# A comprehensive clinical review of Platelet Rich Fibrin (PRF) and its role in promoting tissue healing and regeneration in dentistry. Part 1: Definition, development, biological characteristics and function

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**Summary**

The purpose of Part I of this review is to analyse the available literature on: (i) what is PRF; (ii) how PRF evolved and developed to where it is today; and (iii) what are its biological characteristics and composition and how these key elements function within the clinical environment? The introduction and development of PRF as a biomaterial has set in motion an exciting and promising era in the advancement of tissue healing and regeneration in the fields of dental implantology, periodontology, oral surgery and regenerative endodontics. PRF is an autologous fibrin-based (membrane, matrix or scaffold), living biomaterial, derived from human blood, also referred to as an optimized blood clot. The key elements required to promote tissue healing and regeneration are: the fibrin (serving as a supporting matrix), the platelets (rich in growth factors), and cells (mostly the various populations of leukocytes, and stem cells for their antibacterial, neo-vascularization and regenerative properties). These key elements are all active components of PRF. PRF can be easily prepared at chair-side within a short period of time and provides the surgical wound area or defect not only with a matrix or scaffold permitting cell migration into the defect area but also provides the wound with crucial biological signals or growth factors, that can accelerate the wound-healing and regeneration process. The purpose of PRF technology is to extract from a patients' blood sample these key elements and to prepare it in a clinically usable form such as, a membrane or plug (A-PRF, L-PRF or CGF) or injectable liquid (i-PRF). The function of PRF is to connect the various elements within the fibrin matrix with local tissues (bone and soft tissue) to accelerate neo-angiogenesis within the tissue and to enhance its healing and regeneration potential. The PRF technique continues to develop because it is very easy to prepare, inexpensive, and allows the quick production of natural fibrin membranes, enriched with platelets and leukocytes, that can be used immediately in any clinical situation.

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## Introduction

The prospect of having new therapies, biomaterials and bioactive surgical additives available that will improve success and predictability of patient outcomes in soft and bone tissue healing and regeneration are key treatment objectives in dental implantology, periodontology and oral surgery.

Platelet Rich Fibrin (PRF), a patient blood-derived and autogenous living biomaterial, is increasingly being investigated and used worldwide by clinicians as an adjunctive autologous biomaterial to promote bone and soft tissue healing and regeneration. The gold standard for in vivo tissue healing and regeneration requires the mutual interaction between a scaffold (fibrin matrix), platelets, growth factors, leukocytes, and stem cells.[1] These key elements are all active components of PRF, and when combined and prepared

properly are involved in the key processes of tissue healing and regeneration, including cell proliferation and differentiation, extracellular matrix synthesis, chemotaxis and angiogenesis (neo-vascularization).[2,3] An improved understanding of the development, biological and physiological properties and characteristics of PRF in tissue healing and regeneration over the last two decades, has led to more successful therapeutic applications, especially in the fields of dental implantology, periodontology and oral surgery.

The purpose of this comprehensive review is to analyse the available scientific literature on PRF regarding its: **(Part I)** (i) definition and purpose in the clinical environment; (ii) development and classification of platelet concentrate biomaterials; (iii) biological characteristics and composition and the function of key elements; **(Part II)** (iv) preparation technique, optimizing quality and benefits; and **(Part III)** (v) its clinical applications in implant dentistry, periodontics, oral surgery and regenerative endodontics.

**Part I** of this review will analyse: (i) what is PRF; (ii) how PRF evolved and developed to where it is today; and (iii) what are its biological characteristics and composition, and how these key elements function within the clinical environment?

## Methodology, Search strategy and inclusion criteria

An electronic MEDLINE (PubMed) and Google Scholar search was performed for all articles on Platelet Rich Fibrin (PRF) and Platelet concentrates up to May 2016. The search was complimented by an additional hand search of selected journals in oral implantology, oral surgery and periodontal, as well as gray literature. The reference lists and bibliographies of all included publications were also screened for relevant studies. The search was limited to the English language. Randomized controlled trials (RCT's), controlled clinical trials (CCT's), case reports, case series, prospective, retrospective and in-vitro/in vivo studies were included in the narrative review. Animal studies were excluded from this review.

