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The Platelet Cargo- A Regenerative Cellution

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Abstract

Background: PRP has gained significant traction in clinical settings in recent decades and is now considered a standard procedure in fields like dermatology, cosmetology, wound healing, and orthopedics. The growth factors and other constituents present in platelets play a crucial role in using PRP.

Review results: The therapeutic value of PRP is attributed to the "soup" of factors present in granules of the platelets, namely alpha and dense granules. There is an orchestrated release of over 800 proteins that get involved in tissue regeneration. It is critical to have a harmonized approach for preparing PRP and its derivatives. More important is the development of an acellular approach as opposed to the conventional application of PRP. Platelet activation leads to degranulation which in turn releases these growth factors responsible for regeneration. The presence of RBCs in PRP preparations could be detrimental in certain therapeutic applications, therefore the use of them in an acellular form would be desirable.

Discussion: Since the activation of platelets releases several factors and is an orchestrated play of factors from the various granules present in the platelets, it makes it evident that the removal of cellular debris from the degranulated platelets and having an RBC-free environment would increase the efficacy of this regenerative therapy. An acellular approach to the preparation is rapidly gaining popularity with its ease of use and superior yields of growth factors.

Clinical Significance: PRP has been in clinical practice for the past 7 decades, and the field is rapidly evolving offering newer insights and offering newer therapeutic applications.

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BACKGROUND

The History of PRP (Platelet-rich Plasma)

Haematology is the field where the concept and definition of PRP were initially developed. Originally used as a transfusion product for patients with thrombocytopenia, was coined by haematologists in the 1970s to refer to plasma with a platelet count higher than that of peripheral blood.[1,2] In the 1980s and 1990s, PRP started to be applied during surgical procedures due to its many qualities that aid in healing wounds, decreasing inflammation, and promoting the creation of new cells.[3] It was most frequently employed in aesthetic surgery, skin grafting, maxillofacial surgery, and periodontal surgery. Since then, PRP has been utilized in orthopaedics to treat a variety of conditions, including bone grafts, fractures, and connective tissue damage, as well as heart surgery, sports injuries, plastic surgery, gynaecology, urology, and other conditions.[4,5] PRP is increasingly being utilized to repair burn scars, post-surgical scars, and acne scarring because research has shown it to be beneficial in reducing scarring.[2,6-14] Chronic ulcers have a wound environment that is pro-inflammatory and hinders healing. It also has a high level of protease activity, which lowers the concentration of effective GF.[12] Because PRP possesses growth factors (GFs) that contribute to its mitogenic, angiogenic, and chemotactic properties, it is employed as an intriguing alternative

treatment for wounds that are resistant to healing. In vitro, research has shown that PRP can boost type I collagen synthesis and human skin fibroblast proliferation which has a very significant role in aesthetic dermatology.[13] Additionally, according to histological research, PRP injections into the human deep dermis and immediate sub-dermis cause the creation of new blood vessels, adipose tissue, and soft-tissue augmentation in addition to activating fibroblasts and producing new collagen.[14,15] These studies led aesthetic surgeons, dermatologists and cosmetologists to focus on PRP. Scar tissue is another area of application for PRP and studies have demonstrated the benefits of PRP in areas of burn scars, post-surgical scars and acne scars.[16] Several reports have also been on the use of PRP in androgenic alopecia.[17]

