INTRODUCTION

Platelets are nonnuclear cellular fragments derived from megakaryocytes in the bone marrow; they are specialized secretory elements that release the contents of their intracellular granules in response to activation. It is known, that more than 300 proteins are released by human platelets in response to thrombin activation. In addition to their well-known function in hemostasis, platelets also release substances that promote tissue repair, angiogenesis, and inflammation. Furthermore, they induce the migration and adherence of bone-marrow-derived cells to sites of angiogenesis; platelets also induce differentiation of endothelial-cell progenitors into mature endothelial cells ***(Gonzalez et al., 2012).***

Platelets are key components in haemostasis, and stimulate the construction of new connective tissue, and revascularization. Platelets are derived from the fragmentation of precursor megakaryocytes and have a lifespan of 5–9 days. The physiological range for platelets in humans is between 150 and 400 × 10^9 per litre. They normally circulate in the blood and are involved in the formation of the haemostatic plug, Platelets in the body are activated when they come in to contact with von Willebrand factor, collagen (exposed endothelium), or by the action of thrombin. Once activated, they secrete the contents of their alpha and dense granules. The growth factors released from these granules facilitate the three stages of healing: inflammation, proliferation and remodeling ***(Ahmad et al., 2012).***

As recently as forty years ago, platelets were considered to be exclusively hemostatic cells. Today we know that platelets actually perform myriad diverse functions ***(Textor, 2014)***.

In 1974, Ross et al. determined that the addition of either intact platelets and calcium, or the supernatant derived from thrombin activated platelets, resulted in significant improvements in the mitogenic capacity of ‘‘plasma serum’’, such that it equaled that of the serum derived from whole blood. They concluded that platelets must be the major source of the proliferative effect provided by serum ***(Ross et al., 1974).***

Accumulating evidence suggest that platelets play an important role not only in haemostasis, but also in inflammation and tissue repair through paracrine ways or direct cell-cell interactions. Platelets contain a number of growth factors which are released upon activation and influence the microenvironment and mediate tissue repair ***(Stellos et al., 2007).***

Platelets contain many intracellular structures. For *clinical application*, the most notable of these components are two types of granules – the alpha granules and the dense granules. The alpha granules contain coagulation proteins, growth factors, cytokines, chemokines and various other proteins, including adhesion proteins. The dense granules contain ATP, ADP, serotonin and calcium. Thus, it is the dense granule that provides the factors necessary for platelet aggregation ***(Tate et al., 2010)***.

The concept of using a component of whole blood to augment healing was first promoted by Ferrari et al. in 1987 as an autologous transfusion component after an open heart operation to avoid homologous blood product transfusion ***(Ferrari et al., 1987).***

During the late 1990s the term ‘‘Regenerative Medicine’’ was coined and a new field was born. Regenerative Medicine refers to a strategy whereby the injured site is provided with the raw materials necessary for a ‘‘scarless repair’’, or regeneration, to occur in situ. These therapies provide at least 1 of the 3 components considered essential for tissue regeneration—namely, cells, growth factors and scaffold. In Regenerative Medicine the assembly of these resources into new tissue takes place within the lesion site or in proximity to it, and is directed under local influences. Platelet-rich plasma (PRP) is included within the field of Regenerative Medicine, since it can provide 2 of the 3 components (i.e., growth factors and scaffold) deemed necessary to support true tissue regeneration ***(Textor, 2014).***

Regenerative Medicine is a rapidly evolving field in sports medicine and orthopaedic surgery. The ideal biologic tool would be effective, simple to use, inexpensive, safe and available immediately at the point of care. Platelet rich plasma (PRP) meets many of these criteria. PRP is autologous, therefore, potential for adverse reactions and transmission of infection is very low. It has been used for almost 20 years in several medical specialties without reports of significant complications. PRP is simple to produce from small amounts of peripheral blood in short period of time (15 minutes or less) using desktop sized equipment obviating the need to send the material out for processing or culturing. PRP is inexpensive to produce compared to stem cells or genetically engineered proteins. Over the last several years, many more basic science and clinical investigations have been published supporting the use of PRP for sports related injuries and disorders ***(Mishra et al., 2012).***

