Expert Review of Hematology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ierr20

Evidence-based indications of platelet-rich plasma therapy

Shyla Gupta , Anna Paliczak & Diego Delgado

To cite this article: Shyla Gupta , Anna Paliczak & Diego Delgado (2020): Evidence-based indications of platelet-rich plasma therapy, Expert Review of Hematology, DOI: 10.1080/17474086.2021.1860002

To link to this article: https://doi.org/10.1080/17474086.2021.1860002

Accepted author version posted online: 04 Dec 2020.

Publisher: Taylor & Francis & Informa UK Limited, trading as Taylor & Francis Group

Journal: *Expert Review of Hematology*

DOI: 10.1080/17474086.2021.1860002

Review

Evidence-based indications of platelet-rich plasma therapy

Shyla Gupta[1], Anna Paliczak[2] & Diego Delgado[3]

1. Queen's University, 99 University Ave, Kingston, ON, Canada
2. Vita Medical Aesthetics Center. Medical Aesthetician. 27 Rean Drive, Toronto, ON,
3. University of Toronto. 585 University Ave, Toronto, Ontario, Canada

Corresponding author:

Diego Delgado

University of Toronto. 585 University Ave, Toronto, Ontario, Canada

Tel.: +1 416 340 4800

Email: diego.delgado@uhn.ca

Abstract

Introduction: Platelet-rich plasma (PRP) is an autologous blood-derived product that contains platelet concentrations at least 2/3 times above the normal level and includes platelet-related growth factors. The concept of PRP began in the 1970s in the field of hematology to treat patients with thrombocytopenia. In the 1980s and 1990s, PRP began to be used in surgical procedures such as maxillofacial surgery and plastic surgery. Since then, PRP had been used in orthopedic procedures, cardiac surgery, sports injuries, plastic surgery, gynecology, urology, and more recently in medical aesthetics.

Areas covered: This review analyzes the mechanisms of action, current indications, clinical evidence, safety and future directions of PRP in the management of various medical conditions. The literature search methodology included using medical subject headings terms to search in PubMed. Articles used were screened and critically appraised by the co-authors of this review.

Expert Opinion: Platelet-rich plasma is a therapeutic option used to treat many medical conditions. PRP could be used alone or in combination with other procedures. The effectiveness and safety of PRP has been demonstrated in many medical scenarios, however there is limited availability of large randomized clinical trials.

Key words

Aesthetics, Hematology, Mechanism of action, Platelet-rich plasma, Surgery

Article Highlight Box

1. PRP is an effective technology that is used for a diverse number of medical conditions.
2. The current research surrounding PRP therapy has significant limitations in terms of article bias, lack of standardization in preparation, and not enough multi-centered randomized large clinical trials.
3. PRP has shown potential benefit for certain clinical indications, specifically for wound healing.
4. Because PRP is biologically unique to different people, it is difficult to ascertain the true efficacy of the therapy.
5. More research needs to be done before PRP therapy can become a new standard of care for certain indications.
6. More focus needs to be put on developing methods to standardize PRP preparation and administration to determine true efficacy.

1. Introduction

This review will focus on the clinical applications of platelet-rich plasma (PRP) therapy, which is currently being thoroughly studied to manage various medical conditions. This review aims to analyze the available evidence on plasma-rich therapy, focusing on the current medical indications.

Platelet-rich plasma (PRP) is an autologous derivative of whole blood that contains platelet concentrations above the average level and includes platelet-related growth factors. Platelets or thrombocytes are derived from megakaryocytic cells in the bone marrow and circulate in the bloodstream at a concentration between 150,000 to 400,000 platelets/uL [1]. These cells are responsible for the aggregation process and participate in other processes such as inflammation, cell proliferation, angiogenesis, and wound healing [2].

PRP has been gaining attention in the research community due to its diverse potential as a regenerative therapy. The concept of PRP therapy began in the 1970s in hematology to treat patients with thrombocytopenia [3]. In the 1980s and 1990s, PRP began to be used in surgical procedures such as maxillofacial surgery and plastic surgery. Since then, PRP had been used in many medical fields, including orthopedic procedures, cardiac surgery, sports injuries, plastic surgery, gynecology, urology, and, more recently, in medical aesthetics. Platelet-rich plasma is a therapeutic option that could be used alone or in combination with other procedures. The effectiveness and safety of PRP have been demonstrated in many medical conditions. While there is limited availability of large randomized clinical

trials, understanding the intricacies of PRP therapy can help foster a firmer grasp on healing processes in the body.

It is important to note that although PRP therapy is becoming increasingly popular, the physiological pathways' complexity cannot be overstated. The limited knowledge surrounding the mechanism of action of PRP, limited quantity of large-scale studies, and most rudimentary theories need to be acknowledged. When analyzing studies, the authors took into account the poor standardization of preparation methods and variability regarding PRP context and quality of the products used. Varying numbers of blood cell concentrations such as leukocytes, platelets, and red blood cells are standard, making it challenging to study PRP products' correct biological properties and their respective efficacies. Some of the clinical trials available were carried out without standardized terminology.

This expert review analyzes the mechanisms of action, current indications, clinical evidence, safety, and future PRP directions in the management of various medical conditions.

2. Methods

A review of the literature was conducted using PubMed. The selection of articles of interest was made according to the following criteria: (i) Clinical trials, systematic reviews, and pronouncements of professional associations and scientific societies; (ii) human studies, (iii) English language. Papers were excluded if the full text was not accessible. The keywords used were chosen according to the MESH terminology. Some examples include "plasma-rich therapy", "musculoskeletal", "function", "clinical applications", "preparation", "injury", "stem cells", “aesthetics”.

We adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (PRISMA) statement in conducting and reporting this review.

3. Platelet-Rich Plasma

Platelet-rich plasma (PRP) is an autologous blood-derived product that contains platelet concentrations 2 to 3 times above-average levels [4]. The preparation process starts with a blood collection obtained by venipuncture. Centrifugation of the sample is followed by the extraction of the plasma enriched in platelets. The PRP preparations contain many growth factors, cytokines, chemokine, and cell-adhesion molecules [5]. These lead to the activation and synthesis of essential products involved in the healing process and tissue proliferation and regeneration [6].

Many Food and Drug Administration and Health Canada approved PRP systems available to use in medical practice. Many systems use anti-coagulated tubes (acid citrate or sodium citrate) for blood collection. The blood is then centrifuged at a pre-specified speed defined by the manufacturer. [7].

PRP classifications are available based on different principles such as the presence or absence of leukocytes and fibrin, and the dose, efficiency, purity, and activation of the product [8]. These different classifications will be described in other sections. Currently, there are no scientific studies that exist which compare different PRP systems for different medical conditions.

4. Platelet Biology

Historically, platelets were believed to be solely responsible for the hemostatic activity. Hemostasis is achieved by employing aggregation, adhesion, and activation [9]. Secretory granules of the platelets discharge factors to promote coagulation through a fibrin clot [10].

However, recent developments have suggested that platelets contain cytokines and growth factors [11]. These signaling molecules can alter the mechanism of action of angiogenesis, stem cell migration, cell proliferation, immune response, and inflammation [10].

Pluripotent stem cells all differentiate into blood cells, which then further separate into contrasting established cell lines. These cell lines can then proliferate indefinitely and mature [12].

Thrombocytes (commonly known as platelets) arise from the marrow of the bone. They have the smallest size of all blood cells, only about two µm wide. They contain various sized cellular elements such as mitochondria and endoplasmic reticulum fragments. At any given point in time, platelets counts in 1 µL of blood is approximately 150,000 to 400,000. To function as storage compartments for secretory products, platelets have many secretory granules involved in regulated secretion [13]. Dense granules and lysosomes constitute the types of secretory granules found in platelets. Each platelet comprises of 50 - 80 secretory granules that help with the aggregation process.

Platelet-rich plasma is an organic source of specific signaling molecules, such as growth factors. Platelets contain three major granule types – dense granules, α-granules, and lysosomes [14]. As a result, the pericellular environment is altered, explaining platelets' different perfunctory roles beyond aggregation [15].

The platelet α-granules provide diverse growth factors and cytokines that are vital to healing processes. These α-granules are essential to regular platelet activity. They have secretory granules that get their cargo from regulated secretory and endocytotic pathways in megakaryocytes [16]. These granules have adhesive molecules like the von Willebrand factor and fibrinogen, growth factors, and inflammatory and

angiogenic mediators [17]. These factors essential roles with regards to inflammatory responses and tumorigenesis. The α-granules are made up of a group of subcellular compartments with unique composition and ultrastructure. These growth factors contribute to angiogenesis, cell proliferation, and differentiation. PRP injections have been shown to produce an environment that fosters tissue regeneration [17]. PRP therapy's influence on cells is the migration and proliferation of stem cells to the local area.