PLATELETS AND THEIR GROWTH FACTORS

Platelets are tiny, flat blood cells that have a vital role in preserving the well-being of blood vessels after they are damaged.[18] Platelets play a key role in a variety of activities such as inflammation, innate immunity, growth and development, angiogenesis, wound healing, and cancer metastasis.[19,20] Platelet granule exocytosis is an essential component of platelet function and is intricately connected to a range of platelet activities. Platelets contain several types of secretory granules, including dense granules, alpha granules, lysosomes, and a recently discovered type called T-granules. T granules exhibit the presence of toll-like receptors and protein di-sulphide isomerase (PDI) during pro-platelet production.[21-23] Platelet activation is facilitated by substances that are released and stored in DGs and AGs (Figure 1). These two granules have distinct cargos, biogenesis processes, and functions.[24] The majority of the proteins released by AGs by platelets include coagulation factors, adhesion molecules, growth factors, angiogenic and immunological mediators. DGs contain fewer kinds of small molecules than AGs which include hundreds of proteins. These molecules include serotonin, ADP, ATP, Ca^{2+}, Mg^{2+}, K^{+}, pyrophosphate, and polyphosphate.[25] Platelets that react to low-level agonists like collagen or thrombin are stimulated through autocrine and paracrine routes mediated by DG production of ADP and ATP.[26]

Platelet granules called AGs are found in abundance, with each platelet containing 50-80 of them.[27] These granules vary in size from 200 to 500 nm and make up approximately 10% of platelet volume.[28] The main components are proteins, which include receptors like P-selectin and cargo such as platelet factor 4 (PF4) and fibrinogen. An extensive examination of soluble proteins has uncovered over 300 unique proteins that are essential for a range of biological processes. These proteins are involved in important functions such as hemostasis (e.g., von Willebrand factor [VWF] and factor V), inflammation (e.g., chemokines like CXCL1 and interleukin-8), and wound healing (e.g., VEGF and FGF)[24]. With 3-8 per platelet, DGs are the second most numerous platelet granules.[25] They have a diameter of roughly 150 nm. These platelet-specific granules are a subtype of lysosome-related organelles (LROs), which also contain melanosomes, type II alveolar cell lamellar bodies, and cytotoxic T-cell lytic granules. Bioactive amines (such as serotonin and histamine), adenine nucleotides, polyphosphates, and pyrophosphates, as well as significant amounts of cations, notably calcium, are found in dense granules. DG is composed of various components, including serotonin, calcium, pyrophosphate, and a pool of non-metabolic adenine nucleotides (ADP and ATP). These components have a significant impact on the activation of platelets.[29] Platelets can also become activated via G-protein coupled receptor (GPCR).[30] The soluble agonists, especially ADP released from DGs of activated platelets, result in the swift activation of a significant number of platelets (Figure 2). The restoration of vessel integrity is achieved through the collaboration of various secreted mediators and cells. The release of bioactive compounds is a crucial outcome of platelet activation and is responsible for the various functions of platelets outside of the clot's immediate surroundings. Keeping in mind this orchestrated effort of platelets, platelet secretion is at the heart of the control of vascular integrity, in health and disease.[31,32]

AGs and DGs play vital roles in the process of wound healing, although their functions differ from each other.[29,33] When the vascular endothelium is damaged or in an inflammatory state, platelets gather to create a plug that is abundant in platelets. Platelet function encompasses adhesion, activation, secretion, and aggregation.[34] During adhesion, platelets first attach to collagen that is exposed due to damage to the endothelium. This attachment occurs through a glycoprotein receptor complex called Ib/V/IX, which is present on the surface of platelets. The process is improved by vWF, which is stored and released from AGs and Weibel-Palade bodies (WPBs) of endothelial cells. Platelets adhere to the damaged vascular endothelium, causing DGs to release various stimuli, including ADP, which then activate platelets. Simultaneously, the pathway for platelet activation triggers the release of Ca^{2+} from DGs. As the Ca^{2+} concentration rises, the cAMP concentration decreases. This triggers the activation of protein kinases like Src kinase and protein kinase C (PKC). Consequently, the release of DG contents, ADP, and serotonin is induced, which speeds up the activation and aggregation of platelets. This leads to the formation of a hemostatic plug.[34]

Following aggregation, platelets release the contents of their granules and generate cytokines and intercellular mediators that were previously stored in the cytoplasmic pool. The first hour of this secretion is highly active, with platelets continuously producing additional cytokines and growth factors for at least the following seven days. Over 800 different proteins are released into the environment, and they can interact with various types of cells, such as myocytes, tendon cells, mesenchymal stem cells from different sources, chondrocytes, osteoblasts, fibroblasts, and endothelial cells,

in a paracrine manner. The stimulation of cell migration, angiogenesis, and proliferation leads to tissue regeneration. According to studies, platelets release antimicrobial peptides, which may have an antibacterial effect.

