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Autologous Platelet Concentrates: Their Generations, Forms, Preparation Protocols and Roles in Periodontal Regeneration

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ABSTRACT

The application of autologous platelet concentrates in wound healing and tissue regeneration, especially in the field of dentistry has been developing with time. There are different methods to produce various forms of autologous platelet concentrates by changing centrifugation speed and time used during preparation and, of course, there is a difference in regeneration capacity according to preparation protocol. In general, autologous platelet concentrates contain various growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factors (IGF) and transforming growth factor (TGF-β) which are considered a key factor in the contribution of periodontal soft and hard tissue regeneration. Autologous platelet concentrates have become more efficient in periodontal and implant surgery due to their positive effects in healing acceleration and new soft and hard tissue formation capability in addition to their other favorable advantages, being inexpensive, timesaving and completely taken from patient. The aim of this review article is to briefly explain the forms and generations of autologous platelet concentrates, their applications, potential role in periodontal regeneration, and to compare between their uses, efficiency and preparation methods to qualify clinicians to use them properly according to the case to achieve better results.

Keywords: Autologous Platelet Concentrate; Wound Healing; Growth Factor; Regeneration

Introduction

Periodontitis is a complex multifactorial inflammatory disease described by loss of connective tissue and destruction of alveolar bone, cementum, periodontal ligament and gingiva as a response to inflammation stimulated by microbial accumulations (1). This leads to the initiation of periodontal defects (2). Periodontal defects are divided into suprabony pocket and intrabony pocket. Intrabony pocket is a pocket with the deepest point of the sulcus is more apical than alveolar bone, which generally also called vertical or angular defect. Whereas suprabony pocket is a pocket with the deepest point of the sulcus is more coronal or at the same level with alveolar bone (3).

Treatment of periodontitis includes surgical and non-surgical treatment (4), where surgical treatment has to be performed if inflammation continues after non-surgical treatment (5). One of the most common surgical treatments of periodontal pockets is an open flap debridement (OFD) (fig. 1 a-b) which aims to eliminate infection by removing inflamed tissues (6).

The basic objective of periodontal treatment is to regenerates lost periodontium, improves periodontal health, reduces pocket depth and obtains healthy gingiva (7). In general, periodontal regeneration is defined as the formation of a new periodontium (bone, cementum, gingiva, and periodontal ligaments) on root surfaces that are already lost before and to restore natural anatomy and function (8). Probing depth (PD), clinical attachment loss (CAL), and bone height (BH) are considered as clinical parameters which can be used to evaluate the success of periodontal regeneration. BH could be evaluated by radiographic examination starting at least after 3 months of regeneration surgery (9).

Some types of autologous products which used in regenerative periodontal treatment are autogenous bone grafts, autogenous soft tissue grafts and autogenous platelet concentrates which contain

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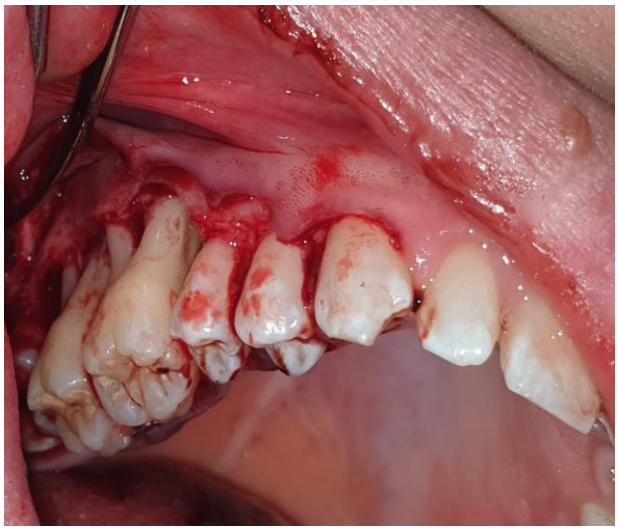