5. PRP Components and Function

Platelet-rich plasma is composed of several central proteins that aid in healing, in addition to those from plasma origin of PRP such as fibrin scaffolds [18]. These proteins are called transforming growth factor-β (TGF-β), epidermal and vascular endothelial growth factors (EGF and VEGF), platelet-derived growth factors (PDGF), fibroblast growth factors (FGF), and insulin-like growth factors (IGF-1).

Secreted by platelets and macrophages, TGF-β performs an anti-proliferative role in healthy epithelial cells [18]. TGF-β targets marrow stem cells, fibroblasts, and pre-osteoblasts. These cells' primary role is to promote the regeneration of bones and aid with long-term healing while also inhibiting the growth of small depressions on the surface of bones called osteoclasts. TGF-β also participates in the growth of epithelial and vascular endothelial cells and collagen production.

EGFs encourage cell proliferation and differentiation, employing the epidermal growth factor receptor [19]. They also have an essential role in angiogenesis and wound healing [19].

VEGFs are signal proteins that encourage the specialization of precursor cells into mature endothelial cells [20]. These signal proteins also encourage the maturation of specific blood vessels (capillaries) from fully grown blood vessels [21].

PDGFs are glycoproteins that arise from the site of injury by the cellular process that releases antimicrobial cytotoxins (degranulation) of platelets [22]. These glycoproteins act on receptors in the target cell's cell membrane to establish high energy phosphate bonds [23]. This activates signal proteins to initiate mitogenesis, angiogenesis, macrophage activation, and collagen production [23].

FGFs participate in the migration of fibroblasts and therefore have an essential role in collagen production and tissue repair. IGF-1s also participate in the cell growth and healing process [24].

Connective tissue growth factor (CTGF) is a secreted matricellular protein. The function of CTGF is dependent on interactions with other molecules. CTGF is not generally expressed in normal tissue. However, it is developed at higher levels during wound healing. It also strongly relates to angiogenesis.

The gene expression of CTGF is tightly regulated. Mechanisms related to CTGF gene expression in cells are dependent on the context of what the cell requires [25].

PRP assists with several biological pathways within the body. The pathologies are complex, and preparation protocols can directly impact the efficacy of healing mechanisms [26]. PRP is thought to stimulate the extracellular matrix and enhance metabolic protein balances [26]. Understanding that PRP therapy and the platelet-rich plasma's direct results are from the vast number of growth factors just mentioned is of utmost importance [26]. The array of growth factors present in the platelets' alpha granules can be linked to their involvement in helping with tissue healing [26]. A traditional saying, the whole is greater than the sum of its parts, can be used to explain why individually, these growth factors are not explanatory of the overall healing mechanism of PRPs [26].

6. Preparation and Composition of PRP

Platelet-rich plasma therapy is relatively new, and there is not much unanimity in the most effective form of preparation. There are validated standard of protocols in transfusion medicine for PRP. However, thre are many variabilities. It is still uncertain whether preparing autologous or allogeneic PRP is efficacious with tissue regeneration. It is relatively sure that sterile preparations of PRP are clinically safe. However, due to the poorly standardized preparation methods and differing opinions on optimal blood components concentrations, it is difficult to ascertain real efficacy.

Additionally, several varying forms of commercialized PRP systems exist on the market [24]. Each commercialized PRP system possesses unique properties based on the differences present in collection protocols and preparation characteristics. These variations are often present in the efficiency of platelet capture, centrifugation, and isolation (one or two steps), the type of collection tube system and operation, and the overall speed of centrifugation [27].

The biological properties of PRP have a vast array of content and purity. PRP regenerative properties are dependent on the release of bioactive proteins after platelet activation. Understanding that the concentration of leukocytes present can have an enormous impact on efficacy is critical. They are vital components in healing and the regulation of growth factor alongside platelet mediators that attracts leukocytes to the site of injury [28]. Additionally, autologous plasma with platelet-derived proteins is implied when PRP therapy is implemented [29]. A fibrin scaffold from this implementation helps to heal injured tissue by acting as a matrix. This scaffold further helps platelets play a fundamental role in thrombosis and hemostasis [30]. Platelets contribute their hemostatic capacity via adhesion, activation, and aggregation, triggered upon tissue injury, and these actions stimulate the coagulation factors and

other mediators to achieve hemostasis [31]. These coordinated series of events are the vital biological processes for wound healing phases.

When analyzing different PRP preparation methods, these bioactive proteins were essential to identify and understand the therapy's potential efficacy.

The overarching process of preparing PRP begins with collecting blood and the combination of an anticoagulant factor. Research regarding specific anticoagulant factors such as anticoagulated whole blood, and citrate-anticoagulated whole blood is still uncertain [32]. This process happens before centrifugation. The preparation differentiates red blood cells from platelet-poor plasma and what is known as the "buffy coat." The buffy coat is composed of concentrated platelets and leukocytes [33]. Platelets are segregated using differing methodologies and can be activated through amalgamation with calcium chloride or thrombin. These two activating agents are specific compounds that cause a degranulation reaction to release growth factors [34]. Platelet activation is correlated with a release of a wide range of chemokines and pro-inflammatory lipids that induce specific effects on a large variety of tissues and cells, including leukocytes [35]. Specifically, during thrombosis, leukocytes' release is considered an important step that links thrombosis to inflammatory responses and increases the procoagulant state. This event is strictly controlled and affected by the delicate interactions between cells at the site of injury. The relationship between platelets and leukocytes corresponds with a vast array of mediators, including adhesion molecules, chemokines, proteins, and various pro-inflammatory lipids [35].

Understanding the variances in PRP therapy needed for each patient explains the difficulties in deciphering the reviews and research on the analytical efficacy of PRP. Preparation styles differ depending on the patient need [33].

The primary process responsible for preparing PRP is called differential centrifugation. Evident by the title, the force of acceleration is adjusted to precipitate various cellular components based on differing specific gravities [36]. Ideally, PRP should be produced by low-g centrifugation in quick time bursts. However, there is not enough clinical evidence to support this just yet. The theory is that shorter periods will help to maximize platelet yield and lower contamination effects. The centrifugation protocol implemented has a direct impact on platelet concentration and yield. The centrifugation process can be done in one or two steps. The double centrifugation process begins with collecting what is known as "whole blood" in tubes that incorporate anticoagulants in the blood volume. The first centrifugation step is executed at a consistent acceleration with the sole purpose of separating red blood cells from the central blood volume [37]. The upper layer of blood is mostly composed of platelets and white blood

cells. The thin intermediate layer, also known as the buffy coat, is plentiful in white blood cells. The bottom layer is made up of just red blood cells [38]. To create pure platelet-rich plasma (P-PRP), the upper layers are separated from the blood volume. This form of preparation also includes a shallow surface of the buffy coat. However, to make just leukocyte rich PRP (L-PRP), the buffy coat is used along with a few red blood cells from the bottom layer, instead of the upper layer [39].

L-PRP plays a critical role in tissue repair and regeneration. L-PRP is a preparation made with leukocytes and low-density fibrin networks after activation. In this family, a large number of commercial and experimental systems exist for regenerative medicine. For the production of L-PRP, the entire layer of the buffy coat and very minimal RBCs are transferred. L-PRP regulates the fundamental mechanisms involved in the healing process. However, more research needs to be conducted to establish L-PRP as a beneficial preparation method [40].

The second centrifugation step is then executed to form erythrocyte-platelets at the bottom of the tube. Platelet-poor plasma makes up the upper portion of the blood volume after this step, while the platelet-rich plasma forms the bottom layer. The upper layer of the blood is removed, leaving behind the erythrocyte-platelets. The bottom layer forms what is called "soft pellets." These pellets are homogenized in plasma to create the final product, platelet-rich plasma.

Differing from the first method of preparation, the buffy coat method differs in a few principles. First, the whole blood volume is centrifuged at high speed, and the intermediate buffy coat is removed. As mentioned previously, this layer contains a volume with a large congregation of leukocytes. Issues arise during the separation of the intermediate thin Buffy coat layer from the red blood cell layer underneath.

PRP needs to be prepared so that it is activated in a way to release α-granules content. The activation method has been debated, with thrombin and re-calcification being two standard options [41]. Platelet activation with thrombin would be associated with fibrin regeneration and clot formation [42].

Platelets in regenerative medicine have a very poorly standardized method of preparation. Anticoagulants, activation methods, gravitational centrifugation techniques, standard cell separators, plateletpheresis, and differences in the g-force and centrifugation time can yield significantly different results. Concentrations of platelets, purity, viability, differences in yield, and platelet activation states all play a role in efficacy. In order to clearly understand PRP therapy and clinically implement the use of this medicine at a more appropriate level, standardization methods need to be put into place. Guidelines for clinical trials involving PRP must be enacted before the authors can make any conclusive statements about the therapy as a relevant form of regenerative medicine [43].