The properties of PRP are derived from the production and release of a wide range of growth and differentiation factors. Platelets have a significant impact on cartilage regeneration as they release specific cytokines, including Platelet-derived Growth Factor (PDGF), which originates from the AGs.

This growth factor is essential for connective tissue healing as it stimulates collagen and protein synthesis. It seems that PDGF mainly promotes the growth of cells derived from mesoderm, such as fibroblasts, vascular muscle cells, glial cells, and chondrocytes. PDGF is essential for promoting angiogenesis, attracting fibroblasts, and facilitating collagen synthesis. There is strong evidence supporting the use of PDGF in cartilage repair, which is derived from its involvement in wound healing and its synthesis in growing chondrocytes.[35]

The members of the TGF-beta (Transforming Growth Factor β) superfamily share a structural similarity and are biologically active when they form homo- or heterodimers connected by a single disulfide bond.[36] Recently, studies have been conducted on cartilage-derived morphogenetic protein (CDMP-1) (also known as GDF-5; growth differentiation factor-5) and CDMP-2 in vitro. These studies have shown that these proteins can stimulate the synthesis of cartilage matrix. TGF-beta 3 has also been found to enhance extracellular matrix (ECM) synthesis. It has been studied in vitro using rabbit models of acute cartilage injury.[37-39] TGF-b1 enhances chondrocyte synthetic activity while reducing the catabolic activity of IL-1.[40] *In vitro* TGF-b1 stimulates chondrogenesis of synovial lining and bone marrow-derived MSCs.[38,41]

Several other growth factors are worth mentioning, such as Fibroblast Growth factor (FGF) and Vascular Endothelial Growth Factor (VEGF). However, due to the scope of this review, we cannot cover them in detail. Platelet-derived basic fibroblast growth factor (bFGF) plays a crucial role in promoting osteogenesis and angiogenesis.[42] VEGFs are a group of signaling proteins that play a crucial role in promoting angiogenesis and vasculogenesis by activating a signaling cascade mediated by tyrosine kinase receptors.[43] Both these GFs play an important role in osteogenesis besides other important regenerative events such as wound healing.

Considering the numerous publications, clinical trial data, and reviews in the field of autologous platelet-rich plasma (PRP), it is clear that these preparations have been widely used and proven effective in certain cases.

REVIEW RESULTS

The therapeutic effect of PRP is thought to result from the activation and release of a range of growth factors, cytokines, and interferons by platelets. These substances contribute to the promotion of various biological processes that aid in the healing of injuries. It is important to understand that, similar to biologics, licensed medications also yield different outcomes, but they are generally favorable and backed by significant scientific evidence, which enhances their credibility as standardized treatments (Figure 3). Due to variations in settings, PRP preparations can differ in quality as different processes are often modified to enhance PRP quality. Differences can be observed in the amount of venous blood collected for processing, the types of collection tubes utilized, the anticoagulant employed, the centrifugation equipment utilized, the level of G force applied, the duration of centrifugation, the temperature regulation, the inclusion of additives/activators in PRP, the techniques used for pipetting (which may be prone to human error), the quantity of PRP obtained, the procedures for storage, and the duration of storage.[44-48] There have been significant efforts in harmonizing and standardization the methodologies for processing PRP, however, a salient point is that it is the GFs and other factors in the granules present in platelets that have the therapeutic value. Wouldn't it be better to isolate these molecules and use them in an acellular, cell-free manner?

