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Review article

PRINCIPLES AND METHODS FOR ISOLATION AND PREPARATION OF PLASMA PREPARATIONS CONTAINING PLATELETS (PR) - HOW TO IMPROVE THE PURPOSE AND EFFECTIVENESS

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Abstract

The clinical use of platelet-rich plasma (PRP) is based on increasing the concentration of growth factors and the secretion of proteins that can maximize the healing process at the cellular level. PRP is an autologous biological material, that involves minimal risk of immune reactions and transmission of infectious and contagious diseases, and it is widely used to repair musculoskeletal lesions and skin. Despite the great potential for applicability, the implementation of therapeutic inclusion of PRP as a clinical alternative has become difficult due to the lack of studies related to the standardization of production techniques, application conditions, and/or insufficient description of the adopted procedures.
Currently, platelet-rich plasma therapy (PRP) is widely used and continues to grow for a variety of clinical applications.
Along with its development, there are various options in the way of obtaining PRP, automatic or manual, while one of the most reliable methods according to the literature is the method of double centrifugation.
Therefore, it is necessary to establish standard criteria to be met to obtain high-quality PRP, as well as several studies to determine the appropriate platelet concentration for different clinical applications. In this context, this paper aims to discuss some methodological aspects used to obtain PRP, as well as to discuss the bioactive properties of PRP and to point out its therapeutic use in various fields of regenerative medicine. Additionally, current techniques, critical points, technology for PRP preparation, and a review of the present deficiencies of this therapy that will need to be overcome if widely accepted, are described.

Keywords: platelet-rich plasma (PRP), centrifugation methods, biological factors-growth factors.

1. Introduction

Platelet-rich plasma (PRP) is becoming increasingly popular as a non-surgical treatment option for a wide range of medical disorders.

PRP is widely used in orthopedic and sports medicine to relieve pain by naturally promoting healing in musculoskeletal disorders such as tendinitis, arthritis, ligament strain, and tearing. In particular, PRP injections are used for athletic injuries, resulting in exceptional healing, rapid return to regular activities, and complete pain relief.

PRP first attempts in esthetic medicine are a long time ago with a famous vampire lift. Today PRP is not only an option for rejuvenation of the face and neck but in a COVID decade is a valuable treatment option for effluvium. PRP injections in the scalp to treat one of the most frequent side effects seen by a dermatologist in post covid period- hair loss is part of the procedures in many dermatology departments. A standardized application regimen for PRP is a meter of discussions. The most frequent protocol in dermatology use is 3 times after a one-month interval. But some doctors are applying every six months.

Autologous PRP is obtained from an individual blood sample that is centrifuged to remove red blood cells.

The remaining plasma has 5 to 10 times the concentration of growth factors than the initial blood.

Many scientists and specialists have found that these growth factors promote the response to natural healing in areas such as dentistry, dermatology, urology and gynecology, and orthopedics (Rubina Alves et al.,2018).

The theory underlying this modality of treatment is derived from natural healing processes, with the body's first response to tissue injury is the delivery of platelets to the injured site. Platelets promote healing and the attraction of stem cells to the site of injury. The transition from basic science to clinical practice involves the application of PRP injections to diseased ligaments, tendons, and joints, with excellent results in terms of healing.

The aim of this paper is to review the studies related to the use of PRP in the field of medicine, the techniques used to obtain PRP, and the critical pharmaceutical-technological conditions required for successful treatment due to the limited experience of using PRP in treatment of various disorders.

PRP is a biological product defined as part of the autologous blood plasma fraction with a platelet concentration above baseline, platelet concentration 4-5 x higher than that in the whole blood sample taken for its preparation, or better 0.5-1 x 10 9 platelets / ml. PRP is obtained from the blood of patients collected before centrifugation (Ion-Bogdan Codorean et al.2017).

Platelets contained in autologous blood play an important role in the healing process because they secrete several growth factors at the site of injury. Shortly, among other roles, these platelets serve to promote the mitogenesis of cells capable of healing and angiogenesis in tissue. Autologous blood that contains such platelets in higher-than-normal concentrations is commonly referred to as platelet-rich plasma (PRP).

For example, the normal platelet count in healthy individuals is about 1.5 to 4.5 x 10 5 / lL; however, to be considered PRP, platelets should be 4-5 times this volume.

It has been observed that this relatively recent biotechnology improves the healing process because the increased platelet count results in an increased number of secreted growth factors, thus theoretically improving the healing process. Some of the growth factors in PRP include platelet-derived growth factor (PDGF), transforming beta growth factor (TGF-b), vascular endothelial growth factor (VEGF), and epithelial growth factor (EGF). In addition to these factors, PRP contains adhesive molecules that promote bone formation. These molecules include fibrin, fibronectin, and vitronectin (José Fábio Santos Duarte Lana et al.,2014).

