Chapter 1

CURRENT KNOWLEDGE AND APPROACHES FOR THE USAGE OF PLATELET-RICH FIBRIN IN PERIODONTAL REGENERATION

Ece AÇIKGÖZ[1]

INTRODUCTION

Periodontal disease is a multifactorial and complex disease characterized by periodontal tissue destruction and the connective tissue attachment loss. The aim of periodontal therapy is to prevent the progression of periodontal disease by eliminating the existing inflammatory process and also to regenerate damaged periodontal tissues.

Periodontal regeneration is a difficult process that involves biological events such as adhesion, migration, proliferation, and differentiation[1]. Periodontal regenerative procedures include bone grafts, guided tissue regeneration, soft tissue grafts, and combinations of these procedures[2]. In addition to autogenic grafts, different biomaterials are used for periodontal tissue regeneration, such as allografts, xenografts, or grafts derived from synthetically produced alloplastic. However, there is no single material which is considered as the gold standard in periodontal intra-bony defects treatment[3]. It is crucial to note that although most of these biomaterials are promising in different aspects of regenerative dentistry, all of them can lead to foreign body reaction[4]. The generally accepted idea so far is that regenerative periodontal treatments can only recover some of the tissue volume

[1] Assistant Professor, Trakya University Faculty of Dentistry, Department of Periodontology, eceacikgoz@trakya.edu.tr. ORCID iD: 0000-0003-4560-6156

and showed limited success for full periodontal regeneration[2]. Nevertheless, recently it was understood that true periodontal regeneration includes not only the periodontal ligament but also the alveolar bone, cementum, epithelium, and connective tissue. All the mentioned reasons lead us to look for new procedures based on new biomaterials, derived from autologous blood[5].

Wound healing is a multifactorial biological process in which many cellular events occur simultaneously and lead to repair or regeneration of damaged tissues[6]. The healing process includes 4 phases as hemostasis, inflammation, proliferation, and remodeling. These four aspects of wound healing have been identified as key ingredients for successful regeneration and various cell types are involved in each phase. One of the most important factors in these stages are platelets, cells that have been proven to be essential regulators of homeostasis through fibrin clot formation[7]. Vascularization, which is impaired in the wound healing process, leads to platelet aggregation, formation of fibrin, and growth factors release from platelets into tissues through molecular signals mediated by cytokines[3]. Key growth factors that are present in thrombocytes are vascular endothelial growth factor (VEGF), transforming growth factorβ- 1 (TGFβ-1) and platelet-derived growth factor AB (PDGF-AB)[8]. Additionally, fibrin, fibronectin, and vitronectin are also secreted from platelets and function as a matrix for connective tissue and adhesion molecules for greater cell migration[9]. All these roles that platelets play during the healing process have raised the question of whether they can be used to improve tissue recover in periodontal regeneration.

FROM FIBRIN GLUES TO PLATELET CONCENTRATES

In 1970, the first article of Matras was published on usage of fibrin glue to improve wound healing on skin. The fibrin glues were applied as tissue sealants, with fairly good outcomes. However, favorable clinical outcomes were not enough to enhance the

advancement of this technique cause of economic concerns. The autologous fibrin glues were time-consuming and complicated to prepare; therefore, these techniques were never widely developed[10].

Evolving of the fibrin glue procedures into the platelet concentrates was a good chance to replace high priced fibrin glues with other autologous preparations. Since the presence of the autologous platelets reinforced with fibrin gel architecture, fibrinogen concentrates (fibrin glues) were impractical. Like fibrin glues, the procedure of platelet concentrate was first described for the treatment of skin ulcers[11]. But the usage of platelet concentrates in oral and maxillofacial surgery did not gain interest until Marx's study. In this study, Marx et al. used a common name as Platelet Rich Plasma (PRP) and offered a new concept named plasma rich growth factors[12].

In the last 20 years, platelet concentrates used alone or as a matrix for other graft materials and have been developed as a potential autogenous biomaterial in regenerative dentistry. Platelet concentrates are blood extracts, provided from processing a blood sample, commonly through centrifugation[13].