7. Clinical Applications of PRP

Over the last ten years, PRP has been used as a therapeutic tool in regenerative medicine. Clinical use was initially mostly for dental and maxillofacial surgery. PRP's healing properties on cartilage, skin, tendons, and muscles have garnered interest in these fields regarding orthopedic and plastic surgery. Currently, it is uncertain what the utility in different clinical scenarios is. Some general points include the fact that PRP can be used in general wound healing. In treating other injuries such as acute muscle wounds, sports injuries, cosmetics, and bone healing, efficacy is still questionable.

7.1 Maxiollofacial/Peridontal Disease

One of the PRP therapy uses in the regeneration of skeletal and connective tissues in maxillofacial or periodontal diseases [44]. High platelet concentrations are often used as forms of medical technology when dealing with regenerative medicine.

However, there is limited available data on the efficacy of PRP for maxillofacial and periodontal uses. Current uses include improving structural integrity and working as a scaffold for tissue ingrowth, causing migration of stem cells that can differentiate and support regeneration and contain factors for regeneration.

Different platelet concentrations, such as P-PRP or L-PRP, are often used for bone grafting in reconstructive surgery. Specifically, during implant surgeries, PRP is used to treat peri-implant bone defects, insufficient bone volumes, or post-extraction.

In maxillofacial reconstructive surgeries, PRP therapy has been studied for the treatment of patients using anticoagulants.

One systematic review looked at the available scientific evidence for applying PRP in oral surgery [45]. A mix of random control trials and meta-analysis studies were analyzed. Periodontal defects, healing of extraction sockets, sinus lift augmentations, and periapical osseous defects were analyzed. The evidence available proved to have low quality due to the imprecision in preparation methods, patient recruitment, and diseases of variable severities. Most of the studies included were deemed a high risk of bias.

There was some evidence to show the improvements in periodontal defects after PRP therapy. Some studies showed a positive effect on soft tissue from PRPs, and others reported a bone gain. The authors deem these conclusions as isolated and in serious need of further investigation.

7.2 Musculoskeletal and Tissue Regeneration caused by a sports injury

PRP has been used in musculoskeletal and tissue regeneration caused by a sports injury [46,47,48]. A meta-analysis conducted by Grassi et al. analyzed PRP usage for muscle injuries [49]. The results showed that patients who were provided with PRP therapy had an increased ability to return to their sport at better function. This result was only noticeable when non-blinded studies were included. When excluded, patient expectations likely skewed the results. This makes a conclusion surrounding the real efficacy of PRP for musculoskeletal indications challenging to ascertain. The author believes that the current literature does not accurately assess pain, healing, strength, and healing effects. These unanswered questions make it difficult to determine efficacy. The authors are still cautious of this therapy being incorporated into mainstream guidelines and should continue to be used with clinical judgment.

7.3 Tendon Injuries

Some examples of successful clinical outcomes are PRP's use to treat leg muscle injuries and Achilles tendinopathy. However, these findings are based on low-quality evidence [50]. Treating tendon injuries from collagen's breakdown in the tendons (tendinopathies) with platelet-rich plasma is being researched slowly right now [51]. PRP has been used widely to treat many tendinopathies; however, modest improvements have been shown [52]. Most studies have used corticosteroids or whole blood to compare the effects of PRP. Due to the diversity in PRP preparation and delivery methods, efficacy has not been established. Theories propose that signaling pathways are activated and progressed by the cytokines found in PRP. These pathways take place during different phases of cell proliferation, inflammation, and tissue remodeling. PRP therapy helps with the natural formation of new blood vessels, neovascularization [53]. This increases functional microvascular networks, which allows cells to have boosted nutrients and blood supply. This works in a two-fold manner; new cells are developed as damaged tissues are excised. This action mechanism will likely prove to be beneficial to chronic cases of tendinopathies because the existing biological circumstances are adverse. A systematic review and meta-analysis showed that PRP therapy was indeed successful in alleviating the symptoms of specific tendon injuries [54].

7.3.1 Lateral Epicondylitis

Lateral epicondylitis is a tendinopathy injury involving the extensor muscles of the forearm [55]. Physical therapy is frequently the course of action for treating the lateral epicondylar region of the distal humerus. However, several patients often fail from this treatment, allowed PRP to be a potential

alternative. In a study conducted by Mishra et al., over 200 physical therapy regimens for treating their lateral epicondylitis showed no progress and were enrolled [56]. The LR-PRP treatment protocol showed considerable advancements in pain compared to the control group. After 24 weeks, enduring sensitivity in the elbow was significantly less compared to the control group. These results show a statistically significant difference between the two groups. LR-PRP was proven to be more beneficial to patients compared to a local anesthetic injection. Other studies have supported these findings [57]. LR-PRP provides elongated and more efficacious symptom relief for lateral epicondylitis. Although corticosteroids are a class of drugs specifically designed to lower inflammation, the LR-PRP treatment method is a more reliable option. The studies that have looked at this treatment option present the medical community with evidence that has been replicated [58]. LR-PRP has shown both long- and short-term effects on treating lateral epicondylitis.

7.3.2 Patellar Tendinopathy

Patellar tendinopathy is a clinical condition where the anterior knee is exposed to pain and sensitivity where the patellar tendon enters [59]. Similar to lateral epicondylitis, LR-PRP has been used to treat chronic refractory patella tendinopathy. Various randomized controlled studies have tested this treatment. Vetrano et al. compared PRP therapy to a control group of extracorporeal shock wave therapy [60]. The PRP injections showed significant improvement at the 6 and 12 months follow up period. This statistical difference was measured using the Blazina scale, with a p-value of less than 0.05. Another study conducted by Dragoo et al. looked at only 23 patients with refractory patella tendinopathy [61]. Physical therapy and conservative treatments were not significant. The control group received ultrasound-guided dry needling, and the treatment group received the control along with LR-PRP therapy. The treatment group showed statistically significant relief in symptoms at the 12-week mark, but not at the 26-week mark. The advantages of LR-PRP for patellar tendinopathy might only be beneficial for the early onset of symptoms. Both studies showed a difference in the time frame that LR-PRP is beneficial. Although PRP seems to act as a feasible substitute treatment method for chronic refractory patellar tendinopathy, there are not enough studies to confirm and replicate findings. More extensive clinical trials must be conducted before the leukocyte-rich preparation is recommended for standard clinical care.

7.3.3 Achilles Tendinopathy

Unlike patellar tendinopathy and lateral epicondylitis, several older trails for Achilles tendinopathy show PRP is insufficient [62]. However, a recent trial compared four leukocytes rich PRP injections against placebo [63]. Alongside this, both treatment groups were enrolled in a rehabilitation program. The PRP

group had a statistically significant benefit to functionality, mobility, and sensation compared to the control group. However, because of the lack of clinical evidence available, the author thinks PRP should not currently be recommended for treatment use.

7.3.4 Rotator Cuff Tendinopathy

Although rotator cuff tendinopathy is often treated with surgery, some studies have analyzed PRP injections' impact on the condition [64]. These studies have compared the impacts of corticosteroids or placebo against subacromial PRP injections. No studies have looked at the impacts of PRP injections into the specific afflicted tendon. No statistically significant difference was found between a saline injection and PRP injection. One randomized controlled trial showed no improvement in pain when investigating PRP injections' effect on pain and shoulder functions in patients with chronic RCT [65]. Another study conducted by Shams et al. used the Western Ontario RC index, Shoulder Pain Disability Index and VAS shoulder pain test to demonstrate an improvement in pain between treatment and control groups [66].

7.3.5 Plantar Fasciitis

Several RCTs have assessed PRP infusion in the treatment plan for chronic plantar fasciitis. Plantar fasciitis is an inflammation of the fibrous tissue along the bottom of the foo [64]. Administered as a local injection might be a possible alternative to lower worry related to corticosteroid injections. Possibilities of steroid injections in this area include atrophy of the muscle or plantar fascia rupture. A meta-analysis assessed PRP infusions against corticosteroid infusion [68]. Efficacy wise, PRP was proven superior. However, the sample size and number of RCTs conducted on the topic was far too small. More studies need to be conducted with long term follow up before clinical use is put into place. However, as of now, PRP injections seem to be a strong potential for better pain management and mobility with patients suffering from chronic plantar fasciitis. Corticosteroids pose numerous safety threats that PRP does not have [69].

7.4. Medical Aesthetics

PRP has become a popular procedure in the medical aesthetics field. PRP has been used for skin rejuvenation, treatment of scars, burn healing, and scar removing wounds due to its ability to activate fibroblasts and synthesize collagen [70]. The clinical evidence shows PRP's use by itself or with lasers or micro-needling, can improve skin texture and minimize side effects of concurrent abrasive therapies. PRP is also widely used to treat alopecia due to the use of growth factors that promote hair regrowth.

More extensive randomized clinical studies are needed to fully understand PRP's long-term effect on skin and hair care [71].

