CASE REPORT

Second-generation Platelet Concentrate (Platelet-rich Fibrin) as a Scaffold in Regenerative Periodontics: A Case Series

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ABSTRACT

Periodontal regeneration is defined as the reproduction or reconstitution of a lost or injured part to restore the architecture and function of the periodontium. The goal of periodontal regeneration is to restore the damaged alveolar bone proper, root cementum, and periodontal ligament with collagen fibers inserted into the root surface. Platelets play a crucial role in regeneration of the bone and maturation of the soft tissue. Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) are autologous platelet concentrates prepared from patient's own blood. Recent researches are being focused on the development of therapeutic alternatives which are easy to prepare, non-toxic, or biocompatible to living tissues and economically cheap that might result in the local release of growth factors accelerating hard- and soft-tissue healing. PRF is a fibrin-based biomaterial prepared from an anticoagulant-free blood harvested without any artificial biochemical modification that allows obtaining fibrin membranes enriched with platelets and growth factors. Evidence from the literature has proven the potential role of PRF in periodontal regeneration and tissue engineering. The slow polymerization during centrifugation and fibrin-based structure makes PRF a better healing biomaterial than PRP and other fibrin adhesives. Hence, the present case series demonstrates the clinical and radiological (bone fill) effectiveness of autologous PRF along with the use of bone graft mineral in the treatment of intrabony defects.

Key words: Bone grafts, growth factors, platelet-rich fibrin, regeneration

INTRODUCTION

Periodontal disease is defined as a complex, multifactorial disease characterized by the loss of connective tissue attachment with destruction of periodontal tissues. The aim of periodontal therapy is to eliminate inflammatory process, to prevent the progression of periodontal disease, and also to regenerate the lost periodontal tissues. Periodontal regeneration is a complex multifactorial process involving biologic events such as cell adhesion, migration, proliferation, and differentiation in an orchestrated sequence. The ultimate goal of periodontal regeneration is to restore the damaged alveolar bone proper, root cementum, and periodontal ligament with collagen fibers inserted into the root surface.[1] Periodontal regenerative procedures include soft-tissue grafts, bone grafts, root biomodifications, guided tissue regeneration, and combinations of these procedures.[2] Numerous therapeutic modalities for restoring periodontal osseous defects have been investigated. For the past few decades, demineralized freeze-dried bone allograft (DFDBA) and beta-tricalcium phosphate with hydroxyapatite (β-TCP with HA) have been used alone and in combination with platelet-rich fibrin (PRF) in the treatment modalities for periodontal regeneration. The presence of bone morphogenic proteins contained within DFDBA aids in mesenchymal cell migration, attachment, and

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osteogenesis.[3,4] DFDBA has both osteoinductive and osteoconductive activities and β-TCP with HA has osteoconductive activity. Evidence shows that the presence of growth factors and cytokines in platelets plays key roles in inflammation and wound healing. Platelets consist of growth factors along with fibrin, fibronectin, and vitronectin, which act as a matrix for the connective tissue and as adhesion molecules for more efficient cell migration which has led to the idea of using platelets as therapeutic tools to improve tissue repair, particularly in periodontal wound healing.[4]

Platelets contain at least three major types of granules – α-granules, dense granules, and lysosomes – which carry distinct cargos and vary in biogenesis, trafficking, and exocytosis. The alpha granules in blood platelets contain growth factors platelet-derived growth factor (PDGF), insulin-like growth factor (IGF)-1, epidermal growth factor, and transforming growth factor (TGF)-β which begin healing of wounds by attracting and activating macrophages, fibroblasts, and endothelial cells.

PRF described by Choukroun *et al.* is a second-generation platelet concentrate which contains platelets and growth factors in the form of fibrin membranes prepared from the patient's own blood free of any anticoagulant or other artificial biochemical modifications. The PRF forms a strong natural fibrin matrix, which concentrates almost all the platelets and growth factors of the blood harvest and shows a complex architecture as a healing matrix with unique mechanical properties which makes it distinct from other platelet concentrates.[5] When periodontal disease results in intrabony defect, the prognosis is considerably altered, as the treatment of intrabony defect affected by periodontal disease is one of the most difficult problems for general dentist and periodontist.

In the present case reports, we present the clinical and radiographic changes of a patient using PRF along with DFDBA and β-TCP with HA as grafting materials in the treatment of periodontal intrabony defect.

CASE REPORTS

A thorough case history and clinical and radiological examinations were carried out. The two cases selected for the procedure were non-smokers and systemically healthy. Phase I therapy included scaling and root planning and reinforcement of oral hygiene instructions were carried out. The patients were informed about the treatment procedure and written informed consents were obtained.

