Hassan SS, Alkhalidi EF, Hamed RS. Platelet concentrates a new renaissance in tissue regeneration. 2025 Jan-Marc;23(1):3104.
https://doi.org/10.18549/PharmPract.2025.1.3104

Original Research

Platelet concentrates a new renaissance in tissue regeneration

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Received (first version): 29-Mar-2024 Accepted: 02-May-2024 Published online: 10-Jan-2025

Abstract

Background: Platelet concentrates inevitably open novel options in regenerative medicine and are commonly used in a variety of medical fields, including medicinal dentistry, thanks to the ability of platelets as key regenerating factors as well as other blood components to enhance tissue regeneration. Various types of autologous platelet concentrate that are typically prepared from patients' blood have been discovered according to their preparation method and centrifugation protocol, type of tubes, and whether they require biochemical additives such as anticoagulants with different structural and regenerative properties. This review article aimed to clarify the generations and forms of platelet concentrates their preparation methods, biological action, composition, and application in dentistry.

Keywords: platelets; autologous platelet concentrates; tissue regeneration; growth factors

INTRODUCTION

Constantly, the researchers are focusing on developing a wonder material with the highest potential for regeneration. The world of medicine became aware of platelets' capacity for regeneration in 1974. Platelet concentrates have been introduced as a blood-derived product, serving as a biological mediator to boost the body's natural healing mechanisms and tissue regenerative processes.[1]

Platelets (thrombocyte) were discovered in 1882 by the pathologist Giulio Bizzozero,[2] are small anucleated megakaryocyte fragments circulating in the blood stream. Platelets contain granules including alpha and dense granules. These granules are crucial to platelets functions. Upon activation, the platelets secrete various biomolecules that are contained in these granules, including angiogenic and growth factors, adhesion molecules, chemokines, cytokines, and other bioactive mediators that are able to respond to a multitude of signals and regulate a diverse array of important biological processes such as inflammatory process, angiogenesis, cell proliferation and differentiation, stem cell migration, and tissue repair and regeneration.[3,4] α-granules store more than 300 different proteins that release them after platelet activation, therefore, using platelet concentrates can accelerate tissue healing and regeneration.[5]

Whole blood is composed of four components: blood cells and platelets make up about 45% of human blood; (white blood cells (1%), red blood cells (40%), and platelets (4%)); and plasma (the liquid part) makes up about 55%,[6] whereas platelet concentrates contain a higher concentration of platelets. The regenerative ability of platelets, the basis of platelet concentrate, is ascribed to their secretomes (growth factors, cytokines, and other signaling molecules), which are released in super-physiological concentration at the target site. Therefore, the development of autologous blood- derived aggregates has attracted considerable attention as a therapeutic option in both medical and dental fields due to its significance for tissue regeneration.[7]

Platelet concentrates

Platelet concentrates are biomaterials of autologous origin containing platelets and growth factors in high concentration, obtained through the centrifugation of the recipient's whole blood. Depending on their characteristics and the preparation protocols needed, these growth factor-based products are simply divided into three generations: platelet-rich plasma (PRP), platelet-rich fibrin (PRF), and concentrated growth factors (CGFs).[8]

The three standard characteristics that make platelet concentrates open a supporting gate for tissue regeneration in clinical practice are: 1) They function as bio-scaffolds; 2) They offer a natural source of growth factors; and 3) They hold live cells.[9]

Because a plethora of natural growth factors are available in platelet concentrates, pricey synthetic growth factors will not be needed.[10] Moreover, Platelet concentrates are autologous, biocompatible, biodegradable, easy to prepare, and cost-effective with no risk of adverse reactions, making them more advantageous than synthetic biomaterials.[11,12]

Platelet concentrates are enriched with numerous growth factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor (TGF-β), basic fibroblast growth factors (bFGF), and insulin-like growth factor (IGF), and cytokines, which trigger biological effects including

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chemotaxis, cell proliferation and differentiation, angiogenesis, and extracellular matrix synthesis, which are key processes that are involved in tissue repair and regeneration.[11,13,14]

