Will Platelets Rich Plasma treatment find a Cesarean Section Scar Niche in reproductive medicine? Still Not ready

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Abstract:
One of the complications of cesarean section (C/S) is related to the incision site. Considering the effectiveness of platelet–rich plasma (PRP) on healing of wounds and management of scars, the present review aimed to inquire the effect of PRP on the thickness and completeness of the uterine scar.

DOINumber:10.48047/nq.2022.20.19.NQ99364 **NeuroQuantology2022;20(19):4008-4020**

per formed platelet. They contain more than 30 bioactive proteins, many of which have a fundamental role in haemostasis and/or tissue healing[(1)].

The platelet cytoplasm contains an open, canalicular system that increases the effective surface area for intake of stimulatory agonists and the discharge of effector secretions. The submembrane region contains microfilaments of actin and myosin that mediate morphologic alterations. These cells possess a tricarboxylic acid cycle and use glucose via the glycolytic and hexose monophosphate shunt pathways. Their function is closely linked to their metabolic activity[(1)].

It has been reported that despite the absence of a nucleus and DNA, platelets have a system for protein synthesis, copies of mRNA for almost one third of the known proteins in the human genome, process mRNA and effectively translate the different proteins. These discoveries have changed the way that platelets are seen[(2)].In addition pro- as well as anti-

Introduction:

Platelets are cytoplasmic fragments of megakaryocytes, a type of white blood cell, and are formed in the marrow. They are the smallest of the blood cells, round or oval in shape, and approximately 2µm in diameter. Electron microscopy shows the cell membrane is trilaminar with a glycoprotein receptor surface overlying and partially interspersed with and penetrating a bilayer of phospholipids and cholesterol. They contain organelles and structures such as mitochondria, microtubules, and granules[(1)].

The latter are divided into three types:

1) Delta or dense: containing ADP, ATP and serotonin that are potent agonists or platelet activators,

2) Lambda: lysosomes that help dissolve the clot once it has served its function and,

3) Alpha:The α granules are bound by a unit membrane and formed during megakaryocyte maturation. They are about 200 to 500 nm in diameter, and number approximately 50 to 80

patients own blood factors and began to be used in many fields of medicine. In odontology, PRP has proven successful in gingival regeneration. In orthopedics, it is used in acceleration of bone fracture healing and in articular cartilage repair. PRP is also applied for the treatment of muscle strain injuries, and interesting results have been reported in the treatment of osteodegenerative diseases and in the management of patients with complex injuries. Ulcer reconstruction especially in diabetic patients was treated effectively by PRP[6].

Components of PRP:

The most important and most abundant component of PRP is the platelets as a 3-7 fold increase in its concentration in relation to that of total blood . Other components are leukocytes that have about 5 fold increase in its percentage than in similar blood sample & plasma which contains clotting factors but in normal physiological concentration[7].

1. Platelets:

Platelets are small, discoid, anucleate cells . Platelets form by budding off from megakaryocytes in the bone marrow, and then enter the circulation[3].

Platelets enter the bloodstream as anuclear cells and, therefore, have a limited life span of only about 7 to 10 days. Throughout their lifespan, they actively synthesize growth factors, especially in response to clotting. Platelets contain a large number of storage granules, predominantly characterized into three types:

1. Lysosomal granules: function as storage for digestive enzymes.
2. Dense granules: store and secrete adenosine diphosphate (ADP), which is a potent recruiter and activator of other platelets.
3. Alpha granules: store growth factors in an incomplete bio-inactive form. Secretion of growth factors is activated by the clotting process[8].

inflammatory, the large number of growth factors contained in platelet granules, the ability of de novo protein synthesis and its antimicrobial activity and inflammation modulator promote cell proliferation and synthesis of extracellular matrix promoting healing, wound repair and other tissue lesions. These features have led to propose the use of autologous PRP for repair and regeneration of different `tissues[2].Platelets reside intravascularly, with high concentration in the spleen. Normal blood contains approximately 140,000 to 400,000 platelets/mm3 which remain in the circulation for an average of about 10 days before removal by macrophages of the reticuloendothelial system. The condition of depressed platelet level iscalled thrombocytopenia, which is characterized by persistent bleeding from cuts and wounds, by petechiae and ecchymoses and oozing of blood from vascular beds[3].