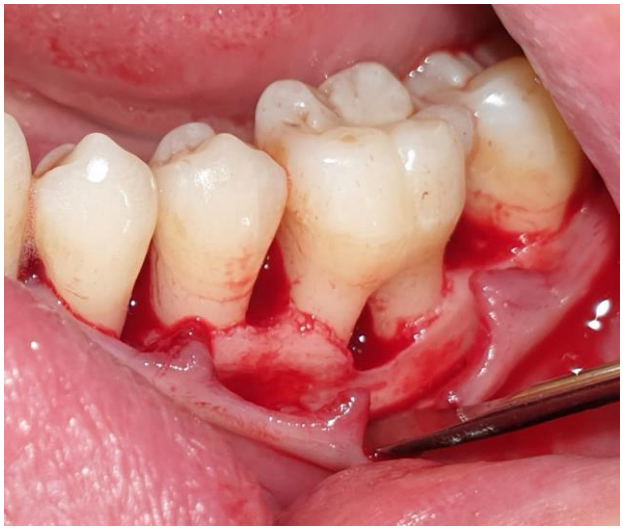

Fig. 1. Open flap debridement after granulation tissue removing shows periodontal defect, affected teeth and raised flap. (a) Maxilla. (b) Mandible

Fig. 2. Centrifugation machine used to centrifuge and produce any types of platelet concentrates

Fig. 3. Platelet rich fibrin (PRF) after centrifugation and taken from blood tube

growth factors (10). Platelet concentrates can be obtained by taking some of the patient's own venous blood and feeding it into special empty tube. Then, the sample should be immediately centrifuged by centrifugation machine (fig. 2), which can produce various platelet concentrates according to centrifugation speed and time used in the preparation protocol.

In general, autologous platelet concentrates are more affordable by patients in periodontal treatment because there is no need to the other operation region to obtain bone or soft tissue graft. So, the operation time, morbidity, pain and patient discomfort will be lesser. Also, autologous platelet concentrates contain multiple growth

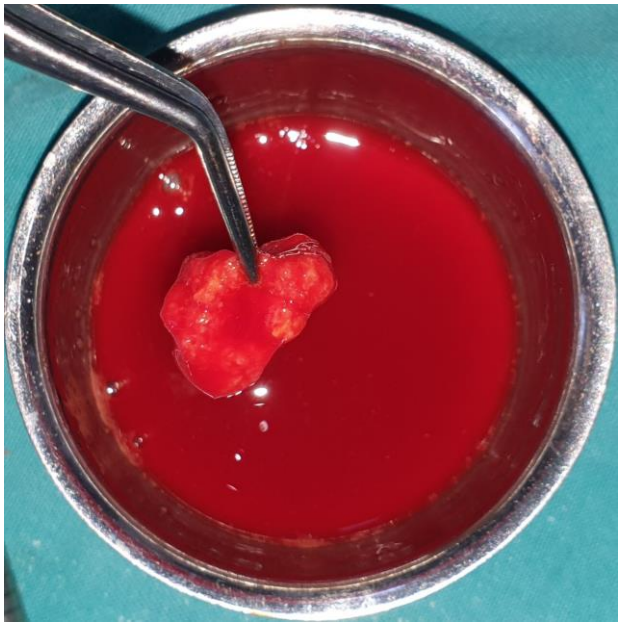

Fig. 4. Sticky bone (MPM) which prepared by mixing i-PRF and bone graft. It is ready to administration into periodontal defect

factors which have tissue regeneration capability. Therefore, this review article will clarify autologous platelet concentrates forms, preparation protocols, their growth factors efficiency, clinical applications, and will show the differences between them.

Autologous platelet concentrates generations: In general, blood compose of four components: platelets, white blood cells (WBCs), red blood cells (RBCs) and plasma. In particular, platelets have a major role in growth factors release in the site of surgery to enhance and accelerate wound healing (11). Platelet Rich Plasma (PRP), the first-generation platelet concentrate composed of limited quantity of plasma which is enriched with platelets obtained from the patient's own blood and can be used in conjunction with bone grafts in the treatment of periodontal defects. Now PRP is not desirable due to its inadequate effects on tissue regeneration and its complicated preparation protocol which is also a bit expensive because of using anticoagulants (12). In order to exceed these limitations found in PRP, the second generation of platelet concentrates (Platelet Rich Fibrin) (fig. 3) have been recently developed, which have desirable properties in comparison to PRP (13).