Platelet rich plasma has emerged as a new treatment modality for regenerative plastic surgery. Preliminary evidence surrounding hair regrowth is also evident [72]. A randomized, placebo-controlled trial compared the hair regrowth of patients given platelet rich plasma injections against placebo injections. At the end of three treatment cycles, patients had a mean increase of 33.6 hairs in the target area and a mean increase in density of 45.9 hairs per cm^2 compared to baseline values. In another study, several patients who displayed male and female pattern hair loss were treated with autologous platelet-rich plasma [73]. The authors found that controlled injections of A- platelet rich plasma in certain regions of the skull also show a reduction of pain for patients during treatment. The protocol included interfollicular A-PRP injections by controlled manual or mechanical injections using a medical injector gun. Patients treated with mechanical injections had a greater increase in hair count and total hair density, compared to patients treated with manual injections. Another study showed that microscopic evaluation of patients who were injected with AA-PRP had an increase in epidermis thickness and the number of hair follicles [74]. After 3 cycles of treatment, patients presented with a mean increase of 18 hairs in the target area, and a mean increase of hair density of 27.7 hairs per cm^2 compared to baseline values. Hair follicular bulge cells and keratinocytes of the epidermis all had a slight increase compared to patients given placebo. A review of 12 studies analyzed the efficacy of platelet rich plasma therapy for androgenetic alopecia [75]. The overarching conclusion showed that platelet rich plasma was effective in augmenting hair growth. 6 out of 12 studies demonstrated an improvement that was statistically significant after platelet rich plasma injections. 4 studies specifically noted the density and diameter of hair follicles, proving platelet rich plasma to be beneficial in increasing these specific markers [76].

Recently, PRP and micrografts that contain human follicle mesenchymal stem cells have also been used as a treatment for androgenetic alopecia [77]. In a recent study, the clinical effectiveness of HF-MSDCs and platelet rich plasma treatment were analyzed. 12 weeks after the last injection with A-PRP, mean hair count and density increased significantly over baseline values. Twenty-three weeks after the last treatment, patients presented with mean hair thickness increments (29 ± 5.0%), and an increased hair density (31 ± 2%) compared to baseline values. It is believed that signalling from platelet-derived growth factors positively influences hair growth by means of cellular proliferation. The prolonging of the anagen phase to stimulate hair follicle development is the mechanism of action for this therapy. In another study, a method to isolate human adult stem cells by mechanical centrifugation of punch biopsy from human hair follicles was conducted [78]. Human follicle stem cells were used to improve the hair density

in 11 patients affected by androgenetic alopecia. Specifically, a 29% ± 5% increase in hair density was present in the treated area, compared to a less than 1% increase in hair density for the placebo area. The study showed that the cells appear to be located in the bulge area of human hair follicle stem cells, and the isolated cells are capable in improving hair density. Another study reported the microscopic and trichoscopic results of human adipose tissue-derived hair follicle stem cells against placebo [79]. There was an evident expansion in the number of hair follicles after 11 months from the last micro-graft application. The patients presented with a mean increase in hair density of 33% ± 7.5% and 27% ± 3.5% respectively, for the treated region. HS-AFSCS in micro-grafts was shown to be a safe and possible effective alternative therapy option to platelet rich plasma therapy. Both are relatively unestablished thus far.

Concentrated growth factors are released from the secretory granules present in platelet-rich plasma. These GFs actively encourage hair regrowth using the seven central growth factors mentioned previously (vascular endothelial growth factor (VEGF), platelet-derived growth factor, fibroblast growth factor, insulin-like growth factor IGF-1, transforming growth factor, connective tissue growth factor, and epidermal growth factors). Proteins secreted by the α-granules in PRP encourage cell proliferation by binding to stem cells' receptors in hair follicles [80]. Associated tissues and stem cells receive signals from ligand binding, producing follicular units to facilitate increased hair regrowth [81].

However, the studies all used unstandardized treatment plans for applying PRP. There is no set evaluation method or uniform parameters to measure impacts. Therefore, the real efficacy of PRP is challenging to ascertain. Difference activators such as calcium chloride and calcium gluconate are used, centrifugation time and speed, and platelet concentrations differ. The author's belief that based on the research, PRP is useful for this indication; however, a more standardized preparation method would help confirm this better.

7.5 Regenerative Plastic Surgery

The authors would like to draw light to the field in which PRP, SVFs, and AD-MSCs improve the wound healing when used alone or in combination with hyaluronic acid [82]. There is a similar biomolecular pathway between wound healing and hair regrowth, both showing promising results. In lower extremity surgery, complex wounds with bone exposure are a difficult problem faced by plastic surgeons. A study analyzed the regeneration of lower extremity complex wounds based on a combined treatment of PRP and hyaluronic acid dressing. The results showed that the re-epithelialization time was 8.1 weeks in patients treated with platelet rich plasma and HA dressing, compared to the 30% of patients treated

with HA dressing only. This data evidently points to the idea that PRP technology can be used in the healing of soft and hard tissue wounds. Furthermore, surgical treatment for hidradenitis suppurativa (HA) involves local excisions and reconstruction using different methods such as skin flaps [83]. A study shows the experience of surgical excision and closing by using PRP with Hyalomatrix PA in patients with severe HA. In this study, no recurrence was observed during the 1-year post-operative monitoring period. Complete wound healing was shown within months. Another study reported on the osteochondral lesions that result in osteoarthritis and progressive joint destruction [84]. Autologous platelet rich plasma causes a release of endogenous growth factors and hormones. This study compared the effects of 3D collage type 1 scaffold culture and combined treatment with platelet rich plasma on osteogenic differentiation of ASCs. The findings showed that 3D collagen scaffold culture with platelet derived growth factors favor osteogenic differentiation of ASCs. This poses a new potential therapy option for managing osteochondral defects.

Furthermore, recent studies have shown that treatment of PRP and ASCs increase survival of grafted adipose tissue [85]. In fat grafting containing adipose derived mesenchymal stem cells and stromal vascular fraction cells, facial rejuvenation, scars on the face, soft tissue defects and breast reconstruction have all benefited from the combined use of platelet rich plasma and SVF/ASCs. The same authors also published data to suggest that PRP plays a role in soft tissue healing [86]. In vivo adipocyte delivery systems by autologous PRP can favor cell survival and can be used to stimulate early microcapillary action to the site of implantation to avoid scars. Because these treatments are all preliminary, the use of platelet rich therapy is still controversial, not suitable for daily practice and still limited by laws and regulation.

7.6 Diabetic Foot Ulcers

As an area of grave public health concern, diabetes mellitus is a highly frequent and fast-spreading disease globally. Almost 15% of individuals with diabetes suffer from diabetic foot ulcers (DFUs), leading to lower limb amputation and gangrene [87]. Ulcers with vascular insufficiencies require vascularization via surgery. Some neuropathic ulcers can be treated with medication. Many studies have recently shown that offloading in areas of ulcers can help decrease tissue destruction and vascularize neural pathways [88]. Growth factors play a central role in these wound healing processes. Because PRPs contain a wide variety of GFs post platelet activation, the use of PRPs is being studied as adjunctive therapy in treating ulcers.

Additionally, up to date, research shows that treatment with only a single growth factor is insufficient due to the lack of healing ability to deal with all the pathological changes caused by DFU. PRP therapy may be inefficient, controversial, and still lacks much evidence. Studies suggest that using certain growth factors may be better [89]. However, a prospective study conducted between 2011 and 2014 showed that wound size reduction was detected in patients after four PRP treatment weeks. The study provides evidence for the use of autologous PRP therapy in dealing with ulcers [90].

Another systematic review with meta-analyses showed that there was a statistically significant benefit to using PRP therapy. However, the methodological bias present in the studies used could have seriously skewed the results [91].

As of now, the authors deem PRP therapy a potential candidate for the healing of diabetic foot ulcers, but more research is needed.

7.7 Intrauterine Infusion in Women Undergoing Assisted Reproduction

Platelet-rich plasma has also been studied as an intrauterine infusion in women undergoing assisted reproduction [92]. A recent systematic review and meta-analysis suggest that PRP is an alternative treatment strategy in patients with thin endometrium and recurrent embryo implantation failure. However, high-quality studies are needed to identify this therapeutic option's role in different subgroups of patients.

8. PRP vs. Stem-cell Based Therapies

It is important to note that PRP treatments and Stem cell (SC)-based therapies are not the same. SC-based therapies use undifferentiated, immature cells that require being isolated and cultured using sophisticated technics [81]. Those cells then divide and differentiate into specific types of cells and tissues used for therapeutics. Stem cells also produce growth factors and cytokines responsible for the repair process in the damaged tissue. SC-based therapies applications are still under investigation in many medical fields and the long-term effects associated with these novel therapies are unknown.

Regenerative medicine is a branch of research that deals with replacing human tissue to restore and establish normal function [93]. It is expeditiously emerging to work on the development of new therapeutic strategies. The advancements made in biomedical research have allowed several medical breakthroughs to prosper. Substitutes to traditional therapies have been developing at increasing rates. Although numerous therapies exist, stem cell and platelet-rich plasma therapy are two primary applied sciences to improve and restore afflicted cells.