DEVELOPMENT OF PLATELET-RICH PLASMA

Platelet-rich plasma (PRP) is the first-generation scaffold and platelet concentrate derived from blood samples. The accepted mechanism of PRP therapy is to achieve the highest and largest quantities of growth factors from alpha granules of platelets. When alpha factors activated through injury and clot formation, they release transforming growth factor, epithelial cell growth factor, vascular endothelial growth factor, fibroblastic growth factor, platelet-derived growth factor, and insulin-like growth factor[14]. According to many studies, PRP has been shown to have an advantage on the tissue healing and regeneration processes[15].

Platelet-rich plasma preparation requires the additional use of coagulation factors and two separate cycles of centrifugation to maintain proper platelet concentration. Two centrifuge cycles can take between 30 minutes to 1 hour. Furthermore, bovine thrombin and calcium chloride must be added to PRP to provide gel form. Weak fibrin network, short releasing time of growth factors, and all mentioned above reduce the regenerative potential and clinical effectiveness of PRP[5, 7, 16].

Platelet-rich growth factor (PRGF) and platelet-rich plasma (PRP) contain secondary by-products known as both unnatural and inhibitors of wound-healing. Therefore, many studies have attempted to remove these anticoagulants (secondary products) and modify the centrifuge protocol. A few years later, platelet-rich fibrin (PRF) began to affect many medical fields significantly, including dentistry[4].

WHAT IS FIBRIN?

Plasma (55%) and cells (45%) are the main ingredients of blood and plasma contains soluble proteins, electrolytes, and metabolic wastes as well as water (92%). The most notable soluble ingredient of plasma is fibrinogen, a clotting protein[17]. When a vascular injury occurs, thrombin enzymatically converts fibrinogen to insoluble fibrin which acts as a matrix containing growth factors, structural glycoproteins, platelets, and cytokines. This three-dimensional structure acts as a network that seems favorable for the growth of periosteal cells and binds platelets and erythrocytes in clot formation. This first step is essential for wound healing and tissue regeneration[16, 18]. Currently, the natural fibrin scaffold formed at the initial stage of wound healing can be used alone or in combination with other biomaterials and grafts[16].

PLATELET-RICH FIBRIN

In 2001, the second-generation platelet concentrates were presented by Choukroun and colleagues due to the inconsistent out-

comes and preparation difficulties of PRP and PRGF[19]. The most common formulation of second-generation platelet concentrates contains leukocytes and platelet-rich fibrin and usually termed L-PRF (leukocyte-and PRF)[16].

Three-dimensional matrix, locally harvested cells, and bioactive growth factors are the essential components to enhance tissue repair and PRF meets all these components. Because, fibrin serves as a scaffold that includes leukocytes, platelets, macrophages, and neutrophils, attracts regenerative cells to the damaged sites, and acts as a reservoir for growth factors that may be released along 10 to 14 days. PRF releases growth factors for a prolonged period of time in comparison with PRP[20]. In addition to these superiorities, PRF increases the proliferation of osteoblasts, gingival fibroblasts, and periodontal ligament cells, while selectively suppressing the proliferation of epithelial cells[21].

Another major superiority of this technique is the simplicity of preparation. Blood samples in 10 ml tubes are immediately centrifuged for 12 minutes at 2700 rpm[7]. Furthermore, the procedure requires neither anticoagulant, calcium chloride nor bovine thrombin like additives. The absence of anticoagulant means the activation of platelets that contact the tube walls and then the coagulation cascades start in a few minutes[9]. When entire blood is centrifuged at high spin without anticoagulants, three layers are obtained. These layers of tubes include platelet-poor plasma on the above, an intermediate layer named "buffy coat", and red corpuscles at the base (Figure 1). The buffy coat is the layer where most leukocytes and platelets are concentrated and a fibrin clot obtained. It should be used immediately after its centrifugation to eliminate the risk of shrinkage of the fibrin clot through diffusion[22] (Figure 2).

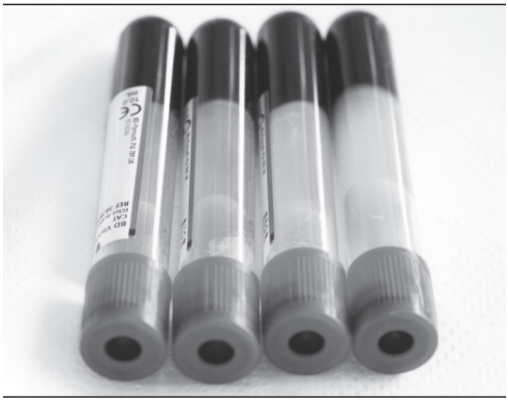

Figure 1. After centrifugation, three tube layers, consisting of PRP, buffy coat, and red corpuscles, are observed from top to bottom.