The growth factors of platelets have numerous functions including:[15]

- Vascular endothelial growth factor: Initiates angiogenesis and vascular permeability, promotes mitogenesis to endothelial cells.
- Platelet-derived growth factor: Mitogenesis for mesenchymal cells and osteoblasts, chemostaxis of neutrophils and macrophages, regulates collagenase secretion and collagen synthesis, and mitogenesis and chemotaxis in fibroblasts and smooth muscle cells.
- Epidermal growth factor: Angiogenesis and endothelial chemotaxis, epithelial and mesenchymal mitogenesis, and regulates collagenase secretion.
- Transforming growth factor beta: Chemotaxis and endothelial angiogenesis, undifferentiated mesenchymal cells prolifieration, Regulates endothelial, fibroblastic, and osteoblastic mitogenesis, Regulation of synthesis and secretion of collagen, Inhibits proliferation of lymphocytes and macrophages, regulate the mitogenic effect of other growth factors.
- Insulin-like growth factor: Chemotaxis of fibroblasts, stimulate protein synthesis, stimulates osteoblast proliferation.
- Connective tissue growth factor: Angiogenesis and regeneration of cartilage, Fibrosis and platelet aggregation.
- Basic fibroblast growth factors: Differentiation and growth of chondrocytes and osteoblasts, mitogenic effect for mesenchymal cells, chondrocytes, and osteoblasts.

Platelet concentrates have been employed in various medical disciplines, including dentistry, dermatology, plastic surgery, and reconstructive surgery, as a way to provide a delivery approach of a copious number of autologous growth factors to their surrounding tissue.[16]

Platelet Rich Plasma (PRP)

PRP represents the first generation of platelet concentrate that was initially defined by Marx and coworkers in 1998.[17] PRP is a platelet-based bioproduct that by definition is a portion of plasma fraction of autologous blood having a platelet concentration above the normal baseline.[18] Normally, the platelet count in humans ranges between 150,000 and 350,000 platelets per microliter of blood. In the PRP, the total platelet count is 2-5 times or more than the baseline, depending on the used PRP preparation protocol. PRP's functional properties are primarily dependent upon integrating the biological impact of growth factors released from activated platelets with the fibrin glue properties. In this way, the processes of wound healing and tissue regeneration have been improved.[19]

High concentrations of different bioactive molecules are contained within platelet alpha granules secreted from PRP, including various growth factors, inflammatory cytokines, and chemokines, which stimulate neovascularization, modulate extracellular matrix (ECM), promote angiogenesis, and accelerate cellular recruitment, proliferation, and differentiation.[20,21] Moreover, PRP has antimicrobial properties, which are attributed to its ability to release various antimicrobial proteins; however, it's of short acting and inferior to those of antibiotics, so PRP can act synergistically with antibiotics, especially in antibiotic- resistant bacteria cases.[20] Additionally, other mediators that are released by activated platelets (specifically from dense granules) are histamine and serotonin, which increase local vascular permeability and thus facilitate the extravasation of leukocytes into the inflammation sites.[22]

PRP treatment has unique advantages. Because PRP is a bio-product of autologous origin, it is safe and demolishes the probability of immunogenic adverse reactions and the transmission of pathogens and infectious diseases. Additionally, PRP can be obtained in a relatively noninvasive technique and is harmless to the patient.[23,24]

PRP has become increasingly popular as a regenerative therapy in a variety of medical fields, as well as in dentistry, involving regenerative periodontics (periodontal plastic surgery and infrabony periodontal defect treatments), endodontics (pulpotomy, apexification and apical surgery), implant dentistry, as well as oral and maxillofacial surgery (tooth extractions and soft and hard tissue surgery).[25]

