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Platelet rich plasma (PRP) enhances wound healing

Ali Abbas Ajeel [1*]; Oune Mansour Qussa ,Sabah Hassan Askar

[1] Department of surgery/ College of Veterinary Medicine/ Al Muthanna University/ Iraq

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***Corresponding author:**
Ali Abbas Ajeel
Email address:
draliabbas1972@yahoo.com

Abstract

Wound healing is a fascinating process directed by a myriad of cellular and molecular mechanisms. Many cells are involved in wound healing, and they produce or are sensitive to many molecules, which allow, in physiologic conditions, the repair, or even the regeneration of injured tissues. Platelets (PLTs) play a central role in the wound healing process since this cytoplasmic fragment possesses not only hemostatic properties, but also pro-inflammatory, regulatory, and regenerative activities mediated by the interaction with cells (neutrophils, and endothelial cells) and by liberating GFs, chemokines and other regulatory molecules. Platelets (PLTs) play a very important role in wound healing since they secrete many growth factors (GFs) and other molecules involved in this process. The main GFs secreted by PLTs include: platelet-derived growth factor (PDGF), transforming growth factor-beta 1 (TGF-β1), TGF-β2, epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), insulin-like growth factor type 1 (IGF-I) and hepatocyte growth factor (HGF). These peptides produce chemotaxis, cellular proliferation and differentiation, neovascularization, and extra-cellular matrix (ECM) deposition, which favors the resolution of inflammation and the healing of the wounds. Review of literature revealed scarce publication regardings applications of platelet-rich plasma in the enhancement of wound healing in Iraq. Therefore, this study aims to review the published articles regarding the role of platelet-rich plasma in the improvement of wound healing.

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Introduction

In human and veterinary medicine, there is an increasing demand for regenerative therapies. The goal of regenerative therapies is to restore the normal architecture and functionality of the injured tissue. This is in contrast to the formation of suboptimal scar tissue that results from the natural tissue repair process (Patterson-Kane and Firth, 2009). A wide variety of regenerative therapies are under investigation, and many are currently being used in clinical practice. An example of such a product is platelet-rich plasma (PRP), a blood product made by concentrating platelets in a small volume of plasma. PRP is

used extensively in human medicine for a wide variety of surgical and sports medicine applications (Sampson *et al.,* 2008; Simman *et al.,* 2008; Nikolidakis *et al.,* 2008). The exact mechanism of action of PRP is unknown; however, the release of high concentrations of growth factors from platelet alpha granules as platelets degranulate is widely believed to play a critical role. Alpha granules contain several growth factors known to have beneficial effects on tissue healing, including platelet-derived growth factor (PDGF), transforming growth factor β-1, vascular endothelial growth factor (VEGF), and insulin-like growth factor(IGF)-I (Arguelles *et al.,* 2008). This study aims to review the published articles regarding the role of platelet-rich plasma in the improvement of wound healing.

1. The Platelet

The studies of platelets were beginning with Giulio Bizzozero, an Italian doctor and medical researcher who is credited with their discovery in 1881. The American pathologist, James Homer Wright, was identified megakaryocytes as the source of platelets in 1906. He found the similarities between the granules of platelets and megakaryocytes using his special stain (Coller, 2002). Later on, a massive expansion of information was built regarding platelet formation and its structure and functions.

1.1. Formation

The platelets are composed of large precursor cells called megakaryocytes. The platelet production and release are mediated by thrombopoietin produced in hepatocytes, renal tubular epithelium, and bone marrow stromal cells (Stockham *et al.,* 2008). Most megakaryocytes are found in the bone marrow, although a small percentage is found in pulmonary capillaries and circulation (Kaufman *et al.,* 1965). The megakaryocyte converts the majority of its cytoplasm into thin cytoplasmic processes called proplatelets. Moreover, each megakaryocyte produces hundreds to thousands of platelets (Italiano and Hartwig,2002;Thon *et al.,* 2010).

