CASE REPORT

Autologous platelet-rich fibrin: a boon to periodontal regeneration - report of two different clinical applications

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Abstract
One of the most important and currently unsolved problems in clinical periodontics is the predictable successful treatment of furcation defects and covering of multiple adjacent recessions. Currently, no single regenerative material can be considered the gold standard in the treatment of periodontal defects. Recently, importance has been given to the use of platelet-rich fibrin (PRF) for predictably obtaining periodontal regeneration. PRF is an intimate assembly of cytokines, glycan chains, and structural glycoproteins, which are enmeshed within a slowly polymerized fibrin network; it has the potential to accelerate soft and hard tissue healing. The purpose of this article is to present the clinical and radiographic results of a Grade II mandibular furcation defect and Millers Class I gingival recession treated with PRF.

Introduction
The primary goal of periodontal treatment is the maintenance of the natural dentition in a healthy state with proper functioning. Regeneration has been defined as the reproduction or reconstitution of a lost or injured part to restore the architecture and functioning of the periodontium. Conventional open-flap debridement falls short of regenerating tissues destroyed by the disease, and current regenerative procedures...
have limited potential in attaining complete periodontal restoration.\textsuperscript{2} Various biomaterials\textsuperscript{3,4} prepared using the endogenous regenerative technology (ERT) have been used for periodontal tissue regeneration in addition to autogenous\textsuperscript{7,8} and allogenic bone grafts,\textsuperscript{9,10} but no single graft material is considered the gold standard.

Choukroun’s platelet-rich fibrin (PRF), which is a second-generation platelet concentrate,\textsuperscript{11} is defined as an autologous leukocyte and PRF biomaterial.\textsuperscript{12-14} PRF consists of an intimate assembly of cytokines, glycanic chains, and structural glycoproteins enmeshed within a slowly polymerized fibrin network. The biologic activity of the fibrin molecule is enough, in itself, to account for the significant cicatricial capacity of the PRF, and the slow polymerization mode confers to the PRF membrane a particularly favorable physiologic architecture to support the healing process.\textsuperscript{15} The beneficial effects of PRF have been studied in various surgical procedures for degree II furcation,\textsuperscript{16} sinus-floor augmentation during implant placement,\textsuperscript{17} use of a coronally displaced flap in multiple gingival recessions,\textsuperscript{18} facial plastic surgery procedures,\textsuperscript{19} etc.; the results of these studies have been promising. In addition, PRF was shown to act as a suitable scaffold for breeding human periosteal cells in vitro with the strongest induction effect on osteoblasts.\textsuperscript{20}

Here, we present two cases in which autologous PRF was used as a biomaterial for achieving periodontal regeneration. Both the patients visited the Department of Periodontics, Government Dental College, Kottayam, Kerala, India, and were otherwise medically fit. In both cases, there were no contraindications for performing periodontal surgeries. All standard pre- and post-surgical protocols were followed, and the patients are on periodontal maintenance care.

**Case 1: PRF in grade II furcation**

A 36-year-old female patient presented with mild pain and sensitivity of a lower left back tooth. Intraoral examination revealed generalized bleeding on probing but no swelling or exudation. The probing pocket depth (PPD) on the mid-buccal aspect of the tooth #36 (first molar) was 7 mm, while the periodontal attachment level (PAL) was 8 mm, without tooth mobility. A Grade II furcation involvement\textsuperscript{21} was noted on the buccal side of #36 with a horizontal probing depth (PD) of 4 mm detected using Naber’s probe. The tooth was vital. A periapical radiograph was taken using the standardized techniques, which revealed the presence of radiolucency in the furcation area of tooth #36 (Figure 1). After a thorough Phase I therapy using ultrasonic scaler and standard Gracey curette, reevaluation was done after 8 weeks. The mid buccal PD of tooth #36 was
5 mm and the PAL was 6 mm. The horizontal PD in the furcation showed no change, with the persistence of Grade II furcation from the buccal side. Therefore, regenerative periodontal surgery using autologous PRF was planned, and informed consent was obtained for the same.