The main downsides of PRP are: (i) PRP's therapeutic efficacy relies upon the platelet concentration in the patient's final platelet concentrate; (ii) The presence of anticoagulants; and (iii) As the PRP is activated to stimulate platelet degranulation, the majority of growth factors (about 95%) release within the first 10 minutes. Lack of uniformity in the PRP isolation techniques and the fast outburst of growth factors bring about the generation of a novel platelet concentrate having the capability to conquer these limitations.[26]

In PRP preparation, the presence of additives, chemical or biological, may adversely affect tissue regeneration and delay the healing process of wounds.[9] Thrombin, calcium, or some biologically acceptable anticoagulants may interfere with the coagulation process and cause an immunological reaction.[27] The anticoagulants have a negative influence on wound healing as they inhibit the genesis of a clot, which is necessary for the physiological process of wound healing.[28,29]

Activation of PRP

In PRP, the platelets are activated that cause degranulation, further releasing numerous growth factors and biomolecules from platelet alpha-granules and to initiate clotting process (form the gel). Generally the platelets are activated via one of existing activation methods. An exogenous activators such as thrombin, calcium chloride ($CaCl_2$), a mixture of thrombin and $CaCl_2$, calcium gluconate, collagen, photoactivation, lysis by freezing, mechanical activation, freeze-thaw cycles.[30–33] However, it is possible to apply PRP without activation, as the endogenous collagen or thrombin released by the injured tissue activates platelets without adding any activators.[34–36] However, calcium chloride is a widely utilized PRP activating agent in order to offset the anticoagulant's effect.[37]

https://doi.org/10.18549/PharmPract.2025.1.3104

Anticoagulant agents employed during PRP preparation

There are several choices of anticoagulants including heparin, sodium citrate, acid citrate dextrose (ACD), ethylenediaminetetraacetic acid (EDTA) and citrate-theophylline-adenosine-dipyridamole (CTAD).[38] The process of anticoagulation occurs by inhibiting thrombin activity (heparinates, hirudin) or by binding plasma calcium ions (EDTA, citrate).[39] The preparation of PRP necessitates the use an anticoagulant agent prior to centrifugation of the blood sample in order to prevent early coagulation and consequently prevent spontaneous activation of platelets.[40]

Regarding the anticoagulant employed during PRP preparation, it's crucial to carefully choose one that has ability to preserve the platelets' best possible functionality, morphology, and integrity.[35] Anticoagulants that are commonly used in PRP preparation include acid citrate dextrose-A (ACD-A) and sodium citrate; it has been reported that EDTA is potentially harmful anticoagulant; it has the potential to damage the platelet membrane.[41]

It is widely acknowledged that citrate-based anticoagulants (Na-Citrate and ACD-A) are appropriate in preparing the PRP as a regenerative therapeutic agent. ACD-A has been demonstrated to preserve platelet morphology (size) and functionality (activation and growth factors retention) more effectively than other anticoagulant agents, such as ethylenediaminetetraacetic acid (EDTA) and heparin. ACD-A is considered a suitable choice as an anticoagulant since its chelating ability is less than that of EDTA and it does not adversely affect platelets, leukocytes, or other cells that are essential for the regeneration process.[42] Furthermore, ACD-A is superior to sodium citrate at preserving platelet physiology.[43]

In ACD-A anti-coagulant, the citrate chelates the free ionized calcium, preventing calcium from being available to the coagulation cascade, while other ingredients including dextrose necessary in supporting platelet metabolism and viability. Moreover, ACD-A formulation, maintains the pH of the PRP at 7.2. Though citrate phosphate dextrose (CPD) is comparable to ACD-A, the former is 10% less effective in preserving platelet viability owing to its fewer supportive ingredients.[44,45]

Also, it has been shown that PRP obtained with EDTA anti-coagulant result in an increase in mean platelet volume, which indicates a change in platelet morphology and platelet activation, while ACD-A and Na-citrate revealed no adverse effect on platelet morphology and greater induction of mesenchymal stromal cell proliferation.[46] Likewise, Rachmi *et al*., 2021 suggested that in the PRP preparation, the ACD is a more effective anticoagulant than EDTA, as the platelet rich plasma based on ACD anticoagulant produced a greater platelet number than EDTA without alterations in the platelet index, including the platelet distribution width and mean platelet volume, in comparison to the EDTA anticoagulant.[47]