Definition of PRP:

Platelet rich plasma (PRP) is an autologous preparation of platelets in concentrated plasma. The concentration of platelets provide a higher amount of several bioactive growth factors reported to promote healing and regeneration process[2].

Because the normal range of platelets in the whole blood of healthy individuals is 150.000 to 350.000 platelets/mL of whole blood, the definition of PRP has evolved to mean a three to seven fold increased concentration of platelets and a 7–30 fold increased concentration of growth factors, compared with whole blood[4].

There are several other terms besides PRP, such as autologous platelet gel, platelet rich concentrate and platelet releasate[1].

History of PRP:

Platelet rich plasma was first promoted by **Ferrari et al**[5]as an autologous transfusion component after an open heart operation to avoid homologus blood product transfusion.

The popularity of PRP grew as physicians began to see clinical results in concentrating a

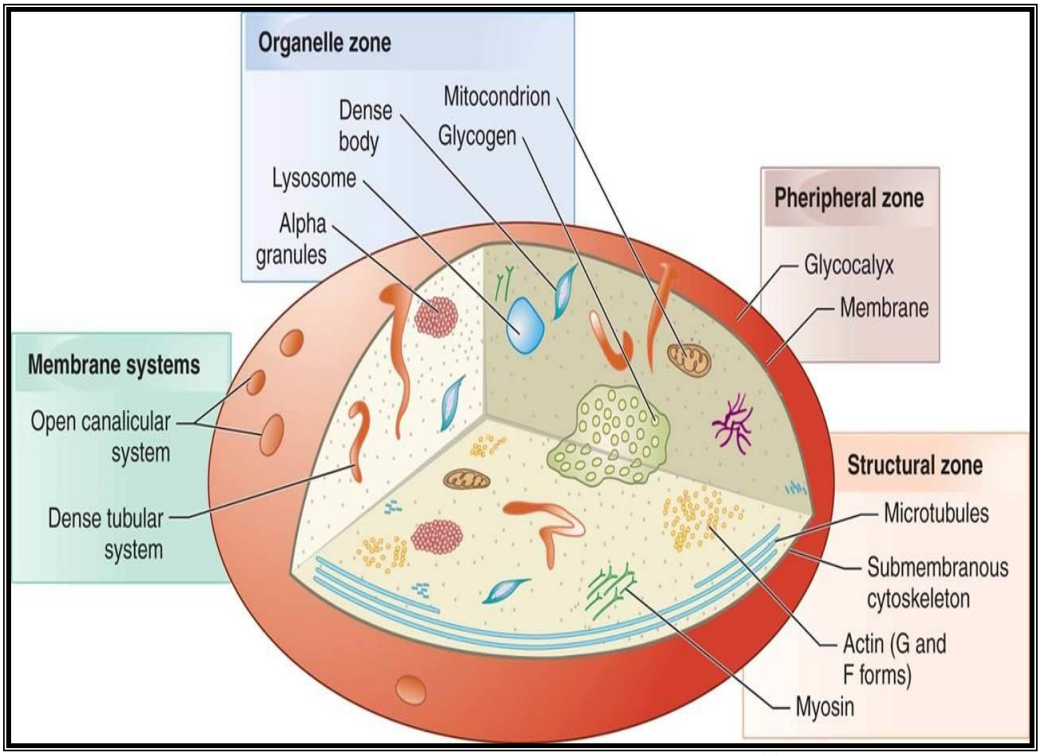

Figure (1): Schematic representation of human platelet[9].

Plasma proteins are also known to be critical components in the healing mechanism of connective tissues. Plasma differs from serum in that plasma still contains fibrinogen as well as a number of clotting factors. Therefore, when plasma is exposed to thrombin either by the addition of exogenous thrombin or by coming in contact with tissue thromboplastin (also known as tissue factor), the clotting cascade is initiated and platelets are activated[11].

The resulting formation of a fibrin clot provides a provisional scaffold for cell migration as well as a reservoir of growth factors. This is similar to what occurs in the formation of a clot after haemorrhage. Although in vitro studies have documented significant differences in cell proliferation between PRP and platelet-poor plasma (PPP) preparations, it is possible that the plasma component of PRP actually plays more significant role in creating a proper local environment for tissue repair[12].

