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Platelet Rich Fibrin - a review and Case report!

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Abstract
Platelets are a key component of blood that plays an essential role in wound healing. They are a reservoir of growth factors and cytokines that are involved in the process of tissue maturation and healing. Platelet rich fibrin (PRF), a second generation platelet concentrate, is an autologous preparation of blood. It contains large quantity of platelets and cytokines. PRF presents a novel therapeutic conception with no biochemical processing of blood. The crux of PRF synthesis lies in the attempt to accumulate platelets and cytokines in a fibrin clot. It is a simple and cost effective method at attempting enhanced healing and recovery of tissues.

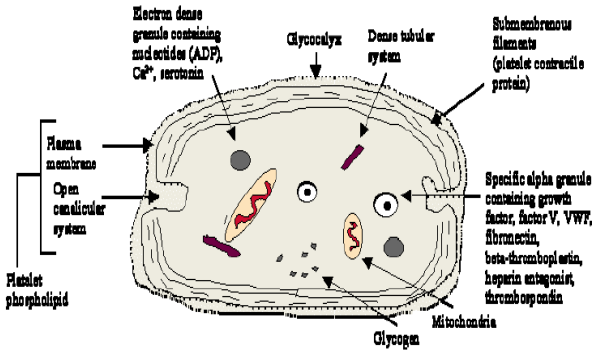
Keywords: Platelet rich fibrin, wound healing, recovery of tissues

Introduction
Periodontal disease is a chronic inflammatory disease characterised by loss of connective tissue attachment and alveolar bone loss. Gingival recession is defined as displacement of marginal tissue apical to cementoenamel junction.[1] It is often a result of periodontal disease as well as periodontal therapy. Patients often have aesthetic complaints or problems due to hypersensitivity, root caries or abrasion of the exposed root surfaces.[2,3]

Platelet concentrates are derived from the blood which is generally used for the prevention and treatment of haemorrhages as a result of thrombopenia.[4] Literature review since the early centuries has documented the importance of several components of blood in healing of wounds. It has been suggested that platelet concentrates when applied locally as surgical additives have the potential to hasten healing. These concentrates can be obtained autologously from the patient or can be obtained commercially (Tissel, Baxter Healthcare).

Platelets
Platelets were discovered by Giulio Bizzozero in 1882, but for many decades the dynamic and multifunctional nature of platelets remained a field of interest only for biologists.[5] Platelets are the cells which are produced in the bone marrow by megakaryocytes after stimulation from a growth factor, thrombopoetin which is produced in the liver and released into the blood circulation. They circulate in the blood for about 10 days after release from the marrow. Normal concentration of platelets is 1,50,000- 3,50,000/ µl. They are 2-3 µm in diameter containing an irregular ring of lobed

nuclei. Platelets play an important role in haemostasis, blood coagulation, phagocytosis and storage and transport of substances. Within the cytoplasm of platelets are found glycogen granules, mitochondria, lysosomes, peroxisomes and various types of inclusions, including alpha and dense granules. Alpha granules constitute 15% of the total volume of platelets. They consist of platelet specific and non platelet specific proteins (fibrinogen, fibronectin, thrombospondin, growth factors etc). Dense granules consist of calcium, inorganic phosphorus, ADP, ATP and serotonin.[6]



Structure of a platelet

Classification of Platelet concentrates[7]

As per the current classification (2009), platelet concentrates can be generally classified into four groups based on the presence of leucocytes and fibrin architecture:

1) Pure PRP/Leucocyte-poor PRP: Absence of Leucocytes and low density of fibrin network after activation. Used in two forms - liquid solution or activated gel.

2) Leucocyte and platelet-rich plasma: Presence of Leucocytes and low-density fibrin network after activation. Also used in two forms - liquid solution or activated gel.

3) Pure platelet-rich fibrin PPRF/Leucocyte-poor PRF: Without leucocytes but with high-density fibrin network. Used in strongly activated gel form.

4) Leucocyte and Platelet-Rich Fibrin: With leucocytes but with high-density fibrin network. Also used in strongly activated gel form.

Platelet rich fibrin (PRF)

Platelet rich fibrin was first prepared by Choukron et al 2001 in France.[8] It is a second generation platelet concentrate.[9] PRF consists of an autologous leukocyte PRF matrix which is a tetra molecular structure consisting of cytokines, platelets and stem cells. This matrix acts as a biodegradable scaffold which allows development of new blood vessels and epithelial cell migration.[10]

Preparation

Preparation of PRF follows the protocol developed by Choukroun et al. in Nice, France.[11] Sufficient quantity of blood is withdrawn into 10 ml test tubes without the addition of an anticoagulant and centrifuged immediately. Blood is centrifuged using a table top centrifuge for 12 minutes at 2700 rpm. As a result of centrifuge three layers are formed- topmost layer consisting of acellular platelet poor plasma, middle layer consisting of platelet rich plasma and bottom layer consisting of red blood cells (RBCs).[12] As there is no anticoagulant in this preparation, blood begins to coagulate as soon as it comes in contact with the glass surface. Therefore for proper preparation of PRF, speedy collection of blood and immediate centrifugation before initiation of clotting process is imperative.