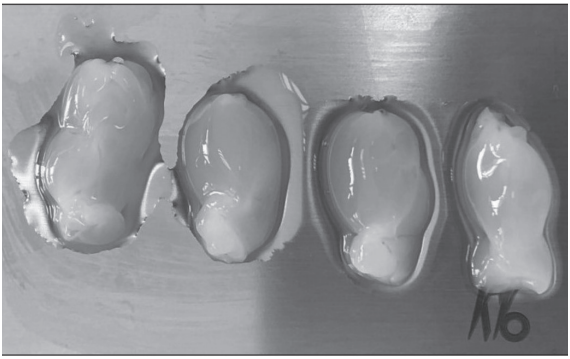

Figure 2. Platelet-rich fibrin obtained from the buffy coat should be applied to the defect sites quickly to prevent shrinkage that will occur over time.

The reduction in the centrifugation forces may lead to greater retention of cytokines and an increase in the number of leukocytes. Moreover, the significant slow release of the main growth factors from 1 week to 28 days has important effects on the healing process[23]. In recent years, alternative procedures based on different relative centrifugation forces including sticky bone, in-

jectable PRF (I-PRF), advanced PRF (A-PRF), and titanium-prepared PRF (T-PRF) were improved upon to original Choukroun's method[24].

Advanced Platelet Rich Fibrin (A-PRF)

A-PRF and A-PRF+ have been improved over L-PRF to support more growth factor release in the wounded tissue (25). In 2014, Ghanaati et al. reported that cells inside the original PRF scaffold were gathered at the bottom. In principle, less centrifugation time increases the total count of cells that remain within the top layer of PRF and enables an advanced number of leukocytes "trapped" within the matrix[5].

Newer formulation of-PRF presented by Choukroun et al. (A-PRF+), does not only lower centrifugation speed but also centrifugation time (1300 rpm for 8 min). It increases the release of TGF-β1, PDGF-AB, PDGF-AA, PDGF-BB, VEGF, epidermal growth factor (EGF) and insulin-like growth factor (IGF) which are necessary for wound healing. Furthermore, A-PRF and A-PRF+ may indicate a significant increase in collagen1 synthesis which is the key factor during wound healing and remodeling[26].

In a study which is conducted by Fujioka-Kobayashi et al.[25], it is concluded that A-PRF demonstrates the highest values of growth factors at 1, 3, and 10th days. Additionally, it is claimed that the release of these factors after 10 days is three times higher when compared to L-PRF. A recently published study reported that the platelet distribution in A-PRF is more widespread and homogeneous compared with L-PRF[27].

Titanium-Prepared Platelet Rich Fibrin (T-PRF)

Although successful results have been reported with L-PRF, in recent years, some researchers have begun to worry about possible health problems that may arise from silica activators in blood collection tubes[28]. Therefore, in 2013, Tunalı et al. modified the primary L-PRF method by changing the glass-evacuated tubes

with titanium which is more biocompatible material and named this modified technique as titanium-prepared PRF.

L-PRF and the T-PRF methods are very alike, but activation of the titanium-induced platelet provides prominent characteristics to T-PRF[8]. Based on scanning electron, light and fluorescence microscopy analysis, it was demonstrated that T-PRF has highly organized network throughout with sustained integrity, and fibrin network which may cover larger areas was thicker compared with L-PRF[24]. In a study conducted by Mitra et al.[29], it is reported that T-PRF had a better fibrin mesh with strong cellular entrapment than L-PRF. Furthermore, in another study published in 2020, it is concluded that T-PRF has a greater cellular distribution for B-lymphocytes, T-lymphocytes, neutrophils, monocytes, and platelets than L-PRF[30]. In conclusion, it can be stated that T-PRF may be an alternative option for L-PRF.

Injectable Platelet Rich Fibrin (I-PRF)

In general, it can be admitted that there are two types of platelet-rich fibrin according to their solid or liquid form. The solid PRF includes the original form of PRF introduced by Choukroun et al. and other PRF types improved in years by other studies. However, the liquid form is called i-PRF[20, 31].