2. Leukocytes:

The role of leukocytes in platelet concentrate is a controversial issue . Leukocytes & L-PRP preparation has been shown to inhibit the growth of Staphylococcus aureus and Escherichia coli in vitro although in the same study it had no effect on Klebsiella, pneumoniae and Ennterococcus faecalis and Pseudomonas aeruginosa activity was actually increased. In another report, platelet-leukocyte gel (a compound rich in both platelets and leukocytes) was shown to have significantly greater antimicrobial activity against S aureus than PRP alone, suggesting that leukocytes may enhance the antibacterial effect of PRP[10].

3. Plasma:

The most consistent component of PRP products is the plasma. Plasma, the fluid portion of blood, is a remarkable liquid containing numerous ions, inorganic and organic molecules, and many of the same proteins found in platelets.

Table(1): Classification and Functions of Bioactive Molecules Present in Platelet-Rich Plasma[3].

Category	Proteins	Functions
Adhesive proteins	Von Willebrand factor, fibrinogen, fibronectin, vitronectin, lamminin-8	Cell interaction, hemostasis, composition of extracellular matrix

Coagulation factor and associated proteins	actor V/Va, multimerin, protein S, high-molecular weight kininogen, antithrombin III, tissue factor pathway inhibitor	Thrombin production and regulation
Fibrinolytic factors and associated proteins	asminogen, α-2 antiplasmin, stidine-rich glycoprotein, α-2 acroglobulin	asmin production and vascular modeling
Proteases and antiproteases	tissue inhibitors of etalloproteases 1-4 (TIMP 1-4), etalloproteases 1, 2, 4, 9, C1 hibitor, α-1 antitrypsin	ngiogenesis, vascular modeling, agulation regulation
Chemokines, cytokines, and others	8, FasL, endostatins, steonectin, bone sialoprotein	egulation of angiogenesis, scular modeling, cell teractions, bone formation
Antimicrobial proteins	hrombocidins	actericidal and fungicidal roperties
Membrane glycoproteins	ost of the components of the asma membrane	atelet aggregation and dhesion, protein endocytosis, flammation, thrombin eneration, platelet-leukocyte teractions
Others	hondroitin 4 sulfate, albumin, mmunoglobulins, semaphoring	romote angiogenesis, cartilage generation, fibrin production, nd platelet adhesion

Table(2): Growth factors present in platelet-rich plasma[3],

Name	Acronym	Function
Platelet-derived growth factor	PDGF	Stimulates fibroblast production, chemotaxis, stimulates transforming growth factor– β1, llagen production, upregulation of proteoglycan synthesis
Transforming growth factor–β1	TGF-β1	Modulates proliferation of fibroblasts, formation of extracellular matrix, cell viability; increases production of collagen from fibroblasts, suppression interleukin1–mediated effects on proteoglycan synthesis in cartilage
Basic fibroblastic growth factor	bFGF	Produces collagen; stimula-tes angiogenesis, proliferation of myoblasts
Vascular endothelial growth factor	VEGF	Promotes angiogenesis
Epidermal growth Factor	EGF	Promotes cell differentiati-on, angiogenesis, rolifera-tion of mesenchymal and epithelial cells

Concentration of PRP:

The mean blood platelet level is 150,000 - 350,000/µL. Although the PRP count

tide). In vitro studies have shown that at lower pH (5.0), platelet concentrate lysate has increased concentrations of PDGF, with an increased capacity to stimulate fibroblast proliferation. TGF-β increases the production of collagen from fibroblasts. Its release (in vitro) is enhanced by neutral or alkaline pHs, which correspond to the later phases of healing. Through modulation of interleukin-1 production by macrophages, PRP may inhibit excessive early inflammation that could lead to dense scar tissue formation[14].

Insulin-like GF-I (IGF-I) has also been extensively studied for its ability to induce proliferation, differentiation, and hypertrophy of multiple cell lines. Separate analyses of GFs in PRP have shown significant increases in PDGF, VEGF, TGF-β1, and EGF, compared with their concentrations in whole blood. However, there are conflicting results with regard to IGF-I, where the majority of studies reported no increase in IGF-I in PRP, compared with whole blood. There are also conflicting results regarding the correlation between the GF content and platelet counts in PRP. The basis of these contradictions are not fully understood and may be related to variability in patient age, health status, or platelet count. Alternatively, differences in GF content and platelet count may be due to the various methods of processing, handling, and storing of samples, in addition to the type of assay performed. The diversity of PRP products should be taken into account when interpreting and comparing results and methods for generating PRP[15].