Platelet-rich plasma is a constituent of plasma that contains a highly concentrated number of platelets. As mentioned in the introduction, platelets are minuscule ingredients in the blood. Because they are abundant in growth factors and are imperative to the formation of clots during injuries, it is established that healing depends on platelets. To have a curative effect, high platelet concentrations are necessary for damaged tissues. PRP is useful because the therapy not only supports cells to heal on their own but also amplifies the healing process to act on tissues affected by injury correctly [94].

As mentioned in this paper, a globally accepted application of PRP is in musculoskeletal and connective tissues. Sports injuries and dermatological conditions are just two examples of efficacious PRP therapy uses. However, stem cell therapy uses embryonic or adult tissue cells [95]. SCs can differentiate into numerous human cell types because of their ability to proliferate independently.

Like PRP, SC can also create specific cytokines and growth factors to rapidly improve mending at sites of injuries. Stem cells are commonly used to manage inflammatory and degenerative diseases such as rheumatic diseases by acting as a substitute for afflicted cells in almost every tissue or organ [96]. Because of SCs differentiation ability, they have a vast range of effectiveness. PRP therapy does not help with ailments like these. Additionally, even though SC and PRP therapy carry out similar roles (augmenting the healing process to bring about normal function), as detailed throughout the paper, several differences are present from the preparation procedures to the perfunctory functionality.

SC is taken from adult tissues, cultured for weeks in carefully monitored settings, and used for therapeutic purposes after a rigorous and lengthy process.

PRP therapy has a relatively straightforward preparation. The centrifugation process is rapid, as the separation from blood is a simple step.

Perhaps due to lack of research or actual efficacy, SC therapies undeniably have a higher curative probability than the medical knowledge available. PRP therapy is a more underdeveloped technology that has a noticeably diminished potential for regeneration and healing. However, several studies have noted a preference for PRP-based therapies because of the ease of PRP therapy preparation [96]. The similarities between the two are real and undeniable.

More extensive research needs to be conducted to appreciate the complexities of tissue regeneration truly. The molecular mechanisms in which PRP and SC therapies act on cells need to be understood better before the benefits of these technologies are established.

The safety, efficacy, and preparation methods of both SC and PRP therapies are quite inconsistent between different people. Conventional standards and common protocols will need to be developed to

determine the differences between the two. Delineations between the SC and PRP technology can only be appropriately analyzed after clinical applications for both are better substantiated.

Additionally, looks into the synergistic effects of using PRP and stem cell therapies, which is of interest. One study looked at the synergistic effects of human platelet-rich plasma combined with adipose-derived stem cells on healing in a mouse pressure injury model [97]. Transplantation of human adipose-derived stem cells (ASCs) is considered an effective treatment for pressure injuries. However, evidence has suggested that the synergistic effect of PRP combined with ASCs can improve wound healing. This combination helps modulate inflammation, increasing collagen depositions, angiogenesis, and neurogenesis, rather than stem cell therapy alone [98]. In a porcine model, PRP and human ASCs have enhanced vascularization of full-thickness wounds. Additionally, in treating radiogenic wounds, the combination of the two has proved effective.

To dig deeper into the pathophysiology, the fibrin network that forms in PRP provides a structure to form cell scaffolds [99]. Studies have reported that PRP promotes the proliferation of ASCs [100]. By providing a highly favorable environment of ASCs, where the scaffold supports attachment of AScs, PRP, and AScs together have proven to be effective. Another study has shown that the adhesive scaffold and growth factors in PRP increase keratinocyte differentiation by ASCs [101].

9. Autologous PRP Vs. Allogeneic PRP therapy

As detailed throughout this paper, autologous platelet-rich plasma therapy has been thoroughly investigated for multiple clinical indications [102]. However, there are known controversial outcomes due to highly variable PRP quality and patients' complicated clinical conditions. Patients usually have very severe complications and comorbidities in certain conditions, such as diabetic lower extremity ulcers. Additionally, the variability and lack of autologous PRP therapy's reproducibility may make it an inferior candidate in patients like this [103]. One study compared the effects of allogeneic platelet-rich plasma with autologous platelet-rich plasma to treat diabetic lower extremity ulcers. The study found that both autologous and al-PRP effects depend on the concentration of various growth factors produced by the degranulation of concentrated platelets.

Additionally, patients treated with al-PRP did not present any apparent local inflammation, allergies, or adverse reactions. Another study showed that al-PRP treatment might play a role in granulation tissue proliferation in the early initiation stage. In the literature searched, in general, al-PRP is less investigated as the risk of immune reactions and crossed contamination is a significant obstacle.

Deactivating the immune response of al-PRP seems to be the critical factor in making this a more appropriate therapy. Al-PRP as a gel can be degraded and absorbed as a topical therapy. This may prevent al-PRP from entering the circulatory system and avoids most alloantigens. This may be something to look into to reduce immunogenicity.

The use of al-PRP could be a new feasible therapy. However, more large-scale investigations need to be done.

10. Safety of PRP

Platelet-rich plasma is obtained from the same individual (an autologous preparation), and there are no concerns about transmittable diseases such as hepatitis and HIV [104]. However, this inherent safety has been called into question based on the analysis of new empirical data.

The blood agar, a general-purpose enriched medium, is used in laboratories to grow and differentiate bacteria. For this reason, PRP therapy may bolster an increased infection rate in patients. Some studies have suggested that PRP may promote infections based on empirical evidence because blood agar is used in microbiological laboratories to culture bacteria [105]. However, this claim is inconsistent with several studies. PRP has been found to have limited differences from the blood clots that are formed in every wound. It has been unable to be linked to increased bacterial growth compared to any other blood clot. A counterargument proposes that PRP is inhibitory to bacterial growth as its pH level is slightly more acidic, 6.5/6.7 compared to 7.0/7.2 in a matured blood clot.

As mentioned previously, the amalgamation of calcium chloride or thrombin to platelets to bring about activation is an accepted PRP preparation method [92]. Reports found in the early-mid-1990s show that the use of bovine products to create a homeostatic environment has brought about the evolution of anti-bovine antibodies that chemically react to human clotting factors [93]. The evidence has not been repeated, so this cross-reaction is not of significant concern yet. Because the report is dated back to the 1990s, modern preparation methods do not utilize as much bovine factor V, reducing potential contamination [93]. Bovine thrombin in PRP usage is used at a low dose as most administration is a topic with no systematic circulation. The bovine is most often already clotted when in contact with other human tissues, reducing cross-reaction potential [107]

11. Forms of PRP Suitable for Different Clinical Applications

Depending on the preparation, pure platelet-rich plasma (P-PRP) are preparations without leukocytes. This product contains a low-density fibrin network post-activation [108]. This therapy's application is common in sports medicine or for skin wounds because of the liquidity of the solution (it can be quickly injected).

Leukocyte or L-PRP products also contain a low-density fibrin network after activation. However, the preparation contains leukocytes. Similar to fibrin glues, this solution can be placed on skin wounds and sutures to promote healing. This form of PRP is the most commercialized. Its uses range from orthopedic surgery to sports medicine, to general surgery.

Pure platelet-rich fibrin (P-PRF) is a preparation without leukocytes and with a high-density fibrin network. This preparation method cannot be injected. Due to the strong fibrin matrix, it is used as a reliable application. It is quite costly and not commercially available on a wide-scale basis.

Leukocyte and platelet-rich fibrin have both leukocytes and a high-density fibrin network. They also cannot be injected due to the strong fibrin matrix.

Further investigations are required to define standardized protocols for preparing different quality PRPs suitable for different clinical applications, thus making it possible to compare results [109].

12. Conclusions

As of now, the authors believe that the literature results show that most uses of PRP therapy will be quite limited in their scope. Only specified benefits have been proven thus far. More research and studies need to be conducted before a formalized conclusion surrounding PRP's effectiveness can be made.

Platelet-rich plasma preparations are being extensively used in many medical fields. However, multicentric, randomized controlled studies with large sample sizes are needed to establish the different PRP systems' therapeutic efficacy in many medical conditions.

Overall, PRP is a safe therapy alone or in combination with other concurrent treatments. The long-term side effects of PRP in many medical fields are unknown.

There are no universally established standards for the collection, quality control, and administration of the product. Therefore, practitioners must follow the rules and regulations of their local or national Public Health authorities.

13. Expert Opinion

Plasma-Rich Platelet is a therapeutic tool that is changing the world of regenerative medicine. Even though the concept of using this therapy had been around for many decades, only recently PRP has been successfully used alone or as part of coadjutant therapies in many medical conditions. New research and clinical advances in this field will significantly impact the treatment of many medical conditions that are currently being undertreated. PRP is currently not a therapy that many practitioners are familiar with, preventing patients from having access to an effective solution for their medical conditions.