Introducing any new method of treatment is associated with high expectations for its success. This is true particularly when it concerns the field of physical activity, where a return to activity is essential for verifying the method’s effectiveness, especially if therapy is based not only on the premise of achieving a symptomatic effect, but also improving functional quality and repairing structurally damaged tissues. This brings hope to the use of autologous platelets as a source of growth factors (GF). This method, known as platelet rich plasma (PRP) application, is becoming a more widely practiced standard in sports medicine, orthopaedics and traumatology ***(Ficek et al., 2011).***

AIM OF THE WORK

To review role of platelet rich plasma, as a safe and effective method, in promoting the natural processes of wound healing, soft tissue reconstruction, and bone reconstruction and augmentation in different orthopedic aspects.

MECHANISM OF ACTION OF PRP

To understand the mechanism of action of PRP It is important to note that growth factors are not the only elements present in significant concentrations in PRP, as proteins of the cytokine and chemokine families are known to be present in varying concentrations as well. The concentrations of the various growth factors increase linearly as platelet concentration increases ***(Wroblewski et al., 2010)***.

PRP initiate wound repair by releasing locally acting growth factors through degranulation of the granules in platelets, which contain the synthesized and prepackaged growth factors (table 1). The active secretion of these growth factors is initiated by the clotting process of blood and begins within 10 minutes after clotting ***(Kaur et al., 2011).***

The network of activated growth factors aid healing by attracting undifferentiated cells in the newly formed matrix and triggering cell division by inducing intracellular signaling pathways that lead to the production of proteins essential to the regenerative processes, such as cell proliferation, matrix formation, osteoid production, and collagen synthesis ***(Wroblewski et al., 2010)***.

Healing cascade involves 3 phases: (1) the inflammatory phase, (2) the proliferative phase, and (3) the maturation and/or remodeling phase.

1. **The inflammatory phase**, occurs in the first week after injury and involves hemostasis and recruitment of inflammatory mediators. Tissue injury activates cyclooxygenase-2 and leads to vasodilation. Growth factors attract macrophages and fibroblasts.
2. **The proliferative and repair phases** follow in the next days to 2 weeks, with formation of extracellular matrix with granulation, contraction, and epithelialization.
3. **The remodeling phase** follows up until about 1 year after injury, when collagen and scar tissue production takes place. Type I collagen replaces proteoglycan and fibronectin to form a more robust matrix with increased tensile strength. ***(Nguyen et al., 2011)***

Role of PRP in tendinopathy:

In tendinopathy, the essential pathology includes chronic microscopic tears occurring in hypovascular tendon tissue. These tears heal by scar formation rather than the normal vascular and inflammatory-driven tendon-healing pathways. Modulation of bioactive factors in the diseased tendon may increase the potential for tendon healing ***(Cole et al., 2010).***

Basic science studies have shown that healing tendon is responsive to the local application of growth factors and describe the role of many of the growth factors contained in the platelet alpha granules in tendon regeneration. TGF-b increases the expression of procollagen types I and III and mechanical properties. PDGF, IGF-1, VEGF and FGF promote tendon cell proliferation and tendon healing. Releasate from PRP has been seen to stimulate the gene expression of the matrix molecules and tendon cell proliferation and promote the synthesis of angiogenic and other growth factors, and also activate circulation-derived cells, that also play an important role in the tissue healing process ***(Kon et al., 2011).***