The mechanism of action of PRP is based on the fact that platelets contain many growth factors in their alpha granules. These factors have a well-known role in the process of tissue repair. Thus, the concentration of these substances in injured tissues could be beneficial to provide more agility to the regeneration process[2].

These growth factors include the three isomers of platelet-derived growth factor (PDGFαα, PDGFββ, and PDGFαβ), two of the numerous transforming growth factors (TGFβ1 and TGFβ2), vascular endothelial growth factor (VEGF) and epithelial growth factor (EGF) [16].

Among the main substance that are released also from these granules are cytokines, has not been optimized, a platelet concentration of more than 1 million/µL (approximately four to seven times the mean levels) is generally regarded as the therapeutically effective Concentration of PRP. Further, a bell-shaped response curve indicating a dose dependant nature has been associated with PRP. It has been demonstrated that lower or higher concentrations than 1.5 million platelets/µL, seemed to inhibit the angiogenic potential in human endothelial cells. All the FDA-cleared PRP separator devices have been shown to achieve this therapeutic concentration of PRP[2].

Mechanisms of action of PRP:

The Role of Platelets

Platelets are the first cell type to arrive at the site of tissue injury and are particularly active in the early inflammatory phases of the healing process. They play a role in homeostasis, through cell membrane adherence, aggregation, clot formation, and release of substances that promote tissue repair and that influence the reactivity of blood vessels and blood cell types involved in angiogenesis and inflammation. Platelets mediate these effects through degranulation, in which platelet-derived GF (PDGF), transforming GF-β1 (TGF-β1), vascular endothelial GF (VEGF), basic fibroblastic GF (bFGF), and epidermal GF (EGF) are released from alpha granules. Platelets also store antibacterial and fungicidal proteins, metalloproteases, coagulation factors, and membrane glycoproteins, which may influence inflammation by inducing the synthesis of other integrins, interleukins, and chemokines. Dense granules in platelets store and release ADP, ATP, calcium ions, histamine, serotonin, and dopamine, which are active in tissue modulation and regeneration. Platelet degranulation begins within 10 minutes of exposure to clotting cascade factors (such as thrombin) or, in their absence, contact to exposed basement membrane. The majority of GF secretion occurs within the first hour, although continued release occurs throughout the period of platelet viability (7 days) [13].

Although many GFs are associated with wound healing, PDGF and TGF-β1 appear to be 2 of the more integral modulators. PDGF has activity in early wound healing (during the acid

Cho et al[21]have shown in vitro that PRP increases the proliferation of dermal papillae cells, and activates the extracellular signaling pathways, signal-regulated-kinase and phosphatidylinositol 3`-kinase (PI3K)/Akt.

Additionally, fibroblast growth factor-7 and beta catenin, which are both stimulators of human fibroblast growth, were stimulated after PRP administration. The study gives further support to the growth-promoting effect of PRP in hair, by providing evidence that levels of Ki-67, a marker for cell proliferation, are increased after PRP administration in humans[20].

Platelet rich plasma may suppress cytokine release and limit inflammation, interacting with macrophages to improve tissue healing and regeneration and promote new capillary growth . Platelets in PRP also play a role in host defense mechanism at the wound site by producing signaling proteins that attract macrophages; PRP also may contain a small number of leukocytes that synthesize interleukins as part of a non-specific immune response . Previous studies of PRP have demonstrated antimicrobial activity against Escherichia coli, Staphylococcus aureus, including methicillin-resistant Staphylococcusaureus, Candida albicans, and Cryptococcus neoformans[22].

Platelet rich plasma has been reported to augment dermal elasticity by stimulating the removal of photodamaged extracellular matrix (ECM) components and inducing the synthesis of new collagen by dermal fibroblasts via various molecular mechanisms[21].

integrins, coagulation proteins and adhesion molecules. These cell adhesion molecules are fibrin itself, fibronectin, and vitronectin[17].

It is important to remember that the skin houses different types of stem cells (including cells present in the hair follicle bulge) and cells of mesenchymal origin, all dispersed in the dermis.After the subcutaneous injections, these proteins and growth factors released by platelets interact with these cells, bind to their specific cellular receptors and activate the intracellular processes that stimulate cell proliferation and differentiation[6].

Fibrinogen contained in PRP preparations and converted fibrin capture not only some of the exogenous growth factors present in the PRP but also endogenously produced growth factors. It gradually releases them as a drug delivery system[18].