Case 1

A male patient of age 26 years reported to the department of periodontology of tertiary care institute with a chief complaint of mobility of lower front teeth for 1 year. Detailed case history revealed that there was gradually increased in mobility to present status along with spacing of lower left lateral incisor (irt 32). The patient was relatively asymptomatic 1 year back when he had noticed pain in lower left lateral incisor, for which he underwent root canal treatment with respect to 32 1 year back. Patient's medical and family history was non-contributory. On intraoral examination, the patient had a fair oral hygiene and gingiva was in bluish-pink color with rolled margin, loss of stippling. Consistency was soft and edematous and mobility seen irt 32. Generalized probing depth of 2–3 mm seen except in 32 where it was 12 mm. On periodontal examination and radiographic evaluation, the patient presented with an intrabony defect extending up to apical third of the root of the left mandibular lateral incisor(#32) with a probing depth of 12 mm using William's periodontal probe [Figure 1a and b]. Routine hematological and urine parameters were within normal limits. Based on the history, clinical and radiological examinations, the patient was diagnosed as a case of localized chronic periodontitis irt 32. A comprehensive treatment plan was formulated and the patient was taken for Phase I therapy and later for surgical therapy. It was decided to perform regenerative therapy in the area of intrabony defect irt 32 using DFDBA graft material along with platelet-rich fibrin. Autologous blood was taken on the day of the surgery according to the PRF protocol with an REMI 2700 RPM and collection kits were used. Briefly, two 6 ml blood samples were taken from the patient without an anticoagulant in 10 ml glass test tubes and immediately centrifuged at 2700 RPM for 12 min. A fibrin clot was formed in the middle of the tube, whereas the upper part contained cellular plasma, and the bottom part contained red corpuscles. The fibrin clot was easily separated from the lower part of the centrifuged blood. One sample of PRF clot was used to make the membrane, and the other was added along with the bone graft (DFDBA) [Figure 1c].

Surgical Procedure

The patient was prepared for surgery and 2% lidocaine with adrenaline (1:80,000) was

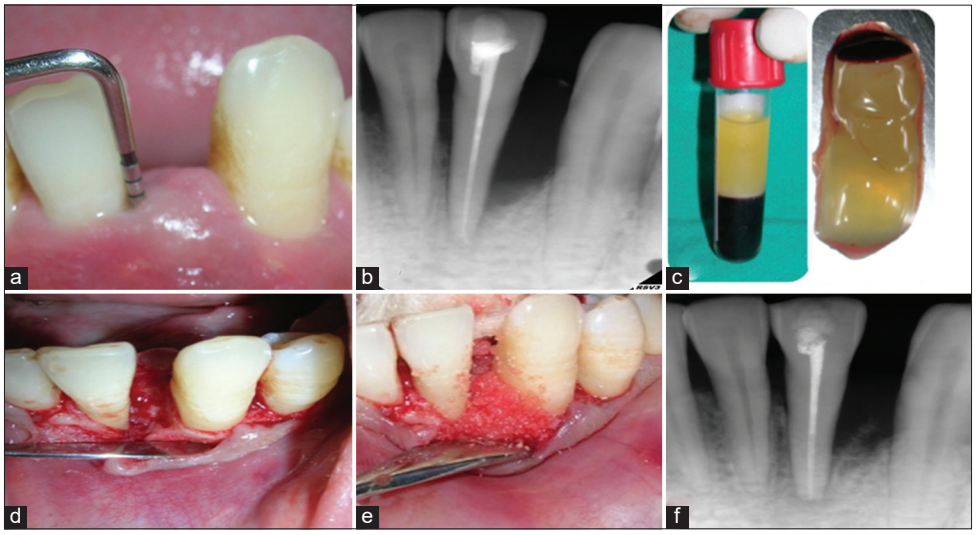

Figure 1: (a) Intrabony defect irt lower left lateral incisor (32); (b) IOPA shows vertical bone loss wrt 32; (c) extraction of platelet-rich fibrin (PRF) gel after centrifugation; (d) reflection of flap exposing the intrabony defect; (e) prepared PRF gel mixed with demineralized freeze-dried bone allograft graft material was condensed into the defect carefully without any void formation; (f) post-operative radiograph of 32 reveals bone fill was maintained satisfactorily

