Blood Products

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Platelet-Rich Plasma

History

The idea of platelet-rich plasma (PRP) was initially introduced in the 1970s, but it was not until the 1990s that PRP began gaining popularity and its applications were being used for sports injuries and other painful conditions [1]. Over the last decade, its applications have been used in multiple fields and there has been a prominent increase in the number of publications focusing on PRP [2].

With the availability of mobile tabletop centrifuges and office-based ultrasound equipment, the application of platelet-rich plasma in the treatment of musculoskeletal issues became more widespread and has allowed for the introduction and expansion of regenerative medicine [3, 4]. In the past, imaging guidance of therapies related to the soft and connective tissues was difficult, but ultrasound imaging has allowed for this to happen. This has resulted in less invasive and additional interventional treatment options for patients [5–8]. With many patients looking for nonsurgical options and the desire to avoid the chronic administration of steroids either within a joint or in a peritendinous location, the application of platelet-rich plasma products has offered another option [5].

This evolution has facilitated the utilization of orthobiologic products that have come to the forefront of multiple medical specialties. Its applications have generated a significant amount of clinical and academic interest and PRP and other orthobiologic products are now employed on the global medical level [9, 10]. Those who are practicing and utilizing regenerative medicine and incorporating it into their practices are often quite optimistic about its use. As the medical treatment paradigm advances, regenerative medicine will continue to propagate and physicians will need to have an awareness of this so that these treatments may be considered in their algorithm of treatment [11].

Preparation and Obtaining PRP

Producing platelet-rich plasma is the process of obtaining blood from a patient and concentrating the platelets to greater than four times the normal level. Often, this can be done with the use of multiple collecting tubes and a centrifuge, but several commercial companies have created systems and kits that can automate the collection, concentration, and separation process [12–14] (Fig. 3.1).

In most cases, the process will involve a sterile venous puncture with a collection of about 40 mL of whole blood in anticoagulated tubes. If the tube does not have an anticoagulant present, the addition of an anticoagulant such as EDTA or acid citrate dextrose (ACD) needs to be done [5, 14]. This will act as an anticoagulant to prevent the platelets from clotting and clumping and preventing it from becoming an unusable sample. Once the sample has been obtained, it is then processed with a centrifuge. This can have some variations in regard to the centrifuge speed and the length of spin time. In general, a first spin or centrifuge process is performed with the initially drawn whole blood. This will often be done at 600*g* for 7 minutes and is considered the first specimen. After the completion of the first spin, the volume below the platelet border is aspirated. This is the upper layer of plasma along with the buffy coat [15–19] (Fig. 3.1).

This first separation will include platelet-poor plasma, the desired portion of PRP, and the buffy coat along with some red blood cells. Then a second centrifuge spin is conducted. This will often be at 2000*g* for 5 minutes. This process will allow for the separation of the desired PRP product from the platelet-poor plasma (PPP) [5, 20].

Often the initial spin is considered a "soft spin" allowing for the blood products to be separated out. The second specimen is then considered a "hard spin" allowing for the

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C. W Hunter et al. (eds.), *Regenerative Medicine*, https://doi.org/10.1007/978-3-030-75517-1_3