**Figure 1.** Intraoral periapical radiograph showing Grade II furcation defect (osseous defect) in the lower left first molar

**PRF preparation:** PRF was prepared in accordance with the protocol developed by Choukroun et al. Just prior to surgery, intravenous blood (by venipuncturing of the antecubital vein) was collected in a 10-ml sterile tube (Figure 2) without anticoagulant and immediately centrifuged in a centrifugation machine at 3,000 revolutions (approximately 400 g) per minute for 10 minutes. Blood centrifugation immediately after collection allows the composition of a structured fibrin clot in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma [platelet-poor plasma (PPP)] at the top (Figure 3). PRF was easily separated from the red corpuscles base [preserving a small red blood cell (RBC) layer] by using sterile tweezers and scissors just after the removal of PPP and then transferred onto a sterile dappen dish (Figure 4).
**Surgical procedure**

After induction of local anesthesia, buccal and lingual sulcular incisions were made, and mucoperiosteal flaps reflected. Care was taken to preserve as much interproximal soft tissue as possible. Meticulous defect debridement and root planing were carried out using ultrasonic instruments and area-specific curettes (Figure 5). No osseous recontouring was done. Autologous PRF and alloplastic bone graft were mixed together at a ratio of 1:1 (Figure 6), and the mixture was introduced into the furcation defect (Figure 7). The mucoperiosteal flaps were repositioned and secured in place by sling sutures by using 3-0 non-absorbable black silk (Figure 8). The surgical area was protected and covered with periodontal dressing. Postoperative instructions were given, and the patient was prescribed amoxicillin 500 mg t.i.d and paracetamol 500 mg t.i.d for 5 days. The sutures were removed after one week. Surgical wounds were gently cleansed with 0.2% of chlorhexidinedigluconate, and patients were given instructions for gentle brushing with a soft toothbrush. Healing was reviewed in subsequent visits at 1, 3, and 6 months.
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**Results**

The patient was examined weekly up to 1 month after surgery and then at 3 and 6 months. No subgingival instrumentation was attempted at any of these appointments. Soft tissues healed within normal limits, and no gingival recession was noted after treatment (Figure 9). Re-examination at 6 months after the periodontal surgery revealed reduction in PPD (from 7 mm to 3 mm) and PAL (from 8 mm to 4 mm), with no sign of bleeding on probing and significant radiographic bone formation in the Grade II furcation defect (Figure 10).

**Case 2: PRF in root coverage**

A 34-year-old man presented with sensitivity and elongated appearance of the upper front teeth. Upon intraoral examination, Miller's Class I recession was noted at #22 and #23 (Figure 11). Probing attachment level (mid-buccal) of about 4 mm was noted on #22 and about 5 mm on #23. Vertical recession, 2 mm (mid-buccal) and PPD of 2 mm on #22 and vertical recession of 3 mm (mid-buccal) with a PPD of 2 mm were noted on #23.
Surgical procedure: Presurgical preparation included scaling and root planning of the entire dentition and provision of oral hygiene instructions. After induction of local anesthesia, sulcular incisions were made labially in relation to #22 and #23 (Figure 12). A vertical releasing incision was made distal to #23. Sulcular incisions were deepened to create a pouch over the root surface on #22 and #23, but the inter-dental papillae between #22 and #23 was not reflected and was left intact. PRF was prepared according to Choukroun’s technique, and membrane of the required size was placed into the pouch created upon the root surface (Figure 13). Suspensory sutures were placed (Figure 14). Tin foil and light cure periodontal surgical dressing (Barricaid) was placed to protect the surgical site (Figure 15). Suitable antibiotics and analgesics were prescribed along with chlorhexidinedigluconate rinses (0.2%) twice daily for 2 weeks. Periodontal dressing and sutures were removed 1 week after the operation. Gentle brushing with a soft toothbrush was advised, and the patient was re-instructed for the maintenance of proper oral hygiene.

Results
Post-operative follow up was done at 1 month and 3 months, and healing was found to be satisfactory. Re-examination at 1 month (Figure 16) after the root coverage surgery revealed reduction in the vertical recession. Further follow up of the patient at 6 months (Figure 17), revealed marked reduction in the vertical recession (with residual recession of only 1 mm on #23).

Figure 11. Pre operative view of Miller’s Class II gingival recession on the upper left lateral incisor and canine

Figure 12. Sulcular incisions placed
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**Figure 13.** PRF membrane placed into the pouch beneath the reflected mucoperiosteal flap

**Figure 14.** Flap advanced and sutured using suspensory sutures

**Figure 15.** Light cure periodontal dressing placed to protect the treated site

**Figure 16.** Gum and teeth during review at 1 month

**Figure 17.** Postoperative view at 6 months

**Discussion**

The aim of periodontal therapy is to arrest and control the periodontal infection and ultimately regenerate lost periodontal structures. Newer approaches to periodontal therapy include regenerative procedures that aim to restore lost periodontal ligament, bone, cementum, and connective tissue. The complete regeneration of the periodontium after periodontal treatment modalities has been difficult to achieve because of differences in
the healing abilities of different periodontal tissues. In recent times, the local application of biologic modifiers, such as growth factors, has been investigated for use in the promotion of periodontal regeneration and healing. These agents act by augmenting the wound healing process through anabolic bone formation, angiogenesis, cementogenesis, osteoblast differentiation, mitosis, chemotaxis, and other processes that improve the healing environment. Biologic modifiers, including enamel matrix derivative (EMD), platelet-derived growth factor (PDGF), bone morphogenetic protein (BMP), platelet-rich plasma (PRP), PRF, fibroblast growth factor (FGF), and parathyroid hormone (PTH), have all shown promise in enhancing regeneration.