PRP preparation

Although the method of preparation varies, PRP preparation protocols involve drawing whole venous blood into an anticoagulant-containing tube, then centrifuging and isolating the plasma fraction containing concentrated platelets.[48]

Regardless of the speed and time of centrifugation, generally, PRP is prepared by a two-step centrifugation (Double-Centrifugation) protocol: separation and concentration. The first spin step (soft spin), which is the separation spin, uses different density gradients of components of blood to separate the blood in the centrifuged tubes into three layers: the bottom layer is composed of red blood cells (RBCs); the middle thin layer is called a buffy coat (BC), which is rich in platelets and leukocytes; and the top layer contains platelet-poor plasma (PPP). Then the PPP layer and the BC layer are carefully aspirated and transferred into another sterile tube (without anticoagulant) and subjected to a second centrifugation (hard spin) (concentration spin), which concentrates the platelets toward the bottom of the tube, resulting in the formation of soft pellets (erythrocyte-platelet) at the tube's bottom. The upper 2/3rd is platelet-poor plasma (PPP), and the lower 1/3rd is PRP. The upper portion of the volume, which is PPP, is taken out while the pellets in the lower 1/3rd are homogenized, forming the PRP.[8,35,49,50]

Platelet-rich plasma can be prepared using commercial kits that employ different centrifugation protocols, and consequently, variability in the final product's characteristic are anticipated.[51] Commercial PRP systems are designed for semi-closed preparation using single-step centrifugation; however, these kits are costly and need specific equipment and consumables.[52]

With the use of commercial kits, PRP could be prepared in a safe aseptic technique and in a steady manner with a consistent platelet count.[53] But the overall platelet concentration is somewhat lower than when double-centrifugation approaches are employed.[31,54]

Furthermore, it would be important to keep in mind that, even though the majority of these kits are cleared with the Food and Drug Administration (FDA) and marketed with FDA 'labels,' these clearances (Medical Device 510 (k) clearance) are only for device safety, not for efficacy. Three basic methods are employed in the most widely utilized commercial kits: the gel separator method, the narrow neck tube method, or the automated cell separators.[55]

PRP commercial kits can usually be divided in relation to platelet concentration into high-yielding systems (5-9 times the basal value concentration) and low-yielding systems (2.5-3 times the basal value concentration). Harvest SmartPRep 2 APC+, Biomet GPS II and III, and Medtronic ArterioCyte Magellan are examples of high-yielding systems. While Arthrex ACP, PRGF, Cascade PPR therapy, and Regen PRP are among the lower concentration systems.[35,56]

Platelets Rich Fibrin (PRF)

Platelets Rich Fibrin is a second-generation platelet concentrate and was introduced by Joseph Choukroun in France in 2001 in the field of oral and maxillofacial surgery. This novel formulation is completely autologous; unlike PRP, the preparation of PRF requires neither an anticoagulant nor bovine thrombin (nor any other activating agent). Simply put, PRF is obtained via a one-step process of immediate centrifugation of the individual's whole blood without any additives, producing a fibrin matrix enriched with platelets as well as other bioactive components of blood.[57–59]

The success of the PRF preparation protocol is dependent on the speed at which the blood is collected and transferred to the centrifugal device to undergo the spinning process. Actually, in the absence of an anticoagulant, the coagulation cascade initiates, and the blood samples start to coagulate almost immediately as they come in contact with the collection tube, and it takes a minimal of a few minutes of centrifugation to concentrate fibrinogen in the upper and middle parts of the tube. So the sole mean to produce a PRF that is clinically usable is through quick handling. Failure will occur if the time required for blood collection and launching centrifugation is too long. The fibrin will polymerize diffusely in the tube and will produce only a small blood clot without consistency. Interestedly, the PRF protocol enables the collection of a fibrin clot loaded with serum and platelets.[60,61] Moreover, PRF is simple to prepare, involves standard protocol production, has reduced expense, and could even be applied outside the hospital setting.[19,26,62]

