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Second generation platelet concentrates in periodontal surgery: A narrative review and clinical perspectives

Emilija Migauskaitė[*], *Jūratė Žekonienė*[*], *Arūnas Rimkevičius*[*], *Giedrius Krukonis*[*]

SUMMARY

Objective. The aim of this article is to analyze the latest scientific literature and focus on the development of second-generation platelet concentrates: platelet-rich fibrin (PRF) and concentrated growth factor (CGF), their fabrication procedure and capability in periodontal surgery.

Material and methods. Scientific articles published in English were selected from PubMed and Cochrane Library databases according to selected keywords. Duplicate and off-topic articles were excluded from further analysis.

Conclusion. PRF and CGF can be considered beneficial biomaterials because they are reported to have favorable biological effects, are low in cost and easier to prepare compared to first generation platelet concentrates and do not cause side effects. Although PRF and CGF demonstrate promising results in the regeneration of intrabony defects, further research is needed. Same as with furcation defect therapy - more evidence of use of PRF or CGF is necessary. In gingival recession treatment PRF or CGF is not yet suggested as first option for treatment as connective tissue graft is still preferable.

Keywords: PRF, CGF, furcation defect, growth factors, intrabony defect, gingival recession.

INTRODUCTION

It has been known for over 20 years that dental practitioners use platelet concentrates to enhance wound healing, improve regeneration, and decrease a risk of post-operative symptoms related to oral surgical treatment. Platelet concentrate is a biological autologous material that is derived from a patient's own blood and contains platelets and growth factors (1). Natural materials often referred as "autologous biomaterials" are present in the body and can provide signals for regeneration, repair, and healing. The process of regeneration involves the reconstruction of periodontal structures. For regenerative procedures to be successful and predictable, a sequence of biological events, such as cell migration, adhesion, growth, and differentiation must be assured (2). As a source of 6–8 times supraphysiological doses of growth factors, platelet concentrates play an important role in wound healing, angiogenesis and hemostasis (3). Platelet concentrates contain many growth factors, including transforming growth factors ß-1 (TGFß-1), platelet-derived growth factors (PDGF), epithelial growth factors (EGF), insulin growth factors-I (IFG-I) and vascular endothelial growth factors (VEGF), which promote cell proliferation and angiogenesis (4). Growth factors found in blood plasma and platelets contain proteins that regulate wound healing processes. The main functions of these proteins are to facilitate cell migration, proliferation, and new blood vessel formation (angiogenesis) during tissue regeneration phase.

Also, various bone substitutes are commonly used in periodontal or oral surgery procedures. Bone substitutes such as alloplastic, allografts or xenografts show promising results, but may cause undesirable foreign body reactions. The advantage of self-derived platelet concentrates is that there is no risk of allergies or rejection and there is no need for antibiotic therapy after use of platelet concentrates.

Many platelet concentrates were introduced since 1998 (5). The method of preparation and biological efficacy of platelet concentrates used today are much better than the ones that were used in the past. There are first generation platelet concentrates such as platelet-rich plasma (PRP) and plasma rich in growth factors (PRGF) and second-generation platelet aggregates including platelet rich fibrin (PRF) and concentrated growth factor (CGF). There was a need to develop a second platelet concentrates generation due to several factors limiting

[*]*Institute of Odontology, Faculty of Medicine, Vilnius University, Vilnius, Lithuania*

Address correspondence to Emilija Migauskaitė, Institute of Odontology, Faculty of Medicine, Vilnius University, Žalgirio g. 117, 08217 Vilnius, Lithuania.
E-mail address: emilija.migauskaite@zalgirioklinika.lt

the use and versatility of PRP and PRGF (6). Firstly, it is well known that thrombin or $CaCl_2$ and coagulation factors must be added to preparation of first-generation platelet concentrates. As a result, anticoagulants found in first-generation platelet concentrates interfere with healing by preventing coagulation and fibrin clot formation. Also, these additives make first generation platelet concentrates a more expensive alternative. Moreover, preparing first generation platelet concentrates takes more time - the solution must be centrifuged twice to increase platelet concentration without incorporating leukocytes (sometimes it might take even up to 1 hour) (6). Lastly, the PRP shows a very short release time for growth factors that, as previously referred, have clinical potential for tissue regeneration. Furthermore, the number of concentrated platelets in PRF is similar as in PRP, but PRF has its own natural fibrin network that protects growth factors from proteolysis (7). In Aizawa *et al.*. study it is said that the main benefits of the introduction of new platelet aggregates generation was to form mechanically tough fibrin matrices using thicker and well-crosslinked fibrin fibers. Secondly, improve the capacity for growth factor retention/release and include more leukocytes. Because of this, many different centrifugal force (speed) and time combinations have been tested (8).