It is worth noting that extensive research has been conducted on the biology of platelet activation, which has greatly enhanced our understanding of the underlying mechanisms. As a result, the isolation of growth factors (GFs) and other components has become comparatively more straightforward. The activation of platelets involves two crucial processes: the release of granules from platelets and the cleavage of fibrinogen to initiate the formation of a matrix. This clotting process enables the creation of a platelet gel, which helps to localize the secretion of molecules to a specific site. Typically, this activation process is often performed by introducing thrombin and/or calcium chloride ($CaCl_2$). However, certain doctors opt to administer PRP in its inactive state, relying on the natural platelet activation that takes place when exposed to the collagen found in the connective tissues. The latter generally would not lead to all the platelets getting activated and also the deposition of the cellular debris at the site of injury. Nonetheless, The investigation and understanding of the direct effects of exogenously added Ca^{2+} on platelets in vitro have been limited. It is now becoming increasingly popular to use kits that produce a "soup" of GFs directly from the patient's blood. The use of diagnostic collection devices containing thixotropic agents to separate the cellular components from the serum/plasma assists in deriving this "soup" of direct-to-inject growth factors. The operation being performed in a closed system adds to the safety benefit of using these kits. There have been several publications ranging from pre-clinical to clinical applications of such a kit that utilizes acellular growth factors. The preclinical study focussed on the use of this acellular growth factor also termed as growth factor concentrate (GFC), for the treatment of wounds in diabetic and non-diabetic rats. This study outcome

demonstrated that topically applied GFC enhanced wound closure in both animals.[49] There are studies published on conditions such as melasma and osteoarthritis (OA) using this process of the preparation of GFC.[50-52] It is important to note here that the use of conventional PRP in osteoarthritis is riddled with controversies that surround the presence of leukocytes and RBCs. Additionally, crucial to the PRP product's effectiveness and the inflammatory response are the concentrations of red blood cells, neutrophils, and mononuclear cells. Leukocyte concentrations in PRP preparations can be either leukocyte-rich or leukocyte-poor, above or below baseline amounts, respectively. Red blood cells and leukocytes, especially neutrophils, are generally thought to need to be reduced because they are pro-inflammatory and produce catabolic cytokines like tumour necrosis factor (TNF) and interleukin-1 (IL-1), which cause cartilage deterioration in OA (Figure 4). Further, the presence of RBCs when delivered to tissues, can cause a local response called eryptosis, which triggers the release of a potent cytokine, macrophage migration inhibitory factor.[53] This cytokine prevents monocytes and macrophages from migrating. It significantly disrupts local cellular function and sends strong pro-inflammatory signals to the tissues around it, preventing stem cells from migrating and fibroblasts from proliferating. So it's crucial to keep RBC contamination in PRP preparations to a minimum.

DISCUSSION

PRP has a long history, and extensive research has been done on this blood component. New therapeutic techniques are being actively sought after by clinicians and researchers. There have been over 12,000 publications related to PRP from 2000 till date. This is evidence of a promising field of regenerative medicine. In the past decade, there have been over 1,100 publications in the area of osteoarthritis itself, establishing the use of PRP in clinical settings. However, there are growing concerns about the method of preparation of PRP. There is no one harmonised approach and added with the fact that when preparing PRP it gets contaminated with RBCs it makes it essential to move towards newer methodologies. Since the activation of platelets releases several factors and is an orchestrated play of factors from the various granules present in the platelets, it makes it evident that the removal of cellular debris from the degranulated platelets and having an RBC-free environment would increase the efficacy of this regenerative therapy. Described is a methodology developed for the preparation of an acellular growth factor mix called GFC. This is rapidly gaining popularity with its ease of use and superior yields of growth factors.

CLINICAL SIGNIFICANCE

This brief history shows that PRP has a minimum of 70 years of history. The procedure is therefore well enough established to permit broad application in medicine, particularly in aesthetic dermatology, orthopaedics, and wound healing.

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