Table 1. Summary of growth factors contained in platelet – rich plasma

Growth factor	Function
Transforming growth factor–β (TGF–β)	Stimulates undifferentiated mesenchymal cell proliferation Regulates endothelial, fibroblastic, and osteoblastic mitogenesis Regulates collagen synthesis and collagenase secretion Regulates mitogenic effects of other growth factors Stimulates endothelial chemotaxis and angiogenesis Inhibits macrophage and lymphocyte proliferation
Fibroblast growth factor (FGF)	Promotes growth and differentiation of chondrocytes and osteoblasts Mitogenetic for mesenchymal cells, chondrocytes, and osteoblasts
Platelet – derived growth factor a and b (PDGF)	Mitogenetic for mesenchymal cells and osteoblasts Stimulates chemotaxis and mitogenesis in fibroblast, glial or smooth muscle cells Regulates collagenase secretion and collagen synthesis Stimulates macrophage and neutrophil chemotaxis
Epidermal growth factor (EGF)	Stimulates endothelial chemotaxis or angiogenesis Regulates collagenase secretion
Vascular endothelial growth factor (VEGF)	Increases angiogenesis and vessel permeability

	Stimulates mitogenesis for endothelial cells
Connective tissue growth factor (CTGF)	Promotes angiogenesis Cartilage regeneration Fibrosis and platelet adhesion
Insulin like growth factor (ILGF 1 and 2)	Chemotactic for fibroblasts and stimulates protein synthesis Enhances bone formation
Platelet factor 4 (PF–4)	Stimulate the initial influx of neutrophils into wounds Chemo–attractant for fibroblasts
Interleukin 8 (IL–8)	Pro–inflammatory mediator Recruitment of inflammatory cells
Keratinocyte growth factor (KGF)	Promote endothelial cell growth, migration, adhesion and survival Angiogenesis

Note. Reused from the article of Moon Hee Kim et al. (J Cosmet Med 2019;3(1):1-13)

After centrifugation, according to the different densities of the constituent components, the different components of the blood are separated (red blood cells, PRP and platelet depleted plasma).

In PRP, in addition to higher platelet concentrations, other parameters should be considered, such as the presence or absence of leukocytes and activation. This will define the type of PRP used in various pathologies (Kim Hrmon et al.)

There are several commercially available kits, which simplify the preparation of PRP. According to the manufacturers, PRP kits usually reach a concentration of PRP 2-5 times higher than the initial concentration. Although logic says that a higher platelet count with higher growth factors would lead to better results, this has not yet been determined. In addition, one study suggests that a PRP concentration 2.5 times higher than baseline may have an inhibitory effect (Nasir Hussain 1 et al.,2017).

2. Use of PRP preparations

PRP's initial popularity grew out of its promise as a safe and natural alternative to surgery. Proponents of PRP have promoted the procedure as an organic-based therapy that enables healing using its own natural growth factors. In recent years, scientific research and technology have provided a new perspective on platelets. Studies suggest that platelets contain an abundance of growth factors and cytokines that can affect inflammation, postoperative blood loss, infection, osteogenesis, wounds, muscle tearing, and soft tissue healing. Studies now show that platelets also release many bioactive proteins responsible for attracting macrophages, mesenchymal stem cells, and osteoblasts that not only promote the removal of degenerated and necrotic tissue but also enhance tissue regeneration and healing (Dohan et al.2009).

The growth of PRP therapy relies primarily on anecdotal or individual patient reports. Historically, there have been several controlled trials to demonstrate the effectiveness of PRP. From these existing studies, the sample size was too small to allow generalization of the findings. In addition, the lack of consensus on the technique, the number of injections, the distance between injections, the platelet count, the platelet concentration above baseline, with or without leukocytes in the injection, the exogenous activation of the injected platelets, and even the definition of suitable candidates is missing and need further definition and evaluation. However, literature has recently emerged on the beneficial effects of PRP for chronic non-healing tendon injuries, including lateral epicondylosis, plantar fasciopathy, and cartilage degeneration.

The current guidelines will focus on the general principles for the use of PRP and its application specifically to musculoskeletal care.