One of PRF's main advantages over PRP is its slower and more gradual breakdown and release of growth factors over time. Because of its clotted formulation, PRF's release of growth factors is much longer and extended over a period of 10 days, whereas PRP releases growth factors within minutes to hours once activated.[63]

PRF promotes cell proliferation, differentiation, migration, and adhesion along with cell signaling activation, also PRF possesses an anti-inflammatory effect, inhibits osteoclastogenesis, and enhances the expression of numerous growth factors in mesenchymal cells.[64]

Biological actions and characteristics of PRF

The biological function of PRF is derived from its composition and structure. The main constituents of PRF are plentiful platelets, an abundance of different autologous growth factors, leucocytes, circulating stem cells, and fibrinogen which is transformed into fibrin, forming a tetra molecular structure and acting as a bio-scaffold.[62,65]

Since platelets are the dominant constituents of PRF, they are considered the key elements accountable for PRF bioactivity. Apart from their crucial role in the formation of blood clots due to their thrombotic action, platelets contain a variety of physiologically active proteins that participate in the signaling cascade of wound healing. All these substances are stored within the platelet granules.[19]

Platelets are important reservoirs of bioactive substances such as growth factors, chemokines, cytokines, and other mediators. After activation, they immediately start releasing these substances that enhance the recruitment of cells, facilitate angiogenesis, and encourage growth and cell morphogenesis, all of which are further involved in the promotion of wound healing and tissue regeneration.[66] Platelets are able to initiate and modulate the host's immunological response by secreting various immunoregulatory cytokines and chemokines that recruit and influence leukocytes, endothelial cells, and lymphocytes to areas of tissue infection or injury.[66,67]

PRF contains a plethora of growth factors, which play a central role in tissue repair and regeneration. Upon platelet activation, they are secreted through exocytosis from platelet granules. The important growth factors of PRF include, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), insulin-like growth factor 1 (IGF1), and epidermal growth factor (EGF).[19,58] It is worth noting that growth factors from PRF are released over a longer time period, releasing steadily over seven or ten days.[68]

Growth factors, which are natural biomolecules, have growth-promoting activities that act as mediators and regulators in cellular processes.[10] These factors capable of promoting neoangiogenesis, revascularization, cell proliferation and differentiation, chemotaxis, and extracellular matrix remodeling, all these are important processes of healing and tissue regeneration.[69]

By attaching to their corresponding receptors on cell membranes, the growth factors trigger downstream signaling pathways and transmit signals into the nucleus to participate in a diversity of cellular processes, such as angiogenesis, inflammation, collagen production, and tissue granulation.[62]

Moreover, among the growth factors, PRF contains numbers of immune cytokines such as:[19]

- Interleukin-1β (IL-1β), which stimulates helper T cells, increased endothelial cell adhesion molecule expression; increased chemotaxis of lymphocytes and phagocytes.
- Interleukin-6 (IL-6), which stimulates differentiation of B-cells, enhances antibodies secretion; and prompts differentiation of naive T cells in cytotoxic T-lymphocytes.
- Interleukin-4 (IL-4) which promotes the activation of macrophages in M2 cells and induces Th2 differentiation, B-cell differentiation into plasmocytes, and B-cell class switching to IgE.
- Tumor necrosis factor-α (TNF-α) which provokes remodeling capacities of fibroblasts, induces neutrophil cytotoxicity, stimulates cell survival and proliferation.

Leukocytes (the body's armed forces) are cells of the body's immune system that function to protect the body against infections or invading foreign substances. PRF contains leukocytes, including monocytes/ macrophages, lymphocytes (T and B-lymphocytes), as well as neutrophilic granulocytes.[66,70] The majority of leukocytes are concentrated in the clot's first 25–30% proximal part.[71] They are key actors in many platelet concentrates that impact their intrinsic biology because of their immune and antibacterial potential, and additionally leukocytes appear to be the source of certain growth factors (namely vascular endothelial growth factors and transforming growth factors β 1).[72]

The fibrin matrix is crucial to PRF's therapeutic outcome.