Table (1): Sources of growth factors and their biological actions on wounds

Platelet Growth Factor Type	Growth Factor Source	Biological Actions
Platelet Derived Growth Factor, PDGF(a-b)	Platelets, osteoblasts, endothelial cells, macrophages, monocytes, smooth muscle cells	Mitogenetic for mesenchymal cells and osteoblasts; stimulates chemotaxis and mitogenesis in fibroblast/glial/ smooth muscle cells; regulates collagenase secretion and collagen synthesis; stimulates macrophage and neutrophil chemotaxis
Transforming Growth Factor TGF (α-β)	Platelets, extracellular matrix of bone , cartilage matrix, activated TH_1 cells and natural killer cells, macrophages/ monocytes and neutrophils	Stimulates undifferentiated mesenchymal cell proliferation; regulates endothelial, fibroblastic and osteoblastic mitogenesis; regulates collagen synthesis and collagenase secretion; regulates mitogenic effects of other growth factors; stimulates endothelial chemotaxis and angiogenesis; inhibits macrophage and lymphocyte proliferation
Vascular endothelial growth factor, VEGF	Platelets, endothelial cells	Increases angiogenesis and vessel permeability, stimulates mitogenesis for endothelial cells
Epidermal Growth Factor, EGF	Platelets, macrophages, monocytes	Stimulates endothelial chemotaxis/ angiogenesis; regulates collagenase secretion; stimulates epithelial/mesenchymal mitogenesis
Fibroblast Growth Factor, FGF	Platelets, macrophages, mesenchymal cells, chondrocytes, osteoblasts	Promotes growth and differentiation of chondrocytes and osteoblasts; mitogenetic for mesenchymal cells, chondrocytes and osteoblasts
Connective tissue growth factor CTGF	Platelets through endocytosis from extracellular environment in bone marrow.	Promotes angiogenesis, cartilage regeneration, fibrosis and platelet adhesion
Insulin-like growth factor-1 IGF-1	Plasma, epithelial cells, endothelial cells, fibroblasts, smooth muscle cells,osteoblasts, bone matrix	Chemotactic for fibroblasts and stimulates protein synthesis. Enhances bone formation by proliferation and differentiation of osteoblasts

(Everts et al., 2012)

Role of PRP in muscle injury:

Muscle injuries in sports may result from a blow, strain, or sometimes a laceration. Similar to tendon healing, muscle healing occurs in an arrangement of overlapping steps. The sequence of these steps is inflammation, proliferation, and remodeling, and they are regulated by growth factors and cell-to-cell interactions. PRP therapy has been shown to enhance both of these processes. B-FGF, IGF-1 and nerve growth factors have been identified as substances capable of enhancing muscle regeneration and improving muscle force in the strained injured muscle ***(Kon et al., 2011).***

Role of PRP in Cartilage Injuries:

Articular cartilage is frequently exposed to multiple macro or microtraumatic events that may lead to the loss of tissue homeostasis, resulting in the accelerated loss of articular cartilage and progressing to oostearthritis ***(Cole et al., 2010).***

GFs play a crucial role in modulating the phenotypic expression of chondrocytes. TGF-β affects cartilage regeneration through increased chondrocyte phenotype expression and matrix synthesis, through chondrogenic differentiation of mesenchymal stem cells, and through suppression of interleukin-1-mediated decrease in proteoglycan synthesis. PDGF helps maintain the hyaline like chondrogenic phenotype; it increases chondrocyte proliferation; and it up-regulates proteoglycan synthesis. IGF-I has been shown to stimulate proteoglycan synthesis and suppress proteoglycan catabolism. Other GFs, including bFGF and VEGF, have chondroinductive roles ***(Cole et al., 2010).***

Role of PRP in bone injury:

Platelets play a major role in fracture healing. Indeed, the alpha granules of platelets, releasing several growth factors in the fracture rim, such as platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-b), fibroblast growth factor (FGF-b), and vascular endothelial growth factor (VEGF) stimulate polymorphonuclear leukocytes, lymphocytes, monocytes, and macrophages. TGF-b and PDGF molecules show in vivo osteoinductive capacity. Moreover, VEGF enhances bone formation and bone healing by improving angiogenesis, and appears to be an appropriate tool to induce bone healing in atrophic non-unions (table 2). **(Galasso et al., 2008)**

Table (2): Role of PRP components in bone remodeling.