As with any musculoskeletal injury, a thorough history and examination are required to make a differential diagnosis. PRP is generally considered the treatment of choice for subacute and chronic conditions. Generally, healing slows or stops 6 to 12 weeks after an acute injury. If the patient does not improve for

more than six weeks, his recovery phase may be interrupted. In conditions of overuse or repetitive conditions, it may be more challenging to isolate the transition from the acute phase.
The following are some of the common orthopedic indications for which PRP is used along with a partial review of the current evidence for each indication (Tidball JG & Wehling-Henricks M.2007)

2.1 Tendinopathies

Tendinopathy refers to a degenerative condition of the tendons marked by chronic loss of collagen, tissue integrity, stability, and strength. Tendinopathy is not an inflammatory condition because histological specimens lack inflammatory cells. The causes are multifactorial, but natural aging, injuries, recurrent stress, and nervous, vascular, and hormonal inputs probably all contribute. Since tendinopathy is almost ubiquitous as we age, pain and dysfunction generally only occur when sufficient stress is applied to the degenerated tendon.
Basic science and animal studies support the use of PRP in tendinopathy. Laboratory studies have shown improved tenocyte proliferation, collagen deposition, and endogenous growth factors. Animal models with surgically induced lesions are common and show good results. However, positive high-level human evidence is still lacking, and others have shown negative results.
Scientists encourage further research into the technique, several injections, the distance between injections, platelet count, platelet concentration above baseline, with or without leukocytes in the injection, exogenous activation of the injected platelets, use of standard measures and patient selection (Kim Harmon, et al.).

2.2 Patellar tendinopathy

The use of LR-PRP (leukocyte-rich PRP) in the treatment of chronic refractory patellar tendinopathy has been supported by randomized controlled trials. One study evaluated 23 patients with patellar tendinopathy who had failed conservative treatment. Patients were randomized to receive a dry ultrasound-guided injection or LR-PRP injection and followed for> 26 weeks. The PRP-treated group had significant improvement in symptoms, measured by VISA-P, at 12 weeks ($P = 0.02$), but the difference was not significant at> 26 weeks ($P = 0.66$), suggesting that the benefit of PRP for patellar tendinopathy may be an early improvement in symptoms.
In another study, the benefits of PRP injections for the comparative treatment of chronic refractory patellar tendinopathy with focused extracorporeal shock wave therapy (ECSWT) have also been reported. While there was no significant difference between the 2-month follow-up groups, the PRP group showed a statistically significant improvement, measured by VISA-P and VAS, through ECSWT at 6-month and 12-month follow-up. PRP appears to be a viable treatment option for chronic refractory patellar tendinopathy and a leucocyte-rich preparation is recommended. Given the small number of studies to support this conclusion, additional clinical trials will be required to recommend general clinical use.

2.3 Achilles tendinopathy

Several historical studies have shown no difference in PRP versus placebo injection for Achilles tendon treatment in clinical outcomes. A more recent randomized controlled trial compared a series of four LP-PRP (leukocyte-poor PRP) injections compared with a placebo in combination with an eccentric rehabilitation program. The PRP-treated group significantly improved pain, function, and activity during the 6-month follow-up compared with the placebo group. This study also found a comparable improvement with a single volume injection (50 mL) of 0.5% bupivacaine (10 mL), methylprednisolone (20 mg), and normal saline (40 mL), although great care should be taken with considering the increased risk of tendon rupture during this

treatment after steroid injection.
Routine use of PRP in Achilles tendinopathy is not supported by the current literature.

2.4 Plantar fasciitis

Several randomized controlled trials have evaluated PRP injection in the management of chronic plantar fasciitis. The potential of PRP as a topical injection treatment alleviates corticosteroid injection-related concerns, such as pillow atrophy or plantar fascia rupture. Two recent meta-analyzes evaluated PRP injections versus corticosteroid injections, concluding that PRP injections were a viable alternative to corticosteroid injections in terms of efficacy, with some studies showing the superiority of PRP.
PRP injections appear to be an effective treatment for improving pain and function in chronic plantar fasciitis and may be superior to corticosteroids, especially given the better safety profile of PRP (Kim Harmon, et al.).

2.5 Ligament strain

Most studies of human ligaments have so far been combined with surgical reconstruction of the anterior cruciate ligament. In general, the evidence suggests improved pain, healing, and graft quality. At this point, there is a lack of literature on sports medicine regarding non-surgical care. Anecdotal evidence from expert sources indicates improved recovery time, reduced pain, and reduced recovery time.

2.6 Muscle stretching

Muscle spasms are a very common source of pain in dysfunction, especially in athletes. Muscles are rich in blood supply and generally heal with normal care, approximately 8 times faster than ligaments. If a subacute or chronic condition develops, it may be acceptable to consider PRP treatment. In rare situations, delivery of an acute injury to facilitate function may be considered, but there is currently insufficient evidence to support this. One study showed faster recovery from acute muscle tears with PRP injection. If applicable to larger-scale use, it is not known whether this is clinically, functionally, or relevant to a return to sport. Treatment of myositis ossificans by barbiturate, aspiration, and PRP injection is an area of particular interest that should be considered.