Platelet rich fibrin (PRF) is a second-generation platelet concentrate as it is a natural concentrate prepared with whole blood taken from a patient without adding any anticoagulants (14). PRF was firstly developed in France by Joseph Choukroun and colleagues in 2001 (15) with the aim of

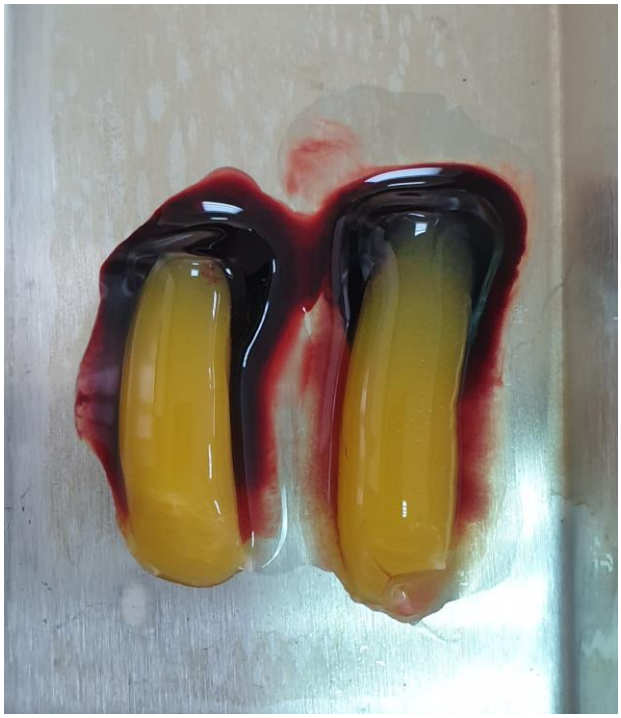

Fig. 5. Concentrated Growth Factor (CGF) after centrifugation. It is thicker, denser and darker in color than classic PRF

simplifying the PRP preparation protocol and overcoming the drawbacks of PRP. PRF has more advantages in compered to PRP, including ease in preparation method which can be prepared without needs to anticoagulant, in addition to its high efficiency in tissue regeneration. It is inexpensive, timesaving, and does not require chemical alteration of the blood, which makes it completely an autologous preparation (16). Also, 70% of the growth factors containing in PRP are released within 10 minutes of activation, and almost 100% are released within an hour. So, it should be used immediately after activation (17). Dohan Ehrenfest *et al (18)* found that PRF membrane can stay intact and release continuously large quantities of growth factors slowly for at least 1 week, due to its fiber network scaffold. Therefore, PRF can promote healing of soft and hard tissues better than PRP. The study also reported that the rate of growth factor release is influenced by the PRF's surroundings. PRF contains growth factors such as platelet-derived (PDGF), vascular endothelial (VEGF), epidermal (EGF), insulin-like (IGF) and transforming growth factor (TGF-β) (19). Growth factors stimulate and attract stem cells to the site of injury, increase proliferation and migration of periodontal fibroblasts, prevent periodontal fibroblast apoptosis and promote angiogenesis, cell mitosis and osteogenesis (20). Also, after

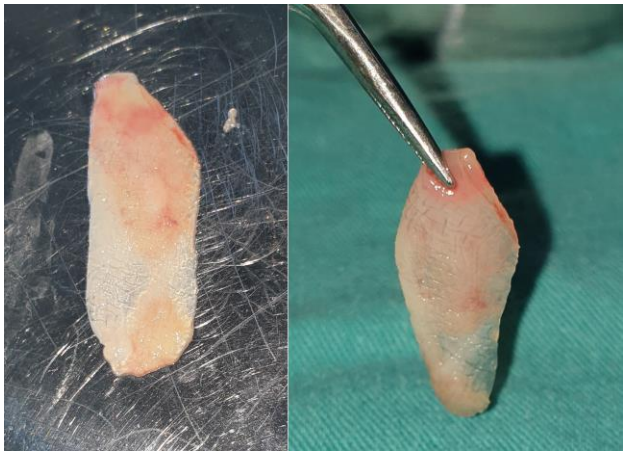

Fig. 6. PRF membrane obtained by PRF compressing. It is ready to use in covering bone graft as barrier membrane in implant and periodontal surgery

activation of growth factors, they stimulate a mitogenic activity of periosteum cells to accelerate bone healing (21). Cytokines (trombospondin-1, fibronectin, vitronectin, osteocalcin, osteonectin) are also released from the platelets, responsible in modulating platelet activation, proliferation and differentiation of leukocytes (22).