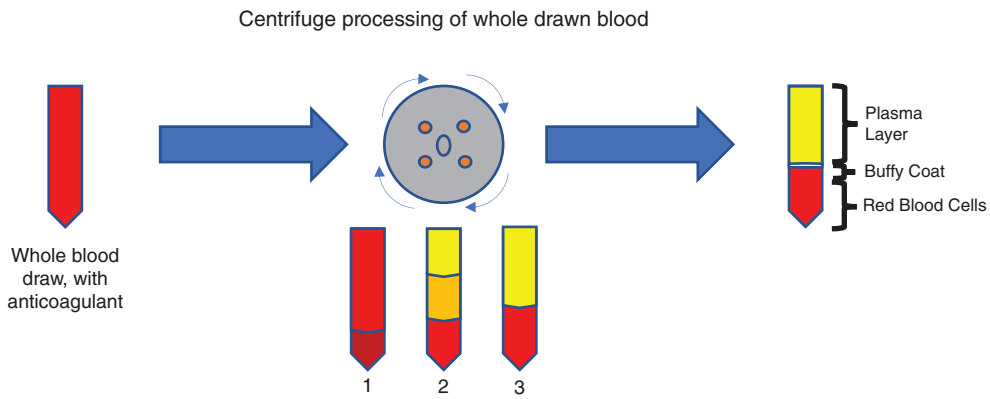

Fig. 3.1 This is the process of blood draw, preparing with an anticoagulant agent, and the centrifuge process. The centrifuge is often done with a 600G spine, followed by a 2000G spin to getting the layers out and a final platelet-rich plasma product

separation of the remaining red blood cells and PPP from the desired platelet-rich plasma [19, 21–23].

After the completion of the second spin, the PPP is often discarded and the remaining product is considered to be the desired platelet-rich plasma. This will require the removal of the upper fluid layer containing the PPP. The volume that is most often leftover is between 2 and 5 mL from the initial draw of 40 mL. The volume that is recovered from the second spin can certainly be variable from individual to individual, technique to technique, and/or system to system [24–27].

When considering blood and its products, a basic understanding of blood components is important. Blood is primarily made up of red blood cells, white blood cells, platelets, and plasma. The volume of plasma is usually about 55% and primarily consists of water. The cellular components will make up about 45% of the blood volume [2, 28–30]. The cellular components are often distributed throughout the buffy coat and among the red blood cells. The buffy coat is the portion of the blood after centrifuging process that remains between the plasma and the red blood cells. This will contain the white blood cells and platelets. The plasma will contain blood proteins, such as fibrinogen, albumin, globulin, electrolytes, hormones, and water [30–33].

Another method to obtain the buffy coat involves storing the whole blood at 20–24°C prior to the centrifuge process. After the room temperature storage, the whole blood is placed in a high-speed centrifuge and the plasma and buffy coat are separated out and subsequently removed. This is then centrifuged at a lower speed to separate the white blood cells from the red blood cells [32, 33].

PRP Types and Classifications

With the different preparation processes for PRP, there can be variability in the type of PRP that is obtained. This may be intentional in many cases and based on the type of injection therapy or treatment the practitioner would like to render. However, in certain cases, this may be purely based on the individual's blood components and concentration along with the type of processing or machine automation [20, 34–36]. The overarching purpose of preparing PRP is to isolate out the platelets and remove the red blood cells. Given this, many practitioners focus on having a very yellow-colored final product indicating very little red blood cell content, although in certain cases a red-tinged or red product may be obtained or desired. There are five main types of platelet-rich plasma (PRP). This is based on the cell content and the presence of other blood products [36, 37].

The first type is red PRP. Platelet-rich plasma that appears red has a low concentration of platelets. It will purposely have a certain content of red blood cells that are thought to have an inflammatory reaction following an injection. It is not clear as to the clinical role of red PRP, but ongoing studies are being conducted to determine its utility in specific conditions [2, 20, 37–39].

When the prepared PRP has more of a yellowish color, the concentration of platelets in the solution can be either high or low and can be called either high-concentration or low-concentration PRP. Low-concentration or low-density PRP is yellowish or amber in color with lower levels of platelets. This solution is typically poor and devoid of white or red blood cells. It is thought that this preparation is less reactive in regard to producing an inflammatory process and results in less of a chemotactic response following injection compared with that of red PRP. This is a newer approach and sometimes is considered to be "pure" PRP or P-PRP. It will also have a low density of fibrin in this preparation [2, 20, 30, 31].

PRP that is prepared with leukocytes present is the most common type of PRP and is often prepared in the automated systems and kits that are available. This will also have a low density of fibrin and is known as leukocyte-rich PRP or L-PRP [36, 40].