2.7 Joints

Osteoarthritis (OA) is a chronic degenerative condition of hyaline cartilage. OP covers the deep costs of morbidity, pain, and health care. The consequences for the individual and for the population are very significant, especially with our aging population. There are several confirmed interventions that improve the clinical condition of the patient as soon as the degenerative process becomes symptomatic. Given the lack of response of the body's healing mechanisms to degenerative conditions in general, injecting growth factors and cytokines is reasonable. There are laboratory and animal models for using PRP in OP with generally favorable results. A recent article points to improved functional results. It is not known whether PRP acts on local paracrine factors to alter pain, with the formation of new hyaline or fibrous cartilage or a combination of both or neither. Further high-quality studies, pre / post imaging, and joint fluid analysis are needed to help clarify the effects.
Animal models describe improved healing of the meniscus and glenohumeral labrum, with induced defects, but human studies in these areas are currently lacking.

2.8 Intervertebral discs

Animal models using different preparations and matrices show encouraging results, however, there are no human studies. Placing PRP in a disc will require disc damage, which is potentially permanent after discography. Due to the proximity of critical neurological structures to the posterior annulus, CT or fluoroscopic guidance would be the preferred method of placing the regenerative factor in the discs.

2.9 Nerves

Trapped neuropathies that have failed in "conservative management" are traditionally treated with surgical release/decompression (neurolysis). With the advancement of musculoskeletal ultrasound, peripheral nerves and their adjacent structures can now be visualized. There is growing experience in performing percutaneous nerve release using a variety of solutions (called hydrodissection or hydroneurolysis). There is not enough information to approve PRP treatment for this use, however, in cases of ischemic nerve damage due to scar tissue lining, there is theoretically a role for PRP during percutaneous procedures and we encourage further investigation.

2.10 Lateral epicondylitis

PRP has been evaluated as a potential treatment option for patients with lateral epicondylitis who have failed to respond to physical therapy. In the largest such study, Mishra et al. evaluated 230 patients who failed to respond to at least 3 months of conservative treatment for lateral epicondylitis in a prospective cohort study. Patients were treated with LR-PRP and at 24 weeks, LR-PRP injection was associated with a significant improvement in pain compared to control (71.5% vs. 56.1%, P = 0.019) as well as a significantly lower proportion of patients who reported residual elbow tenderness (29.1% vs. 54.0%, P = 0.009). There was a clinically significant and statistically significant improvement at 24 weeks in patients treated with LR-PRP versus active control injection of a local anesthetic.
Previous studies have suggested that LR-PRP may also provide longer continuous relief of symptoms for lateral epicondylitis than corticosteroid injections and therefore have a more sustainable treatment effect. PRP appears to be an effective treatment for lateral epicondylitis with high quality evidence for short- and long-term efficacy, and the best available evidence specifically suggests that LR-PRP should be the treatment of choice.

2.11 Osteoarthritis

Osteoarthritis (OA) has unique characteristics in terms of joint biology, homeostasis, and levels of metalloproteases and inflammatory cytokines, which contribute to the patient's symptoms. Clinical reports of PRP use for cartilage injury have primarily involved patients with osteoarthritis of the knee or hip.

2.12 Osteoarthritis of the knee

There has been a growing interest in the efficacy of intra-articular PRP injection for the non-surgical treatment of osteoarthritis of the knee. A meta-analysis was performed looking at 14 randomized clinical trials (RCTs) involving 1,423 patients, comparing PRP with a variety of controls including placebo, hyaluronic acid, corticosteroid injections, oral medications, and homeopathic treatments. The meta-analysis showed significant improvement in Western (PRFM), while others injected PRP directly into the correction site. Significant heterogeneity of PRP or PRFM preparations was present. Patient-oriented outcomes such as

the University of California-Los Angeles (UCLA), American Shoulder and Elbow Association (ASES), Constant shoulder scores, Simple Shoulder Test (SST) scores, and VAS pain were provided as an objective clinical data such as the strength of the rotational cuff and shoulder ROM collected to measure differences in functional outcomes. Most individual studies have shown little difference in these outcome measures for PRP as an adjunct to arthroscopic rotator cuff repair compared to repair alone. Additionally, major meta-analyzes and recent critical examination have shown no significant benefit from PRP augmentation of the arthroscopically corrected rotator cuff. However, there were limited data to show some effect in reducing perioperative pain, most likely attributed to its anti-inflammatory PRP properties (Kim Harmon, et al.).