During the four phases of the wound-healing process hemostasis, inflammation, proliferation, and remodeling, platelet growth factors regulate a well- orchestrated and complex series of events involving cell–cell and cell–matrix interactions, ultimately resulting in the promotion of mesenchymal stem cell proliferation at the wound site[19].

Platelet-rich plasma has been shown to promote significant changes in monocyte-mediated cytokine release by decreasing the levels of monocyte chemoattractant protein-1 (MCP-1) and increasing the levels of “regulated activation normal T-cells expressed and secreted” (RANTES) from the ß-granules of platelets. Thus, PRP paves the way towards wound healing[20].

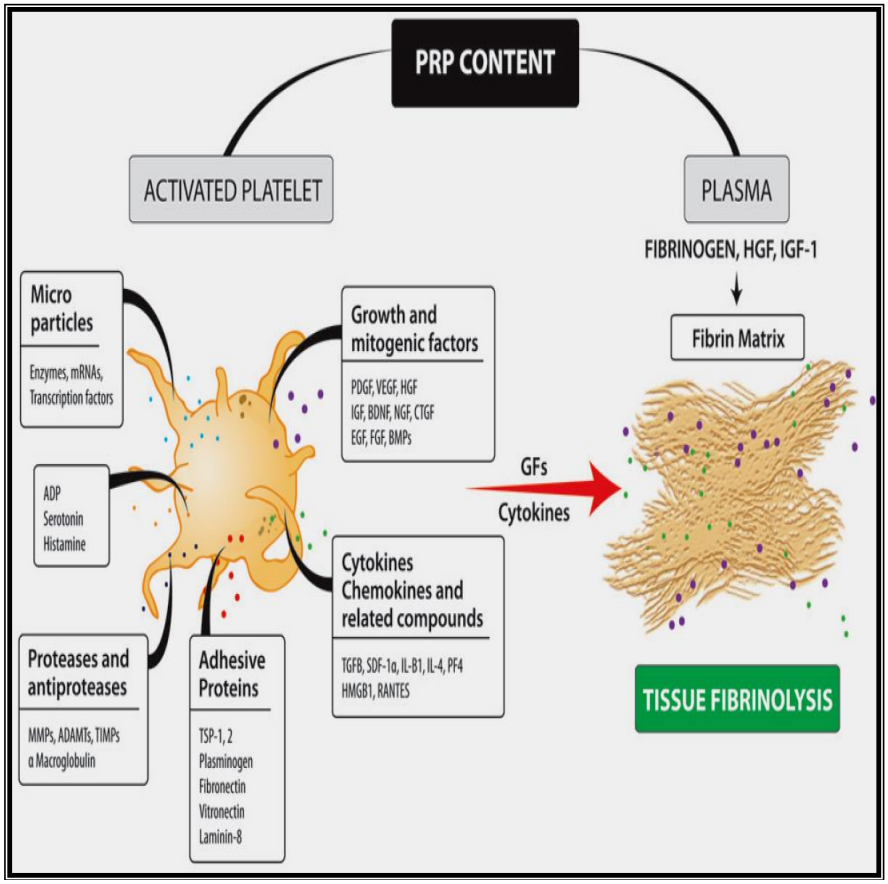

Fig. (2): Illustration of some biological mediators of platelet-rich plasma (PRP) that helps tissue repair[23].

them to sink to the bottom of the centrifuge tube more rapidly than do the platelets and leukocytes[26].

The first spin (called the soft spin) will separate the red blood cells from the plasma, which contains the platelets, the white blood cells, and the clotting factors. The second spin (called the hard spin) finely separates the platelets and white blood cells together with a few red blood cells from the plasma. This second spin produces the PRP and separates it from the PPP free from the obstruction provided by a large number of red blood cells[24].

Once the PRP is separated from the whole blood, it is stable for about 8 hours. However, because these procedures are considered an autograft, the plasma should be prepared and used immediately at the point of care, and should not be stored. Prior to application, platelets can be slowly activated by initiating the coagulation cascade with the addition of calcium chloride, a necessary cofactor for prothrombin conversion into thrombin[27].

Preparation of PRP:

Platelet rich plasma is prepared either manually or by the use of automated device. The process must be carried out under strict aseptic conditions as well as optimum temperature regulations i.e., 20-22°C. In order to inhibit platelet aggregation, it is prepared with an anticoagulant. The platelets need to be sequestered in high concentrations, enough for achieving therapeutic benefit and in a viable state at the same time, so that they can actively secrete their GFs[24].

