of care over a 5-year period than other treatment modalities for patients with non-healing diabetic foot ulcers. Although actual treatment outcomes may differ from those modeled, PRP treatment represents a potentially attractive treatment alternative for insurers and health care providers to address the cost burden and health effects of non-healing diabetic foot ulcers.

(4)PRP In cosmetic Surgery

(4a) Haemostatic Properties of PRP

In the relm of plastic surgery theuse of autologous PRP was first introduced 20 years ago. It was used as haemostatic agent to promote adherence of skin flaps. PRP undergoes significant decrease in hematoma formation and ecchymosis as well as the elimination of the need for drains.

(4b) PRP for the treatment of rhytids

In addition to haemostatic properties PRP has significant role in facial skin rejuvenation and the treatment of rhytids. The PRP is well efficacious in the treatment of skin wrinkles. The PRP undergoes increased fibroblast and collagen formation as well as increased dermal thickness by that they undergoes clinical improvement in skin tonicity and reduction of facial rhytids.

(5) PRP in the treatment of Allopecia

The most common cause of hair loss in men & women is androgenetic alopecia (common baldness) which is an inheritant disorder. Men and women inherit the gene for hair loss from either or both parents. Every 4 men amongst 10 are affected with androgenetic alopecia. Despite this condition being genetic in nature, it is a treatable condition. In allopathic practice, there are medications available which have proven beyond doubt to be effective in regrowth of hair. However these medications take 6 to 9 months to induce hair regrowth. Therefore there has been a constant quest for the search of newer modalities of treatment for hair loss. Microneedling, meso-therapy and platelet rich plasma are newer modalities.

Platelet rich plasma (PRP) is a unique technique where platelets are separated and concentrated from the patient's own blood by an intricate method. It is then used on the bald areas on the head. PRP is rich in PDGF (platelet derived growth factors), TGF (transforming growth factor), VEGF (vascular endothelial growth factor), EGF (epithelial growth factor), and IGF (insulin like growth factor). These growth factors transform thin hair into stronger and thicker hair.

Microneedling is a simple procedure used to activate platelets present in bald areas capillaries and thereby release its growth factors. Microneedling also increases the penetration of substances into the skin. In our experience the combination of microneedling, and PRP is synergistic in hastening hair regrowth in individuals with androgenetic alopecia with high rates of satisfaction.

(6) PRP In Ophthalmology

Another remarkable application of PRP is in ophthalmology. Several successful examples include the use of PRP releasates as eye drops for the treatment of a broad spectrum of corneal persistent epithelial defects. Furthermore, the use of autologous platelet rich plasma was shown to be very effective in the treatment of patients suffering from dry eye symptons; it improved both patient symptoms and major clinical signs platelet rich plasma also promotes healing of dormant corneal ulcers even in eyes that are threatened by corneal perforation, and it is a reliable and effective therapeutic tool for the enhancement of epithelial wound healing on the ocular surface.[15]

CONCLUSION

Platelet Rich Plasma is a new application of tissue engineering and a developing area for clinicians and researchers. It is a storage vehicle for growth factors, especially PDGF and TGF-b, both of which influence bone regeneration. Although the growth factors and the mechanisms involved are still poorly understood, the ease of applying PRP and its beneficial outcomes, including reduction of bleeding and rapid healing. Most important, this autologous product eliminates concerns about immunogenic reactions and disease transmission. PRP may become a routine treatment modality for regeneration in future.

Because of the safety of these products, basic science, clinical discovery and patient-oriented research should be interdependent rather than successive steps. The substantial challenges of incorporating such research into clinical care must be pursued if the potential of PRPs is

to be realized. Although PRP therapies have many compositions and procedures for application, they all try to maximize the cell signals that may enhance tissue healing. Our increased understanding of the healing mechanisms that result in tissue repair is paving the way towards the optimization of healing therapies.

REFERENCES

1. Mikel Sanchez, Isabel Andia, Eduardo Anitua, Pello Sanchez. Platelet Rich Plasma (PRP) Biotechnology: Concepts and Therapeutic Applications in Orthopedics and Sports Medicine. Innovations in Biotechnology, 2013; 1(3): 114-134.
2. Jamie Textor. Platelet Rich Plasma: The Rest of The Story Science Direct University of California, 2012; 2(1): 107-116.
3. Rani Soman, Iram Zaidi, Shipra Jaidki. Platelet Rich Plasma - A Healing Aid and perfect Enhancement Factor. IJCPD Journals, 2010; 3(4): 1005-1085.
4. De Jonge S, de Vos RJ, Weir A. Clinical Review Criteria of Platelet Rich Plasma. Am J Sports Med, 2011; 4(1): 625-635.
5. Wes Sutter. PRP: Platelet Rich Plasma. J Orthop Res, 2007; 23(2): 281-6.
6. Steven Sampson, Michael Gerhardt, Bert Mandelbaum. Platelet Rich Plasma injection grafts for musculoskeletal injuries: a review. Curr Rev Musculoskelet Med, 2009; 1(7): 37-42.
7. Robinder S Dhillon, Edward M Schwarz, Michael D Maloney. Platelet Rich Plasma therapy – future or trend? Arthritis Research & Therapy, 2012; 1(4): 219.
8. David Crane, Peter A.M. Everts. Platelet Rich Plasma (PRP) Matrix Grafts. Practical Pain Management, 2008; 9(3): 174-183.
9. Robert.S.Krang, Mathew K Le, Rahul Set. Platelet Rich Plasma in Cosmetic Surgery. Int J Otorhinolaryngol Clin, 2013; 5(1): 24-28.
10. Rick.G, Craig.J, Gassmann, Mark.S. Platelet Rich Plasma –A new treatment for Hair Loss. The journal of Lancaster General Hospital, 2012; 2(2): 34-40.
11. Ruiz-Colecha.J, Rodriguex A, Artola.S. Treatment of ocular surface syndrome after LASIK with autologous Platelet Rich Plasma. Journal of Refractive Surgery, 2007; 23(6): 617.
12. Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. Thrombosis and Haemostasis, 2004; 91(1): 4-15.
13. De Vos RJ, Weir A, van Schei. Platelet Rich Plasma Injection for Chronic Achilles Tendinopathy A Randomized Controlled Trial. Jama-Journal of the American Medical Association, 2010; 303(2): 144-149.
14. Lopez S, Morales MC, Freire V. Plasma rich in growth factors as a therapeutic agent for persistent corneal epithelial defects, 2010; (8): 843-8.
15. Nurden P, Sanchez M, Andia I, Anitua E. Platelets and wound healing. Frontiers in Bioscience, 2008; 1(3): 3532-48.