3. PRP in skin lesions and wound healing

Due to the ability of PRP to promote angiogenesis and wound healing, it is widely used in dermatology including the treatment of ulcers, scars, and alopecia. PRP has been tested in wound healing in high-risk women undergoing caesarean section. PRP was used in 70 patients and compared with 71 control cases without PRP. Criteria for PRP inclusion were body mass index (BMI)> 25 kg / m2, previous caesarean section, section, diabetes, twin pregnancy, corticosteroid use, and anemia. The result of the study was a greater reduction of redness, edema, ecchymosis, discharge, compared to the control group (85.5% reduction in the PRP group versus 72% in the control group) ($p < 0.001$). It has been concluded that PRP is an effective therapeutic approach for wound healing, and faster wound healing can be expected when using PRP due to the presence of multiple platelets and growth factors.
Another study was conducted on 55 patients who underwent major gynecological surgery, in which PRP was applied directly to the site of the procedure. It was observed that the applied autologous platelet grafts in gynecological surgery were effective in reducing pain and were not associated with any adverse effects (Lin MY, Lin CS, et.al,2020).

4. PRP in reproductive medicine

PRP in premature ovarian failure
Premature ovarian failure (POF) refers to the loss of normal ovarian function before the age of 40, accompanied by loss of fertility. A team of researchers from Harvard University injected the ovaries of mice with growth factors, and it was shown that mature eggs develop from ovarian stem cells. They noted that the introduction of isolated growth factor-carrying platelets directly into the ovaries could reactivate oocyte production.
PRP therapy has been studied in women with POF, infertile women over the age of 35, and women with low ovarian reserve.
PRP treatment is called ovarian rejuvenation; In this procedure, PRP is injected into the ovaries under ultrasound guidance, like in vitro fertilization (IVF). This modality of treatment is still being explored. Scientists at the Conference of the European Society of Human Reproduction and Embryology held in 2016 in Helsinki, Finland, introduced this modality (ovarian rejuvenation). They injected PRP into eight perimenopausal / POF women with poor ovarian reserve. This resulted in successful ovarian rejuvenation 1-3 months after PRP treatment. All cases underwent natural IVF cycles with follicles 15.20 ± 2.05 mm in diameter, the resulting oocytes were fertilized by intracytoplasmic sperm injection (ICSI), and all resulting embryos were cryopreserved (Toliopoulos IK and Papageorgiou 2018).

5. Materials and methods

Data obtained by searching and processing the relevant scientific literature that covers the given topic was used to prepare this paper.
A systematic search of the literature was performed through the free PubMed research machine which has access primarily to the MEDLINE databases with references and abstracts from the biomedical sciences. An extensive search of relevant books in the field of medicine, and veterinary medicine dealing with PRP was also conducted.
A systematic review of the literature from 2006 to 2021 was made. Inclusion criteria were human clinical and animal examinations, English literature, and manuscripts reporting on the use of PRP in musculoskeletal/orthopedic conditions.
Keywords used: techniques, centrifugation, isolation, platelets, growth factors, plasma, enriched, medicine, veterinary, pharmacy, regenerative medicine, standardization, principles.
A descriptive method was used to describe and explain in detail the characteristics of the methods and conditions used to obtain a PRP preparation, as well as a comparative method for comparing their advantages and disadvantages.
A review of the scope of comparative studies evaluating at least two alternatives in one or more stages of preparation, storage and/or administration of PRP or its related products.

6. Results and discussion

An overview of the techniques used to obtain PRP

PRP must contain a higher platelet concentration than the initial one, however, an increase in platelet concentration is a very large description of PRP and does not accurately describe the variability between different types of PRP. There are several parameters to consider when making PRP, including platelet concentration above baseline, whether leukocytes are present, whether PRP is anticoagulated, and whether it requires exogenous activation.
Platelet count is the first variable to consider. The absolute platelet count varies depending on the concentration of platelets in the peripheral blood of the subjects. PRP systems can usually be divided into lower (2.5 - 3 times higher than the initial concentration) and higher (5-9 times higher than the initial concentration).
It is assumed that higher platelet counts will bring more growth factors and better clinical outcomes, however, this has not yet been determined.
Several experts in this field suggest that the optimal PRP concentration is 2.5 x the initial concentration and above this may have an inhibitory effect. Therefore, more research is needed in this area.
White blood cells containing PRP will have a different biological activity from PRP in which they are absent.
A system with a lower platelet concentration separates whole blood into two components: one with cellular components and the other with a serum in which platelets are suspended (Dohan et al.2009).
Systems with higher platelet concentrations separate whole blood into three fractions: red blood cells, serum, and the buffy (yellow surface) layer. The buffy layer contains platelets and white blood cells.
WBC can further be classified into different types. These include neutrophils, monocytes/macrophages, and lymphocytes. Their role in tissue healing is different. Neutrophils are phagocytic and contain over 40 hydrolytic enzymes. Their activation leads to residual phagocytosis and the release of oxygen-free radicals and proteases. This release of toxic molecules from neutrophils can lead to secondary tissue damage. It is not yet known whether the presence of neutrophils has a negative or positive effect on acute or chronic soft