A fourth type is a pure platelet-rich fibrin or leukocyte-poor/platelet-rich fibrin preparation that is thought to have no leukocytes but has a high density of fibrin products in it. This is called pure platelet-rich fibrin or P-PRF [20, 40].

Finally, there is a leukocyte-rich and platelet-rich fibrin PRP. This is thought to represent the second generation of PRP products with a high density of fibrin and leukocytes mixed in with the platelets and its other factors. This is known as L-PRF [2, 19, 30].

Proposed Mechanisms of Action

The purpose of injecting or providing platelet-rich plasma is to increase the concentration of growth factors and platelets at that site compared to what is available in whole blood [32, 35]. The injected PRP is thought to have a healing and/or regenerative effect on the environment it is injected into. There remain many unknowns regarding the appropriate concentrations of blood products in the PRP preparations that would produce the maximum desired effect on the different tissues [19].

The PRP preparation also contains many growth factors as well as platelets. Platelets have a combination of dense and alpha granules that, when activated, will release these factors into their medium or surrounding environment [11]. The growth factors that are considered to be important are transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor II (FGF2), and insulin growth factor (IGF). These factors are considered to have stimulating properties for enhancing both tissue and bone healing. Others are thought to stimulate osteoblast proliferation and differentiation, as well as to stimulate epidermal cell proliferation. It is also believed that these factors are involved in stimulating collagen synthesis, as well as angiogenesis and revascularization of tissue [22, 31, 32, 41] (Table 3.1).

Table 3.1 Common factors present in platelet-rich plasma and the proposed mechanism of action

Name		Function
Platelet-derived growth factor	PDGF	Enhances collagen synthesis, proliferation of bone cells, fibroblast chemotaxis, and proliferative activity; macrophage activation
Transforming growth factor β	TGF-β	Enhances synthesis of type I collagen; promotes angiogenesis; stimulates chemotaxis of immune cells; inhibits osteoclast formation and bone resorption
Vascular endothelial growth factor	VEGF	Stimulates angiogenesis, migration, and mitosis of endothelial cells; increases permeability of the vessels; stimulates chemotaxis of macrophages and neutrophils
Epidermal growth factor	EGF	Stimulates cellular proliferation and differentiation of epithelial cells; promotes cytokine secretion by mesenchymal and epithelial cells
Insulin-like growth factor	IGF	Promotes cell growth, differentiation, and recruitment in bone, blood vessel, skin, and other tissues; stimulates collagen synthesis together with PDGF
Fibroblast growth factor	FGF	Promotes proliferation of mesenchymal cells, chondrocytes, and osteoblasts; stimulates the growth and differentiation of chondrocytes and osteoblasts

Further understanding of the properties of platelet-rich plasma is facilitated by adequate comprehension of the normal healing process. The repair response in musculoskeletal injuries will often start with a blood clot and degranulation of the platelets [8]. This process will then release the platelet growth factors present in the alpha and dense granules after they burst and will, in turn, result in the chemotactic effect, which promotes the migration of inflammatory cells and proliferation of pro-generator cells [2, 11, 31] (Fig. 3.2).

When we consider this and look at structures in the musculoskeletal system, one can see that vascular supply plays a very important role in this process. The muscle tissues are very well vascularized and often demonstrate a remodeling and rapid healing process similar to that of other organ systems with an optimal vascular supply such as the integumentary system [14, 16]. When we consider other connective tissues, however, vascular supply is often not as prevalent as in muscle or skin tissue. Tissues with a vascular supply that is less replete will often heal slower and are more prone to chronic inflammatory processes and slower healing [9, 25]. With the application of platelet-rich plasma, the growth factors present can increase the cell activity and products that are locally available in those tissue environments that are otherwise limited or devoid of an ample vascular supply [29].

Applications in Musculoskeletal Medicine

Platelet-rich plasma is most commonly used for musculoskeletal applications. Although the number of overall treatments for all organ systems is vast, its utilization in the musculoskeletal accounts for the greatest number of therapies by far. The applications of PRP in the musculoskeletal tissues can be segmented into regions or tissue types, including injections into muscles, tendons, ligaments, and joints [12, 19, 40].