There have been many applications of platelet concentrates in bone and soft tissue healing, including alveolar ridge augmentation, periodontal surgery, socket preservation after tooth extraction, implant surgery, endodontic regeneration, maxillary sinus floor augmentation, osteonecrosis of the jaw caused by bisphosphonates, osteoradionecrosis and oral ulcers (1).

The purpose of this review is to analyze the latest scientific literature on second generation platelet concentrates development and to provide clinically related information about use of two of the platelet concentrates – PRF and CGF – in periodontal surgery.

MATERIAL AND METHODS

The Pubmed and Cochrane Library databases were used to find studies published from 2016 to 2022 about the effects of different autologous platelet concentrates, their fabrication procedure and application potential in periodontal surgery. The keywords used in the preliminary search were as follows: (PRF) OR (CGF) AND (intrabony defect) OR (furcation) OR (gingival recession). Selections of studies were based on English-language. To identify potentially eligible full-text papers, abstracts and titles were reviewed by two independent researchers. Laboratory and clinical studies that included use of at least one of the platelets concentrates for periodontology surgery were selected for the review.

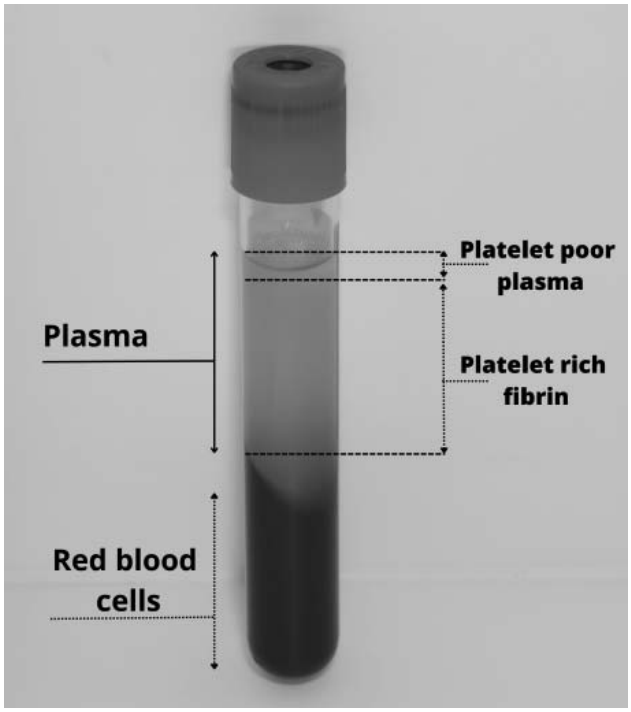

Fig. 1. After centrifugation, blood is separated into 3 layers: red blood cells, platelet rich fibrin and poor platelet plasma

DISCUSSION

PRF

Platelet-rich fibrin is a fibrin matrix enriched with platelets and their growth factors (9). This second-generation platelet aggregation gel is produced from venous blood by single centrifugation without the use of anticoagulant (10). As a result, wound healing cascades are not inhibited by anticoagulants, and clot formation is natural.

Upon centrifugation, blood is separated into two layers: a lower layer containing almost no platelets, mostly made of red blood cells and an upper layer containing plasma (Figure 1). A few minutes after contact with the tube walls, most platelets of the blood sample activate and the coagulation cascades are released - therefore, it is necessary to begin the centrifugation process as soon as possible after blood collection. Fibrinogen in the upper layer combines with circulating trombin and transforms into fibrin. A fibrin clot is then in the middle of the tube between red blood cells on the bottom and acellular plasma – platelet-poor plasma in the upper part. The vast majority of platelets are theoretically trapped in the fibrin meshes (10).