Mourão et al.[32] was developed an injectable platelet-rich fibrin (i-PRF) based on the idea of slow centrifugal force that preparing PRF at higher speed but in a shorter time. This procedure provides a liquid that can be injected directly into the tissue and periodontal pockets or mixed with particulate bone grafts to increase an agglutinated "sticky bone". Sticky bone maintains stabilization of the graft in the bone defects, allows the graft to be easily handled and therefore, reduces bone loss during the healing period by supporting tissue healing[24].

The most significant outcome of this technique is being a suspension that can be manipulated like PRP without anticoag-

ulation. Nevertheless, this type of PRF retains the capability to configure a slow-release matrix once applied to the tissue[31]. In 2017, Miron et al. have recommended that utilizing specific centrifugation tubes at a low speed of 700 rpm (60 g) with a shorter centrifugation time (3 minutes). In this study comparing PRP and i-PRF, it is demonstrated that i-PRF has the capability to release higher numbers of various growth factors, induce migration of fibroblasts and increase expression of PDGF, TGF-ß, and collagen1. Furthermore, they stated that PRP had dissolved entirely following 10 days whereas a further release of growth factors could still be expected from i-PRF[33].

The major advantages of i-PRF are forming fibrin clot, remaining an autologous product with the benefit, and sustaining comparable growth factor release. The only disadvantage of i-PRF is the requirement to apply within 15 minutes of collection[26].

CURRENT CLINICAL APPROACHES OF PRF IN PERIODONTOLOGY

Regeneration of destroyed tissue as a result of periodontal disease is the ultimate goal of periodontal therapy. Periodontal regeneration can be defined as the thorough restoration of lost or injured tissues to their unique structure and function by repeating the wound healing events associated with tissue formation[34].

PRF is an effective biomaterial for wound healing with native regenerative capacity that can be applied in various cases such as furcation involvements, periodontal intra-bony defects, peri-implant defects, gingival recessions, and sinus lift procedures. Also, in the periodontal tissue engineering field, it can be utilized as a scaffold for periosteal cells[24]. In the literature, many studies are investigating the application areas of PRF in periodontology, proposing new procedures, and stating the superiority of different techniques to another[5, 8, 13].

Recently, clinical trials about PRF have shown significant outcomes of osseous growth in intra-bony defects. Since PRF is already a natural matrix for osteoblastic conduction, it can be used with or without bone graft materials and can also stimulate tissue regeneration 3-6 months after application to the periodontal pocket. In two different studies, Thorat et al.[35] and Sharma and Pradeep[36] reported that PRF-treated sites had greater reduction in pocket depth, greater clinical attachment gain, and greater intra-bony defect filling than only open-flap-debridemented sites. Furthermore, Mitra et al.[29] demonstrated that noticeable clinical and radiographic improvements according to baseline values at both PRF and T-PRF treated intra-bony defects after 9 months. A recent meta-analysis is reported that conventional flap surgery with L-PRF compared to conventional flap surgery alone showed significant differences such as greater bone filling in intra-bony defects (1.7 mm), clinical attachment gain (1.2 mm), and probing depth reduction (1.1 mm). Besides, the positive effects of L-PRF on soft and hard tissue healing and reduction of postoperative discomfort were reported[13]. In a research that investigates the effect of PRF in extraction sockets treated with or without membrane, Simon et al. found that sites treated with PRF alone or PRF with membrane showed more rapid healing and had osseous filling by 3 weeks[37]. Similar to intra-bony defects, many studies indicated that peri-implant defects treatment with PRF provides greater bone formation and better clinical outcomes than conventional flap surgery[38, 39].

In guided tissue regeneration, the membrane does not only act as a shield against penetration of epithelial cells but also can release growth factors that improve the osteoblasts activity and promote the healing of the gingival tissue. When PRF is flattened, it can be applied as a barrier membrane in bone grafting procedures[31]. L-PRF membranes do not have any contraindications, they can be recommended in all cases (even in patients under

anticoagulant therapy), they always promote soft tissue healing and reduce the necrosis risk of the flap after surgery. As opposed to other guided bone regeneration membranes, L-PRF membranes act as competitive barriers, that's why they should not be used as a cell-proof shield. L-PRF membranes let cells migrate through, and as a consequence promote angiogenesis and interactions between the gingival flap and the bone[10]. The number of membranes within a site and the convenient blood volume may affect the clinical outcome[13]. Simonpieri et al. [40, 41], in a two-part publication, introduced an innovative technique with using PRF membranes, freeze-dried bone allografts, metronidazole solution (0.5%) together. In this technique, small quantity of 0.5% metronidazole solution provides effective protection to the graft material against bacterial contamination while the membrane component of PRF preserves the surgical site and enhances soft tissue healing.