When considering skeletal muscle injuries such as sprains, strains, and muscle tears, there are certain barriers to recovery that exist. Some limitations can be from scar formation or fibrosis development within the muscle as well as repeated stress and use that prolongs the period of recovery [40, 41]. Platelet-rich plasma has been demonstrated to have a role in modulating the inflammation and healing process in muscle. There have been studies that have demonstrated the application of PRP compared with saline for muscle tears in animal models showing an increase in satellite cell activation

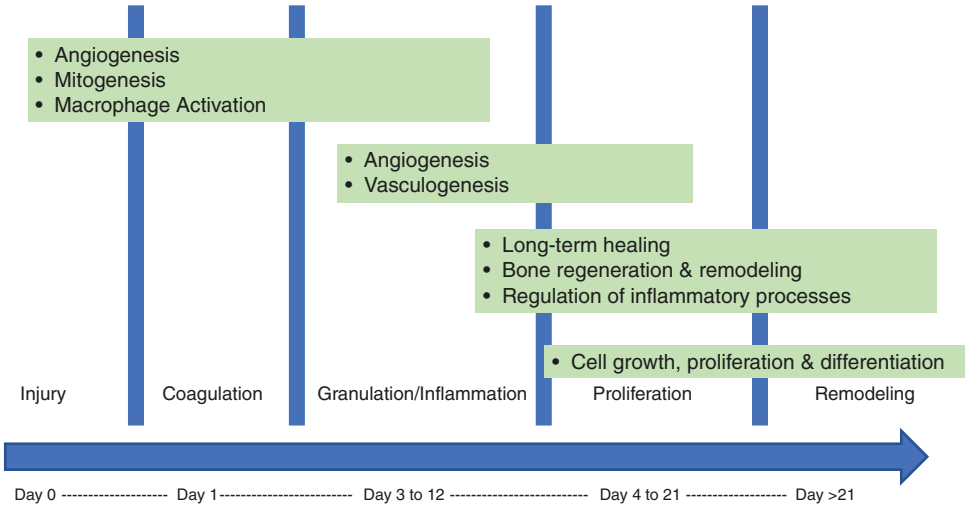

Fig. 3.2 Demonstration of the healing cascade and timeline for tissue remodeling

postinjury for those animals injected with PRP [36, 39]. Several studies have demonstrated the different factors that may be responsible for this. This certainly can have an impact on certain types of injuries particularly to the muscle that demonstrate delayed healing or are refractory to other certain types of therapies [30, 32, 36, 39].

Tendons and ligaments are often subject to high stress and shear forces and can be injured with areas of rupture, partial tear, or complete tear. These injuries are often seen in a background of chronic inflammation. Several laboratory studies have shown beneficial effects of PRP on the tendon healing process [21–23, 28, 31–33]. The healing mechanism involves stimulation of cell proliferation and total collagen production in those injected with PRP or PPP compared with those that were not injected. Much of the literature regarding the tendons and ligaments has been primarily focused on lateral epicondylitis. These data have been applied to other tendon injuries with the hope that analogous outcomes would occur given the findings and the research that supports lateral epicondylitis. Several studies have demonstrated histologic changes and extracellular matrix responses following PRP injections [32]. These have been demonstrated at the 7–10-day postinjection interval as well as at histological evaluations at the 6–12 week postinjection timeframe. These histologic evaluations showed robust cellular responses to the injections and overall increased cellular activity [27, 28, 30, 31, 34].

There are several studies ongoing for different applications of platelet-rich plasma. Many of these studies are focused on cartilage and joint injury and/or degeneration [31, 33, 34, 36]. There has been good literature support for PRP injections to treat intra-articular injuries as well as for tendon, ligament, and muscle use. In the intra-articular applications, the exact mechanism of action and the appropriate concentration of blood products are not well understood nor are they well known [36]. Further research including randomized controlled trials are needed to determine the effects and impact of regenerative techniques on musculoskeletal injuries. What is known is that the use of platelet-rich plasma in different environments has demonstrated some clinical promise and positively affects the injured tissue in a way that is conducive to healing [2, 20, 33].