PRF is removed from the tube and separated from the lower red part with sterile scissors before use (Figure 2). At this point PRF is almost ready – the last step is compression with sterile gauze or special fibrin compressor plate as it removes acellular plasma. Now a strong PRF membrane is ready for use (Figure 3). It has

Fig. 2. Plasma removed from the tube

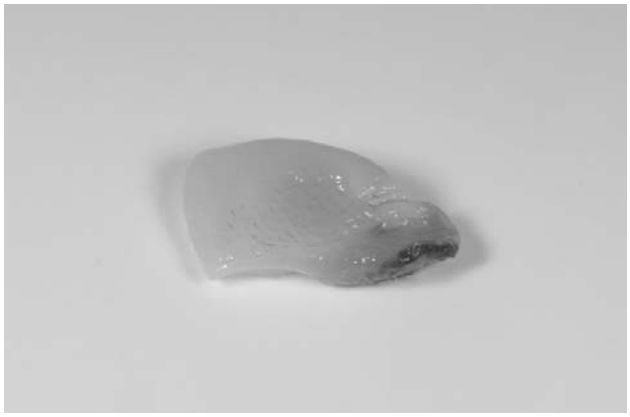

Fig. 3. PRF membrane

demonstrated clinically good handling properties such as flexibility and elasticity, but most importantly - easy suturing ability (11). PRF can be used separately, in combination with bone substitutes or as a membrane.

The disadvantage of PRF is that only a limited amount of PRF can be used. Due to the fact that it is derived from an autologous blood sample, only small quantities can be produced (6). Moreover, not only one more procedure - venipuncture is required but also special equipment is needed. Furthermore, blood samples must be centrifuged immediately. The idea of establishing PRF tissue banks is impractical due to dehydration. Fibrin matrix contains all circulating immune cells and molecules with high antigenicity. Consequently, PRF membranes cannot be used as allogenic graft tissue since they are only specific to the donor (6). While it is an inexpensive method and requires a simple two-step procedure - drawing patient's blood and centrifuging the tube, it is important to keep in mind that blood sampling can only be done by a nurse, anesthesiologist or another licensed specialist. In addition, PRF preparation extends time of surgical treatment.

The preparation of blood concentrates is mainly determined by three parameters: first - on most centrifuges, the round per minute (RPM) parameter appears and can be adjusted, second – the applied relative centrifugal force (RCF) that is calculated based on the centrifuge radius, and the centrifugation time, which is the third parameter.

Many different centrifugation protocols were reported in the literature (10, 11). The following second-generation platelet concentrates are most commonly used in periodontal surgery procedures: leukocyte-rich platelet rich fibrin (L-PRF), advanced platelet rich fibrin (A-PRF) and concentrated growth factor (CGF).

L-PRF

First platelet-rich fibrin protocol was introduced in France by Choukroun *et al.*. in 2001 – a standard leukocyte-rich platelet rich fibrin (L-PRF). Blood samples are taken without anticoagulant in 10-ml tubes and immediately centrifuged at 2700 round per minute (RPM) for 12 minutes (10).

This protocol is called L-PRF because it contains more leukocytes than first-generation PRP. A high concentration of leukocytes promotes wound healing as well as immune and antibacterial reactions (10). Using a high RCF when centrifuging PRF generates a significantly lower concentration of platelets, leukocytes, and growth factors in PRF matrices than when using a low RCF (12). In contrast, by reducing the RCF, PRF based matrices can release more growth factors and increase cell number (13).

While L-PRF is mainly used in scientific research, several changes have been made to the PRF protocol over the years.

A-PRF

Due to the fact that high centrifugal forces shift cells to the bottom of tubes, it was suggested to decrease centrifugation speed to prevent cell loss and increase growth factor numbers. In 2014 a new form of PRF was introduced – advanced platelet rich fibrin (A-PRF), which was obtained by a lower speed centrifugation (13). The modification of the preparation setting based on the low-speed centrifugation concept is also a first step toward reducing RCF. During this step, slower speeds 1300/1500 rpm for 14 min resulted in A-PRF (12, 13). In comparison to L-PRF, A-PRF showed a more porous structure, which leaves more space for trapped platelets and immune cells, also higher and more sustained release of growth factors (13). However, according to Shah *et al.*. A-PRF releases fewer growth factors than L-PRF. A-PRF also showed significantly more neutrophilic granulocytes than L-PRF based on histomorphometric analysis, which means a better antimicrobial function (14). These immune cells influence macrophage differentiation and maturation. By releasing growth factors, macrophages may promote bone and soft tissue regeneration (13).