Furcation defects are considered difficult areas for the treatment due to low accessibility to the operation region and anatomical irregularities. Therefore, these defects are generally treated surgically to allow appropriate root planing, osseous recontouring, and periodontal regeneration. In many studies, it is reported that the combination of PRF and bone grafts has remarkable outcomes in periodontic-endodontic furcation defects. Especially with early grade II furcation treatment usually shows great results[24, 42]. A meta-analysis published in 2019 showed that autologous platelet concentrates may be advantageous in the treatment of furcation defects in adjunct to bone graft or open flap debridement[43]. However, in another meta-analysis published in 2020, Tarallo et al. stated that all the studies reported favorable results with the addition of PRF to conventional flap techniques for the treatment of grade 2 furcation defects. According to these meta-analyses, PRF has positive effects on soft tissue and hard

tissue healing such as reducing vertical furcation depth, vertical pocket depth, and vertical clinical attachment loss[42]. Additionally, in another meta-analysis, statistically significant differences are found in favor of L-PRF again[13].

Manipulation of the tissue is the basic concept in soft tissue surgery. The L-PRF membrane can be adapted and sutured to soft tissue, therefore can open *new horizons in gingival surgery.* The slowly release of growth factors and blood proteins derived from the L-PRF membrane during root covering promotes two important biological mechanisms such as impregnation and induction. Initially, the surface layer of the root is impregnated with blood proteins and secondly, the release of growth factors continues long enough to stimulate cell induction phenomenon. The short term outcome is a rapid wound closure with the reduction of post-surgical edema and pain. Subsequently, the long term result occurs not only as a firm root covering but also as thicker gingiva[10, 44]. Many studies have suggested the use of PRF membranes as an alternative approach to connective tissue grafts for the treatment of gingival recessions. Uzun et al.[45] reported that T-PRF is a safe and effective material for the treatment of multiple Miller Class I and II gingival recession defects. Eren and Atilla[46] treated gingival recessions with coronally advanced flap procedure by using PRF or subepithelial connective tissue graft and they announced development in all parameters with both techniques. In a meta-analysis conducted by Castro et al.[13], stated that L-PRF may be an alternative to a connective tissue graft (CTG) when it was compared with a connective tissue graft and similar results were noted for pocket reduction, clinical attachment level gain, gingival recession reduction and enhancement of keratinized tissue width. Also, in a recent study, the histological assessment revealed earlier angiogenesis and tissue maturation at PRF compared with CTG[47]. At the same time, in a study

published in 2020, a statistically significant difference was detected in favor of subepithelial CTG only in keratinized mucosa width thus PRF membranes were decided to be a promising option to autogenous gingival grafts in the treatment of Miller class I and II gingival recessions[48]. Similarly, according to the results obtained from another meta-analysis, it is indicated that the use of PRF in combination with coronally advanced flap (CAF) significantly improves relative root coverage when compared with CAF alone. However, it did not improve the keratinized mucosa width[49]. Considering all the studies mentioned above, PRF may be preferred as an alternative approach to connective tissue grafts for the treatment of gingival recessions and only in specific cases with narrow keratinized mucosa, the use of CTG might be selected instead of PRF.

CONCLUSION

Platelet-rich fibrin has gain popularity in periodontal regeneration due to ease of application, low costs, and providing a completely autologous reservoir for growth factors. PRF is an effective healing biomaterial with native regenerative capacity that can be applied in various cases in periodontology such as furcation involvements, periodontal intra-bony defects, peri-implant defects, and gingival recessions. T- PRF is recently developed by changing the glass-evacuated tubes with titanium which is a more biocompatible material. Furthermore, recent modifications of the centrifugation speeds and times (A-PRF) improved the regenerative potential of PRF and provided a liquid formulation for injection (i-PRF). Nowadays, current knowledge and approaches are being developed continuously to improve the clinical results of regenerative procedures utilizing platelet concentrates but further studies are needed in order to achieve satisfactory outcomes.

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