However, PRF has some drawbacks such as storage difficulty, as it could not be stored after preparation and needs to be used as quickly as possible after centrifugation. For obtaining better therapeutic benefits from PRF, it requires speed handling because it is strongly depending on the time between blood collection, centrifugation, and usage. So, PRF membrane should be immediately utilized after centrifugation, otherwise it will get smaller, dry and change the structural component of PRF. Dehydration also leads to decrease the amount of growth factor in PRF (18), the leukocytes life will be negatively affected, and their biological characteristics will be altered. PRF if stored in the refrigerator can lead to a risk of bacterial contamination of the membranes (23).

Effects of autologous platelet concentrates in periodontal regeneration: Kim et al showed that the use of bone graft materials with PRP appeared positive results and increased mature trabecular bone density by 15-30% (24). Although some histological studies claim that PRP increases local bone formation (24,25), there are also studies advocating the opposite (16,26). Consequently, PRP slowly disappearing due to some disadvantages, such as their complexity of use (sensitive technique), cost of production, mixed clinical results and time consuming (requires at least 30 minutes) (12).

PRF is considered a healing biomaterial and can be successfully used in different fields of medicine, especially in dental implant and periodontal surgery to promotes bone regeneration and soft tissue wound healing (27). PRF is the source of a wide range of growth factors which are considered the key factor of soft and hard tissue regeneration capacity (15). Therefore, treatment of periodontal and peri-implant intrabony defects by combining open flap debridement and PRF gives better results than OFD alone (28). In surgical procedures, PRF can serve as a resorbable membrane for guided bone regeneration (GBR) (29), blocking non-desirable cells from migrating into the bone defect and creating a place for osteogenic and angiogenic cells to migrate, as well as allowing the underlying blood clot to be mineralized. However, PRF membrane degrades quickly (1-2 weeks) (30).

Recently, researchers developed new products of PRF which aims to improve the properties of PRF and obtain better autogenous biological material by changing the centrifugation time and speed (31) (injectable-PRF, Advanced-PRF, Titanium-PRF and Concentrated growth factor).

Forms of Platelet-Rich Fibrin (PRF)

4. Injectable Platelet-Rich Fibrin (i-PRF) is a liquid form of PRF that can be injected and includes stem cells with a high regeneration potential. The blood centrifuged at low centrifugation speed in non-glass (plastic) tube result in a liquid-form platelet rich fibrin called i-PRF. An injectable PRF has several advantages in relation to PRP (32). Because i-PRF does not include anticoagulants, coagulation occurs quickly after injection or while mixing with biomaterials, allowing for a longer time and more steady releasing of growth factors over than PRP (33). It is usually used in the preparation of growth factors-enriched bone graft which called Mineralized Plasmatic Matrix (MPM) (also known as "sticky bone") (fig. 4) which can easily be handled and manipulated as a desired shape into bone defects. Otherwise, especially in large bony pockets, the handling of bone graft become so difficult. Out of dentistry field, i-PRF has also excellent regenerative potential in hair growth treatment (34).

Advanced Platelet-Rich Fibrin (A-PRF) is a variation of PRF that employs a reduced rotational speed idea. A-PRF has the advantage of maintaining more progressive release of growth factors up to 10 days. So, when compared to standard PRF it has more time of growth factor releasing (33). Slow and more centrifugation time process of A-PRF exhibits more equally distributed granulocyte neutrophils and looser fibrin matrix. Because it did not settle to the bottom of the tube due to slow centrifugation, the number of leukocytes collected in A-PRF is larger (31).

Table 1. Centrifugation Protocols of Platelet Concentrates

Platelet Concentrate Type		Centrifugation Protocol	
Platelet-Rich Plasma (PRP)	Whole blood	(first soft spin) 1200 rpm	12 min
	Plasma	(second hard spin) 3300 rpm	7 min
Platelet-Rich Fibrin (PRF) or (L-PRF)		2700-3000 rpm	12 min
Injectable Platelet-Rich Fibrin (i-PRF)		700-800 rpm	3-4 min
Advanced Platelet-Rich Fibrin (A-PRF)		1500 rpm	14 min
Titanium Platelet-Rich Fibrin (T-PRF)		2700 rpm	12 min
Concentrated Growth Factor (CGF)		2700-2400-2700-3000 rpm	2-4-4-3 min

Leukocytes, particularly macrophages, play a significant role in bone repair by improving the differentiation of osteoblasts, which increase bone production and stimulate tissue integration. In addition, leukocytes limit pathogen invasion, thereby reduce the chance of infection risk (35). According to a research that evaluates growth factor release in PRP, PRF, and A-PRF, A-PRF releases considerably more amount of growth factor than the others (33). Later, A-PRF may shows more future clinical benefits for regeneration treatment, so more researches are needed.