Unfortunately, there are still many challenges in this field. In many clinical scenarios, the research supporting the use of PRP is scarce. There is a consistent lack of well-designed large randomized clinical

trials. Therefore, this therapy's implementation as first-line therapy in most medical conditions is far from optimal. Well-designed research is needed. Future research should be directed to compare the different PRP systems currently available and compare the use of PRP alone versus PRP in conjunction with well-established therapies. This field's ultimate goal is to position PRP as a treatment of choice in many medical conditions, avoiding unnecessary surgical interventions or costly prolonged treatments.

In many medical conditions, it is unknown the effect of PRP in specific subgroups of patients. The use of PRP in the pediatric population is minimal. Therefore, more research and clinical experience are needed. Also, research has to focus on evaluating the ideal formulations and long-term outcomes and possible long-term complications. Another up-and-coming research area is PRP's use in cardiovascular surgery to facilitate cardiac tissue repair alone or in conjunction with conventional cardiac surgery.

Platelet-rich plasma holds promise as a potential treatment option for various musculoskeletal conditions. In the field of medical aesthetics, it is expected that PRP will have exponential growth over the next five years for the treatment of multiple skin conditions, skin rejuvenation, and alopecia.

Considering all of the research conducted about platelet-rich plasma therapy, the therapy's efficacy is still very questionable. Because PRP is biologically unique to different people, it is difficult to ascertain the therapy's real efficacy. The authors believe that due to different immune responses, medical background, and demographics, different patients will respond uniquely to PRP therapy.

Through analysis of the literature, the authors believe that currently, PRP should be utilized in a "trial-and-error" approach.

Established from the literature are theories as to why PRP therapy is inconsistent with different patients. Leaving aside the molecular complexity of PRP therapy and the complex mechanism of action (more than just a release of growth factors), PRP quality should be taken under consideration.

Demographics such as age will likely play a role in how effective PRP therapy is. Impairment due to age in cell function is often induced because of the molecular composition of the environment. If the host tissue is significantly older than platelet injections, or vice versa, complications may ensue.

The immune status of each patient is also quite essential to consider when choosing between treatment options. The immunologic, hormonal, and metabolic homeostasis of host tissue has unique fingerprints to which microbiota are crucial. However, changes to microbiota are linked to wound healing. Symbiosis must exist for PRP therapy to be effective in different environments.

Additionally, medications that patients take can have adverse effects with PRP therapy. Administration of anesthetics, prescription drugs, and steroids can all compromise cell viability.

Recipient tissue is essential to consider when administering PRP therapy. The interactions between host tissue and PRPs are essential to consider and explain why patients have adverse effects.

Accessibility and affordability are two critical barriers to the use of PRP in many medical conditions. The hope is that as the technology and clinical experience evolve, PRP's cost and access will significantly improve.

It is possible that within the next five years, PRP alone or together with other conventional therapies will be the treatment of choice for many musculoskeletal and dermatologic conditions.

Funding

This paper was not funded.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

References

Papers of special note have been highlighted as:

* of interest

** of considerable interest

1. Sim X, Poncz M, Gadue P, French DL. Understanding platelet generation from megakaryocytes: implications for in vitro-derived platelets. Blood. 2016;127(10):1227-1233.
2. Gonzalez AC, Costa TF, Andrade ZA, Medrado AR. Wound healing - A literature review. An Bras Dermatol. 2016;91(5):614-620.
3. **** Alves R and Grimalt R. A review of platelet-rich plasma: history, biology, mechanism of action and classification. Skin Appendage Disord. 2018;4:18-24. This paper provided an in depth review of the biology and mechanisms of action of PRP and review of the most common indications.
4. Pochini AC, Antonioli E, Bucci DZ, et al. Analysis of cytokine profile and growth factors in platelet-rich plasma obtained by open systems and commercial columns. Einstein (Sao Paulo). 2016;14(3):391-397.
5. ** Ramaswamy Reddy SH, Reddy R, et al. Stem-cell therapy and platelet-rich plasma in regenerative medicines: A review on pros and cons of the technologies. J Oral Maxillofac Pathol. 2018;22(3):367-374. This paper was a comprehensive review of the preparation, classification and clinical applications of PRP.
6. Farias RJ, da Silva NP, Haddad NF, et al. Platelet-Rich Plasma Obtained with Different Anticoagulants and Their Effect on Platelet Numbers and Mesenchymal Stromal Cells Behavior In Vitro. Stem Cells Int. 2016; 2016:7414036.
7. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol. 2009;27(3):158-67.
8. Periayah MH, Halim AS, Mat Saad AZ. Mechanism Action of Platelets and Crucial Blood Coagulation Pathways in Hemostasis. Int J Hematol Oncol Stem Cell Res. 2017;11(4):319-327.
9. Palta S, Saroa R, Palta A. Overview of the coagulation system. Indian J Anaesth. 2014;58(5):515-523.
10. Masuki H, Okudera T, Watanebe T, et al. Growth factor and pro-inflammatory cytokine contents in platelet-rich plasma (PRP), plasma rich in growth factors (PRGF), advanced platelet-rich fibrin (A-PRF), and concentrated growth factors (CGF). Int J Implant Dent. 2016;2(1):19.

11. Slukvin II. Generation of mature blood cells from pluripotent stem cells. Haematologica. 2010;95(10):1621-1623.

12. Pavlovic V, Ciric M, Jovanovic V, Stojanovic P. Platelet Rich Plasma: a short overview of certain bioactive components. *Open Med (Wars)*. 2016;11(1):242-247.

13. Sharda A, Flaumenhaft R. The life cycle of platelet granules. *F1000Res*. 2018;7:236.

14. Andia I, Abate M. Platelet-rich plasma: underlying biology and clinical correlates. Regen Med. 2013;8(5):645-658.

15. Blair P, Flaumenhaft R. Platelet alpha-granules: basic biology and clinical correlates. *Blood Rev*. 2009;23(4):177-189.

16. Heijnen H, van der Sluijs P. Platelet secretory behaviour: as diverse as the granules … or not?. J Thromb Haemost. 2015;13(12):2141-2151.

17. Everts PA, Knape JT, Weibrich G, et al. Platelet-rich plasma and platelet gel: a review. J Extra Corpor Technol. 2006;38(2):174-187.

18. Li J, Ballim D, Rodriguez M, et al. The anti-proliferative function of the TGF-β1 signaling pathway involves the repression of the oncogenic TBX2 by its homologue TBX3. J Biol Chem. 2014;289(51):35633-35643.

19. Ai G, Shao X, Meng M, et al. Epidermal growth factor promotes proliferation and maintains multipotency of continuous cultured adipose stem cells via activating STAT signal pathway in vitro. Medicine (Baltimore). 2017;96(30):e7607.

20. Shibuya M. Vascular Endothelial Growth Factor (VEGF) and Its Receptor (VEGFR) Signaling in Angiogenesis: A Crucial Target for Anti- and Pro-Angiogenic Therapies. Genes Cancer. 2011;2(12):1097-1105.

21. Brudno Y, Ennett-Shepard AB, Chen RR, Aizenberg M, Mooney DJ. Enhancing microvascular formation and vessel maturation through temporal control over multiple pro-angiogenic and pro-maturation factors. Biomaterials. 2013;34(36):9201-9209.

22. Andrae J, Gallini R, Betsholtz C. Role of platelet-derived growth factors in physiology and medicine. Genes Dev. 2008;22(10):1276-1312.

23. Jain NK, Gulati M. Platelet-rich plasma: a healing virtuoso. *Blood Res*. 2016;51(1):3-5. doi:10.5045/br.2016.51.1.3

24. Yun YR, Won JE, Jeon E, et al. Fibroblast growth factors: biology, function, and application for tissue regeneration. J Tissue Eng. 2010;2010:218142.

25. Cicha I, Goppelt-Struebe M. Connective tissue growth factor: context-dependent functions and mechanisms of regulation. *Biofactors*. 2009;35(2):200-208.

26. Andia I, Maffulli N. Blood-Derived Products for Tissue Repair/Regeneration. *Int J Mol Sci*. 2019;20(18):4581.

27. Oudelaar BW, Peerbooms JC, Huis In 't Veld R, Vochteloo AJH. Concentrations of Blood Components in Commercial Platelet-Rich Plasma Separation Systems: A Review of the Literature. Am J Sports Med. 2019;47(2):479-487.

28. Ghasemzadeh M, Hosseini E. Intravascular leukocyte migration through platelet thrombi: directing leukocytes to sites of vascular injury. Thromb Haemost. 2015;113(6):1224-1235.

29. Gato-Calvo L, Magalhaes J, Ruiz-Romero C, Blanco FJ, et al. Platelet-rich plasma in osteoarthritis treatment: review of current evidence. Ther Adv Chronic Dis. 2019;10:2040622319825567.

30. Barbon S, Stocco E, Macchi V, Contran M, et al. Platelet-Rich Fibrin Scaffolds for Cartilage and Tendon Regenerative Medicine: From Bench to Bedside. Int J Mol Sci. 2019;20(7):1701.

