

New Understanding of Platelet-Rich Plasma (PRP)

Therapy-Part 1

The emerging autologous cell therapy using platelet-rich plasma (PRP) may play an auxiliary role in various regenerative medicine treatment plans. There is a global unmet demand for tissue repair strategies for treating patients with musculoskeletal (MSK) and spinal diseases, osteoarthritis (OA) and chronic complex and refractory wounds. PRP therapy is based on the fact that platelet growth factor (PGF) supports wound healing and repair cascade (inflammation, proliferation and remodeling). A number of different PRP formulations have been evaluated from human, in vitro and animal studies. However, the recommendations of in vitro and animal studies usually lead to different clinical results, because it is difficult to translate non-clinical research results and method recommendations into human clinical treatment. In recent years, progress has been made in understanding the concept of PRP technology and biological agents, and new research instructions and new indications have been proposed. In this review, we will discuss the latest progress in the preparation and composition of PRP, including platelet dose, leukocyte activity and innate and adaptive immune regulation, 5-hydroxytryptamine (5-HT) effect and pain relief. In addition, we discussed the PRP mechanism related to inflammation and angiogenesis during tissue repair and regeneration. Finally, we will review the effects of some drugs on PRP activity.

Autologous platelet-rich plasma (PRP) is the liquid part of autologous peripheral blood after treatment, and the platelet concentration is higher than the baseline. PRP therapy has been used for various indications for more than 30 years, resulting in great interest in the potential of autogenous PRP in regenerative medicine. The term orthopedic biological agent has recently been introduced to treat musculoskeletal (MSK) diseases, and has achieved promising results in the regeneration ability of heterogeneous bioactive PRP cell mixtures. At present, PRP therapy is an appropriate treatment option with clinical benefits, and the reported patient results are encouraging. However, the inconsistency of patient results and new insights have posed challenges to the practicability of clinical application of PRP. One of the reasons may be the number and variability of PRP and PRP-type systems on the market. These devices are different in terms of PRP collection volume and preparation scheme, resulting in unique PRP characteristics and biological agents. In addition, the lack of consensus on the standardization of PRP preparation scheme and the full report of biological agents in clinical application led to inconsistent report results. Many attempts have been made to characterize and classify PRP or blood derived products in regenerative medicine applications. In addition, platelet derivatives, such as human platelet lysates, have been proposed for orthopedic and in vitro stem cell research.

One of the first comments on PRP was published in 2006. The main focus of this review is the function and mode of action of platelets, the effect of PRP on each stage of the healing cascade, and the core role of platelet-derived growth factor in various PRP indications. In the early stage of PRP research, the main interest in PRP or PRP-gel was the existence and specific functions of several platelet growth factors (PGF).

In this paper, we will extensively discuss the latest development of different PRP particle structures and platelet cell membrane receptors and their effects on innate and adaptive immune system immune regulation. In addition, the role of individual cells that may exist in the PRP treatment vial and their influence on the tissue regeneration process will be discussed in detail. In addition, the latest progress in understanding PRP biological agents, platelet dose, specific effects of specific white blood cells, and the effects of PGF concentration and cytokines on the nutritional effects of mesenchymal stem cells (MSCs) will be described, including PRP targeting different cell and tissue environments after cell signal transduction and paracrine effects. Similarly, we will discuss the PRP mechanism related to inflammation and angiogenesis during tissue repair and regeneration. Finally, we will review the analgesic effect of PRP, the effect of some drugs on PRP activity, and the combination of PRP and rehabilitation programs.

Basic principles of clinical platelet-rich plasma therapy

PRP preparations are increasingly popular and widely used in various medical fields. The basic scientific principle of PRP treatment is that the injection of concentrated platelets at the injured site may initiate tissue repair, the synthesis of new connective tissue and the reconstruction of blood circulation by releasing many biologically active factors (growth factors, cytokines, lysosomes) and adhesion proteins responsible for initiating the hemostatic cascade reaction. In addition, plasma proteins (e.g. fibrinogen, prothrombin, and fibronectin) are present in platelet-poor plasma components (PPPs). PRP concentrate can stimulate the hyperphysiological release of growth factors to start the healing of chronic injury and accelerate the repair process of acute injury. At all stages of the tissue repair process, a variety of growth factors, cytokines and local action regulators promote most basic cell functions through endocrine, paracrine, autocrine and endocrine mechanisms. The main advantages of PRP include its safety and the ingenious preparation technology of current commercial equipment, which can be used to prepare biological agents that can be widely used. Most importantly, compared with common corticosteroids, PRP is an autogenous product with no known side effects. However, there is no clear regulation on the formula and composition of injectable PRP composition, and the composition of PRP has great changes in platelets, white blood cell (WBC) content, red blood cell (RBC) pollution, and PGF concentration.

PRP terminology and classification

For decades, the development of PRP products used to stimulate tissue repair and regeneration has been an important research field of biomaterials and drug science. The tissue healing cascade includes many participants, including platelets and their growth factors and cytokine granules, white blood cells, fibrin matrix and many other synergistic cytokines. In this cascade process, a complex coagulation process will occur, including platelet activation and subsequent densification and α - The release of the contents of platelet particles, the aggregation of fibrinogen (released by platelets or free in plasma) into fibrin network, and the formation of platelet embolism.

“Universal” PRP simulates the beginning of healing

At first, the term “platelet-rich plasma (PRP)” was called platelet concentrate used in blood transfusion medicine, and it is still used today. Initially, these PRP products were only used as fibrin tissue adhesive, while platelets were only used to support stronger fibrin polymerization to improve tissue sealing, rather than as a healing stimulant. After that, PRP technology was designed to simulate the initiation of the healing cascade. Subsequently, the PRP technology was summarized through its ability to introduce and release growth factors into the local microenvironment. This enthusiasm for PGF delivery often hides the important role of other components in these blood derivatives. This enthusiasm is further intensified due to the lack of scientific data, mystical beliefs, commercial interests and lack of standardization and classification.

The biology of PRP concentrate is as complex as blood itself, and may be more complex than traditional drugs. PRP products are living biomaterials. The results of clinical PRP application depend on the intrinsic, universal and adaptive characteristics of the patient’s blood, including various other cellular components that may exist in the PRP sample and the local microenvironment of the receptor, which can be in acute or chronic state.

Summary of confusing PRP terminology and proposed classification system

For many years, practitioners, scientists and companies have been plagued by the initial misunderstanding and defects of PRP products and their different terms. Some authors defined PRP as platelet-only, while others pointed out that PRP also contains red blood cells, various white blood cells, fibrin and bioactive proteins with increased concentration. Therefore, many different PRP biological agents have been introduced into clinical practice. It is disappointing that the literature usually lacks a detailed description of biological agents. The failure of product preparation standardization and subsequent classification system development led to the use of a large number of PRP products described by different terms and abbreviations. It is not surprising that changes in PRP preparations lead to inconsistent patient outcomes.

Kingsley first used the term “platelet-rich plasma” in 1954. Many years later, Ehrenfest et al. The first classification system based on three main variables (platelet, leukocyte and fibrin content)

was proposed, and many PRP products were divided into four categories: P-PRP, LR-PRP, pure platelet-rich fibrin (P-PRF) and leucocyte rich PRF (L-PRF). These products are prepared by fully automatic closed system or manual protocol. Meanwhile, Everts et al. The importance of mentioning white blood cells in PRP preparations was emphasized. They also recommend the use of appropriate terminology to denote inactive or activated versions of PRP preparations and platelet gel.

Delong et al. proposed a PRP classification system called platelets, activated white blood cells (PAW) based on the absolute number of platelets, including four platelet concentration ranges. Other parameters include the use of platelet activators and the presence or absence of white blood cells (i.e. neutrophils). Mishra et al. A similar classification system is proposed. A few years later, Mautner and his colleagues described a more elaborate and detailed classification system (PLRA). The author proved that it is important to describe the absolute platelet count, white blood cell content (positive or negative), neutrophil percentage, RBC (positive or negative) and whether exogenous activation is used. In 2016, Magalon et al. The DEPA classification based on the dose of platelet injection, production efficiency, purity of PRP obtained and activation process was published. Subsequently, Lana and her colleagues introduced the MARSPILL classification system, focusing on peripheral blood mononuclear cells. Recently, the Scientific Standardization Committee advocated the use of the classification system of the International Society for Thrombosis and Hemostasis, which is based on a series of consensus recommendations to standardize the use of platelet products in regenerative medicine applications, including frozen and thawed platelet products.

Based on the PRP classification system proposed by various practitioners and researchers, many unsuccessful attempts to standardize the production, definition and formula of PRP to be used by clinicians can draw a fair conclusion, which is likely not to happen in the next few years. In addition, the technology of clinical PRP products continues to develop, and scientific data shows that different PRP preparations are needed to treat different pathologies under specific conditions. Therefore, we expect that the parameters and variables of ideal PRP production will continue to grow in the future.

PRP preparation method is in progress

According to PRP terminology and product description, several classification systems are released for different PRP formulations. Unfortunately, there is no consensus on the comprehensive classification system of PRP or any other autologous blood and blood products. Ideally, the classification system should pay attention to various PRP characteristics, definitions and appropriate nomenclature related to the treatment decisions of patients with specific diseases. At present, orthopedic applications divide PRP into three categories: pure platelet-rich fibrin (P-PRF), leucocyte-rich PRP (LR-PRP) and leucocyte-deficient PRP (LP-PRP). Although it is more specific than the general PRP product definition, LR-PRP and LP-PRP categories obviously lack any specificity in white blood cell content. Due to its immune and host defense mechanisms, white blood cells have greatly affected the intrinsic biology of chronic tissue diseases. Therefore, PRP biological agents containing specific white blood cells can significantly promote immune regulation and tissue repair and regeneration. More specifically, lymphocytes are abundant in PRP, producing insulin-like growth factor and supporting tissue remodeling.

Monocytes and macrophages play a key role in the process of immune regulation and the mechanism of tissue repair. The importance of neutrophils in PRP is unclear. LP-PRP was determined as the first PRP preparation by systematic evaluation to achieve effective treatment results of joint OA. However, Lana et al. The use of LP-PRP in the treatment of knee OA is opposed, which indicates that specific white blood cells play an important role in the inflammatory process before tissue regeneration, because they release pro-inflammatory and anti-inflammatory molecules. They found that the combination of neutrophils and activated platelets had more positive effects than negative effects on tissue repair. They also pointed out that the plasticity of monocytes is important for the non-inflammatory and repair function in tissue repair. The report of PRP preparation scheme in clinical research is highly inconsistent. Most published studies have not proposed the PRP preparation method required for the repeatability of the scheme. There is no clear consensus among treatment indications, so it is difficult to compare PRP products and their related treatment results. In most reported cases, platelet concentration therapy is classified under the term "PRP", even for the same clinical indication. For some medical fields

(such as OA and tendinosis), progress has been made in understanding the changes of PRP preparations, delivery routes, platelet function and other PRP components that affect tissue repair and tissue regeneration. However, further research is needed to reach a consensus on the PRP terminology related to PRP biological agents in order to fully and safely treat certain pathologies and diseases.

Status of PRP classification system

The use of autologous PRP biotherapy is troubled by the heterogeneity of PRP preparations, inconsistent naming and poor standardization of evidence-based guidelines (that is, there are many preparation methods to produce clinical treatment vials). It can be predicted that the absolute PRP content, purity and biological characteristics of PRP and related products vary greatly, and affect the biological efficacy and clinical trial results. The selection of PRP preparation device introduces the first key variable. In clinical regenerative medicine, practitioners can use two different PRP preparation equipment and methods. A preparation uses a standard blood cell separator, which operates on the complete blood collected by itself. This method uses continuous flow centrifuge drum or disk separation technology and hard and soft centrifuge steps. Most of these devices are used in surgery. Another method is to use gravity centrifugal technology and equipment. High G-force centrifugation is used to separate the yellow layer of ESR from the blood unit containing platelets and white blood cells. These concentration devices are smaller than blood cell separators and can be used beside the bed. In difference g – Force and centrifugation time lead to significant differences in the yield, concentration, purity, viability, and activated state of isolated platelets. Many types of commercial PRP preparation equipment can be used in the latter category, resulting in further changes in product content.

The lack of consensus on the preparation method and validation of PRP continues to lead to the inconsistency of PRP treatment, and there are huge differences in PRP preparation, sample quality and clinical results. The existing commercial PRP equipment has been verified and registered according to the specifications of the proprietary manufacturer, which solves the different variables among the currently available PRP equipment.

Understand platelet dose in vitro and in vivo

The therapeutic effect of PRP and other platelet concentrates stems from the release of various factors involved in tissue repair and regeneration. After the activation of platelets, platelets will form platelet thrombus, which will serve as a temporary extracellular matrix to promote cell proliferation and differentiation. Therefore, it is fair to assume that higher platelet dose will lead to higher local concentration of platelet bioactive factors. However, the correlation between the dose and concentration of platelets and the concentration of released platelet bioactive growth factor and drug may be uncontrollable, because there are significant differences in the baseline platelet count between individual patients, and there are differences between PRP preparation methods. Similarly, several platelet growth factors involved in the tissue repair mechanism are present in the plasma part of PRP (for example, liver growth factor and insulin-like growth factor 1).

Therefore, higher platelet dose will not affect the repair potential of these growth factors. In vitro PRP research is very popular because the different parameters in these studies can be accurately controlled and the results can be obtained quickly. Several studies have shown that cells respond to PRP in a dose-dependent manner. Nguyen and Pham showed that very high concentrations of GF were not necessarily conducive to the process of cell stimulation, which might be counterproductive. Some in vitro studies have shown that high PGF concentrations may have adverse effects. One reason may be the limited number of cell membrane receptors. Therefore, once the PGF level is too high compared with the available receptors, they will have a negative impact on cell function.

Significance of platelet concentration data in vitro

Although in vitro research has many advantages, it also has some disadvantages. In vitro, due to the continuous interaction between many different cell types in any tissue due to tissue structure and cellular tissue, it is difficult to replicate in vitro in a two-dimensional single culture environment. The cell density that can affect the cell signal pathway is usually less than 1% of the tissue condition. Two-dimensional culture dish tissue prevents cells from being exposed to extracellular matrix (ECM). In addition, the typical culture technology will lead to the

accumulation of cell waste and continuous nutrient consumption. Therefore, in vitro culture is different from any steady-state condition, tissue oxygen supply or sudden exchange of culture medium, and conflicting results have been published, comparing the clinical effect of PRP with the in vitro study of specific cells, tissue types and platelet concentrations. Graziani and others. It was found that in vitro, the greatest effect on the proliferation of osteoblasts and fibroblasts was achieved at the PRP platelet concentration 2.5 times higher than the baseline value. In contrast, the clinical data provided by Park and colleagues showed that after spinal fusion, the PRP platelet level needs to be increased by more than 5 times than the baseline to induce positive results. Similar contradictory results were also reported between the tendon proliferation data in vitro and the clinical results.

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