Uses of PRF[13]

- PRF can be used in concurrence with bone grafts which has many advantages including promotion of wound healing, bone growth and maturation, graft stabilization, wound sealing haemostasis and improving the handling properties of graft materials.
- PRF can also be used as a membrane for root coverage procedures.
- In perio-endo lesions and furcation defects.
- Sinus lift procedures.
- Socket preservation.
- Filling of cystic cavities.

Advantages of PRF over PRP

- The redundant process of adding an anticoagulant and the need to neutralize is eliminated.
- Bovine derived thrombin is not added for conversion of fibrinogen to fibrin. The use of bovine thrombin may result in the formation of antibodies to factors V, XI and thrombin, resulting in the risk of life threatening coagulopathies. Thus the risks associated with bovine derived thrombin are eliminated. The conversion of fibrinogen to fibrin takes place with the small quantity of physiologically available thrombin present in the sample itself.
- Thus, these steps reduce the biochemical handling of blood.[14]

Limitations of PRF[15]

- As PRF is an autologous product, it cannot be procured in larger amounts if indicated in a case.
- It cannot be used as an allogenic material as it contains immune cells and antigenic molecules. Therefore there will be an increased risk of infectious transmission.

Difference between PRF and blood clot

PRF is more homogenous and more stable, easy to handle and consign in a local place.[16]

Studies comparing PRF with PRP

- **Witfang et al 2005[17]-** The findings of Wiltfang et al 2005 from a series of clinical trials are encouraging, in that they show improved properties of PRF as compared with PRP.
- **Saluja H et al 2011[18]-** This study aimed to assess the potential use and benefits of Platelet-Rich Fibrin (PRF) over Platelet-Rich Plasma (PRP). PRF, which belongs to a new second generation of platelet concentrates, with simplified processing, and not requiring biochemical blood handling, has several advantages over traditionally prepared PRP, which has been widely used for accelerating soft tissue and hard tissue healing. However, the preparation being strictly autologous, the amount of PRF obtained is limited.
- **Kobayashi E et al 2016[19] -** The aim of the present study was to compare growth factor release over time from platelet-rich plasma (PRP), platelet-rich fibrin (PRF), and a modernized protocol for PRF, advanced-PRF (A-PRF). Based on the findings of this study, PRP can be recommended for fast delivery of growth factors whereas A-PRF is better-suited for long-term release.

Studies on PRF

- **Choukroun J et al 2006[15]** - This study aimed to evaluate the potential of PRF in combination with FDBA to enhance bone regeneration in sinus floor elevation. 9 cases were performed. This study showed that sinus floor augmentation with FDBA and PRF leads to a reduction of healing time prior to implant placement.
- **Dohan et al 2006[20] -** This is a retrospective analysis to evaluate the biochemical properties of 3 generations of surgical additives, respectively fibrin adhesives, concentrated platelet-rich plasma (cPRP) and PRF. The slow polymerization during PRF preparation seems to generate a fibrin network very similar to the natural one. Such a network leads to a more efficient cell migration and proliferation and thus cicatrization.
- **Sasha Jankovic et al 2010[21]** - The objective of this study was to evaluate the clinical effectiveness of PRF with coronally advanced flap and to compare it with EMD with coronally advanced flap in gingival recession treatment. The study did not show any advantage of the use of PRF compared to EMD in the treatment of gingival recession.
- **Chang YC et al 2011[22]** - This report was to present the clinical and radiographic changes of a patient with periodontal intrabony defects treated with PRF. From a clinical and radiologic point of view at 6

months after surgery, the use of PRF as the sole grafting material seems to be an effective modality of regenerative treatment for periodontal intrabony defects.

- **Simonpieri A et al 2012[16] -** He reported and confirmed the validate usage of PRF membranes in reconstruction protocols along with FDBA, 0.5% metronidazole solution in about 20 patients who were treated using this new technique and followed-up during 1-5 years, and finally 184 dental implants were placed and they found no implant or graft loss in a case series.
- **Joseph VR et al 2014[23] -** The aim of this study was to evaluate the mechanical properties of the platelet-rich fibrin (PRF) membrane and to compare these properties with that of commercially available collagen membranes used for guided tissue regeneration (GTR) procedures. The preliminary findings from the assessment of the mechanical properties of PRF membrane showed that it was lacking in several desired properties when compared to commercially available collagen membranes. Lack of rigidity and faster degradation may limit its application in GTR procedures.