Platelet-Poor Plasma with Alpha-2-Macroglobulin

Introduction

As previously described in the preparation of platelet-rich plasma, there are many factors that are present within whole blood. Each component can have a specific regenerative medicine role, but it is not always clear as to what the right concentration needs to be to accomplish a specific purpose or which component is most helpful to accomplish a certain treatment goal [42, 43].

Platelet-poor plasma (PPP) is the preparation that is devoid of platelets and is defined as plasma with a concentration of platelets of less than 10×10^3 per mL. Platelet-poor plasma was often used as a control in comparison to platelet-rich plasma, but during its utilization, the studies have demonstrated the PPP solution to have elevated levels of fibrinogen. This high fibrinogen content has shown to have the ability to form and activate fibrin-rich clots and to assist with wound healing [43, 44].

As more techniques developed different filters to be used in isolation processes, specific proteins and factors were successfully isolated and extracted from various PPP and PRP products. Alpha-2-macroglobulin is a plasma protein found in the blood, concentrated in the plasma, and is mainly produced by the liver as well as synthesized by macrophages, fibroblasts, and adrenocortical cells [42, 44].

Properties

Platelet-poor plasma is often discarded when the preparation of PRP is obtained, but it has shown to have a role in certain cases of wound healing. It also has a role in the promotion of hemostasis and blood clotting [43].

Alpha-2-macroglobulin (A2M) is a large plasma protein present in the blood. It requires a special filtration process to obtain and concentrate A2M. Alpha-2-macroglobulin is primarily present in the plasma portion of PRP and PPP. To obtain the A2M product, a special filtration process must be employed to obtain a higher concentration of this product and isolate it from its PRP counterpart [44].

It acts as an anti-protease and is able to inactivate many different kinds of proteinases. It primarily functions to inhibit plasmin and kallikrein that are responsible for degrading many blood plasma proteins including fibrin clots. It also functions to inhibit thrombin that has a crucial role in both the coagulation cascade and in the activation of platelets [42]. Its application to regenerative medicine thus far has been specifically focused on the matrix of cells responsible for the breakdown of cartilage within joints. The use of A2M has been shown to inhibit the action of matrix metalloproteinases (MMPs), which have been shown to break down tissues within the joint capsule [43]. These MMPs have been demonstrated to have a role in the process of developing osteoarthritis in joints by breaking down the articular cartilage. The application of A2M can interfere with this and may have some beneficial properties in preventing the progression of osteoarthritis [44].

Indications

The applications of PPP have been primarily in wound management and cosmetic surgery patients. Additional uses are being studied with applications in patients with degenerative arthritis, degenerative disc disease, and numerous other conditions.

The application of A2M is currently primarily intra-articular, and its role is thought to be specifically to prevent the progression of osteoarthritis. In many cases, a fibronectin aggregation (FAC) G3 complex test is conducted prior to A2M injection to determine if a patient is eligible for the therapy. This is done by first doing a joint aspiration and then sending the fluid off for FAC testing [44]. If this determination is positive, then early intervention with A2M can be conducted for the purpose of trying to mitigate the arthritic process and preserving the integrity of the articular cartilage. Its application as of broad-spectrum protease inhibitor has demonstrated good results and efficacy as a treatment for osteoarthritis. At present, there are limited data and a few manuscripts that have examined the use of A2M in humans. Given its promising early results, additional studies are important to demonstrate its full spectrum of use and applications [42–44].