As it was mentioned before, RCF is calculated based on the centrifuge speed and the centrifugation time. Due to this Fujioka-Kobayashi *et al.*. introduced another A-PRF preparation protocol where not only slower speed of centrifugation but also a shorter centrifugation time was used - 1300 rpm for 8 min (14). The updated protocol A-PRF has a higher level of released growth factors compared to previous PRF protocols (14). Various RCF values result in different second-generation platelet concentrates. There is no one strict PRF preparation protocol encompassing blood collection, centrifugation, and application. As a result, it is difficult to compare studies that have different variations of PRF preparation protocols.

CGF

In 2006, Sacco reported on the newest platelet concentrate - concentrated growth factor (CGF) (15). CGF preparation differs from PRF preparation in terms of the centrifuging. Using a specific centrifuge - Medifuge (Italy), CGF is isolated from the rest of the blood sample using a simple and standardized protocol without any additives. To prepare CGF, cells in the venous blood are centrifuged according to variable value - revolutions per minute, from 2400 to 2700 rpm, resulting in fibrin rich blocks that are larger, denser and are richer in growth factors compared to PRF (16). During the CGF manufacture protocol, acceleration for 30 seconds is followed by 2700 rpm for 2 minutes, 2400 rpm for 4 minutes, 2700 rpm for 4 minutes, 3000 rpm for 3 minutes, and a 36-second deceleration, then a stop. CGF might be called an upgraded form of PRF, with a strengthened fibrin matrix, in which growth factors are closely bound to one another (17). This provides the slow release of growth factors, which might facilitate wound healing and regeneration (18).

As an organic matrix rich in fibrin, CGF has a higher density than other platelet concentrates such as platelet-rich plasma (PRP) or plasma rich in growth factors (PRGF), and is very similar to L-PRF (19). Although in Isobe *et al.*. study it was concluded that CGF membranes were almost identical to A-PRF membranes (16). The fibrin fiber thickness or cross-link density do not differ between A-PRF and CGF clots (16). Also, both A-PRF and CGF preparations contained significant amounts of growth factors (20). The characteristics of CGF enable it to be used in a variety of ways, alone or in combination with other materials (21).

Based on morphological analysis Enrico *et al.*. suggested that PRF and CGF are reliable materials to use in guided tissue regeneration techniques in periodontology since they are suitable for fibroblast cell culture as scaffolds (22).

Platelet concentrates in intrabony defects treatment

Vertical bone defect regeneration potential is considered to be good, especially with three or two walls intrabony defects. Various surgical techniques have been used to regenerate periodontal tissues, including guided tissue regeneration with barrier membranes, bone substitutes, enamel matrix proteins, or their combinations. PRF and CGF not only has a suitable structure for intrabony defects but it also acts as an immune regulator over a 14 day period, controlling inflammation and slowly releasing growth factors which indicates regeneration (23).

The phosphorylated extracellular signal-regulated protein kinase (p-ERK), osteoprotegerin (OPG) and alkaline phosphatase (ALP) activities of periodontal ligament fibroblasts were evaluated for responses to PRF. Amounts of all three of the materials were increased by PRF in periodontal ligament fibroblasts and this enhancement may promote periodontal regeneration (24). Shirakata *et al.*. reported the first study of histological evaluation in gingival and intrabony defects using PRF in dogs (25). As a result of PRF application to two-wall intrabony defects, new bone and new cementum were formed and resulted in periodontal regeneration. In this study intrabony defects were created by using a fissure bur and treatment was done in two groups: open flap debridement (OFD) and PRF group. A limited amount of spontaneous bone formation occurred in the open flap debridement group, but new cementum formation was restricted below the bone crest in most cases. Also, a new cellular cementum was observed, with or without collagen fibers. PRF groups showed new bone formation to varying degrees. However, when compared to the OFD group, new cementum with collagen fibers running perpendicular to root surfaces dominated in all PRF groups. It was noted that when PRF was applied the highly vascular and dense ligament-like tissue between new cementum and bone maintained its width all the way to the coronal portion. While in histomorphometric analysis there was found no difference between OFD and PRF groups comparing defect height, junctional epithelium length, new cementum and new bone parameters. Certainly, regeneration of periodontal tissue in human periodontal defects requires further study.