31. Periayah MH, Halim AS, Mat Saad AZ. Mechanism Action of Platelets and Crucial Blood Coagulation Pathways in Hemostasis. Int J Hematol Oncol Stem Cell Res. 2017;11(4):319-327.

32. Periayah MH, Halim AS, Mat Saad AZ. Mechanism Action of Platelets and Crucial Blood Coagulation Pathways in Hemostasis. *Int J Hematol Oncol Stem Cell Res*. 2017;11(4):319-327.

33. Weibrich G, Kleis WK, Hitzler WE, Hafner G. Comparison of the platelet concentrate collection system with the plasma-rich-in-growth-factors kit to produce platelet-rich plasma: a technical report. Int J Oral Maxillofac Implants. 2005;20(1):118-123.

34. Martineau I, Lacoste E, Gagnon G. Effects of calcium and thrombin on growth factor release from platelet concentrates: kinetics and regulation of endothelial cell proliferation. Biomaterials. 2004;25(18):4489-4502.

35. Ghasemzadeh M, Hosseini E. Platelet-leukocyte crosstalk: Linking proinflammatory responses to procoagulant state. Thromb Res. 2013;131(3):191-197.

36. Perez AG, Lana JF, Rodrigues AA, et al. Relevant aspects of centrifugation step in the preparation of platelet-rich plasma. ISRN Hematol. 2014; 2014:176060.

37. Kuffler DP. Variables affecting the potential efficacy of PRP in providing chronic pain relief. J Pain Res. 2018;12:109-116.

38. Livshits MA, Khomyakova E, Evtushenko EG, et al. Isolation of exosomes by differential centrifugation: Theoretical analysis of a commonly used protocol Sci Rep. 2015;5:17319.

39. Shin HS, Woo HM, Kang BJ. Optimisation of a double-centrifugation method for preparation of canine platelet-rich plasma. BMC Vet Res. 2017;13(1):198.

40. ** Dhurat R, Sukesh M. Principles and Methods of Preparation of Platelet-Rich Plasma: A Review and Author's Perspective. J Cutan Aesthet Surg. 2014;7(4):189-197. This paper provided an in-depth understanding of PRP therapy preparation methods.

41. Pavlovic V, Ciric M, Jovanovic V, Stojanovic P. Platelet Rich Plasma: a short overview of certain bioactive components. Open Med (Wars). 2016;11(1):242-247.

42. Crisci A, Marotta G, Licito A, et al. Use of Leukocyte Platelet (L-PRF) Rich Fibrin in Diabetic Foot Ulcer with Osteomyelitis (Three Clinical Cases Report). *Diseases*. 2018;6(2):30.

43. Harrison P; Subcommittee on Platelet Physiology. The use of platelets in regenerative medicine and proposal for a new classification system: guidance from the SSC of the ISTH. *J Thromb Haemost*. 2018;16(9):1895-1900

44. Simonpieri A, Del Corso M, Vervelle A, et al. Current knowledge and perspectives for the use of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in oral and maxillofacial surgery part 2: Bone graft, implant and reconstructive surgery. Curr Pharm Biotechnol. 2012;13(7):1231-1256.

45. Franchini M, Cruciani M, Mengoli C, et al. The use of platelet-rich plasma in oral surgery: a systematic review and meta-analysis. *Blood Transfus*. 2019;17(5):357-367.

46. Mosca MJ, Rodeo SA. Platelet-rich plasma for muscle injuries: game over or time out?. *Curr Rev Musculoskelet Med*. 2015;8(2):145-153.

47. Halpern BC, Chaudhury S, Rodeo SA. The role of platelet-rich plasma in inducing musculoskeletal tissue healing. *HSS J*. 2012;8(2):137-145.

48. Cruciani M, Franchini M, Mengoli C, et al. Platelet-rich plasma for sports-related muscle, tendon and ligament injuries: an umbrella review Blood Transuf 2019;1:465-478.

49. Grassi A, Napoli F, Romandini I, et al. Is Platelet-Rich Plasma (PRP) Effective in the Treatment of Acute Muscle Injuries? A Systematic Review and Meta-Analysis. Sports Med. 2018;48(4):971-989.

50. Billesberger LM, Fisher KM, Qadri YJ, Boortz-Marx RL. Procedural Treatments for Knee Osteoarthritis: A Review of Current Injectable Therapies. Pain Res Manag. 2020;2020:3873098

51. Yang G, Rothrauff BB, Tuan RS. Tendon and ligament regeneration and repair: clinical relevance and developmental paradigm. Birth Defects Res C Embryo Today. 2013;99(3):203-222.

52. Martin JI, Atilano L, Merino J, et al. Platelet-rich plasma versus lidocaine as tenotomy adjuvants in people with elbow epicondylopathy: a randomized controlled trial. *J Orthop Surg Res*. 2019;14(1):109.

53. Bosch G, Moleman M, Barneveld A, et al. The effect of platelet-rich plasma on the neovascularization of surgically created equine superficial digital flexor tendon lesions. Scand J Med Sci Sports. 2011;21(4):554-561.

54. Setayesh K, Villarreal A, Gottschalk A, et al. Treatment of Muscle Injuries with Platelet-Rich Plasma: a Review of the Literature. Curr Rev Musculoskelet Med. 2018;11(4):635-642.

55. Vaquero-Picado A, Barco R, Antuña SA. Lateral epicondylitis of the elbow. EFORT Open Rev. 2017;1(11):391-397.

56. Mishra AK, Skrepnik NV, Edwards SG, et al. Efficacy of platelet-rich plasma for chronic tennis elbow: a double-blind, prospective, multicenter, randomized controlled trial of 230 patients. Am J Sports Med. 2014;42(2):463-471.

57. Kwapisz A, Prabhakar S, Compagnoni R, et al. Platelet-Rich Plasma for Elbow Pathologies: a Descriptive Review of Current Literature. Curr Rev Musculoskelet Med. 2018;11(4):598-606.

58. Ben-Nafa W, Munro W. The effect of corticosteroid versus platelet-rich plasma injection therapies for the management of lateral epicondylitis: A systematic review. *SICOT J*. 2018;4:11.

59. Rath E, Schwarzkopf R, Richmond JC. Clinical signs and anatomical correlation of patellar tendinitis. Indian J Orthop. 2010;44(4):435-437.

60. Vetrano M, Castorina A, Vulpiani MC, et al. Platelet-rich plasma versus focused shock waves in the treatment of jumper's knee in athletes. Am J Sports Med. 2013;41(4):795-803.

61. Dragoo JL, Wasterlain AS, Braun HJ, Nead KT. Platelet-rich plasma as a treatment for patellar tendinopathy: a double-blind, randomized controlled trial. Am J Sports Med. 2014;42(3):610-618.

62. Krogh TP, Ellingsen T, Christensen R, et al. Ultrasound-Guided Injection Therapy of Achilles Tendinopathy With Platelet-Rich Plasma or Saline: A Randomized, Blinded, Placebo-Controlled Trial. Am J Sports Med. 2016;44(8):1990-1997.

63. Boesen AP, Hansen R, Boesen MI, et al. Effect of High-Volume Injection, Platelet-Rich Plasma, and Sham Treatment in Chronic Midportion Achilles Tendinopathy: A Randomized Double-Blinded Prospective Study. Am J Sports Med. 2017;45(9):2034-2043.

64. Rha DW, Park GY, Kim YK, et al. Comparison of the therapeutic effects of ultrasound-guided platelet-rich plasma injection and dry needling in rotator cuff disease: a randomized controlled trial. Clin Rehabil. 2013;27(2):113-122.

65. Kesikburun S, Tan AK, Yilmaz B, et al. Platelet-rich plasma injections in the treatment of chronic rotator cuff tendinopathy: a randomized controlled trial with 1-year follow-up. Am J Sports Med. 2013;41(11):2609-2616.

66. Shams A, El-Sayed M, Gamal O, Ewes W. Subacromial injection of autologous platelet-rich plasma versus corticosteroid for the treatment of symptomatic partial rotator cuff tears. Eur J Orthop Surg Traumatol. 2016;26(8):837-842.

67. Neufeld SK, Cerrato R. Plantar fasciitis: evaluation and treatment. J Am Acad Orthop Surg. 2008;16(6):338-346.

68. Jain K, Murphy PN, Clough TM. Platelet rich plasma versus corticosteroid injection for plantar fasciitis: A comparative study. Foot (Edinb). 2015;25(4):235-237.

69. Shetty SH, Dhond A, Arora M, Deore S. Platelet-Rich Plasma Has Better Long-Term Results Than Corticosteroids or Placebo for Chronic Plantar Fasciitis: Randomized Control Trial. J Foot Ankle Surg. 2019;58(1):42-46.