With regard to the type of anticoagulant for use, most authors agree on not using Ethylenediaminetetraacetic acid (EDTA) because it could damage the platelet membrane. Therefore, anticoagulants with citrate and dextrose of sodium citrate are recommended[25].

Manual double spin method

A sample of peripheral venous blood is drawn and immediately spun in a centrifuge to separate the erythrocytes from the platelets and leukocytes. The increased density of the erythrocytes causes

tubes (usually with acid citrate dextrose or sodium citrate solution).The blood is then centrifuged with single- or a double spin centrifugation, depending on the device[24].

After centrifugation, the tube shows 3 basic layers: at the bottom of the tube, there are red blood cells with leukocytes deposited immediately above; the middle layer corresponds to the PRP and at the top, there is the PPP. The PPP is removed, and PRP is obtained[29].

- quantity of plasma (2-4 mL) by gently shaking the tube.

The active secretion of prepackaged GFs begins within 10 minutes of clot initiation and 95% of the secretion is completed within 1 hour. Hence, PRP must be used on the treated site within 10 minutes of activation[28].

Automated devices

Numerous commercial devices of varying standards are now available for the preparation of PRP. The preparation of PRP depends on the type of device chosen and should be done according to the manufacturer's instructions. The procedure requires the use of relatively small volumes of blood. The whole blood is obtained by venipuncture in anticoagulated

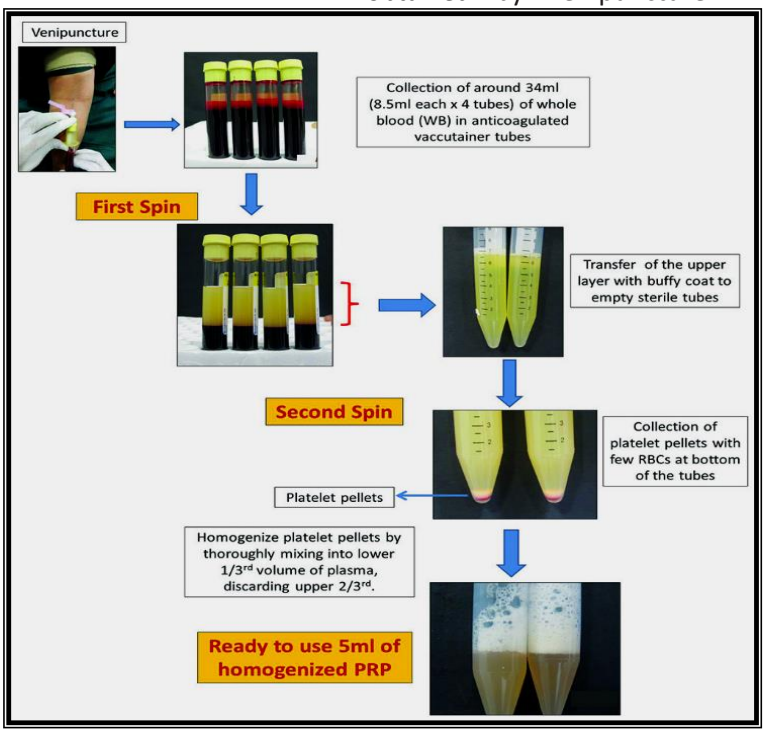

Fig. (3): Flow chart describes a double centrifugation process of PRP[24].

However, some authors state that the addition of an activator to PRP can diminish efficacy by decreasing the long-term availability of growth factors. Thedelivery of PRP without an activator is feasible because platelets are triggered to release GFs and cytokines by exposure to the target tissue[29].

Factors affecting the efficacy of PRP:

Various factors influence the yield of PRP such as draw of blood, speed, time and temperature of centrifugation and use of anticoagulants.

1. Draw of blood

The clotting process is influenced from the time of the draw. To avoid unintentional

Activation of PRP*:*

The PRP application requires initiating the coagulation process in order to be activated and release the growth factors contained within. Calcium chloride or calcium gluconate solution is added after the second centrifugation step. Activation of PRP with calcium modulates the release of PDGF, TGF-β, VEGF, IGF-1, FGF and interleukin-1 β (IL-1β). These molecules are markedly involved in endothelial cell proliferation which is subsequently greatly enhanced with the PRP supernatants. The amount of calcium added is to activate PRP and regulate endothelial cell division[29].

added after the second centrifugation step. Activation of PRP with calcium modulates the release of PDGF, TGF-β, VEGF, Insulin-like growth factor (IGF-1), basic fibroblast growth factor (bFGF) and interleukin-1 β (IL-1β). These molecules are markedly involved in endothelial cell proliferation which is subsequently greatly enhanced with the PRP supernatants. The amount of Ca added to activate PRP regulate endothelial cell division[33].