Case- Root coverage with PRF

A 30 year old male patient presented with mild sensitivity in upper right front region of jaw. Patient also complained of long appearance of tooth. Intraoral examination revealed gingival recession in upper right canine. (Fig.1) Gingival recession when measured was 2mm and the clinical attachment loss (CAL) was 3mm without mobility. A periapical radiograph was taken with standard techniques which revealed sound bone structure around the tooth. Phase I therapy was done which included thorough scaling and root planning. Re-evaluation was done after 3 weeks. A coronally positioned flap with PRF was planned in upper right canine.

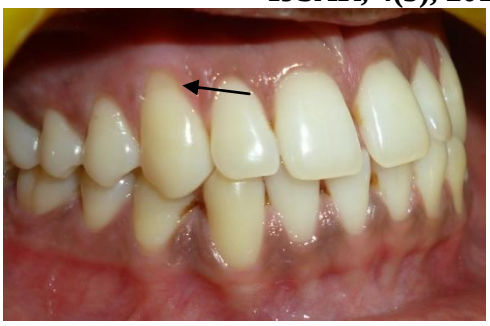


Figure1: Gingival recession seen in upper right canine.

Surgical procedure:

Presurgical procedure included scaling and root planing and oral hygiene instructions. Local anaesthesia was given in upper right canine. Sulcular incisions were given labially in relation to #12, #13 and #14. A vertical releasing incision was given distal and mesial to #13 extending beyond the mucogingival junction (Figure 2). A partial thickness flap was reflected beyond the mucogingival junction (Figure 3). The surgical site was cleaned off all the tissue tags.

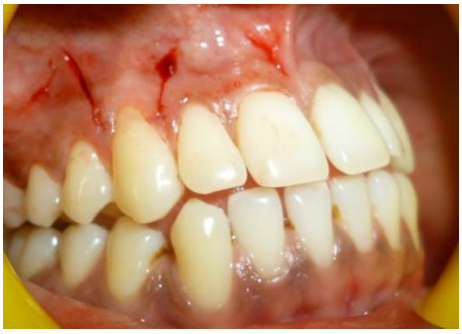


Figure 2: Sulcular and vertical incisions given.

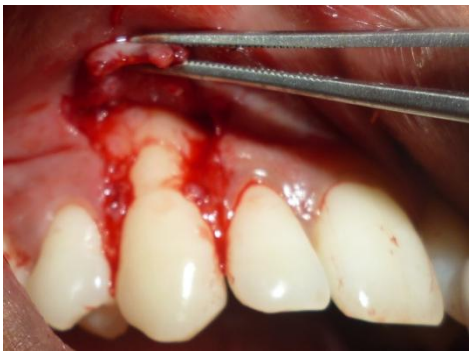


Figure 3: Flap reflected beyond mucogingival junction.

PRF preparation:

PRF was prepared in accordance with the protocol developed by Choukroun et al 2001. Prior to surgery intravenous blood was collected in a 10ml sterile tube without the addition of any anticoagulant and immediately centrifuged in a centrifugation machine at 3000 revolutions per minute for 10 minutes. Immediately after centrifugation, the tube showed three layers- the middle layer being the fibrin clot, lower layer comprising the red blood corpuscles (RBCs) and the acellular plasma (platelet poor plasma) being the uppermost layer (Figure 4). The middle layer was then separated using a pair of sterile tweezer and scissor (Figure 5). The PRF was then squeezed in a sterile gauze piece to form a thin membrane (Figure 6). It was then transferred to a dappen dish.

Figure 4: After centrifugation.

Figure 5: Separation of PRF with a sterile tweezer.

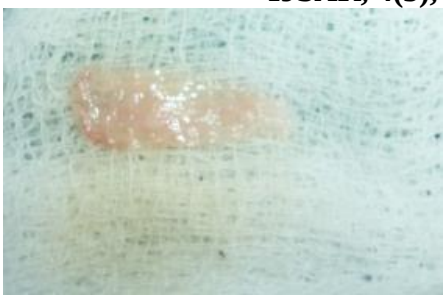


Figure 6: PRF squeezed into a membrane.