## What is PRF?

### Definition

PRF is an autologous fibrin-based (membrane, matrix or scaffold), living biomaterial, derived from human blood, [4,5,6,7,8] also referred to as an optimized blood clot.[9] In essence, PRF is a natural (autologous) composite biomaterial, consisting of fibrin, platelets, growth factors and various cell types including leukocytes and stem cells.

The blood concentrate which is obtained after centrifugation has 3 distinct layers: a red blood cell (RBC) base at the bottom, a PRF clot in the middle, and an acellular plasma (platelet-poor plasma [PPP]) supernatant layer at the top. (Figure 1) The PRF clot is composed of two main parts observable with the naked eye: a fibrin yellow portion, constituting the main body, and a red portion located at the end of the clot (full of RBCs). (Figure 2a and 2b) Between these two areas, a whitish layer called the ''buffy coat'' can be observed with the naked eye. PRF can be used directly as a clot (Figure 2a and 2b), or after compression, as a membrane (Figure 3a an 3b) or plug (Figure 4). Alternatively, the supernatant can be aspirated from the vacutube (i-PRF) and used in injectable form.

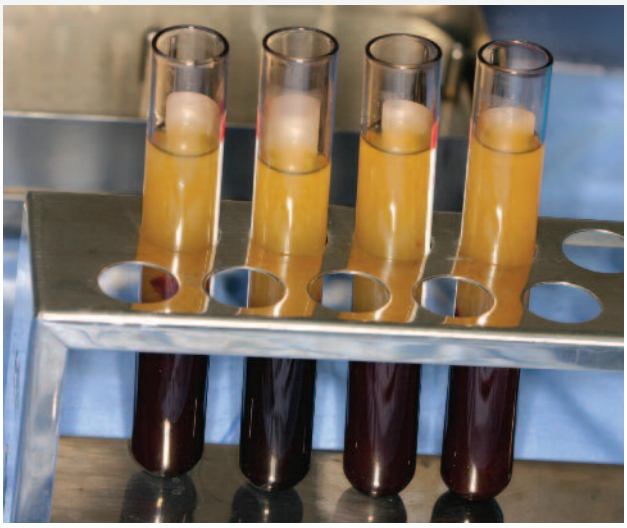

*Figure 1: The blood concentrate after centrifugation has a visible yellow fibrin portion at the top and a red blood cell portion at the bottom.*

## Purpose

The purpose of PRF technology is to extract from a patients' blood sample the essential elements that could be used to improve healing and promote tissue regeneration,[10] and to prepare it in a clinically usable form such as a membrane (A-PRF, L-PRF or CGF) or injectable liquid (i-PRF). The key elements required to improve healing and to promote tissue regeneration are: the fibrin (serving as a supporting matrix), [11] the platelets (rich in growth factors),[7] and cells (mostly the various populations of leukocytes, and stem cells for their antibacterial, neo-vascularization and regenerative properties).[7]

The three-dimensional fibrin membrane is capable of mimicking the extracellular matrix in terms of its structure, and creates the environment or scaffold for cells to function

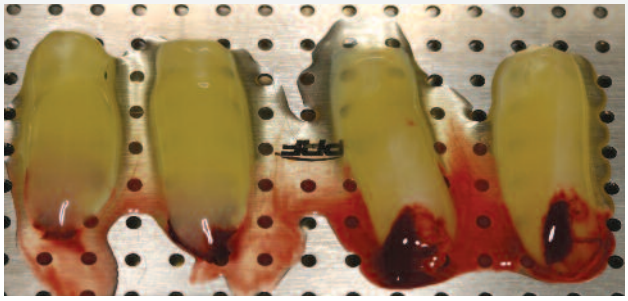

*Figure 2a : PRF clots before compression with distinct yellow fibrin portion at the top and a red blood cell portion at the bottom.*

optimally during healing and regeneration. The fibrin matrix contains the platelets, leukocytes, growth factors and stem cells that act naturally and in synergy to stimulate, improve and accelerate tissue healing and to regenerate soft or bone tissue,[3] including cell proliferation and differentiation, extracellular matrix synthesis, chemotaxis and angiogenesis.[1]

The purpose of the PRF membrane is to connect the various elements within the fibrin matrix with local tissues (bone and soft tissue), to accelerate neo-angiogenesis thereby enhancing healing and regeneration.

## The evolution and classification of patient blood-derived biomaterials

### • Fibrin glue era

Platelet Rich Plasma (PRP) was first applied as a "glue" in surgical procedures in the 1970s [12] and is essentially identical to the present-day fibrin glue (Tisseel™, Baxter, USA)."[13, 14], except that it prepared from platelet-poor plasma (PPP).[15] and that several different protocols for this preparation now exist. Fibrin glue is generally considered to have a positive effect on tissue repair and regeneration.[16, 17] however, their use remains limited owing to the complexity and cost of production. The autologous fibrin glues (with or without platelets) were too complicated and time-consuming to prepare[18] and these techniques were therefore never widely developed.

### • Growth factor era - Platelet-Rich Plasma (PRP) and Plasma Rich in Growth Factor (PRGF)

Breakthrough studies in the late 1990s and early 2000s identified PRP as a promising reservoir for growth factors that could facilitate wound healing and bone regeneration.[19,20,21]

Because of its liquid form, PRP has to be converted to a liquid-form prior to clinical use. This conversion was achieved by adding bovine thrombin to PRP preparations to minimize the rapid diffusion of growth factors at the surgical site. [1, 22,23]

*Figure 2b: PRF clot before compression.*

The concept of "regeneration through growth factors" seduced many clinicians into using this product. However, the key role of fibrin was almost completely neglected for many years. The disadvantage of PRP preparation is that it is technique sensitive and time consuming (requires at least 30 minutes). The use of bovine thrombin for clotting the liquid preparation of PRP also raised the concern of transmission of unknown infections from bovine thrombin to recipients.

The term PRP was used to name all kinds of preparations and techniques without proper characterization of the content and architecture of the tested concentrates leading to contradictory and controversial data. This resulted into a state of confusion that led to the general feeling that PRP's are not so useful in the clinical setting. [24,25] Consequently, PRP's are slowly disappearing due to their complexity of use, cost of production, and mixed clinical results.[26]

Plasma rich in growth factors (PRGF) was subsequently developed by Anitua and co-workers in 1999 [27,28] and is characterized by the elimination of leukocytes to suppress their pro-inflammatory effect.[27]

### • The fibrin and leukocyte era – Choukroun's PRF

In 2006, Choukroun and co-workers developed a novel

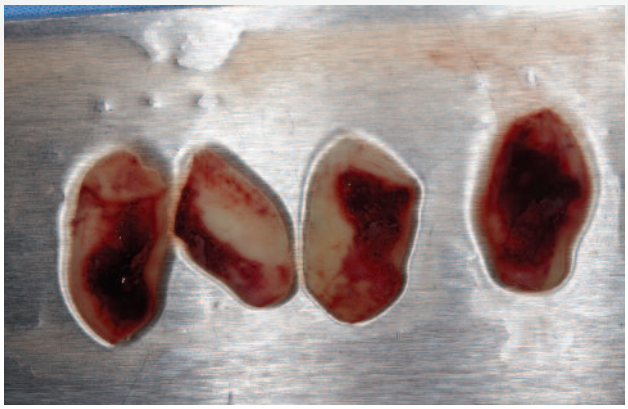

*Figure 3a: PRF clots transformed into membranes after compression in the PRF Box.*

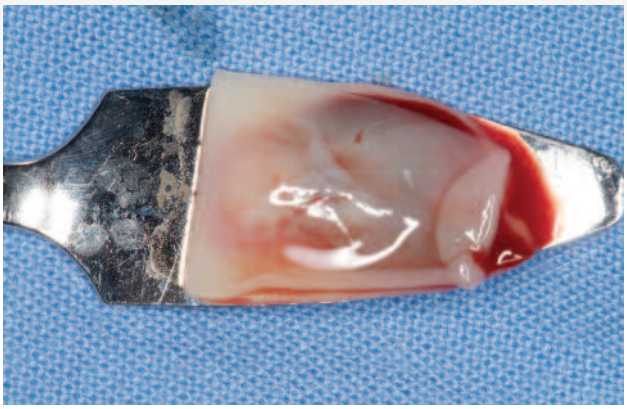

*Figure 3b: PRF membrane after compression of the clot in the Box.*

technique with the aim of simplifying the PRP preparation protocol and to eliminate xenofactors (bovine thrombin).[4,5,29,30,31,32] This lead to the co-called "second generation platelet-derived biomaterials" designated as platelet-rich fibrin (PRF) or Choukroun's PRF. Choukroun's PRF is derived from the patients own blood and contains a variety of blood cells – including platelets, B- and T-lymphocytes, monocytes, stem cells, and neutrophilic granulocytes – as well as growth factors.[33,34]

### • Blood concentrate era

Recently, numerous techniques using blood-derived concentrates have been developed to optimize the different ratios of platelets, growth factors, leukocytes, and other cells within the fibrin matrix.[5,35,36]

A recent study showed that specific cell types are distributed differentially depending on the (cumulative) centrifugal force.[37] This concept has paved the way for preparation of optimal PRF composite biomaterials to be tailored for specific clinical applications. Subsequently, various parties have developed their own centrifuges and kits and associated protocols with the view of improving and streamlining preparation protocols and furthering their commercial interests in PRF.

Choukroun further modified his PRF to produce A-PRF (leukocyte-enriched, advanced type) and i-PRF (injectable PRF)[37,38] by reducing the centrifuge speed; leukocyte infiltration into the red blood cell fraction is minimized. Both A-PRF and i-PRF preparations are characterized by platelets, leukocytes and circulating stem cells, and endothelial cells concentrated in the fibrin clot.[38] Leukocytes are enriched in A-PRF[38] and L-PRF[39] (Choukrouns original protocol used by the Dohan Ehrenfest), to exploit their antibacterial and osteoconductive activity.[1]

In 2006 Sacci developed another preparation called CGF (concentrated growth factors). A centrifuge device that has a special programmed spin cycle is used for the production of CGF. (Medifuge Silfradent srl, Italy).[35] The different centrifugation speed permits the isolation of fibrin matrix that is markedly larger, denser and richer in growth factors as compared to PRF.

### • The future - Stem cell and bone regenerative era

Preparation and application of PRF is, and will in future, increasingly be focused on not only its healing properties, but also how to harness its bone and soft tissue regenerative potential. It is hypothesized that PRF can be a unique source/carrier of hematopoietic stem cells (HSCs), which can potentially play a major role in tissue regenerative dentistry.[40,41]

## Open Access technique

Although this technique is open-access, the initial developers carefully optimized this technique, in order to get the best possible and most reproducible fibrin clots, membranes and clinical results.[39] The PRF technique[4] continues to develop because it is very easy to prepare, inexpensive, and allows the quick production of natural fibrin membranes enriched with platelets and leukocytes.[5]

## Classification of platelet concentrates

Platelet concentrate preparations used in tissue healing and guided tissue regeneration therapy differ according to their preparation from a patients peripheral blood through adding

chemicals, centrifugation speeds and times, and in the selection of supernatants and precipitates. These variations can cause differences in fibrin network structures and in platelets, leucocyte and growth factors content. Therefore the term PRP alone can be non-specific, because it does not define the actual preparation protocol. Recently a full classification of platelet concentrate technologies was suggested[6] that allowed classifying the main available techniques in 4 families depending on their leukocyte content and fibrin architecture:

A. Pure Platelet-Rich Plasma (P-PRP) (i.e. Vivostat PRF, Anitua's PRGF; PRGF-Endoret or E-PRP) – Liquid suspension without leukocytes before activation. (Can be activated and transformed into a gel form – P-PRP gel)

B. Leukocyte- and Platelet-Rich Plasma (L-PRP) (i.e. Curasan, Regen, Plateltex, SmartPReP, PCCS, Magellan) – Liquid suspension with leukocytes before activation. (Can be activated and transformed into a gel form – L-PRP gel)

C. Pure Platelet-Rich Fibrin (P-PRF) (i.e. Fibrinet) – Solid fibrin material without leukocytes.

D. Leukocyte- and Platelet-Rich Fibrin [i.e. Choukrouns PRF; Advanced PRF, (A-PRF), and injectable i-PRF, (Duo Process, Nice, France); L-PRF (Intra-Spin, IntraLock, Boca-Raton, FL, USA); and Concentrated Growth Factors (CGF) – Solid fibrin material with leukocytes.

## The biological characteristics and composition of PRF - how do these key elements function within the clinical environment?

Promoting tissue healing and regeneration is an important goal in surgical disciplines. This healing and reparation process is totally dependent on the initial mechanisms of haemostasis. [42] PRF technology draws on the following three fundamental principles and biological processes of haemostasis[42] and wound healing:[43]

Principle 1: The presence of a fibrin matrix at the surgical site acts as a scaffold for recruiting and migration of cells (epithelial, fibroblast, endothelial) throughout the wound healing and reparation process.

Principle 2: Platelets, leukocytes neutrophils and monocytes within the fibrin matrix (release) secrete growth factors and chemotactic proteins that recruit epithelial, fibroblast, and endothelial cells to the surgical site to facilitate wound healing and reparation.

Principle 3: Angiogenesis (neovascularization) relies on a fibrin matrix (extracellular matrix) and stimulation of endothelial cell recruitment through growth factors (VEGF).

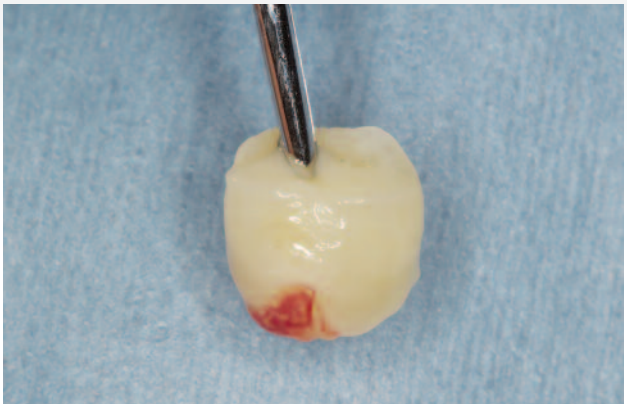

*Figure 4: PRF plug made from compression of the clot in the compression well.*

New blood vessels in the wound bed are essential for carrying oxygen and nutrients to sustain cell metabolism and regeneration.

The concept of generating a fibrin-based cell-seeded matrix solely by drawing blood and the slow polymerization process during centrifugation for 8-12 minutes is truly revolutionary in terms of clinical practicability and simplicity. PRF can be easily prepared at chair-side within a short period of time and provides the surgical wound area or defect not only with a matrix or scaffold permitting cell migration into the defect area but also provides the wound with crucial biological signals or growth factors, potentially accelerating the wound-healing and regeneration process. The PRF membrane is therefore an ideal source of all key elements involved in the processes of tissue healing and regeneration. [5,37]

The combination of the fibrin membrane for a scaffold, leukocytes, stem cells and growth factors are collectively involved in the key biological processes of tissue healing and regeneration, including cell proliferation and differentiation, extracellular matrix synthesis, chemotaxis and angiogenesis (neo-vascularization). [1,31,47]

### • The Fibrin matrix

The slow polymerization mode confers upon the fibrin matrix its favourable physiologic architecture, loaded or seeded with platelets, leukocytes (B- and T-lymphocytes), monocytes, and neutrophilic granulocytes and mesenchymal stem cells, that is required to support and enhance the healing process. [4,5,7,33,34,38]

The strong 3-dimensional fibrin network functions as an "adhesive" scaffolding material for endothelial cells involved in angiogenesis (new blood vessel formation) to adhere to,

proliferate and concentrate at the site of wound healing and tissue regeneration.[1,48,49] Additionally, the fibrin matrix functions as an "adhesive" carrier for growth factors and matrix glycoproteins, and controls their release and sustains their bioactivity for ≥ 7 days.[11,39,49,50,51]

It is these growth factors [i.e. vascular endothelial growth factor (VEGF)] that attract endothelial cells into the fibrin to stimulate formation of new blood vessels.[49] In the field of tissue healing and regeneration, vascularization plays a crucial role as it ensures a continuous supply of nutrients to, and the removal of waste products from, the scaffold and the implanted or wound region. It is theorized, that the overall quality and quantity of fibrin fibers, in addition to growth factors (GF), may potentially affect the potency and efficacy of PRF, both directly and indirectly, in tissue healing and regeneration.[1] A recent study concluded that age could potentially play a significant role in altering fibrin network patterns and hence, its interaction with platelets thus, influencing the quality of the PRF clot expected treatment outcomes.[52]

### • Platelets

Platelets in the fibrin matrix play a crucial role not only in haemostasis, but also in the wound healing process. Platelets are distributed throughout the entire clot and merged within the fibrin-rich scaffold or mesh like a cement.[53] After activation; they become trapped within the fibrin matrix and immediately start releasing growth factors.[34,54]

Platelets are important reservoirs for growth factors since they release high concentrations of these biologically active proteins that support recruitment of cells from the surrounding host tissue, and stimulate growth and cell morphogenesis, thus promoting bone and soft tissue healing.[37,49,55,56]

Entrapped platelets, releases a broad spectrum of cytokines, chemokine's, growth factors, and other mediators that facilitate angiogenesis and tissue healing and regeneration. With these different growth factors, adhesion molecules, and other mediators, platelets have the ability to initiate and modulate host immune responsiveness through recruiting and influencing leukocytes, neutrophils, monocytes, and endothelial cells, as well as lymphocytes to sites of tissue damage or infection. Upon stimulation, platelets actively participate in pathogen detection, capturing, and sequestration. They can even induce the death of infected cellular targets.[37,57]

### • Release of Growth factors

After platelets are activated they start releasing high concentrations of growth factors.[34,54] The PRF membrane stays intact for at least 7 days and releases continuously large quantities of growth factors (such as transforming growth factor-β 1 [TGFβ1], platelet-derived growth factor-AB [PDGF-AB], vascular endothelial growth factor [VEGF]), and key coagulation and healing matrix proteins or cytokines (trombospondin-1, fibronectin, vitronectin, osteocalcin, osteonectin).[11,37,39,58,59,60]

A recent study showed that Choukroun's new formulation of PRF called advanced PRF (A-PRF) had a more gradual release of growth factors up to a 10-day period. A-PRF stimulated significantly higher growth factor release over time when compared to standard PRF and may prove clinically beneficial for future regenerative procedures.[61]

It is generally accepted that growth factors have an essential role in influencing processes of healing and tissue regeneration, including angiogenesis, chemotaxis, cell proliferation and differentiation, and the synthesis and degradation of extracellular matrix proteins (matrix remodelling).[37,39,49,55,62,63]

Platelets are not the only blood cells that release growth factors, but also leukocytes and erythrocytes contain TGF-β1[64] and VEGF.[65,66]

The presence of these growth factors (TGF-β & VEGF) are important for stimulating cell proliferation, matrix remodelling and angiogenesis during healing processes and tissue regeneration.[63,64,67] In-vitro studies suggest PRF is capable of increasing osteoblast attachment, proliferation and simultaneously up-regulating collagen-related protein production. These actions in combination could potentially effectively promote bone regeneration.[68]

### • Leukocytes

Leukocytes are the cells of the immune system that are involved in protecting the body against infections or foreign bodies. Different types of leukocytes are concentrated in the fibrin matrix, namely Lymphocytes (T-lymphocytes, B-lymphocytes), Monocytes and Neutrophilic Granulocytes.