That is the PRF's main advantage over the PRP. It serves as a three- dimensional biological scaffold for platelets and their secretomes, and leukocytes. Moreover, in order to prolong the favorable therapeutic effects, this biological matrix permits a delayed release of its contents.[73] Fibrin is an activated form of fibrinogen. The soluble fibrinogen is converted into insoluble fibrin, which polymerizes into a cicatricial matrix. In PRF, this process is carried out with the physiological thrombin that is available in the blood sample. During PRF preparation's centrifugation, the slow and natural fibrin polymerization leads to its homogenous three-dimensional organization. This results in the intrinsic incorporation of cytokines and glycan chains in the fibrin networks that are released slowly, giving a long-term beneficial impact of cytokines at the target area.[60] In PRF, its fibrin matrix is elastic, flexible, and very strong, with equilateral junctions due to low thrombin concentration and slow polymerization rate. These interconnected junctions allow the establishment of a fine and flexible fibrin network capable of supporting cytokines and cellular migration to occur. Whereas in PRP, it is characterized by the formation of a fibrin matrix with bilateral junctions and rigid fibrin networks, which are induced by high thrombin concentration and rapid polymerization. This sudden polymerization permits the cytokines and growth factors to become trapped extrinsically between the fibrin network and their massive release within the first hour.[74,75] The undifferentiated mesenchymal cells use the fibrin matrix as a bio-scaffold that facilitates their differentiation, consequently aiding in tissue regeneration.[76] The fibrin matrix serves as an "adhesive" scaffolding material that enables the adherent cells, including endothelial cells engaged in angiogenesis, to adhere, proliferate, and concentrate at the tissue regeneration site. Furthermore, the 3-dimensional fibrin network serves as an "adhesive" carrier for growth factors that regulates their release as well as prolongs their biological activity.[77]

Mesenchymal stem cells in PRF, which is a source of hematopoietic stem cells, and multipotent cells can be of great significance for regeneration of tissue and bone.[66,78–80]

Types of PRF

Standard platelets rich fibrin (S-PRF)

The original protocol for PRF production, presented by Choukroun *et al*. and known as Choukroun's PRF or leukocyte and PRF (L-PRF).[76] This PRF protocol requires the collection of a 10 mL blood sample in a glass-coated plastic tube free from anticoagulant (the glass surface allows the activation of blood coagulation cascade to produce a solid fibrin matrix); the tube is then subjected to immediate centrifugation for 12 minutes at 2,700 rpm (400 g).[81]

Titanium platelets rich fibrin (T-PRF)

T-PRF was produced in response to the idea that titanium could be more effective in stimulating clotting factors, hence platelet activation, than glass tubes. T-PRF is prepared by centrifugally spinning the venous blood sample in medical-grade titanium tubes and setting the centrifugation at 2800 rpm for 12 minutes. For T-PRF, the fibrin matrix is thicker and tighter than L-PRF.[82]

Advanced platelets rich fibrin (A-PRF)

High centrifugal forces are known to cause cells to settle at the bottom of the tube; nevertheless, it has been suggested that decreasing the speed of centrifugation could enhance the cellular content, such as leukocytes in the PRF matrix, while preventing cell loss. Using plain glass-based vacuum tubes, advanced PRF was obtained by employing a reduced relative centrifugation force of 230 g (1,500rpm) for 14 minutes.[71] This centrifugation protocol in A-PRF results in increasing the quantity of neutrophils and macrophages in the distal region of the fibrin matrix. Additionally, A-PRF provides a significantly larger total number of important biological factors, including various growth factors, osteonectin, osteocalcin, thrombospondin, vitronectin, fibronectin, and fibrinogen.[71] The availability of growth factors along with immune cells accelerates the potential for tissue regeneration through promoting intercellular signaling and tissue-specific macrophage.[26]