PDGF	Mesenchymal stem cell (MSC) and progenitor cell recruitment, proliferation, migration, and osteogenic differentiation. Osteoblast proliferation and ECM ossification
TGF-β	MSC recruitment and differentiation. Increased production of collagen and mineral matrix. Inhibits osteoclast formation and bone resorption
TGF-β1	MSC recruitment, proliferation, and osteogenic differentiation
IGF-1	Stimulates bone formation via cellular proliferation, differentiation, and synthesis of Type I collagen
IL-1, IL-6, TNF-α	Promotes early responses of bone repair, endochondral bone formation, and bone remodeling
Basic FGF	MSC growth and differentiation. Osteoblast proliferation
Fibronectin, vitronectin	Enhances formation of focal adhesions by osteoblasts, osteoblast migration
VEGF	Promotes angiogenesis and endochondral ossification
VGF, platelet microparticles	Promotes angiogenesis

(Rodriguez et al., 2014)

Role of PRP in nerve injury:

In normal intact nerves, trophic factors are produced in the target organs and conveyed to the cell body retrogradely. If the communication of axons with the cell body is interrupted by injury, Schwann cells (SCs) in Wallerian degeneration produce neurotrophic factors including neurotrophins such as NGF, brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNF) and glial cell line-derived neurotrophic factor (GDNF). Neurotrophins are released from SCs and dispersed diffusely in gradient fashion around regenerating axons, Regenerating axons extend along the density gradient of neurotrophins to the distal segment. Alpha granules in platelet have been shown to contain mitogenic and chemotactic growth factors such as IGF-1, bFGF and TGF-beta. Although they are not classical neurotrophic factors, the effects of these growth factors on nerve regeneration have been comprehensively studied ***(Yu et al., 2011).***

Preparation

Platelet concentrates are first of all blood extracts obtained after various processing of a whole blood sample, mostly through centrifugation. The objective of the processing is to separate the blood components in order to discard elements considered as not usable (mostly the red blood cells, heavy and easily separated) and to gather and concentrate the elements that may be used for therapeutic applications (fibrinogen/fibrin, platelets, growth factors, leukocytes and other forms of circulating cells, in solution in liquid plasma) ***(Ehrenfest et al., 2014).***

Blood withdraw:

The patient's blood is typically drawn from the antecubital region using aseptic technique. It is recommended that an 18-gauge needle be used in an effort to reduce irritation and trauma to the platelets such that they remain in a relative "inactive" state ***(Foster et al., 2009).***

A 30 cc venous blood draw will yield 3-5 cc of PRP depending on the baseline platelet count of an individual, the device used, and the technique employed ***(Dhurat et al., 2014).***

Anticoagulation:

PRP can only be made from anticoagulated blood. It cannot be made from clotted whole blood because platelets become part of the clot. When whole blood is drawn, many PRP kits will use an anti-coagulant to prevent it from clotting. Most kits use anticoagulant citrate dextrose (ACD) to inhibit clotting. ACD binds calcium preventing the coagulation proteins from using it and initiating the clotting cascade. The addition of citrate to the blood prevents clotting, but also makes it more acidic than is physiologic. Some growth factors can be influenced by the pH of the tissue, thus some protocols recommend buffering the PRP back to a physiologic range prior to injection ***(Mishra et al., 2012).***

The PRP nonaggregated platelet collection rates were significantly higher when EDTA rather than ACD was used as the anticoagulant. Thus, EDTA was apparently superior to ACD in preventing blood coagulation and platelet aggregation. The preservation of nonaggregated platelets is critical in maximizing the concentration of platelets and platelet-derived factors ***(Araki et al., 2012).***

Methods of centrifugation:

PRP is prepared by a process known as differential centrifugation. In differential centrifugation, acceleration force is adjusted to sediment certain cellular constituents based on different specific gravity.

There are many ways of preparing PRP. It can be prepared by the PRP method or by the buffy-coat method: