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Platelet-rich fibrin (PRF) in dentistry

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Abstract
Platelet rich fibrin (PRF) is an improved version of Platelet Rich Plasma (PRP), a fibrin matrix which consists of growth factors and cytokine, can serve as a resorbable membrane facilitating wound healing. It is a second-generation platelet concentrate which is prepared from the patient's own blood free of any anticoagulant. This article enriches the benefits and role of plasma-rich fibrin in dentistry.

Keywords: Dentistry, fibrin, platelet concentrate, platelet-rich fibrin

Introduction
The growing interest in the use of plateletrich plasma (PRP) to optimize the healing response of tissues has sparked the development and marketing of a plethora of commercial procedures that are designed to concentrate platelets and suspend them in plasma or a fibrin construct of varying densities [1]. Although these techniques and their resulting products have been summarily grouped under the generic term "platelet-rich plasma", their precise makeup and, therefore their potential efficacy, can vary widely. For example, some PRP products include white blood cells, whereas others do not. In some techniques, exogenous thrombin or calcium chloride is added to activate platelets or to initiate the clotting cascade. Finally, variations in the initial volume of whole blood used as well as the efficiency of platelet recovery varies markedly among PRP techniques and has resulted in a high variation (3- to 27-fold) of growth factor concentration and availability [1]. Therefore, because all PRP products are not the same, the success or failure of a specific PRP or PRP-related product for a specific pathologic indication cannot be universally applied to all PRP products. PRP is obtained by centrifugation of autologous blood of the patients. The result of this centrifugation is a large concentration of platelets in a small volume of plasma. There are many methods for obtaining PRP, each one with specific properties as to capacity of concentration of the platelets and release process of certain growth factors. In order for PRP to have greater efficacy, the ideal concentration of platelets should be roughly 1,000,000μL in a standard aliquot of 6mL [2]. Figure 1
In order to inform our patients we explain them what PRP is and consent them for venous blood colection. PRP has been described in the literature under different names and abbreviations. Some authors define PRP as only platelets, whereas others note that PRP nalso contains increased concentrations of leukocytes, fibrin, and some bioactive proteins. Based on these descriptions, PRP was classified into pure plateletrich plasma (P-PRP), leukocyte- and platelet-rich plasma (L-PRP), and leukocyte- and platelet-rich fibrin (L-PRF) [3, 4]. Still, some authors regard autology and growth factors as unique advantages of PRP and prefer the terms autologous platelet concentrate (APC), or plasma-rich growth factors (PRGFs) [5]. Based on whether PRP is activated to form gel or not, PRP was also divided into platelet plasma (non-activated platelet) and platelet gel or PRP clot (activated platelet) [6]. Various other terms are also used in the literature, including autologous growth factors (AGF) [7], platelet-leukocyte gel (PLG) [8], autologous platelet gel (APG) [9], platelet-rich gel (PRG) [10], and platelet-rich fibrin (PRF) [11]. In view of the general confusion over PRP terminology, it should be emphasized that different preparations of PRP may have the same name despite having different properties, while similar preparations of PRP might have different names despite having the same properties. We find ethical to inform our patients about the commercial name or the product, the biological type of the product, and the content of the product [12]. This article enriches the benefits and role of plasma-rich fibrin in dentistry.

Fig 1: The four centrifuges used to produce L-PRF clots and tested in this study. From left to right: original L-PRF centrifuge

Growth factors in blood

It is also important to understand that inflammation and wound healing are controlled under high regulation by an array of growth factors. Growth factors can either stimulate or inhibit cellular migration, adhesion, proliferation, and differentiation. While growth factors exist in all tissues, it is important to note that blood serves as the main reservoir of numerous growth factors and cytokines promoting angiogenesis and tissue regeneration for wound healing. Growth factors usually exist as inactive or partially active precursors that require proteolytic activation, and may further require binding to matrix molecules for activity or stabilization. Growth factors also typically have short biological half-lives. For example, plateletderived growth factor (PDGF) has a halflife of less than 2 minutes when injected intravenously [13]. Namely, as many cellular processes involved in morphogenesis require a complex network of several signaling pathways and usually more than one growth factor, recent research efforts have focused on schemes for sequential delivery of multiple growth factors [14]. Unlike recombinant growth factors, platelet concentrates create the opportunity to deliver many autologous growth factors simultaneously. Platelets and macrophages release an abundance of factors including transforming growth factor beta-1 (TGF-β1), PDGF, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and insulin- like growth factor (IGF) [13.15]. Below their individual roles are briefly described. Table.1

Table 1: Summary of Growth Factors Released From Platelets

Growth Factor	Origin Cells	Recipient	Action
PDGF	Platelets, endothelial cells, macrophages, monocytes, Smooth muscle cells.	Fibroblasts, glial cells, macrophages/neutrophils smooth muscle cells	Collagenase Secretion Collagen Synthesis Stimulates macrophage and neutrophils
TGF-b	Platelets, Macrophages/monocytes, T-lymphocytes, neutrophils.	Fibroblasts, endothelial cells, epithelial cells, preosteoblasts, stem cells (marrow)	Stimulates osteoblasts, fibroblasts Collagen synthesis Collagenase secretion
PDEFG	Platelets, monocytes macrophages	Fibroblasts, Endothelial cells, epithelial cells.	Collagen secretion Mitogenesis of epithelial cells Chemotaxis
PDAF	Platelets, Endothelial cells.	Endothelial cells	Increases permeability of vessels Increases angiogenesis
IGF-1	Osteoblasts, macrophages, monocytes, chondrocytes	Fibroblasts, osteoblasts, chondrocytes.	Cartilage growth, Replication of preosteoblasts and osteoblasts, Bone matrix formation,
PF-4	Platelets	Fibroblasts, neutrophils	Attracts neutrophils and fibroblasts

TGF-β1: Transforming growth factor β (TGF-β) is a superfamily of more than 30 members described in the literature as fibrosis agents [15]. Platelets are known to be a major source of TGF-β production. The role of TGF-β mediates tissue repair, immune modulation, and extracellular matrix synthesis. Bone morphogenetic proteins (BMPs) are also part of the TGF subfamily. TGF-β1, the predominant isoform, is important in wound healing, with roles in inflammation, angiogenesis, re-epithelialization, and connective tissue regeneration [16].

This growth factor is crucial during bone mformation contributing to osteoblast precursors in chemotaxis and mitogenesis, and stimulates osteoblast deposition of mineralized tissue on the bone collagen matrix. It is also reported that TGF-β1 can upregulate VEGF, thereby favoring angiogenesis and recruitment of inflammatory cells. Although its effects in terms of proliferation are highly variable, for the great majority of cell types, it constitutes the most powerful fibrosis agent among all cytokines and the growth factor commonly released from autogenous bone during tissue repair and remodeling [18].

PDGF: Platelet-derived growth factors (PDGFs) are essential regulators for the migration, proliferation, and survival of mesenchymal cell lineages and promotes collagen production for remodeling of ECM during wound healing [15-18]. Platelets are the major source of PDGF with various groups divided into homo- (PDGF-AA, PDGF-BB, PDGF-CC, and PDGF-DD) and hetero-dimeric (PDGFAB) polypeptide dimers linked by disulfide bonds. They are present in large amounts in platelet α-granules. Interestingly, PDGF is accumulated in high quantities in the PRF matrix and are considered one of the important released molecules over time from PRF. It is important to note that since PDGF has an extremely short half-life, the PRF matrix acts to support its slow and gradual release over time. PDGF is also a major mitogen for osteoblasts and undifferentiated osteoprogenitor cells, fibroblasts, smooth muscle cells, and glial cells. Since it plays such a critical role in the mechanisms of physiologic healing, a commercially available recombinant source (rhPDGF-BB) was made available having received FDA approval for the regeneration of various defects in medicine and dentistry [19].

EGF: The EGF family stimulates chemotaxis and angiogenesis of endothelial cells and mitosis of mesenchymal cells. It further enhances epithelization and markedly shortens the overall healing process when administered. EGF is upregulated after acute injury and acts to significantly increase the tensile strength of wounds. EGF receptor is expressed on most human cell types including those that play a critical role during wound repair such as fibroblasts, endothelial cells, and keratinocytes [15].

IGF: Insulin-like growth factors (IGFs) are positive regulators of proliferation and differentiation of most cell types, which act as cell-protective agents [16]. This growth factor is released from platelets during their activation and degranulation and stimulates differentiation and mitogenesis of mesenchymal cells. Although IGFs are cell proliferative mediators, they also constitute the major axis of programmed cell apoptosis regulation, by inducing survival msignals protecting cells from many apoptotic stimuli [16].

VEGF: Vascular endothelial growth factor (VEGF) is secreted by activated thrombocytes and macrophages to damaged sites to promote angiogenesis. The VEGF family is related to PDGF, and includes VEGFA, -B, -C, -D, and -E. VEGF has previously been isolated and described as the most potent growth factor leading to angiogenesis of tissues, stimulating new blood vessel formation and, therefore, for bringing nutrients and increased blood flow to the site of injury [15, 18]. It has potent effects on tissue remodeling and the incorporation of recombinant human VEGF into various bone biomaterials has been demonstrated to increase new bone formation, thereby pointing to the fast and potent effects of VEGF [19].

Classification

From last few years several techniques for platelet concentrates are been invented. However, their applications have been confusing because each method leads to a different product with different biology and potential uses. The platelet concentrates are procured by the process of centrifugation. The final product is formed of mainly of biological regenerative material i.e. platelets and fibrin. On the basis of leukocyte and fibrin content, a classification was given in which platelet concentrates was placed into four categories [6] Figure 2, 3.

- Pure platelet rich plasma (PRP), such as cell separator PRP
- Vivostat PRF (Vivolution, Alleroed, Denmark)
- Leukocyte and platelet rich plasma (L-PRP)
- Pure platelet rich fibrin (P-PRF)
- L-PRF, such as Choukroun's PRF.

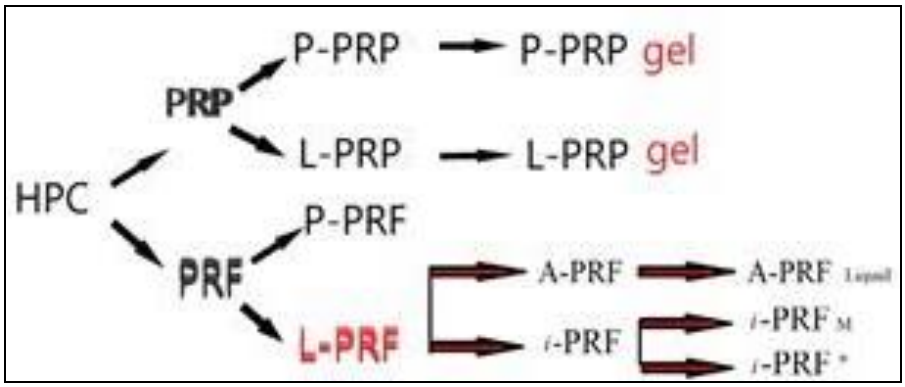

Fig 2: Different types of human platelet concentrates (HPCs): PRP (Platelet-rich plasma); PRF (Plateletrich fibrin); P-PRP (Pure Platelet-rich plasma); L-PRP (Leukocyte and Platelet-rich plasma); P-PRF (Pure Platelet-rich fibrin); L-PRF (Leukocyte and Platelet-rich fibrin); i-PRF (Injectable Platelet-rich fibrin);

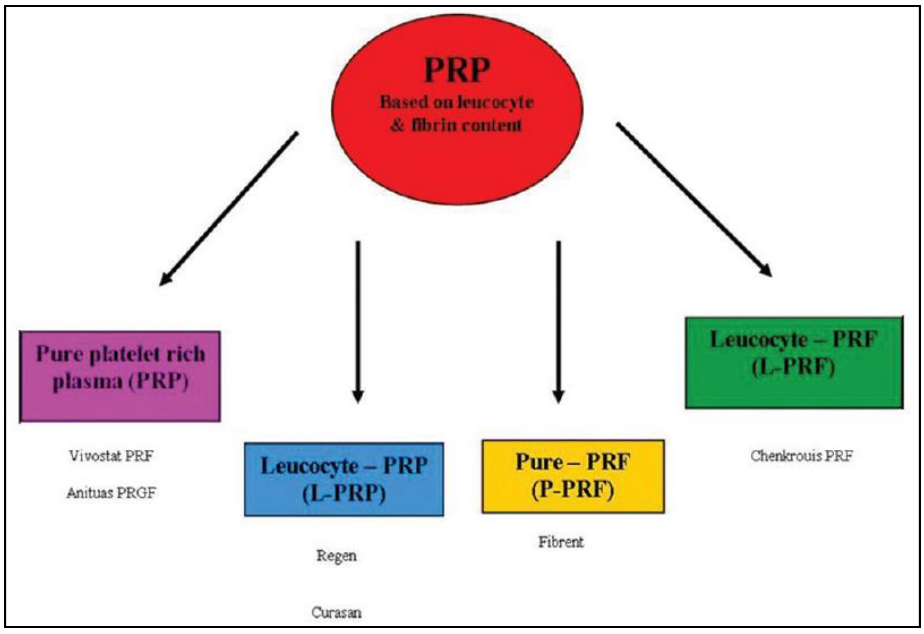

Fig 3: Classification of platelet concentrates

Advantages and disadvantages of its uses

Some studies have demonstrated that PRF is a healing biomaterial with a great potential for bone and soft tissue regeneration, without inflammatory reactions and may be used alone or in combination with bone grafts, promoting hemostasis, bone growth, and maturation [19, 20]. This autologous matrix demonstrated in the *in vitro* studies a great potential to increase cell attachment [7]. Simonpieri., *et al*. reviewed advantages of the use of PRF as it acts as a stabilizing sheath and offers mechanical sustenance, such as the regeneration through PRF membranes both the bone volume and gingival tissue. They also reported satisfactory clinical results related to reshaping the whole alveolar bone and the restoration of gingival volume and peri-implant bone, achieving adequate mechanical and aesthetic properties [15]. Table. 2

Table 2: The advantages of Platelet-rich fibrin over Platelet-rich plasma and disadvantages of Platelet

Advantages of Platelet-rich fibrin over Platelet-rich plasma	Disadvantages of Platelet-rich fibrin
No biochemical handling of blood	Amount available is low, because of autologous blood
Simplified and cost effective process and use of bovine thrombin and anticoagulants not required	Quick handling of blood is needed, immediately after collection
Favorable healing due to slow polymerization	
More efficient cell migration and proliferation	
PRF has supportive effect on immune system	
PRF helps in haemostasis	

PRF = Platelet-rich fibrin

Advantages and disadvantages of its uses

Comparing to PRP it has several advantages over it: it's a minimized autologous blood manipulation [19] including easier preparation. This entire process is natural, without any external manipulation leading to the absence of any immunological reaction [8]. So, it's not required a chemical manipulation of the blood, which makes it strictly an autologous leukocyte-platelet-rich fibrin matrix [8, 11]. Which acts as a biodegradable scaffold [20] that favors the development of microvascularization and is able to guide epithelial cell proliferation and migration to its surface [8]. It has a natural fibrin mesh with growth factors within that may keep their activity for a relatively longer period and stimulate tissue regeneration [7]. Used as a membrane, it avoids a donor site surgical procedure and results in a reduction in patient discomfort during the early wound-healing period [12]. It is an economical and quick option compared with recombinant growth factors when used in conjunction with bone grafts [11]. It can be used in combination with bone grafts or as one layer, depending on the manipulation [21]. Comparing to PRP, it's more efficient and shows better clinical results [13]. While talking about disadvantages: The success of the PRF protocol depends on blood collection time and its way on to the centrifuge [8]. The final amount available is low because it is an autologous blood [10] and for the process is needed a glass-coated tube to achieve clot polymerization [3, 4].

Method for Formation of Platelet-Rich Fibrin

As Chououran *et al* described PRF preparation, neither anticoagulant nor bovine thrombin were used and it was just to avoid all the restrictions of French law regarding blood derived product. The protocol for PRF preparation is very simple; however it has to be prepared just prior to its use. Requirements [19]: Figure 4

- Blood collection armamentarium
- Glass test tube (without anticoagulant)
- Table centrifuge.

The main advantages in PRF preparation are the single stage centrifugation. The blood obtained from the subject is placed into the sterile test tube and centrifuged immediately for 10 minutes at 3000 rpm. 7 Others have used 2700 rpm for 12 minutes with similar findings [8]. Figure 5

The steps involved are as follows [19, 22, 23]:

- Blood specimen is collected or drawn from the patient intravenously.
- The blood specimen is drawn in the test tube and centrifuged immediately (REMI Laboratories) and allowed to spin immediately for the stipulated time.
- Following this the blood sample settles into various layers.
- Three different layers are formed which are as follows:
- Red coloured lower fraction containing the RBCs,
- Middle fraction containing the fibrin clot,
- Straw-colored upper fraction contains acellular plasma or platelet poor plasma.

The results of this technique depends on speed of blood collection and its transfer to centrifuge. The upper portion of the test tube contain the acellular plasma which is removed. The middle portion containing the fibrin clot is then removed by the help of tweezer and is been squeezed off from the lower part that contain the red blood cells [24, 25]. Figure 6

Three different layers are formed which are as follows:

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- Middle fraction containing the fibrin clot,
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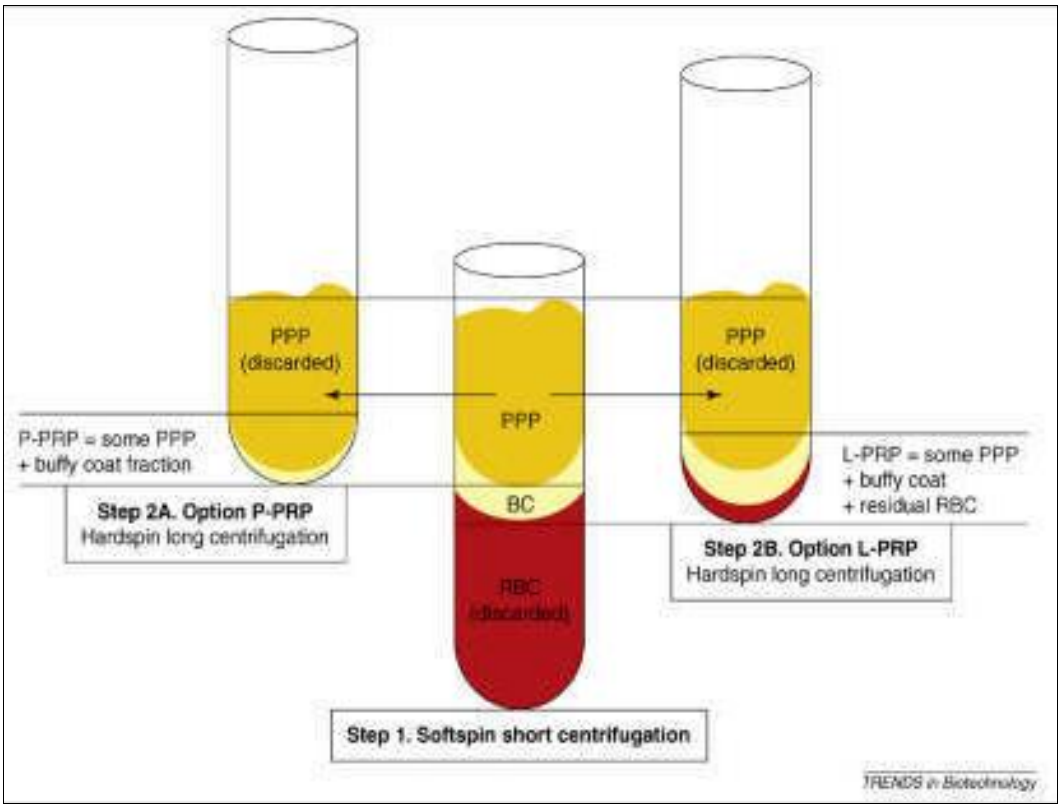

Fig 4: Classical manual platelet-rich plasma (PRP) protocol using a two-step centrifugation

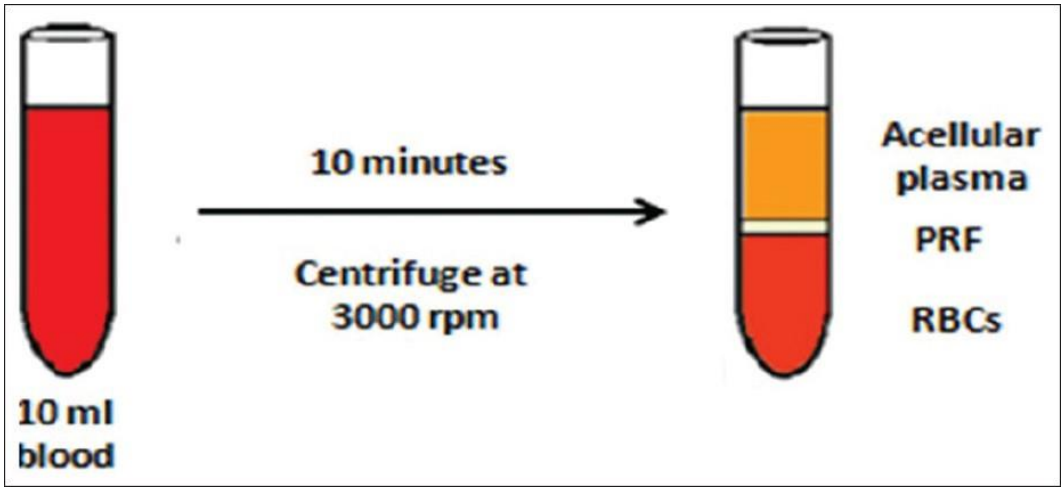

Fig 5: Processing of platelet-rich fibrin

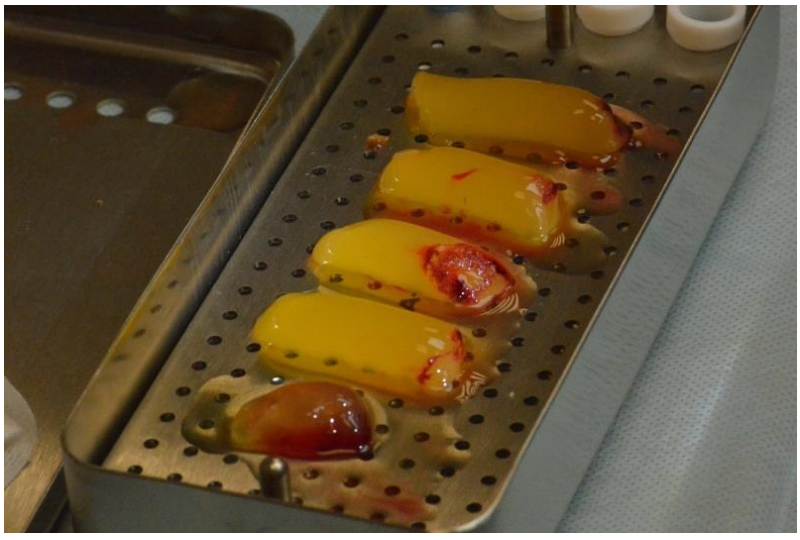

Fig 6: PRF clot, when placed on bone or soft tissue, will gradually excrete growth factors

Role of PRF in dentistry

In recent times a lot of research has been done on PRF, and numerous cases have been reported regarding the use of PRF clot and PRF membranes. Majority of the research has been concentrated on the use of PRF in oral surgery for bone augmentation, sinus lifts, avulsion sockets and in periodontics to correct intrabony defects, gingival recession, guided bone regeneration, periapical lesions, etc. It has also been used for regenerative pulpotomies, periapical surgeries, open apex regeneration, etc. [26] Table.3

Table 3: Clinical applications of PRF

Various fields	Clinical application
Periodontal regeneration	Root coverage procedures
	Clinical conditions requiring good bone fill along with gain in clinical attachment
Oral surgery	In extraction sockets
	Preprosthetic surgery
	Periapical surgery
Implants	To enhance osseointegration of implants
Endodontics	For regeneration of the pulp tissue,

In periodontics

In periodontics, PRF has been used to treat gingival recession, intrabony defects and periapical lesions. Some case reports show the use of a combination of PRF gel, hydroxyapatite graft and guided tissue regeneration membrane to treat intrabony defect. Some studies show the use of PRF gel and PRF membrane in combination with a bone graft for treating a tooth with a combined periodontal-endodontic lesion. Some studies show the use of two layers of PRF membrane with to cover the defect. The membranes are very thin and inhomogeneous, and leukocytes and platelet aggregates are believed to be concentrated in end of the membrane. Therefore, two layers of membrane in opposite sense can be used to prevent the resorption of the thin membrane and to allow the entire surgical area to be exposed to same components (leukocytes and platelet aggregates) [25, 26, 28, 29]. PRF as a potential novel root coverage approach has been reported by Anil Kumar *et al*. For covering localized gingival recession in mandibular anterior teeth using combined laterally positioned flap PRF can promote the healing of osseous defects by the following mechanisms. According to Chang *et al*. the expression of phosphorylated extracellular signal-regulated protein kinase are promoted by PRF. Moreover, it stimulates the production of osteoprotegerin (OPG) which in turn causes proliferation of osteoblasts. Another study by Huang *et al*. reported that PRF stimulates the osteogenic differentiation of the human dental pulp cells by up-regulating OPG and alkaline phosphatize expression. Growth factors such as platelet-derived growth factor and TGF which promote periodontal regeneration are released by PRF [29].

In Endodontics

PRF can be used as a scaffolding material in an infected necrotic immature tooth for pulpal regeneration and tooth revitalization. Other than that, it also can be used in a combined form with mineral trioxide aggregate (MTA) as an alternative for creating artificial root-end barriers and to induce faster periapical healing in cases with large periapical lesions. Usage of PRF in the form of a membrane can prevent the extrusion of material. PRF also can be used in regenerative pulpotomy procedures where the coronal pulp is removed, and the pulp wound is covered by PRF followed by sealing it with MTA and glass ionomer cement. PRF has also been used to fill in the bony defects after periapical surgeries like root-end resection [30].

PRF also act as a scaffold in revascularization of immature permanent teeth with necrotic pulps as it is rich in growth factors which enhances cellular proliferation and differentiation, and acts as a matrix for tissue growth. In a study by Huang *et al*., they concluded that PRF causes proliferation of human dental pulp cells and amplifies the protein expression of these dental pulp cells which differentiate into odontoblast-like cells. This has validated the success of PRF usage in open apex treatment [31, 32].

In tissue engineering

PRF usage as tissue engineering scaffold has been in a constant investigation by the researchers. Gassling *et al*. reported PRF appear to be superior to collagen as a scaffold for human periosteal cellular proliferation, and PRF membranes can be used effectively for *in vitro* cultivation of periosteal cells for the purpose of bone tissue engineering. Thus, PRF is a beneficial in tissue engineering, but clinical aspects of PRF in this field requires further investigation [33].

In oral and maxillofacial surgery

Studies show that PRF can be used as filling material in extraction sockets especially during filling material in extraction sockets; PRF will act as a stable blood clot for neovascularization and accelerated tissue regeneration. This can be used to improve wound healing in immunocompromised and diabetic patients. Besides that, PRF can be used as an adjuvant in patients on anticoagulant therapy as it stimulates coagulation and wound closure [13, 34].

PRF has been commonly used in sinus lift procedures. Besides that, the use of PRF as the sole filling material during sinus lift and implantation. Besides that, studies have proven the use of PRF in combination with other bone graft materials in various direct and indirect sinus lift techniques such as bone-added sinus floor elevation, osteotome-mediated sinus floor elevation, and minimally invasive antral membrane balloon elevation. Some studies also show the use of PRF in combination with beta TCP without bone graft in sinus lift procedures and chronic periodontal lesions [34].

Its proven that in condition with bony walls are intact shows favorable results during filling of avulsion sockets with PRF. A combination of PRF with bone substitutes and other

adjuncts may be necessary in residual defects where one or several walls are missing or damaged to provide an adequate reconstruction of bone volume. Cohesion between the graft materials is increased by PRF as fibrin act as physiological glue between the wound tissues [35].

In case of complicated conditions such as wide sockets and lesions where primary closure is difficult, PRF membrane can be used as a covering and protective membrane that promotes re-epithelialization of the site and accelerates the merging of the wound margins. The elasticity and strength of PRF fibrin membrane make it easy to suture [36].

Use of PRF in various other aspects of dentistry and medicine

The use of PRF in various other facets of dentistry and medicine where PRF has been seldom studied. This includes the use of an injectable PRF into osteoarthritic knees as a second-generation platelet concentrate without use of anti-coagulants. Furthermore, the development of injectable-PRF is also now utilized in the field of facial aesthetics in a similar manner to PRP.

Additionally, this technology is being studied in various other fields including temporomandibular joint disorders, pulp regeneration, treatment of osteonecrosis of the jaw, as well as in the field of orthopaedic medicine [36].

Conclusion

PRF is the new generation of platelet concentrates and has potential applications in medicine and dentistry too. It can be used alone or in combination with grafts or other biomaterials. Although The mechanisms how growth factors work is not clearly understood yet, PRF shows to have beneficial outcomes and satisfactory clinical results giving new perspectives of treatment in dentistry field.

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