tissue injury.
Macrophages are a tissue form of circulating monocytes. Their role is to remove debris and they are primarily phagocytic. They also play a role in balancing the pro-inflammatory and anti-inflammatory aspects of healing. Because it is not possible to fractionate different types of white blood cells from PRP, the absence of macrophages may be detrimental to healing from any secondary damage caused by neutrophils. More research is needed in this area as well. When the full amount of blood is extracted, many PRP kits use anti-coagulants to prevent it from clotting (Tidball JG & Wehling-Henricks M.2007).
Most kits use the anticoagulant citrate dextrose (ACD) to inhibit clotting. ACD binds calcium and prevents coagulation proteins from initiating the coagulation cascade. It should be noted that the addition of citrate to the blood makes it more acidic than physiological. Because some growth factors are influenced by tissue pH, some protocols recommend adjusting PRP to the physiological range before injection.
Activation of PRP before the injection is another parameter that requires further discussion.
PRP can be activated exogenously by thrombin, calcium chloride, or mechanical trauma. Once PRP is activated, a fibrin network begins to form, solidifying the plasma and creating a fibrin clot or membrane. If PRP is too strongly activated, the fibrin network will be a bivalent, unstable network. If activated more physiologically, a stable tetramolecular network is formed that improves cell involvement and growth factors. Although this may be useful for surgical procedures, when PRP is injected into soft tissue, excessive viscosity is not desired.
Activation of PRP results in rapid release of growth factors, with 90% of prefabricated factors released within ten minutes. Many growth factors have short half-lives, so the greatest effectiveness may result if PRP is activated at or just before injection. The variable half-life of growth factors also contributes to the differential preparation of PRP depending on how quickly it is used after activation. Most commercial PRP kits are not activated. Some replace calcium that has been bound to ACD to create a physiological state. The use of inactivated PRP may result in more normal physiological activation by the tissue being injected (Ayman Shehata Dawood, et al.2018).
To avoid inadvertent platelet activation, most protocols use large needles to draw blood and re-inject PRP. In addition, there are different spin protocols with different spin speeds and times.
Some centrifuges offer special braking mechanisms to prevent inadvertent activation.
There is no consensus on the number of centrifuges required, nor on their duration.
Collagen is a natural activator of PRP, which is why, when PRP is injected into soft tissue, exogenous activation is not required.
Once the injection site is activated, the release of growth factors initiates an inflammatory reaction that lasts approximately 3 days. Fibroblasts accumulate at the injection site, marking the beginning of a proliferative healing phase lasting several weeks. Thereafter, remodeling of the collagen matrix formed by fibroblasts occurs. This phase of remodeling that leads to the formation of mature tissue lasts about 6 months. All three stages are required to form new tissue and ensure long-term tissue stability (Burkay Utku, et al.2014).
The PRP application needs to be performed by licensed doctors, pharmacists who are licensed to work or are specialist surgeons, and have undergone training for proper PRP application. They should be familiar with the correct choice of putty to be used, how it will be prepared and whether additional means will be used (such as activator, anticoagulant), complications that may occur after application of injections and how to deal with them as well as with the post-procedural onset of pain in patients.
Successful and safe performance of PRP procedures requires knowledge of the diagnosis, standard treatments, benefits, risks, contraindications, methods of preparation and delivery to the appropriate patient in the appropriate situation (Ion-Bogdan Codorean, et al.2017)(Kim Harmon, et al.).
Regarding the preparation of PRP, there are 2 techniques:

1. Open technique: the product is exposed to the environment in which the PRP procedure is performed comes in contact with various materials to be used for its production, such as pipettes, test tubes. In

such a procedure it should be ensured that the product will not be microbiologically contaminated during the procedure.

2. Closed technique: involves the use of CE-marked commercial kits (including centrifuge and application equipment) in which PRP is not exposed to the conditions of the working environment.

Several CE medical systems are available to produce autologous PRP. Most of them are included in one of the following 3 types of devices:

1. Blood is obtained with a test tube containing anticoagulant, and this test tube can be used for any type of centrifuge.
2. Medical devices that collect blood in a tube that already contains anticoagulant; centrifugation can then be done in any type of centrifuge.
3. Medical devices that collect blood in a syringe previously filled with anticoagulant; use - most often the blood is transferred to a secondary device whose form imposes the use of a centrifuge manufactured by the same manufacturer (Guide to the preparation, the use and quality assurance of blood components,14th edition, 2008)

The blood is then centrifuged with a single or double-spin centrifuge, depending on the device. The basic centrifuge settings to obtain PRP with the required platelet concentration are defined by the manufacturer and cannot be changed by the physician/pharmacist.