Orthokine, Regenokine, Interleukin Receptor Antagonist Protein

Introduction

With many of the autologous products including preparation of platelet-rich plasma, other filtering and isolation processes can be done to obtain concentrations of other factors that may be more helpful in specific injuries and environments [45]. Interleukin receptor antagonist protein (IRAP) is one product that can be obtained from whole blood and the PRP preparation process. It is also commercially known as Orthokine and/or Regenokine [46].

The process of using this product was initially introduced in the early 2000s and first made its presence in a high level playing athletes who sought out this treatment for chronic injuries that were hindering their play. Initially developed and offered in Germany, IRAP therapy is now utilized worldwide, but many athletes and patients still travel to Europe to be treated [47].

History

The IRAP production is an isolation process that focuses on isolating anti-inflammatory factors. Dr. Peter Wehling pioneered the process of isolating this factor in the 1980s. In 2003, the process was approved for use in Germany [45].

It is less invasive than surgical treatments and has anti-inflammatory properties making it a less painful treatment option for those receiving it. The popularity of IRAP was facilitated by its use in high-level athletes who would initially fly from the United States of America to Germany for treatment and then return home to play [44, 46].

Properties and Relation to PRP

Orthokine is a product that is obtained from PRP. As mentioned previously, PRP is a blood product obtained from whole blood drawn from the patient to be treated. In the process of obtaining Orthokine, 60 cc of the patient's blood is obtained and then incubated at a body temperature for 24 hours. Following this, it is centrifuged at 2100*g* for 10 minutes. Then, through a filtration process via syringes, the product is obtained and utilized [44].

Table 3.2 Comparison of several commercial systems

Company	Blood volume (mL)	PRP volume (mL)	WBC concentration factor	PRP concentration factor	Centrifuge time (min)
Peak	27	3	5×	6.8×	1
Arthremex	11	4	0.5×	2.0×	5
Biomet	27	3	5×	4.7×	15
Harvest	27	3	3.5×	4.9×	15
Arteriocyte	27	3	2×	6.0×	17

The systems noted here have been compared with one another and have published data on their results and concentrations of PRP. Several other companies are present in the market; however, not all have been compared with a standard or with other systems available on the market
PRP platelet-rich plasma, *WBC* white blood cells

The Orthokine product and procedure are closely related to the preparation of PRP but with a few more steps and modifications. The final product obtained is the Interleukin 1 receptor antagonist protein (IRAP). The function of IRAP is to bind to the IL-1 receptor and to block signaling, which promotes an immune and inflammatory response. The IRAP binds the IL-1 receptor and prevents inflammatory cascades and potentially harmful inflammatory responses in the environment being used (i.e., within a joint) [44, 45].

Role and Applications

In its purest form, IRAP is thought to be only anti-inflammatory. Its uses have shown good clinical outcomes in patients who have undergone the treatment. There are strong 2-year data demonstrating beneficial outcomes for the treatment of osteoarthritis of the knees, and most of the applications have been focused on intra-articular use and the treatment of degenerative arthritis [47].

That being said, it is not clear if its use is disease modifying, chondroprotective, or chrondroregenerative. Further data are needed to demonstrate the specific circumstances this product would best be used in [46, 47].

Commercially Available Products

Introduction

Several systems exist in the commercial market space for platelet-rich plasma. Certain factors should be present when considering a system, and these may include cost, whether the system is open versus closed, and the ability to manipulate the product for a higher or lower number of platelets that can be obtained. One should evaluate each system and make their own determination based on the specific clinical practice needs [2, 20, 31, 48].

Available Products and Vendors

Several vendors and companies have systems that can produce platelet-rich plasma. When considering a system, the platelet concentration is certainly the most important thing. However, other factors such as whether the system is closed or open are important to consider [2, 3, 31, 49]. An open system is one in which the blood is drawn and transferred to the machine for processing and then removed from the machine for patient use. A closed system is one where the blood is drawn from the patient into the system where it is not touched and does not leave the container until it is utilized for the patient. Additionally, certain systems can be adjusted to alter the concentration of white cells, blood cells, platelets, and other factors based on different filtration processes [50] (Table 3.2).

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