Advanced platelets rich fibrin plus (A-PRF+)

Based on the low-speed concept, another protocol called A-PRF+ was introduced. A-PRF+ is a modification of A-PRF protocol. Because the centrifugation force directly impacts the total count of cells trapped in the PRF matrix, researches aimed to lower the centrifugation time, consequently reducing the forces that could cause cell loss. Fujioka-Kobayashi et al. introduced A-PRF+ preparation protocol by decreasing the centrifugal speed to 1,300 rpm (200 g) and the centrifugal time to 8 min. A-PRF+ revealed more growth factor release, including (VEGF, PDGF, TGF-β1, IGF, and EGF) in comparison to A-PRF and L-PRF, thanks to a reduction in pull- down force during the A-PRF+ centrifugation protocol.[83]

Injectable platelets rich fibrin (i-PRF)

The main drawback of PRF in comparison to PRP is its availability just in gel form, which makes it unsuitable for injection. Due to its capacity to be administered as a liquid, PRP could be employed alone or in conjunction with other biological materials in a variety of regenerative therapies.[19]

Injectable PRF is a fully autologous biomaterial. Using plain plastic tubes, its preparation is based on the low-speed centrifugation concept (LSCC), allowing it to be enriched with platelets, growth factors, and white blood cells, all of which are crucial for tissue regeneration process.[84]

LSCC results in the highest release of VEGF and TGF-β1.[84] VEGF is a signaling molecule of great importance for angiogenesis/blood supply;[85] also, TGF-β1 advantageous in tissue regeneration, facilitating the recruitment of keratinocytes, specifically in the early phase of wound healing.[86]

The unique property of i-PRF is that it remains liquid for about 15-20 minutes, during which fibrinogen has not yet been transformed into a fibrin matrix, and following which, by the action of physiological thrombin, fibrin polymerization occurs, forming a gel-like consistency, having a three-dimensional fibrin network enriched with the cellular substances derived from peripheral blood without immunogenicity. This contributes to the release of growth factors more slowly and gradually over time, consequently prolonging their beneficial biological effect.[87,88]

Following the LSCC, i-PRF can be prepared by immediately centrifuging the tube at a speed of 700 rpm (60 g) for three minutes.[7,16,88–90] In this protocol, plain plastic tubes are utilized, since they possess a hydrophobic surface, do not effectively activate the coagulation process during centrifugation.[91] Whereas the glass- based collecting tubes are required to produce solid PRF matrices as they support the activation of the physiological coagulation cascade, forming solid physiological clot. Because of this, plastic tubes that favor the liquid phase after centrifugation allow the production of injectable blood concentrate without the need for an anticoagulant.[90] Accordingly, this centrifugation protocol permits the components of blood to be separated in a few minutes, forming a yellow layer (plasma, platelets, leukocyte, and clotting factors) at the tube's top and other components at lower level. This yellow layer is then aspirated by a small syringe.[19,88]

However, only a 2- to 3-fold increase in platelets and a 1.5-fold rise in leukocyte concentration compared to baseline concentration could be obtained with i-PRF due to the shorter centrifugation time and speed in.[92] During the preparation and while withdrawing i-PRF, the top of the vacutainer should not be opened because the air exposure may lead to the initiation of clotting. I-PRF should be drawn out through the rubber seal itself using a 1.5-inch, 18 gauge needle. This will enable i-PRF to remain liquid for some time.[55]

Applications of i-PRF in dentistry

In orthodontic dentistry, several researches confirmed that i-PRF was effective for improving orthodontic tooth movement. Since PRF has been proven to accelerate the metabolic activity of numerous cells, it was postulated that using i-PRF would enable tooth movement to be attained in a shortened period of time.[93–96]

In the field of dental pulp regeneration, Chai *et al*. (2019) examined the human dental pulp cells in terms of their cellular regenerative capacity when they were cultured with i-PRF or PRP.[97] Compared to PRP, it was shown that i-PRF significantly enhances the migration of pulp cells. Furthermore, in contrast to PRP, i-PRF significantly promotes higher alkaline phosphatase activity and expression of genes encoding collagen type 1, dentin sialophosphoprotein (DSPP), and dentin matrix phosphoprotein 1 (DMP-1). As a result, it was proposed that the regenerative potential of i-PRF could stimulate odontoblastic differentiation in human dental pulp cells and reparative dentin.