Titanium Platelet-Rich Fibrin (T-PRF) is a new development of PRF that can be prepared by changing the tube material using a more biocompatible material (Titanium). This is the only difference between classic PRF and T-PRF (13). Titanium may be more efficient in activating platelets than silica activators which usually used with glass tube in L-PRF preparation method. The use of titanium material is a try to eliminates the speculations about the undesirable possible effects of silica from dry glass or glass-coated plastic tubes (36). Although the preparation protocol for T-PRF and classic PRF are quite similar, T-PRF has distinctive features due to the titanium-induced platelet activation. It could be hypothesized that T-PRF may persists little longer in the tissue (36), because the fibrin of T-PRF seemed more tightly woven, thicker and have more polymerized fibrin than that of the classic PRF (37).

Concentrated Growth Factor (CGF) is an advanced second-generation platelet concentrate produced by using different continued centrifugation protocol and consists of a variety of growth factors that can stimulate and accelerate bone formation and soft and hard tissue healing. Furthermore, CGF can also improves the quality of the produced new bone. CGF is a relatively new invention at the field of regenerative treatment. It is different from platelet-rich plasma (PRP) in the preparation method, because no additives are added during preparation. It has a different preparation protocol by alternating and controlling centrifugation speeds using a special centrifuge machine, which permits the formation of a considerably bigger and denser fibrin matrix, which is richer in growth factors than those observed in PRP and PRF (38) (fig. 5). CGF contains fibrinogen, coagulation factors, leukocytes, growth factors, and platelets for angiogenesis and tissue remodeling in addition to providing a matrix for cell migration. CGF increases releasing of fibroblast growth factors-β (FGF-β) and vascular endothelial growth factors (VEGF), which are essential for angiogenesis and migration enhancement of neutrophil by performing integrin release (39).

Clinical applications of autologous platelet concentrates: PRF has a wide uses in the fields of oral and maxillofacial surgery, periodontology, plastic surgery, orthopedics, urology, gynecology and dermatology and can be used to treat nerve injuries, hair loss, tendonitis, osteoarthritis, burn wounds, and chronic leg ulcers (40,41). In general, PRF can be useful wherever regeneration is required. In periodontics, PRF can be used in regenerative treatment of both soft tissues and mineralized hard tissues (42) as a result of releasing growth factors (43). Some surgical clinical applications are socket preservation after extraction, root covering procedure, guided tissue regeneration and sinus elevation. It also shows effective outcomes in treatment of periodontal intra-bony and furcation defects that lead to a noticeable reduction of probing depth and attachment loss (20).

PRF also has a wide scope in implant surgery that is considered a healing biomaterial in implant and plastic periodontal treatment procedures due to stimulating hard tissue regeneration and soft tissue healing (44). PRF can be used with grafting materials and as a barrier membrane (Compressed PRF), (fig. 6) as an alternative to collagen membrane in managing of periodontal and peri-implant pockets. Growth factors which released from PRF can promote osteogenesis and osseointegration (45). So, PRF has the ability to enhance osseointegration of implants (46). i-PRF is usually used in the preparation of sticky bone, which is used in the regeneration of periodontal and peri-implant intrabony defects. Sticky bone can be prepared by mixing i-PRF with bone graft granules and allows for about 5 min for

polymerization to produce a sticky bone. To accelerate the polymerization of i-PRF and to utilize obtaining more growth factors, exudate gotten from PRF or CGF membrane after compression can be added. After placing a sticky bone in the target area, a barrier membrane is used to support and protect the grafted bone in the defect (47). PRF has also some clinical applications in dental prosthetics and endodontics, because the fact that PRF has regeneration capacity of pulp tissue, so can be used successfully in periapical and preprosthetic surgery (48).