However, some authors state that the addition of an activator to PRP can diminish efficacy by decreasing the long-term availability of growth factors. Thedelivery of PRP without an activator is feasible because platelets are triggered to release growth factors and cytokines by exposure to derived collagen[34].

Classification of Platelet Concentrates:

In 2009**, Ehrenfest et al.**[35] have proposed a classification of plateletconcentrates into four categories depending upon their leucocyte and fibrin content as follows:

1. P-PRP (Pure platelet-rich plasma):

The P-PRP concentrate consists of an undetermined fraction of buffy coat, containing a large number of platelets, but most leucocytes are not collected. After the first slow spin centrifugation, only the superficial buffy coat layer is pipetted out and prepared for next centrifugation[36].

2. L-PRP (Leukocyte- and platelet-rich plasma):

L-PRP consists of most of the platelets, along with leucocytes and some residual RBCs, suspended in fibrin-rich plasma. It differs from P-PRP only in the means of buffy coat layer collection in which PPP along with the entire buffy coat layer and superficial 1-2 mm layer of RBCs are pipetted out. The manual PRP preparation process (as described above) is not clearly defined, it might randomly lead to P-PRP or L-PRP[32].

3. P-PRF (Pure platelet-rich fibrin):

The term PRF is used synonymously with platelet-rich fibrin matrix (PRFM). When P-PRP is mixed with activator and allowed to incubate for some time, a stable PRFM clot can be collected. Very low amounts of leucocytes are collected owing to a specific separator gel used in the device[36].

4. L-PRF (Leucocyte- and platelet-rich fibrin):

activation of platelets, most protocols use large bore needles (>22 µm) to draw the blood[30].

2. Temperature

When analyzing temperature influence, no effect was detected when centrifugation times were short; but when the centrifugation steps took 16 minutes or more, temperature had a positive effect and increased the platelet yield.

Many authors recommend a temperature level of 12°C-16°C during centrifugation for best platelet recovery[31].

3. Anticoagulants

The importance lies in choosing an anticoagulant capable of preserving the platelet's best possible functionality, integrity, and morphology.

With regard to the type of anticoagulant for use, most authors agree on not usingEDTA because it could damage the platelet membrane. Therefore, anticoagulants with citrate and dextrose of sodium citrate are recommended[25].

4. Number of spins, speed and duration

Perezet al[32]demonstrated that the processing of 3.5 mL of blood at 100×g for 10 min (1st spin), 200×g for 10 min (2nd spin) and withdrawing 2/3 of remnant plasma, promoted high platelet recovery (70%-80%) and concentration (5 folds the baseline) maintaining platelet integrity and viability. Longer time periods slightly increased platelet recovery and decreased the concentrations ofWBCs in the upper layer.

In addition, various factors contribute to platelet concentration gradient such as the size of platelets, the biological difference among individuals and hematocrit variability. However, this gradient is more critical after the second spin step because some erythrocytes are inevitably present in the volume that was transferred from the first spin. The presence of these remaining RBCs can generate a pellet at the bottom of the tube, which adsorb platelets and WBCs on its surface. The manual mixing for a short period of time is insufficient to completely resuspend the platelets, and a large variability in platelet counting is observed[32].

5. Activation of PRP

The PRP application requires initiating the coagulation process in order to be activated and release the growth factors contained within . Calcium chloride or calcium gluconate solution is

biochemical modification of the blood, i.e. no anticoagulants, thrombin or $CaCl_2$. When pressed between two gauzes, the PRF clot becomes a strong membrane which also has potential applications described in oral, maxillofacial, ENT (ear, nose, and throat)and plastic surgery[36].

