A Clinical and Histological Evaluation of Platelet-Rich Fibrin and CGF for Root Coverage Procedure using Coronally Advanced Flap: A Split-Mouth Design

Abstract
The management of gingival recession associated with esthetic concerns and root hypersensitivity is challenging and its sequelae are based on the assessment of etiological factors and the degree of tissue involvement. Procedures using pedicle flaps, free soft-tissue grafts, combination of pedicle flaps with grafts, barrier membranes, and the use of platelet concentrates are all effective for this purpose. The use of the second-generation platelet concentrate, platelet rich fibrin (PRF) has been widely used. Lately, concentrated growth factor (CGF) has evolved as a promising regenerative material, wherein it also acts as a scaffold and accelerates wound healing due to its dense fibrin meshwork. A 21-year-old male patient presented with bilateral multiple gingival recessions due to faulty tooth brushing. Coronally advanced flap with Zucchelli's technique was planned as a treatment modality. Platelet concentrates PRF and CGF were placed bilaterally during the procedure, and the outcome of the treatment was compared. The percentage of root coverage was clinically evaluated, and histological evaluation was also done to assess the density of fibrin meshwork in the platelet concentrates. Nearly 100% of root coverage was achieved with both PRF and CGF membrane 3 months postoperatively. However, CGF showed satisfactory wound healing by the 10th-day postoperatively compared to PRF. As CGF operates on varying centrifugation to separate cells in the venous blood, thereby resulting in fibrin-rich blocks that are much larger, denser, and richer in growth factors as also shown histologically.

Keywords: Concentrated growth factor, coronally advanced flap, gingival recession, platelet concentrates, platelet-rich fibrin

Introduction
Gingival recession is a common clinical finding in periodontal disease which is frequently associated with esthetic concerns, root hypersensitivity, and root caries.[1] One of the most predictable procedures for gingival recession management is the coronally advanced flap (CAF),[2] with subepithelial connective tissue graft.[3] Owing to the second surgical donor site and difficulty in procuring a sufficient graft for multiple bilateral recessions, various alternative additive membranes are being used. The use of growth factors, application of extracellular matrix proteins, attachment factors, and use of bone morphogenetic proteins and platelet concentrates are known to bring about integrated tissue formation.

Platelet concentrates and its regenerative potential are known since 1974,[4] as they aided in the improvement of repair and regeneration of the soft and hard tissues after various periodontal surgical procedures.

They harbor growth factors, including platelet-derived growth factor (PDGF), transforming growth factor (TGF-β), and insulin-like growth factor-I, epithelial growth factor, insulin growth factor-I, and vascular endothelial growth factor, which are known to stimulate cell proliferation and upregulate angiogenesis.[5] Numerous techniques of autologous platelet concentrates have been developed and applied in dentistry. The first generation incorporates the platelet-rich plasma (PRP), whereas the second generation involves the platelet-rich fibrin (PRF) and CGF.

PRF is a fibrin-based biomaterial harvested from the patient's blood without adding any anticoagulant to obtain fibrin membranes enriched with platelets and growth factors.[6] The potential role of PRF in periodontal regeneration and tissue engineering is evident in the literature.[7,8] The slow polymerization during centrifugation and fibrin-based structure makes PRF a better healing biomaterial than PRP and other fibrin adhesives.[9]
CGFs, unlike PRF, utilize altered centrifugation speed to produce much larger, denser, and richer fibrin matrix-containing growth factors than PRF and have shown better regenerative capacity and higher versatility as a regenerative material due to the concentration of fibrinogen, Factor XIII, and thrombin.\(^{[10]}\)

Owing to the better healing and regenerative property of the platelet concentrates, this case report aims in treating bilateral multiple Miller’s Class I recession with a split-mouth design using CAF with CGF membrane in teeth #13, 14, 15 compared to CAF with PRF membrane in teeth #3, 4, 5. The percentage of root coverage and resolution of dentinal hypersensitivity associated with gingival recession with both the membranes was assessed. The fibrin network patterns of both the membranes were also histologically compared to assess the density of their fibrin meshwork pattern.

**Case Report**

A 21-year-old male patient reported to the Department of Periodontology, Meenakshi Ammal Dental College and Hospitals, Chennai, India, with a chief complaint of sensitivity in relation to the upper right and left posterior region. His history revealed the presence of sensitivity for the past 5 months that aggravated on tooth brushing and intake of hot and cold foods. His personal history revealed the habit of vigorous tooth brushing twice daily. Clinical examination showed healthy periodontium with no signs of inflammation. Gingival recession was seen at the sites of sensitivity, and the patient was diagnosed with bilateral multiple Miller’s Class I recession in maxillary left molar and premolar (teeth #13, 14, 15) [Figure 1] and maxillary right molar and premolar (teeth #3, 4, 5) [Figure 2]. Hence, a split-mouth design using CAF with CGF membrane in tooth #13, 14, 15 and CAF with PRF membrane in teeth #3, 4, 5 was planned. Before the surgical procedure, informed consent was obtained from the patient, scaling and root planing was done, and oral hygiene instructions were given. Proper brushing technique was also advised. Apart from assessing the percentage of root coverage, the fibrin clots were also histologically assessed for the density of the fibrin meshwork.