CGF improves implant stability and speeds up osseointegration by stimulating osteoblast differentiation and bone healing after implant placement. So, it positively affects the healing time of the implants due to the fact that CGF has larger growth factors amount than other platelet preparations. The new bone formation rate around the CGF-treated implant is higher than that treated with classic PRF. So, implant cavity can be lined with CGF membrane before placement of implant (49). For all of the above reasons, CGF has a major importance in implants world because osseointegration is considered a vital factor for stability and success for a longer shelf life (50).

Preparation protocols (Table. 1)

PRP: There are multiple methods of preparing PRP, which differ from an author to another. Despite these differences, all procedures describe two centrifugation process which follow a general sequence that include blood collection, first centrifugation to separate RBCs, second centrifugation to concentrate platelets and then activation the sample. The classical method of PRP preparation starting by taking venous blood from the patient and feeding it into anticoagulant-containing (sodium citrate) tubes. The blood is centrifuged (first soft spin) at 1200 rpm for 12 minutes; the blood separates into three layers: an upper layer that consist of platelets and WBCs; an intermediate thin layer (the buffy coat) which is rich in WBC; and a bottom layer that consist of RBCs. For preparation of pure PRP (P-PRP), the upper and middle buffy coat layer are moved to other empty sterile tube. For preparation of leukocyte rich PRP (L-PRP), few RBCs can also be transferred. The plasma is centrifuged again (second hard spin) for 7 minutes at 3300 rpm to facilitate the formation of soft pellets at the bottom of the tube (erythrocytes and platelets). The upper two-thirds of the plasma (platelet-poor plasma (PPP)) will be eliminated. Pellets are homogenized in the lower third of the plasma producing final form of PRP (51).

The procedure of preparing classic PRF: Firstly, blood will be taken from a patient fed into glass or glass coated plastic tube without anticoagulant and centrifuged immediately at 2,700 rpm for 12 mins, which results in PRF clot in the middle between acellular plasma and RBCs (52). For patient taking anticoagulant medication, up to 18 minutes centrifugation time is recommended (53). Some factors can affect fibrin clot formation are genetic factors, acquired factors (an abnormal concentration of thrombin and factor XIII in the plasma, platelet activation, blood flow, hyperglycemia, oxidative stress, cigarette smoking, and medications), and other parameters (microgravity, pH and temperature) (54).

Preparation of i-PRF: Blood will be collected using empty plastic tubes without any additional material and centrifuged at 700-800 rpm for 3-4 min. The resulted upper yellow fluid (i-PRF) should be collected and used immediately. The aim of using plastic tubes in i-PRF is to prevent clotting during centrifugation, which is intended to be administered to patients while in liquid form (55).

The procedure of preparing A-PRF: A-PRF can be obtained by collection blood from a patient without any anticoagulant in glass tube or glass coated plastic tube, and setting the centrifugation at 1500 rpm for 14 minutes (35).

The processes of preparation of T-PRF: After blood will be drawn from the patient, it will be fed to titanium tube without any anticoagulant, and setting the centrifugation at 2700-2800 rpm for 12 minutes (36).

CGF preparation: After the patient's venous blood will be collected in an empty glass tube without anticoagulant solution, special centrifugation protocol should be followed: 2min 2700 rpm, 4min 2400 rpm, 4min 2700 rpm, 3min 3000 rpm. All these processes can be adjusted automatically by "preprogramming" in special machine or can be manually performed in classic centrifugation machine (38).

PRF has productive results in healing and regeneration of hard and soft tissue; Therefore, it has wide clinical applications in all treatment fields wherever regeneration is required. The use of autologous platelet concentrates in surgical treatment is acceptable by patients due to several reasons, where the sample is taken from the patient; So, there is no risk of infection, no cost is needed, and lesser discomfort suffered by patients. There are multiple forms of PRF used in surgical producers which can be obtained by changing the centrifugation speed and time, and all of them have wide applications in periodontics and implant dentistry due to their leukocytes, platelets, and growth factors. Recent

studies proved that all forms of PRF are more efficient in the regeneration treatment than PRP, which almost has been eliminated from current clinical applications due to some important drawbacks include complicated and long preparation protocol, anticoagulant need and inadequate regeneration capacity in comparison to PRF. Since PRF has promising results, further researches are also needed to find more different formulations which may have more advantages than those currently used.

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