administered. Sulcular incision was placed extending from 31 to 33. The full-thickness flap was then reflected. Granulation tissue was removed and intrabony defect exposed. The dimensions of the defect were examined and the length and depth of defect was found to be 12 mm [Figure 1d]. The prepared PRF gel mixed with DFDBA graft material was condensed to the defect carefully without any void formation. Over the defect, PRF membrane made was used as a barrier membrane [Figure 1e]. Closure of the flap was done using 3–0 silk suture and periodontal pack was placed. Antibiotics and analgesics were prescribed. The patient was put on chemical plaque control using 0.12% chlorhexidine mouth wash. Immediate post-operative radiograph revealed adequate bone substitute fill in the defect. The patient was recalled after 1 week and suture was removed. The patient was revaluated after 1, 3, and 6 months and periodontal parameters were compared to baseline. A significant gain in the attachment level and reduction in the intrabony defect probing depth was noted. Six months post-operative radiograph of 32 revealed that bone fill level was maintained satisfactorily [Figure 1f].

Case 2

A male patient of age 38 years old reported to the department of periodontology of tertiary care institute, with a chief complaint of food impaction in the left lower back tooth region and bleeding from the gums for 6 month. History revealed that the patient noticed food impaction from the past 6 months and was associated with dull aching pain. On examination, the patient was systemically healthy and had not taken any long-term anti-inflammatory medications or antibiotics. On periodontal examination and radiographic evaluation, the patient presented with an intrabony defect extending up to apical third of lower left first molar (#36) with a probing depth of 8 mm using William's periodontal probe [Figure 2a and b]. The patient did not present with pain in relation to number 36 tooth and had no pain on percussion. There was positive electric pulp test response, suggesting that the concerned tooth was vital. The diagnosis was made to be localized chronic periodontitis with periodontal abscess. Initial therapy consisted of incision and drainage of the abscess, with antibiotic regimen for 5 days followed by oral hygiene instructions and scaling and root planning of the teeth were performed. Four weeks following Phase 1 therapy, a periodontal reevaluation was performed to confirm the suitability of the tooth for periodontal surgery. Before surgery, 6 ml of blood was taken from patient and PRF gel was extracted using centrifugation [Figure 2c]. An intrasulcular incision was made on buccal and lingual aspect of the tooth of the left mandibular teeth (#35, 36, and 37). The flap was reflected, granulation tissue was removed, and intrabony defect was exposed [Figure 2d]. The dimension of the defect was examined and; the

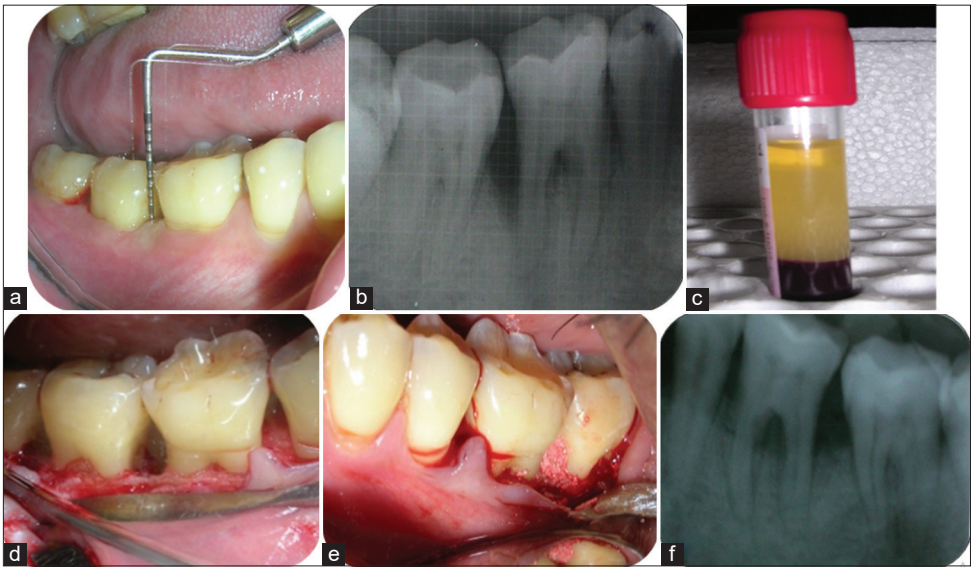

Figure 2: (a). Intrabony defect irt lower left first molar 36; (b) IOPA shows vertical bone loss wrt 36; (c) extraction of platelet-rich fibrin (PRF) gel; (d) exposure of the intrabony defect i.r.t 36; (e) PRF gel mixed with beta-tricalcium phosphate with hydroxyapatite graft material was condensed into the defect and PRF membrane was used as a GTR membrane; (f) post-operative radiograph of 36 revealed satisfactory bone fill

length and depth of defect was found to be 11 mm. The PRF gel mixed with bone graft (β-TCP with HA) material along with PRF membrane made was used as a GTR membrane [Figure 2e]. The flaps were repositioned with 3-0 silk and periodontal pack was placed. Periapical intraoral radiographs were taken at baseline, 3 months, and 6 months which showed appreciable bone fill at the periodontal defect site postoperatively [Figure 2f].