Leukocyte enriched PRF (A-PRF and L-PRF) is reported to be an ideal provider of leukocytes.[37] Leukocytes are enriched in A-PRF and L-PRF, primarily to exploit their antibacterial and osteoconductive actions.[1] Most leukocytes are found in the first 25– 30% proximal part of the clot.[37] The leukocytes enmeshed into the dense fibrin network are alive and functional as an immune node that is able to stimulate defence mechanisms.[30, 53] Leukocytes living in the fibrin matrix are also involved in the production of significant amounts of growth factors, particularly TGFβ1.[11,50,58,63,69]

### • Neutrophilic granulocytes

Neutrophilic granulocytes are considered to be early inflammatory cells due to their phagocytic capacity, participating in the process of wound debridement and revascularization.[70, 71] Macrophages also support cell proliferation and tissue restoration following injury through expression of VEGF, PDGF, FGF, TGF-α and TGF –β, and other biologically active molecules (e.g., BMP-2). [72,73]

Neutrophils also facilitate trafficking of monocytes into the wound to phagocytise inflammatory remnants (necrotic and apoptotic cells). [70,74]

A recent study found that changing the centrifugation protocol in terms of centrifugation time and speed leads to a different distribution pattern for neutrophilic granulocytes.[37] Accordingly, a higher presence of these cells might be able to influence the differentiation of host macrophages and macrophages within the clot after implantation. It is therefore hypothesized that A-PRF might influence bone and soft tissue regeneration, especially through the presence of monocytes/macrophages and their growth factors. However, the relevance and feasibility of this tissue-engineering concept has to be proven through in vivo studies.[37]

### • Monocytes

Monocytes are in essence the 'vacuum cleaners' of the body. They migrate into the wound or inflamed area after the influx of neutrophils, where they then become macrophages.[75] The macrophages collect and remove all the dead, necrotic, bacterial, or foreign particles in the wound site. This function is essential for healing and regeneration of tissue. Monocytes also have a beneficial effect on healing and tissue regeneration bone growth, the production of vascular endothelial growth factor (VEGF) and neovascularization. [42,43] This process however needs to be validated through histochemical and histological studies.

### • Mesenchymal stem cells (MSC)

It is hypothesized that PRF may be a unique source or carrier of hematopoietic stem cells (HSCs) that potentially may be of major importance in bone regenerative procedures. Recently, studies highlighted the differentiation potential of HSCs.[40] Ling He and co-workers were also able to show that different cell types, such as rat osteoblasts, could differentiate and proliferate when cultured on the leukocyte-rich PRF (L-PRF).[41]

Stronger focus will in future be placed on the up-regulation of MSC and osteoblasts in bone healing, osseointegration, and in particular, guided bone regenerative procedures.

## Conclusion

The introduction of PRF as a autologous biomaterial has set in motion an exciting and promising era in the advancement of tissue healing and regeneration in the fields of dental implantology, periodontology, oral surgery and regenerative endodontics. PRF is an autologous fibrin-based (membrane, matrix or scaffold), living biomaterial, derived from human blood, also referred to as an optimized blood clot. The key elements required to promote tissue healing and regeneration are: the fibrin (serving as a supporting matrix), the platelets (rich in growth factors), and cells (mostly the various populations of leukocytes, and stem cells for their antibacterial, neo-vascularization and regenerative properties). These key elements are all active components of PRF. The purpose of PRF technology is to extract from a patients' blood sample these key elements and to prepare it in a clinically usable form such as, a membrane (A-PRF, L-PRF or CGF) or injectable liquid (i-PRF) that can be used immediately in any clinical situation to improve healing and to promote tissue regeneration. The concept of generating a cell-seeded fibrin matrix, solely by drawing the patients' own blood and centrifugation for 8–14 minutes is truly revolutionary in terms of clinical practicability, as it can be made easily at chair side in a short period of time. More importantly, the use of PRF enables local delivery of a fibrin matrix, cells, growth factors and proteins that provide unique biological properties and cues for promoting new blood vessel formation, and potentially accelerating wound healing and tissue regeneration, whilst at the same time reducing morbidity due to its antibacterial and anti-haemorrhagic effects, with virtually no risk of rejection. The future of PRF and it applications in clinical dentistry, especially in the field of soft tissue and bone regeneration, has enormous therapeutic implications, but developing and strengthening its role in dentistry is dependent on its coherence and scientific clarity. Independent and robust scientific studies are needed to validate and standardize PRF processes and that will enhance therapeutic outcomes.

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