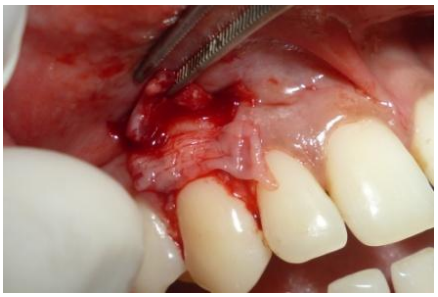


Figure 7: PRF membrane placed beneath the flap on the exposed root surface.

PRF membrane was then placed at the surgical site, flap was advanced and site was sutured with 3-0 silk sutures (Figure 8). A tin foil was then placed over the operated site and a periodontal dressing was applied. Suitable analgesics and antibiotics were prescribed along with chlorhexidine rinses (0.2%) for 2 weeks. A gentle toothbrushing technique was prescribed with a soft toothbrush. Periodontal dressing was removed after 1 week and sutures after 10 days.

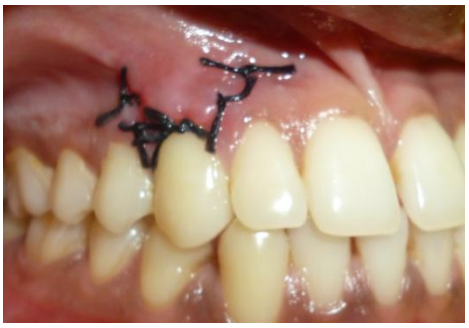


Figure 8: Sutures placed.

Results

Postoperative follow up was done at 15 days, 1 month and 3 months and healing after the root coverage procedure was found to be satisfactory. Follow up at 1 month showed a

reduction in recession. Further follow up at 3 months showed a marked decrease in recession (Figure 9).

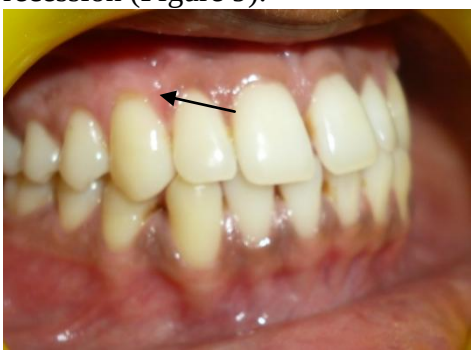


Figure 9: Follow up at 3 months.

Discussion

Treatment of gingival recession has become essential nowadays due to the increased aesthetic demands. There are many different types of procedures available for the procedure of root coverage. Bernimoulin et al 1975 first suggested the coronally advanced flap as a part of a two step procedure. First step involved a free gingival autograft placed apical to the margins of recession to be treated and the second step involved a coronally positioned flap carried out after a few months to cover the exposed root surfaces.[24] Tarnow in 1986 suggested the semilunar coronally positioned flap for root coverage.[25] Allen and Miller in 1989 suggested the use of one stage coronally positioned flap along with root biomodification with citric acid for coverage of recession defects.[26] A modified coronally advanced procedure was surggested by Zucchelli and De Sanctis in 2007. The main modifications were in flap thickness and dimension of surgical papillae during flap elevation.[27]

PRF is a recent innovation in dentistry, which is a concentrated suspension of growth factors in platelets that are involved in healing and tissue regeneration. These growth factors include Platelet derived growth factor (PDGF), Transforming growth factor (TGF) and many others. Growth factors are mitogenic (proliferative), chemotactic and angiogenic (stimulate the formation of new blood vessels). Healing of any wound starts with formation of clot, epithelialisation, angiogenesis, granulation tissue formation and collagen deposition and finally collagen maturation and contraction. PRF is a fibrin matrix containing platelet cytokines, growth factors and cells, trapped and released with time and can serve as membrane. PRF can be used as a membrane in cases of gingival recession to restore the gingival tissues.

In periodontics, PRF has been used to treat gingival recession, intra-bony defects and periapical lesions. Some case reports show the use of a combination of PRF gel, hydroxyapatite graft and guided tissue regeneration (GTR) membrane to treat IBD.[28] Some studies show the use of PRF gel and PRF membrane in combination with a bone graft for treating a tooth with a combined periodontic- endodontic lesion.[29] A report of clinical trials comparing the growth factors content of PRF and PRP was presented by Dohan and Diss at the second international Symposium on growth factors held in May 2005. Combining the growth factors has been shown to accelerate bone repair and promote fibroblast proliferation, and increase tissue vascularity, rate of collagen formation, mitosis of mesenchymal stem cells and endothelial cells, as well as osteoblasts, playing key roles in the rate and extent of bone formation.[20]

Conclusion

The use of Platelet Rich Fibrin for enhancing the healing of periodontal tissues could present new possibilities for regeneration. However, the clinical effectiveness of PRF should be evaluated in randomized clinical trials and studies involving larger group of subjects.

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