At present, one of the most widely used periodontal regenerative modalities is bone graft therapy. Unfortunately, the application of bone graft materials derived from the host or other living tissues may be limited by their inherent limitations. Finally, it is also important to weigh the cost/benefit ratio because many of these biologic adjuncts have additional costs associated with their use. Consequently, over the past two decades, dental research and industry have increasingly focused on biologically inert, synthetic, and autologous materials.

The present paper evaluates the clinical efficacy of PRF in the treatment of Grade II furcation defect and Miller’s Class 1 gingival recession. The uneventful healing in the patients was in agreement with the findings of previous studies, thus supporting the excellent ability of autologous PRF to enhance periodontal wound healing. PRF afforded a great improvement in soft and hard tissue regeneration. The findings in the presented cases were in accordance with those of previous studies done by Pradeep et al. in the treatment of mandibular Grade II furcation and those of Aroca et al. in the treatment of multiple adjacent gingival recession.

In assessing the success of the treatment methods, complete closure of the periodontal defect is desirable. Therapeutic results can be measured by PD and CAL, bone regeneration, and evidence of histologic periodontal regeneration. Although histologic evaluation is most accurate, surgical closure of the furcation defect and improvements in PD and clinical attachment level serve as suitable and practical outcome measures. The current case report assesses the clinical and radiographic parameters useful for evaluating the effect of autologous PRF on soft and hard tissue.

Similarly, multiple gingival recessions may be a concern for patients with a high lip smile line. Studies on this surgical challenge mostly concern the treatment of multiple Miller Class I and II recession defects. Suspensory sutures around the contact point were used in our case to allow stabilization of the flap margin at or above the CEJ during the first 2 weeks of wound healing. This is to promote full root coverage with the coronal stabilization of the flap margin.
with suspensory sutures during initial wound healing. In addition to the claimed benefits of using the PRF membrane in soft tissue wound healing, our results show the beneficial effects of using a PRF membrane for root coverage procedures. It has been found that PRF consists of a fibrin matrix polymerized in a tetramolecular structure; the incorporation of platelets, leukocytes, and cytokines; and circulating stem cells. Slow fibrin polymerization during PRF processing leads to the intrinsic incorporation of platelet cytokines and glycan chains in the fibrin meshes. This result implies that PRF, unlike the other platelet concentrates, can progressively release cytokines during fibrin matrix remodelling. This mechanism might explain the clinically observed healing properties of PRF. In addition, PRF slows down the blood activation process, which could induce an increased leukocyte degranulation and cytokine release from proinflammatory mediators, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α, to anti-inflammatory cytokines, such as IL-4. It has also been reported that PRF organizes as a dense fibrin scaffold with a large number of leukocytes concentrated in one part of the clot, with a specific slow release of growth factors (e.g., transforming growth factor-1β, platelet-derived growth factor-αβ, and vascular endothelial growth factor) and glycoproteins (e.g., thrombospondin-1) over ≥7 days. Leukocytes seem to have a strong influence on growth factor release, immunoregulation, anti-infectious activities, and matrix remodelling during healing. As a healing material, it stimulates the gingival connective tissue on its entire surface with growth factors and impregnates the root surface with key matrix proteins for cell migration (fibronectin, vitronectin, and thrombospondin-1). Moreover, the fibrin matrix itself shows mechanical adhesive properties and biologic functions like fibrin glues: it maintains the flap in a high and stable position, enhances neoangiogenesis, reduces necrosis and shrinkage of the flap, and guarantees maximal root coverage. PRF preparation is simple, easy, fast, and cost-free, without the use of any anticoagulant. It causes sustained release of growth factors. Therefore, PRF is considered the leader in fibrin technology.

Conclusion

Due to its peculiar properties, the natural fibrin biomaterial PRF has great potential for surgical wound healing. PRF has been shown to be an effective regenerative material in the management of Grade II furcation, displaying greater reduction in pocket depths and gain in clinical attachments with significant radiographic evidence of bone fill. Similarly, for the treatment of multiple gingival recessions, PRF can be considered as a viable cost-effective option. Further studies are necessary to assess the histology of the regenerated tissue and mechanisms to maximize the growth factor delivery while using PRF.
References


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