DISCUSSION

Periodontal disease is among the most prevalent diseases worldwide and is characterized by the presence of gingival inflammation, periodontal pocket formation, loss of periodontal attachment, and alveolar bone around the affected teeth.[6] The goal of periodontal therapy includes not only the arrest of periodontal disease progression but also the regeneration of structures lost due to disease. Bone grafting is one of the most common forms of regenerative therapy and is usually essential for restoring periodontal supporting tissue. A wide range of bone grafting materials, including autografts, allografts, xenografts, and alloplasts (synthetic/semisynthetic materials), has been applied and evaluated clinically.[7]

DFDBA and β-TCP with HA are widely used in periodontal therapy and have been demonstrated to be safe and capable of inducing new bone formation. In the first case, DFDBA was used which has both osteoconductive and osteoinductive.[4,5] Urist *et al.* showed through numerous animal experiments that DFDBA could stimulate the formation of new bone by osteoinduction. This type of graft material induces host undifferentiated mesenchymal cells to differentiate into osteoblasts with subsequent leading formation of new bone.[6,7] Moreover, DFDBA also provides a scaffold for osteoconduction. DFDBAs have repeatedly demonstrated signi cant improvements in soft- and hard-tissue clinical parameters for the treatment of intraosseous periodontal defects. β-TCP with HA (Biograft HT)® was used in the second case, which has property of osteoconductivity and ability to integrate with the host bone. It acts as scaffolds with a foam type of structure that contains 60% HA and 40% TCP.[8]

Recently, the use of growth factors in periodontal regeneration has shown promising results. Growth factors are a class of natural biologic mediators that regulate key cellular events in tissue regeneration including cell proliferation, chemotaxis, differentiation, and matrix synthesis through binding to specific cell surface receptors. Alpha (α) granules present in platelets form an intracellular storage pool of growth factors including PDGF, transforming growth factor-β (including β-1 and β-2isomers), vascular endothelial growth factor, epidermal growth factor, and insulinlike growth factor-1. PRF is a matrix of autologous fibrin, in which is embedded a large quantity of platelet and

leukocyte cytokines during centrifugation. The intrinsic incorporation of cytokines within the fibrin mesh allows for their progressive release overtime (7–11 days), as the network of fibrin disintegrates. The easily applied PRF membrane acts much like a fibrin bandage, serving as a matrix to accelerate the healing of wound edges. It also provides a significant post-operative protection of the surgical site and seems to accelerate the integration and remodeling of the grafted biomaterial.[4]

In the present case reports, the decision to utilize PRF as defect fillers in combination with bone graft was made due to its ease of manipulation and delivery to the surgical site. The intended role of the PRF in the intrabony defect was to deliver the growth factors in the early phase of healing.

According to Simonpieri *et al.*, the use of this platelet and immune concentrate during bone grafting offers the following four advantages: First, the fibrin clot plays an important mechanical role, with the PRF membrane maintaining and protecting the grafted biomaterials and PRF fragments serving as biological connectors between bone particles. Second, the integration of this fibrin network into the regenerative site facilitates cellular migration, particularly for endothelial cells necessary for the neoangiogenesis, vascularization, and survival of the graft. Third, the platelet cytokines (PDGF, TGF-β, and IGF-1) are gradually released as the fibrin matrix is resorbed, thus creating a perpetual process of healing. Finally, the presence of leukocytes and cytokines in the fibrin network can play a significant role in the self-regulation of inflammatory and infectious phenomena within the grafted material.[9]

In the present study, reduction in pocket depth and gain in clinical attachment level were found after 6 months follow-up. These are the important clinical outcomes for any periodontal regenerative procedures. Radiographs revealed significant bone fill in the intrabony defect compared to measurements at baseline.

CONCLUSION

The principle objective of all surgical procedure therapies is to preserve the dentition in a state of health and comfort throughout life. A combination of PRF with DFDBA and β-TCP with HA (Biograft HT) demonstrated better results in probing pocket depth reduction and clinical attachment level gain alone in the treatment of periodontal intrabony defects. This result may be attributed to beneficial effects of PRF. The main advantage is that PRF preparation utilizes the patient's own blood reducing or eliminating disease transmission through blood. In the future, potential applications of PRF in the field of periodontal regeneration and tissue engineering can be extend into clinical applications.

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