In the field of oral medicine, treatment of erosive lesions of lichen planus with i-PRF showed a reduction in lesion size and pain; therefore, injection of i-PRF instead of depot-corticosteroid injection can be used in treatment of oral lichen planus.[82]

In periodontology, several studies have investigated i-PRF in terms of its potential for periodontal regeneration. Promising results were observed upon the use of i-PRF, and demonstrated improved periodontal ligamental/gingival cell proliferation, differentiation, migration, and mineralization after treating with i-PRF biomaterial.[88,89,98] I-PRF revealed an advantageous therapeutic impact on root coverage in a free gingival graft surgical operation and increased new gingival tissue formation.[99] Moreover, in the case of thin gingival phenotypes, the combination of i-PRF with micro-needling showed the highest capacity to increase the thickness of gingival tissue,[100] also injecting of i-PRF into attached gingiva with thin gingival biotypes is promising for improving gingival tissue thickness.[101] I-PRF application following gingivectomy and gingivoplasty is considered a positive influencing factor in terms of wound healing and epithelialization.[102]

Research showed an improvement when i-PRF was used as an injection modality in an attempt to treat various disorders affecting temporomandibular joint. Intra-articular injections of i-PRF resulted in a significant decrease in the pain value also increased the maximal mouth opening.[103,104] Moreover, in the management of Wilkes stage III internal derangement, it was demonstrated that injection of i-PRF after arthrocentesis has superior performance than when the arthrocentesis alone or with hyaluronic acid had been performed.[105]

In the field of dental implantology, it was found that i-PRF can enhance the osseointegration and stability of implant, consequently increasing the success rate of implant treatment,[106,107] due to its quick angiogenesis, tissue regeneration capacity, quicker bone remodeling, and cost effectiveness, so allowing earlier implant treatment.[108] I-PRF is a safe and reliable biomaterial that can be utilized for sinus lifts and reduce the healing period also increase the osteogenisis.[109]

In addition to its uses in the field of dentistry, i-PRF is also used across various medical fields. It is employed for a variety of clinical conditions, including ostheoarthritis, tendon injuries, sports injuries, musculoskeletal regenerative producers, heart surgery, knee arthroplasty, diabetic ulcers, alopecia, acne, and facelift surgery.[110]

Concentrated growth factors (CGFs)

CGF is the third and latest generation platelet concentrates, introduced in 2006 by Sacco. CGF has cross-linked fibrin glue rich with platelets and growth factors. It is a 100% blood -derived product; does not require any exogenous additives. To prepare CGFs, autologous blood samples are centrifuged at alternating and controlled speeds with a specific programed centrifuge (Medifuge, Silfradent Srl, Italy).[111,112]

CONCLUSION

The body is a biological machine that God created in an amazing way. Modern medicine hopes to exploit the body's physiology for self-healing and regeneration, far from synthetic medical materials. Platelet concentrates in their different forms are one of these ways that have great biological benefits (owing to their autologous biological cellular components and their bioactivity); perhaps not all of them have been discovered. They have the ability to treat a wide range of medical conditions in the body with much greater effectiveness than medications and other therapeutic methods. On the other hand, they do not have any side effects, allergies, or immune reactions; they are also inexpensive, safe, noninvasive, and easy to prepare in a clinical setting.

AUTHORS CONTRIBUTIONS

All authors contributed equally in this manuscript. All authors have read and agreed to the final version of this manuscript.

FUNDING

This work did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGEMENTS: We would like to thanks "College of Dentistry at Mosul University" for its help and support.

CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest.

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