After centrifugation, 3 basal layers are observed in the test tube: at the bottom, there are red blood cells with leukocytes deposited just above; the middle layer corresponds to PRP, and the top is PPP. PPP removes and isolates PRP. Platelets can be activated before PRP administration, although there is no consensus on whether or not platelets should be activated before administration and with which agonist.

Thrombin and calcium chloride, which are inducers of aggregation, are used to activate platelets and stimulate degranulation, causing the release of GFs.

Some researchers activate platelets before application while others say it is unnecessary because without activation better results are obtained (Oriol Mirallas, et al.2018).

Recent studies suggest that there is no need to use aggregators because during administration the platelets are automatically released and ready to perform their function.

The ideal PRP volume for administration, the frequency of application, the exact site of PRP administration, and which preparation technique/system to use are still a topic of discussion (Adrian D. K. Le, et al.2018); (Amy S. Wasterlain, et al.2016); (Zimmermann R, et al.2001).

Table 2. Blood collection and centrifugation protocols from different medical devices to obtain platelet – rich plasma

Devices	Blood collection/ anticoagulant	Centrifugation		
		number of times	speed/time	centrifuge
Selphyl®	Tube 9 mL/sodium citrate	1	1,100 g/6 min	Classic
PRGF Endoret®	Tube 9 mL/sodium citrate	1	270 g/7 min	Classic
Cascade®	Tube 9 mL/sodium citrate	2	1,100 g/6 min 1,450 g/15 min	Classic
Plateltex®	Tube 9 mL/ACD	2	180 g/10 min 1,000 g/10 min	Classic
Regenkit®	Tube 9 mL/sodium citrate	1	1,500 g/9 min	Classic
ACP Athrex®	Syringe 15 mL/ACD or no anticoagulant	1	1,500 rpm/5 min	Adapted
GPS III®	Syringe 30 or 60 mL/ACD	1	3,200 rpm/15 min	Adapted
Genesis®	Syringe 12 mL/ACD	1	2,400 rpm/12 min	Adapted
SmartPrep 2®	Syringe 20 or 60 mL/ACD	2	2,500 rpm/4 min 2,300 rpm/10 min	Adapted

Proteal®	Syringe 20 mL/sodium citrate	1	1,800 rpm/8 min	Adapted
Magellan®	Syringe 30-60 mL/ACD	-	-	Adapted device
Angel®	Syringe 40-180 mL/ACD	-	-	Adapted device

ACD, acid citrate dextrose.
Note. From "Alves R, Grimalt R. A Review of Platelet-Rich Plasma: History, Biology, Mechanism of Action, and Classification. Skin Appendage Disord". 2018 Jan;4(1):18-24. doi: 10.1159/000477353. Epub 2017 Jul 6. PMID: 29457008; PMCID: PMC5806188.

Critical points for the success of PRP treatment

Despite the widespread use of PRP preparations in the treatment of various injuries, there is a lack of solid knowledge about the real benefits of these treatments. This gap is due to several criticisms of clinical and experimental approaches to this topic. What are the main critical points for the success of PRP treatment:

1. Nomenclature. Indeed, the term PRP encompasses several different biological preparations that are not well characterized.
2. Processing. Often, the details of the techniques used to obtain and apply PRP are not well defined. Platelet counts are often unreported, as is the number of centrifuges used to enrich platelets. The number of injected or administered PRPs is often left to the clinician to decide, as is the frequency of PRP applications. For these reasons, it is difficult to compare methods and outcomes and the required standardization is hampered.
3. Formulation. Published information on the formulation of various PRP-derived products in terms of cell content and bioactive factors is missing. The content of platelet growth factors varies greatly from person to person, regardless of platelet count. In addition, platelets are extremely sensitive to any kind of stress, from drawing blood from the patient to producing PRP gel. Thus, the number of platelet-derived factors available at the end of the process probably depends on the cumulative effects present throughout the preparation process.
4. Exploitation. To date, only a few randomized controlled trials have been performed in humans to provide evidence of level I efficacy of PRP use, and a standardized application protocol is also not yet available. Most published data come from small trials, often without a control group. Large methodologically rigorous, randomized controlled trials are needed, using clear primary clinical and biological endpoints.

Although PRP is a daunting tool for clinical application, many questions remain open, including the appropriate indications for its clinical use, as well as the effective concentration and amount of each product used in a different therapeutic situation.

Although PRP is currently processed at 37 ° C or room temperature (RT) (20-25 ° C), it is well known that low temperatures increase platelet activation, improving the release of dense and alpha granules. Thus, scientists evaluated the effect of cold preconditioning on the release of platelet-derived growth factors.