**Case Management**

**Zucchelli’s technique**

A modification of CAF for multiple recession coverage was planned and performed on both sides. A characteristic feature of this technique is oblique submarginal incisions in the interdental area.\(^{[11]}\) Disinfection of the surgical site was done with 2% betadine. The procedure was carried out under local anesthesia (lignocaine HCl with 2% epinephrine 1:20,000). The incision outline was first marked with a sterile indelible pencil. An intrasulcular incision was made at the buccal aspect of the involved tooth. Oblique horizontal incisions were given connecting the cementoenamel junction of one tooth to the gingival margin of the adjacent tooth interdental papillae 1 mm apical to the level of the coronal border. Two oblique vertical incisions were extended beyond the mucogingival junction and a trapezoidal mucoperiosteal flap was raised up to the mucogingival junction [Figures 3 and 4]. Following this, the full-thickness flap was extended apically, releasing the tension and favoring coronal positioning of the flap [Figures 5 and 6]. Interdental papilla was completely deepithelialized to expose the underlying connective tissue and to eliminate the epithelium that might interfere with healing. The root surface was examined for remnant calculus, and thorough scaling and root planing was done. Following this, CGF membrane was placed to cover the recession in relation to maxillary left site [Figure 7] and PRF membrane in relation to maxillary right site [Figure 8], the flap was coronally advanced and made to rest on the prepared connective tissue beds of the papillae with continuous sling sutures [Figures 9 and 10] followed by periodontal dressing.

**Postoperative Care**

The patient was instructed not to remove the pack or disturb the surgical site until the sutures were removed. Antibiotics (amoxicillin 500 mg TD) and analgesics (aceclofenac + paracetamol, BD for 3 days) were prescribed. The use of 0.12% chlorhexidine rinse was also advised. After 2 weeks, the periodontal dressing and the sutures were removed. Healing was satisfactory and 100% root coverage was obtained on both the sites which remained stable through the 3\(^{rd}\)-month review, along with the resolution of dentinal hypersensitivity [Figures 11a, b and 12a, b].

**Histologic Evaluation**

The PRF and the CGF membranes were also histologically assessed for its fibrin matrix density. The venous blood that was obtained from the patient was dispersed into two test tubes with 5 mL in each one half was used as membrane, and the other that was procured was placed in 10% formalin for further slide preparation. The isolated PRF and CGF clots were prepared into slides by cell block cytology according to Yajamanya et al.\(^{[12]}\)

**Histological Outcome**

The histological analysis of CGF showed a dense fibrin network and the presence of inflammatory component, whereas PRF showed loose and scarce fibrin network and the presence of inflammatory component [Figures 13 and 14].

**Clinical Outcome**

100% root coverage and resolution of dentinal hypersensitivity were achieved with CAF and PRF and CAF and CGF. Although both sites healed uneventfully, there was less postoperative discomfort and accelerated healing.
by the 10th day on the CAF + CGF site [Figure 11a]. The better regenerative capacity may be attributed to the denser fibrin matrix which was appreciated in the histological section as well [Figure 13].

**Discussion**

The most important factor in the etiology of dentin hypersensitivity is the exposure of root surfaces from the gingival recession.[13] Treatment modality includes an attempt to cover the exposed root surfaces. Over the past decades, numerous periodontal plastic surgery procedures have been described in an attempt to cover exposed root surfaces. Among these techniques, the most commonly used is the “coronally repositioned flap” introduced by Bruiest in 1970 and modified by Allen and Miller in 1989. Zucchelli and Sanctis modified this technique further in 2000.[11]

In our study with Zucchelli’s technique, adequate root coverage with stable results for over 3 months was achieved. No scar formation was observed, and the color match of the tissue was excellent. We compared CAF and PRF with CAF and CGF in multiple bilateral recessions. The chief complaint of hypersensitivity was resolved completely following the surgery. The clinical outcomes
for the treatment of gingival recession using both the membranes showed similar results, except that the site where CGF was used showed better and faster healing by the 10th-day postoperatively. Apart from assessing the clinical outcomes, both the PRF and CGF fibrin meshwork pattern were histologically analyzed. The fibrin matrix
structure of PRF and CGF differed with more denser fibrin meshwork in CGF [Figures 13 and 14]. Figure 13 shows dark eosin staining areas denoting dense fibrin along with some lighter eosin staining areas, denoting lesser fibrin. Apart from this, the second half of Figure 13 shows some inflammatory cells in hematoxylin staining seen close to the dense fibrin. Whereas in Figure 14, the eosin staining is much lighter denoting less fibrin meshwork and in the second half of Figure 14, red blood cells are seen with a very thin area of dark eosin (fibrin meshwork) staining. On comparing both PRF and CGF slides, the fibrin meshwork pattern is thicker and more along with inflammatory cells in concentrated growth factor than PRF. This might be one of the contributory factors for the better healing capacity of CGF over PRF. CGF having denser fibrin meshwork compared to PRF would have led to faster healing at the CAF with CGF site compared to CAF with PRF site.

Platelet concentrates are concentrated suspensions of growth factors, and these stimulate healing and regeneration of tissues. They are autologous sources of PDGF and TGF-β that is obtained by appropriating and concentrating platelets by gradient density centrifugation. They have been shown to stimulate healing and renewal of tissues. Unlike PRF which uses constant centrifugation speed, CGF utilizes altered centrifugation speed to produce much larger, denser, and richer fibrin matrix-containing growth factors. The CGF is an autologous platelet concentrate, developed by Sacco, in 2006 and obtained from blood samples through a simple and standardized separation protocol without the addition of exogenous substances. The main feature of CGF resides in its consistency. It is an organic matrix rich in fibrin, able to trap platelets, leukocytes, and growth factors; elements that play a major role in the regenerative process. This shows better regenerative magnitude and increased versatility when using the fibrin-rich block. The resulting fibrin clot/block is of a higher quality due to the concentration of fibrinogen, Factor XIII, and thrombin that is obtained. Factor XIIIa, activated by thrombin, cross-links the fibrin clot to increase stability, strength, and protection against plasmin-mediated degradation.

To the best of our knowledge, this is one of the first studies to compare the outcome of healing using both the membranes clinically as well as histologically.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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