70. O'Connell B, Wragg NM, Wilson SL. The use of PRP injections in the management of knee osteoarthritis. Cell Tissue Res. 2019;376(2):143-152.

71. Shin M, Lee J, Lee S, et al. Platelet-rich plasma combined with fractional laser therapy for skin rejuvenation. Dermatol Surg. 2012;38(4):623-630.

72. Gentile P, Garcovich S, Bielli A, Scioli MG, et al. The Effect of Platelet-Rich Plasma in Hair Regrowth: A Randomized Placebo-Controlled Trial. Stem Cells Transl Med. 2015;4(11):1317-23.

73. Gentile P, Garcovich S, Scioli MG, et al. Mechanical and Controlled PRP Injections in Patients Affected by Androgenetic Alopecia. J Vis Exp. 2018;(131):56406.

74. Cervelli V, Garcovich S, Bielli A, et al. The effect of autologous activated platelet rich plasma (AA-PRP) injection on pattern hair loss: clinical and histomorphometric evaluation. Biomed Res Int. 2014;2014:760709.

75. Owczarczyk-Saczonek A, Krajewska-Włodarczyk M, Kruszewska A, et al. Therapeutic Potential of Stem Cells in Follicle Regeneration. Stem Cells Int. 2018;2018:1049641.

76. Cervantes J, Perper M, Wong LL, et al. Effectiveness of Platelet-Rich Plasma for Androgenetic Alopecia: A Review of the Literature. *Skin Appendage Disord*. 2018;4(1):1-11.

77. Gentile P, Scioli MG, Bielli A, De Angelis B, et al. Platelet-Rich Plasma and Micrografts Enriched with Autologous Human Follicle Mesenchymal Stem Cells Improve Hair Re-Growth in Androgenetic Alopecia. Biomolecular Pathway Analysis and Clinical Evaluation. Biomedicines. 2019;7(2):27.

78. Gentile P, Scioli MG, Bielli A, Orlandi A, Cervelli V. Stem cells from human hair follicles: first mechanical isolation for immediate autologous clinical use in androgenetic alopecia and hair loss. Stem Cell Investig. 2017;4:58.

79. Gentile P. Autologous Cellular Method Using Micrografts of Human Adipose Tissue Derived Follicle Stem Cells in Androgenic Alopecia. Int J Mol Sci. 2019;20(14):3446.

80. Giordano S, Romeo M, Lankinen P. Platelet-rich plasma for androgenic alopecia: does it work? Evidence from metaanalysis. J Cosmet Dermatol J. 2017;16(3):374-381.

81. Maria-Angeliki G, Alexandros-Efstratios K, Dimitris R, Konstantinos K. Platelet-rich Plasma as a Potential Treatment for Noncicatricial Alopecias. Int J Trichology. 2015;7(2):54-63.

82. Cervelli V, Lucarini L, Spallone D, Palla L, et al. Use of platelet-rich plasma and hyaluronic acid in the loss of substance with bone exposure. Adv Skin Wound Care. 2011;24(4):176-81

83. Nicoli F, Balzani A, Lazzeri D, Gentile P, et al. Severe hidradenitis suppurativa treatment using platelet-rich plasma gel and Hyalomatrix. Int Wound J. 2015;12(3):338-43.

84. Scioli MG, Bielli A, Gentile P, Cervelli V, et al. Combined treatment with platelet-rich plasma and insulin favours chondrogenic and osteogenic differentiation of human adipose-derived stem cells in three-dimensional collagen scaffolds. J Tissue Eng Regen Med. 2017;11(8):2398-2410.

85. Gentile P, Scioli MG, Bielli A, Orlandi A, et al. Concise Review: The Use of Adipose-Derived Stromal Vascular Fraction Cells and Platelet Rich Plasma in Regenerative Plastic Surgery. Stem Cells. 2017;35(1):117-134.

86. Azzena B, Mazzoleni F, Abatangelo G, Zavan B, et al. Autologous platelet-rich plasma as an adipocyte in vivo delivery system: case report. Aesthetic Plast Surg. 2008;32(1):155-8; 159-61.

87. Pendsey SP. Understanding diabetic foot. *Int J Diabetes Dev Ctries*. 2010;30(2):75-79.

88. ** Del Pino-Sedeño T, Trujillo-Martín MM, Andia I, et al. Platelet-rich plasma for the treatment of diabetic foot ulcers: A meta-analysis. *Wound Repair Regen*. 2019;27(2):170-182. This paper contained a lot of information about diabetic foot ulcers and PRP therapy. As an important public health concern, the article had a lot of mileage.

89. Gonchar IV, Lipunov AR, Afanasov IM, et al. Platelet rich plasma and growth factors cocktails for diabetic foot ulcers treatment: State of art developments and future prospects. *Diabetes Metab Syndr*. 2018;12(2):189-194.

90. Babaei V, Afradi H, Gohardani HZ, et al. Management of chronic diabetic foot ulcers using platelet-rich plasma. *J Wound Care*. 2017;26(12):784-787.

91. Maleki-Hajiagha A, Razavi M, Rouholamin S, et al. Intrauterine infusion of autologous platelet-rich plasma in women undergoing assisted reproduction: A systematic review and meta-analysis. J Reprod Immunol. 2020;137:103078.

92. ** Ramaswamy Reddy SH, Reddy R, Babu NC, Ashok GN. Stem-cell therapy and platelet-rich plasma in regenerative medicines: A review on pros and cons of the technologies. J Oral Maxillofac Pathol. 2018;22(3):367-374. This paper provided a clear understanding regarding the differences between stem cells and PRP therapy.

93. Pietrzak WS, Eppley BL. Platelet rich plasma: biology and new technology. J Craniofac Surg. 2005;16(6):1043-1054.

94. Biehl JK, Russell B. Introduction to stem cell therapy. J Cardiovasc Nurs. 2009;24(2):98-105.

95. Franceschetti T, De Bari C. The potential role of adult stem cells in the management of the rheumatic diseases. Ther Adv Musculoskelet Dis. 2017;9(7):165-179.

96. Choi J, Minn KW, Chang H. The efficacy and safety of platelet-rich plasma and adipose-derived stem cells: an update. Arch Plast Surg. 2012;39(6):585-592.

97. Zhang ZY, Huang AW, Fan JJ, et al. The potential use of allogeneic platelet-rich plasma for large bone defect treatment: immunogenicity and defect healing efficacy. *Cell Transplant*. 2013;22(1):175-187.

98. He M, Guo X, Li T, et al. Comparison of Allogeneic Platelet-rich Plasma With Autologous Platelet-rich Plasma for the Treatment of Diabetic Lower Extremity Ulcers. *Cell Transplant*. 2020;29:963689720931428.

99. Marx RE. Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg. 2004;62(4):489-496.

100. Choi J, Minn KW, Chang H. The efficacy and safety of platelet-rich plasma and adipose-derived stem cells: an update. *Arch Plast Surg*. 2012;39(6):585-592.

101. Gonshor A. Technique for producing platelet-rich plasma and platelet concentrate: background and process. Int J Periodontics Restorative Dent. 2002;22(6):547-557.

102. Zhang ZY, Huang AW, Fan JJ, et al. The potential use of allogeneic platelet-rich plasma for large bone defect treatment: immunogenicity and defect healing efficacy. *Cell Transplant*. 2013;22(1):175-187.

103. Jo CH, Shin JS, Lee SY, Shin S. ALLOGENEIC PLATELET-RICH PLASMA FOR ROTATOR CUFF REPAIR. *Acta Ortop Bras*. 2017;25(1):38-43.

104. Rapaport SI, Zivelin A, Minow RA, Hunter CS, Donnelly K. Clinical significance of antibodies to bovine and human thrombin and factor V after surgical use of bovine thrombin. Am J Clin Pathol. 1992;97(1):84-91.

105. Zehnder JL, Leung LL. Development of antibodies to thrombin and factor V with recurrent bleeding in a patient exposed to topical bovine thrombin. Blood. 1990;76(10):2011-2016.

106. Mao AS, Mooney DJ. Regenerative medicine: Current therapies and future directions. Proc Natl Acad Sci U S A. 2015;112(47):14452-14459.

107. Christie RJ, Carrington L, Alving B. Postoperative bleeding induced by topical bovine thrombin: report of two cases. Surgery. 1997;121(6):708-710.

108. Dohan Ehrenfest DM, Andia I, Zumstein MA, et al. Classification of platelet concentrates (Platelet-Rich Plasma-PRP, Platelet-Rich Fibrin-PRF) for topical and infiltrative use in orthopedic and sports medicine: current consensus, clinical implications and perspectives. *Muscles Ligaments Tendons J*. 2014;4(1):3-9.

109. ** Andia I, Maffulli N. Some patients (and some of us) respond better to some biological therapies: the as yet unsolved conundrum. *J Orthop Traumatol*. 2018;19(1):1. This paper provided guidance as to why patients respond differently to PRP, thus explaining the difficulty of ascertaining the true efficacy of the therapy.