1.2. Microstructure

The plasma membrane is composed of a phospholipids bilayer with a hydrophobic core. A vast array of protein receptors, are located on the plasma membrane and are essential for platelet adhesion and activation (Boudreaux, 2010). Platelets contain three types of secretory granules: alpha granules, dense granules, and lysosomal granules. Alpha granules are the largest and most numerous of the platelet granules (Gader *et al.,* 2008). Alpha granules acquire proteins by both endogenous synthesis and uptake and packaging of plasma proteins by receptor-mediated endocytosis and pinocytosis (Handagama *et al.,* 1987). Human platelet alpha granules contain 284 different proteins, including Von Willebrand factor(vWF), factor V, proteoglycans, thrombospondin, fibronectin, fibrinogen, albumin, immunoglobulin, and numerous growth factors (Maynard *et al.,* 2007)

Platelet-derived growth factor (PDGF), transforming growth factor (TGF)- β1, TGF- β2, epidermal growth factor, vascular endothelial growth factor, insulin-like growth factor-I, and hepatocyte growth factor are found in platelet alpha granules and are believed to promote tissue repair . The dense granules, less numerous and smaller than alpha granules, contain 40 known proteins, including cell signalling proteins, molecular chaperones, cytoskeletal proteins, proteins involved in glycolysis, and proteins involved in platelet functions (Hernandez-Ruiz *et al.,* 2007). Dense granules also serve as a storage site for calcium and serotonin, important for initial vasoconstriction and formation of the platelet plug. The third type of platelet granule, lysosomal granules, contains acid-dependent hydrolases, including glycosidases, proteases, and lipases (Dell'Angelica *et al.,* 2000). Lysosomal granules are likely vestigial remnants left over from the megakaryocyte cytoplasm and have no known function in platelet function (Coller, 2002).

1.3. Activation

Platelet activation is an essential component of hemostasis. Platelet activation is stimulated by exposure to collagen, adenosine 5'-diphosphate (ADP), proteolytic enzymes (e.g., thrombin, trypsin), antigen-antibody complexes, and platelet-activating factor secreted by leukocytes (Zucker and Nachmias, 1985). Regardless of the stimulus, platelets undergo a specific sequence of events following activation that includes adhesion, aggregation, and the release reaction where granule contents are released to the extracellular environment. Coagulation factors within alpha granules ensure fibrin formation and promote platelet plug stabilization. Calcium and ADP from dense granules recruit additional platelets to the aggregate, forming the platelet plug (Rendu and Brohard-Bohn,2001; Baker,2004). Following the formation of the platelet plug, clot retraction occurs via platelet myosin filament contraction (Hartwig, 2002).

2. Platelet-Rich Plasma

Platelet concentrates created using different techniques contain variable numbers of platelets and leukocytes and may be classified as leukocyte-and platelet-rich plasma (L-PRP) or "pure" PRP (P-PRP) (Dohan *et al.,* 2009). In L-PRP, both platelets and leukocytes are in higher concentration than that found in whole blood. Producing P-PRP involves an additional step of filtering to remove leukocytes or a technique that allows the harvest of platelets without the white blood cells (WBC) (McCarrel *et al.,* 2011). For most applications, anti-coagulated blood is collected, processed into PRP, and injected intralesionally (tendon or ligament injuries) or applied topically (wounds or surgical sites). PRP may also be used intra-articularly for the treatment of osteoarthritis (Carmona *et al.,* 2007). Platelets can be activated prior to use by exposure to substances such as bovine thrombin or calcium chloride (Foster *et al.,* 2009). Almost 100% of stored growth factors are released within one hour of exogenous activation (Marx, 2001). The use of platelet concentrates has shown beneficial effects on wound healing. The use of platelet-fibrin glues in cosmetic surgeries resulted in decreased drain usage, post-operative pain and swelling, and operating time (Man *et al.,* 2001; Oliver *et al.,* 2001). The donor sites for split-

thickness skin grafts treated with PRP demonstrated more rapid epithelialization and less scarring and depigmentation than control donor sites and demonstrated increased epithelial budding and a more mature dermis histologically (Marx, 2004). In patients with chronic, non-healing wounds of the distal extremities, twice daily application of platelet concentrate resulted in epithelialization in 93% of wounds (Knighton *et al.,* 1986), and a 78% rate of limb salvage in patients for which amputation had previously been recommended (Ganio *et al.,* 1993). Chronic wounds are commonly encountered in equine medicine, and wounds on the distal limb are often slow to heal. Therapies that promote faster, more cosmetic wound healing result in decreased economic burden to the client and a faster return to use for the horse. Biopsies of surgical wounds treated with PRP combined with ascorbic acid contained more densely arranged collagen bundles (Carter *et al.,* 2003), and more rapid epithelial differentiation than controls (DeRossi *et al.,* 2009).