In 2022 Pepelassi *et al.*. reported a systematic review and meta-analysis of treatment of two or three wall intrabony defects with the use of PRF (26). There were 16 studies with a healthy non-smoker patients and 6-12 months follow up period. Clinical improvements were observed at 0,86 mm in probing depth (PD) reduction, and 1,02 mm in clinical attachment level (CAL) gain in contrast to open flap debridement (OFD). Also, radio-

graphic defect depth by adding L-PRF was reduced by 1,82 mm after surgical treatment. A reduction of almost 2 mm in intrabony radiographic defect depth might be more critical than improving probing depth and clinical attachment level by 1 mm. Also, when radiographs were taken after surgical treatment to evaluate defect fill, resolution, and changes in alveolar crest height, measurements and results might have turned out misleading due to varying angles of radiographs. Also, it is important to note that only two or three wall intrabony defects were included in the study so there are no recommendations for one wall intrabony defect treatment with PRF. Moreover, only L-PRF is included in this systematic review. Yet the protocols of L-PRF defined 2700 rpm or 3000 rpm and 10 or 12 minutes meaning that there were 4 different L-PRF preparation methods the resulted in non-identical materials. Another Miron et. al intrabony defects treatment with PRF systematic review and meta-analysis referred similar results in PD reduction (1,26 mm) and CAL gain (1,39 mm) compared to OFD (27). The results in using PRF with open flap debridement might be more beneficial in this review because of more studies included. Also, protocols of PRF preparation defined 2700 or 3000 rpm and a volume of blood drawn was 5 ml or 10 ml. Therefore, these materials might be considered non-identical. As a result, more research is needed to compare different second-generation platelet concentrates in osseus defects treatment.

An American Academy of Periodontology concluded that first generation platelet concentrate PRP was deemed a weak recommendation for the treatment of periodontal intrabony defects, whereas enamel matrix derivative (EMD) and PRF were highly recommended (28). Nevertheless, intrabony defects treated with PRF had less of gingival recessions after treatment (28). It might be due to the release of growth factors and improved angiogenesis. Moreover, A-PRF seems to be as clinically effective as EMD during surgical treatment of intrabony defects as A-PRF showed significant PD reductions and CAL gains six months post-operatively (29). Yet there is still a need for more evidence, as according to the latest European Federation of Periodontology guidelines, either barrier membranes or EMD should be considered the treatment of choice for intrabony defects (30).

Only two studies were found that introduced using CGF in intrabony defects (29, 31). Compared to OFD alone, CGF significantly reduced mean PD and increased mean CAL (29). After 12 months mean PD reduction in the group where CGF was used was 2,45±0,76, compared to OFD – 1,55±0,93 mm and respectively mean CAL gain was 3,09±1,14 versus 2,36±0,92. Yet clinical improvement of probing depth and clinical attachment level by almost one millimeter is not crucial. CGF and CGF with bone substitute demonstrated significant improvements in clinical and radiographic parameters compared to open flap debridement. However, adding CGF to demineralized freeze-dried bone allograft did not increase benefits (29, 31). Lei *et al.*. compared the level of growth factors released by A-PRF and CGF, as well as their clinical efficacy in regenerating intrabony defects (24). While there was no difference between using A-PRF or CGF in reducing PD, increasing CAL, radiographic bone level height change or defect filling, both groups achieved better clinical outcomes in intrabony treatment with defect depth reduction and defect filling than the OFD alone. Although the results in intrabony defects treatment using PRF or CGF seems promising, more clinical research is still needed.

Platelet concentrates in furcation defects treatment

Periodontal therapy can be challenging when it comes to regeneration of the periodontium within the furcation defect. It is usually difficult to achieve adequate professional debridement of furcation defect because their entrances might be too small for periodontal instruments, and the defects are difficult to instrument because they have ridges, convexities, and concavities. Use of platelet concentrates in periodontal surgery of second grade furcation defects is becoming common (32).

The use of PRF for the surgical treatment of grade II furcation defects had positive effects on both hard-tissue (vertical furcation depth, percentage of bone defect fill and soft-tissue (PD, clinical attachment level (CAL), gingival recession) healing (33). In 2022 systematic review and meta-analysis concluded with the improvement in mean PD reduction (1.20 mm), mean CAL gain (1.06 mm) and radiographic defect depth reduction (1.72 mm) with the addition of L-PRF compared to OFD alone (27). However, this systematic review included only 4 randomized controlled trial studies with furcation defects.