Here, blood is collected without any anticoagulant and immediately centrifuged. A natural coagulation process then occurs and three layers are formed: the RBC base layer, acellular plasma top layer and L-PRF clot in the middle, which harvests platelet and leucocyte growth factors into the fibrin matrix. There is no

Table (3):Type of platelet-rich plasma/fibrin[36].

ype of platelet-richPlasma	nal components
ure platelet-rich plasma	brin-rich plasma with concentrated atelets. Leukocyte poor
eukocyte- and atelet-rich plasma	brin-rich plasma with concentrated atelets, leukocytes, and red blood cells
atelet-rich fibrin	brin polymerized clot that is rich in atelets and variably rich in ukocytes.
eukocyte- and atelet rich fibrin	brin polymerized clot that is rich in atelets and leukocytes

diminished, the clinical outcome is significantly compromised for this reason, the use of anti-inflammatory drugs is not recommended. This restriction should be in place for about 1 to 2 weeks[29].

Adverse effects of PRP injection

Adverse effects of PRP treatment may occur, some of which are significant. The most common adverse effects are infection, skin discoloration and bruising, occasional swelling, pain in the injected area, allergic reaction (a rare occurrence), and blood clotting (because PRP therapy uses a needle, a vein could be damaged).

Contraindications for PRP injection[38]:

- ***Absolute contraindications:***
  - Platelet dysfunction syndrome.
  - Critical thrombocytopenia (<105/µl).
  - Hemodynamic instability.
  - Septicaemia.
  - Local infection at the site of the procedure.
- ***Relative contraindications[38]:***
  - Consistent use of non steroid anti-inflammatory drugs (NSAIDs) within 48 hours of procedure.
  - Corticosteroid injection at treatment site within 2 weeks.
  - Systemic use of corticosteroid within 2 weeks
  - Haemoglobin < 10gm/dl.

Advantages of PRP injection[37]:

PRP may be considered an alternative promising new therapeutic modality as it has the following advantages:

a) Being autologous, avoiding the allergic and foreign body reactions like encapsulation and granuloma formation.

b) It is simple, ease and inexpensive to use.

c) Has a very low rate of accompanied infection as some researches documented that it has an anti-inflammatory and anti-infectious properties.

d) There is no accompanied downtime or abstaining from normal daily activities after its treatment.

e) Its marvelous property of regeneration and healing.

f) Being beyond the great concerns about transmissible diseases such as HIV, hepatitis.

Precautions:

Certain factors (e.g, smoking and alcohol intake) diminish stem cell release. Avoiding these will increase the success of the PRP procedure. The platelets work by causing an inflammatory reaction. If this inflammatory reaction is

factor proteins by the addition of carbohydrate side chains and intact plasma membranes, any damage to platelets during the isolation procedure will result in the final PRP product most likely becoming inactive[39].

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As summarized by Sharara, PRP started to be used in reproductive medicine only in the past 3–5 years. However, “therapeutic” applications of PRP are not new to gynecology. Among the more sordid examples, some maverick gynecologists injected PRP into the vagina, in a procedure called “O-shot” or “orgasm shot” with the alleged benefit of improving orgasm.So, what is the magic composition of PRP and why could its use be beneficial for infertility management? And specifically, could direct ovarian injection restore follicular growth especially in older patients? [29].

Platelet alpha granules contain multiple factors including at least 7 critical growth factors: 3 isomers of platelet-derived growth factor, 2 of transforming growth factor β, vascular endothelial growth factor, and epithelial growth factor. In addition, since platelets are bathed in plasma, 3 adhesion molecules are also present: fibrin, fibronectin, and vitronectin. Given this payload of growth-promoting factors, there is good cause to believe these powerful biological components may play a role in activating dormant follicles or stem cells, resulting in their assumed therapeutic role in the wide arrays of pathologies for which they are proposed[39].

But why are the results of studies utilizing PRP treatments of a controversial or inconsistent nature? And why, when a benefit is observed, this applies only to a subgroup of patients?The fact that PRP consists of many growth factors, with their exact concentrations unknown and potentially varying from patient to patient (could composition change in different phases of the cycle? with advancing age? with different disease states?) raises doubts about the uniformity of each PRP preparation. In addition, the quality of PRP preparations is often unpredictable, since different devices used to extract PRP have different efficiency with some being consistently less “bioactive” than others. Moreover, since secretion of growth factors begins 10 minutes after blood clotting and more than 95% of growth factors are secreted within 1 hour of clotting, in theory at least, PRP should be used rapidly after clot activation. Studies that fail to use anticoagulated whole blood are therefore not using PRP. Finally, since secretion of the alpha granules requires activation of the growth

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