3. Methods use for Preparing Platelet-rich Plasma

As the popularity of PRP use has increased, commercial systems have been developed to make the clinic preparation of PRP more practical and available. Commercial systems can be divided into automated/semi-automated systems and manual systems. The manual systems depend on the operator to determine what portion of the centrifuged blood is collected as PRP, while automated systems use infrared light, a density buoy, or shelf to sequester the platelet fraction (Sutter, 2007). Many commercial systems require special centrifuges designed to accommodate the disposable. The cost and need for special equipment to produce PRP can be avoided by centrifuging blood collection tubes using a variety of protocols (Arguelles *et al.,*2006; Textor *et al.,* 2011, 2006).
Blood is collected into syringes containing anticoagulant and transferred to empty blood collection tubes (Arguelles *et al.,* 2006), or collected directly into evacuated tubes containing sodium citrate. Single, double, and triple centrifugation protocols have been reported (Arguelles *et al.,* 2008; Textor *et al.,* 2011).

4. Quality Assessment Parameters

4.1. Platelet Numbers

One of the particular clinical importance is the question of how many platelets are needed to produce a beneficial treatment effect. Several studies have investigated this question. Some authors recommend maximal platelet concentration to achieve PRP with very high platelet numbers (Marx, 2004). An early in vitro study demonstrated that PRP, at platelet concentrations greater than four times baseline, enhanced proliferation and differentiation of adult human mesenchymal stem cells in a dose-dependent manner (Haynesworth *et al.,* 1996). Extrapolation from this study led to the commonly recommended target of 1 million platelets (equal to four to five times the average human platelet count of 200,000 ±75,000/μl) (Sampson *et al.,* 2008; Marx, 2004). Not all studies support the idea that very high platelet numbers are desirable in PRP. Fibroblasts demonstrated a dose-dependent increase in type I collagen

production when exposed to platelet lysate; however, there was an inhibitory effect observed at the highest concentrations (Liu *et al.,* 2002). PRP with a platelet concentration 2.5 times that of baseline promoted proliferation of cultured human osteoblasts and fibroblasts; however, PRP with higher platelet concentrations (5 times that of baseline) had an inhibitory effect (Graziani *et al.,* 2006). A similar study of cultured canine osteoblasts demonstrated a marked cytotoxic effect at high PRP concentrations (Choi *et al.,* 2005). Several possible explanations exist for the inhibitory effect of platelets at high concentrations. Increasing the platelet concentration of platelet lysates leads to an increase in pH that is suboptimal for wound healing (Liu *et al.,* 2002). Along with beneficial growth factors, PRP may also contain inhibitory substances that hurt healing in high concentrations. Thrombospondin in-1 (TSP-1), a large extracellular matrix protein, inhibits cell adhesion, proliferation, and neovascularization (Adams, 2001). TSP-1 is found in higher levels in concentrated PRP and inhibits cell proliferation in a dose-dependent manner (Hsu *et al.,* 2009) High concentrations of growth factors may have cytotoxic or anti-mitogenic effects (Weibrich *et al.,* 2004).

4.2. Growth Factor Levels

The positive effects of PRP are believed to be due to the release of growth factors from platelet alpha granules (Bielecki *et al.,* 2007; Sampson *et al.,* 2008). Growth factors enhance healing by recruitment of cells to the wounded area, stimulation of cell proliferation, and enhancement of matrix synthesis (Schmidt *et al.,* 2006). Some investigators suggest that the levels of growth factors present in PRP may be more important than platelet numbers (McLellan, 2001). Several growth factors demonstrate a dose-response curve that reaches a point of diminishing returns as cell surface receptors for that growth factor are completely occupied (Ranly et al., 2005 ; Schmidt *et al.,* 2006). Some growth factors have an inhibitory effect on cell functions once a high enough concentration is reached (Weibrich *et al.,* 2001).

Conclusions

In conclusion, review of literature showed that platelet-rich plasma has been used for a wide range of applications in human and veterinary medicine. Although large, controlled, clinical studies are needed, the body of literature does support a positive effect. PRP has several advantages over other therapies. It is a regenerative therapy and therefore promotes restoration of normal tissue architecture and function. Platelet-rich plasma is used in an autologous manner in medicine; therefore, the risk of disease transmission or immune rejection is avoided. Platelet-rich plasma treatments are applied in a point-of-care manner, avoiding the hospitalization costs and processing delays associated with other regenerative therapies. Many questions remain regarding the mechanism by which PRP may support healing.

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