It was also suggested that PRF could be used as a membrane for treating furcation defects of grade II. PRF used as membrane compared to collagen membrane along with demineralized freeze-dried bone allograft made no significant difference and both groups showed better results in PD reduction and CAL gain compared to OFD alone (34). Despite of this, PRF offers the advantages of low costs, good biological effects, and ease of preparation that make it an attractive option compared to collagen membrane. To protect the periodontal tissue regeneration area from epithelial downgrowth, membranes must be maintained for at least 4-8 weeks while PRF membranes biodegrade after approximately 2-3 weeks. Overall, there are no histological evidence of re-

generation with the PRF application in class II furcation defects and there are not enough evidence-based studies. According to the latest European Federation of Periodontology guidelines of Class II furcation treatment, it is recommended to choose periodontal regenerative therapy using enamel matrix derivative alone or bone-derived graft with or without resorbable membranes.

Also, even PRF added to a bone graft did not prove to be advantageous in PD, CAL measurements or gingival recession over bone grafting alone for the surgical treatment of grade II furcation defects (33).

Platelet concentrates in gingival recession treatment

Mucogingival plastic surgery provides complete root coverage, with keratinized and attached tissues. A variety of periodontal plastic surgical procedures have been proposed to recover mucogingival defects using biomaterials or connective tissue grafts (CTG). In addition, L-PRF can provide benefits to coronally advanced flap (CAF) in terms of mean root coverage and gingival thickness gain, while CAF + CTG showed superior results for mean root coverage and keratinized tissue width gain compared to CAF + L-PRF (35). The same results were given in 2020 systematic review and meta-analysis, it was also concluded that it is recommended to use CTG rather than PRF because of keratinized tissue gain (36). According to Anegundi *et al.*., CTG provides superior root coverage and increases keratinized tissue width compared to A-PRF. Although this study suggests that A-PRF can be used in treating gingival recessions (37). As it is not always possible to take a CTG or a patient refuses another surgical intervention due to post-operative pain, PRF might be an alternative. In spite of no clinical differences between PRF and xenogenic collagen matrix, use of PRF may be a good alternative in gingival recession treatment because it is also cheaper and poses no risk of rejection (38). Comparison of the efficacy of L-PRF and A-PRF when combined with CAF for gingival recession defects did not reveal clinical differences (39). Moreover, the use of platelet concentrates in gingival recession treatment does not provide any clinical improvement when keratinized tissue width is less than 2 mm (28).

Bozkurt *et al.*. reported that 6 months after maxillary gingival recession was treated with CGF combined with CAF, keratinized gingiva had significantly increased in width and thickness compared to CAF alone (40). Although CGF is well known for its use as membrane in recession treatment and is preferable due to decreased postoperative pain, CTG is still superior to CGF with coronally advanced flap for increasing keratinized tissue thickness, keratinized tissue width, and root coverage (41). On the other hand, there was no significant difference between groups in terms of plaque index, gingival index, probing depth, recession depth, clinical attachment level (41). Use of tunnel technique and CGF did not improve the results as much as tunnel technique and CTG (42). In short, use of CGF in gingival recession therapy cannot yet be recommended as a first option (42, 43). Dede *et al.*. studies have shown that the use of CGF and A-PRF membranes in gingival recession treatment could increase gingival thickness (44). There was no difference between PRF and CGF in gingival recession therapy (44, 45). All in all, there are not enough evidence-based studies on PRF or CGF usage in gingival recession treatment.

CONCLUSION

PRF and CGF can be considered as beneficial biomaterials because they are reported to have favorable biological effects, are low in cost, easier to prepare compared to first generation platelet concentrates and do not cause side effects. Also, both substances can maintain the release of growth factors for 7-14 days, which is longer than first generation platelet concentrates, making PRF and CGF more important in regeneration. Moreover, there is no need for antibiotic therapy after use of platelet concentrates. Although PRF and CGF demonstrate promising results in the regeneration of intrabony defects, further research is needed. More evidence of successful use of PRF or CGF in furcation defect therapy is necessary as well. Whereas in gingival recession treatment it is not yet suggested to use PRF or CGF as first option treatment as CTG is still preferable.

STATEMENT OF CONFLICTS OF INTEREST

The authors state no conflict of interest.

LIST OF ABBREVIATIONS

PRP	Platelet-rich plasma
PRGF	Plasma-rich in growth factors
PRF	Platelet-rich fibrin
L-PRF	Leukocytes-rich platelet-rich fibrin
A-PRF	Advanced Platelet Rich Fibrin
CGF	Concentrated growth factor
RPM	Round per minute
RCF	Relative centrifugal force
CAF	Coronally advanced flap
CAL	Clinical attachment level
PD	Probing depth
CTG	Connective tissue graft
OFD	Open flap debridement

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