For this purpose, in one study PRP was incubated at 37 ° C, 23 ° C and 4 ° C for 30 min before PRP generation.

Next, levels of several proangiogenic molecules, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), primary fibroblast growth factor (bFGF), inter- leukin (IL) -17 and IL-8 are determined in PRP and are compared with the total amount of each protein in platelet lysates. The result showed that the secretion of VEGF, EGF, bFGF, IL-17 and IL-8, but not PDGF, was induced in this temperature-dependent preparation.

While VEGF, EGF, bFGF, IL-17, and IL-8 were partially released when PRP was incubated at 37 ° C or 23 ° C (20–60% of total intra-platelet count), total secretion of these molecules was achieved only when PRP was incubated at 4 ° C, indicating that cold preconditioning maximizes the release of platelet-derived proangiogenic molecules. Consistent with these results, the proliferation of PRP-activated HMEC-1 obtained from PRP prerequisite at 4 ° C is increased compared with that induced by PRP prerequisite at 37 °

C (Julia Etulain, ET AL.2018); (Araki J, et al.2012).

Parameters used to evaluate the quality of the PRP kit

1. Platelet count in the blood sample
2. Platelet concentration in the final PRP product
3. Platelets regenerated by method (total platelet count in blood sample/total platelet count in PRP).
4. The number of white blood cells in PRP should be also considered.

Commercial kits

Advantages
- the manufacturer provides materials and tools that are necessary for the preparation of the product
- kits are easy to use and need to be easily applied
- the preparation takes place in sterile conditions
- and usually have high recurrence

Disadvantages
- limited possibility for modification of the volumes and modes of the operating process.
- need to buy special instruments (e.g., centrifuge).
- the cost can be high.

(Prof. Stefano Grolli, et al.2020).

Platelet-rich plasma: contraindications

Absolute contraindications:
- Platelet dysfunction syndrome
- Critical thrombocytopenia
- Hemodynamic instability
- Septicemia
- Local infection at the site of the procedure
- A patient who does not want to take risks

Relative contraindications:

- Consistent use of NSAIDs within 48 hours of the procedure
- Corticosteroid injection at the treatment site within 1 month
- Systemic use of corticosteroids within 2 weeks
- Tobacco use
- Recent fever or illness
- Cancer- especially hematopoietic or bone
- HGB <10 g / dl
- Platelet count <105 / st
- (WHO Technical Report Series,2011)

Safety of PRP treatment

Generally, the use of platelet-rich plasma (PRP) is considered safer and more practical than other cell-based therapies, as they are autologous.
The mechanism of action of PRP is still unclear, preparation standards and methods have not been unified, and the clinical efficacy of PRP in many clinical applications is controversial. Therefore, many studies objectively evaluate the efficacy and safety of PRP and provide a scientific reference for the clinical application of PRP in several indications.
Universal precautions at any time during the procedure and immediately after the procedure.
PRP is antimicrobial and effective against most classes of bacteria except Klebsiella, Enterococcus, and Pseudomonas. Standard skin disinfection should be used before injection. This is a completely autologous graft that eliminates the worry of transmitting the disease unless the graft has been contaminated.
Risks for the patient from the procedure as infection, bleeding, nerve damage, pain, lack of result, limb loss, and death are very rare but possible (Ion-Bogdan Codorean, et al.2017).

7. Conclusion

Platelet-rich plasma (PRP) is an evolving therapeutic option in human and veterinary medicine. In musculoskeletal medicine, PRP is a promising treatment with clear evidence of safety.
However, the evidence for its effectiveness is mixed and depends on the composition and the specific indication.
The heterogeneity of PRP preparations, both now and historically, has made interpretation of the existing literature difficult and limits our ability to make definitive treatment recommendations.
Various procedures for PRP preparation are available. Clinicians should be aware that different preparation methods may result in different final products. Careful consideration of all available alternatives is recommended to determine the best PRP preparation procedure for the appropriate indication.
Many factors influence and should be considered when preparing a quality PRP product.
Because PRP is autologous, there are fewer safety concerns than other cell-based regenerative therapies. Regenerative treatments can even replace surgical treatment for some injuries, reducing recovery time.
Having the advantages of biocompatible safety, low cost, simple preparation, and clinical effectiveness more clinical randomized controlled trials should focus on the use of platelet-rich plasma as adjuvant therapy in the management of chronic conditions and in discovering various conditions in which its use has merit.
This field of work aims to standardize the protocol for obtaining autologous PRP from healthy blood donors, standardize the sample volume, platelet count, and concentration, and characterize growth factors after platelet activation.

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