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Platelet rich fibrin and nanocrystalline hydroxyapatite: hope for regeneration in aggressive periodontitis: A novel clinical approach

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Abstract

Periodontists have been experimenting various modalities for regeneration in Aggressive periodontitis with varying degrees of success. Periodontal surgery along with platelet rich fibrin (PRF) has yielded promising results. The purpose of this clinical trial was to evaluate the surgical outcome of an autologous growth factor obtained by sequestering and concentrating platelets by gradient density centrifugation in periodontal regeneration in AgP. Three patients diagnosed as AgP were selected for this study. Baseline, clinical and radiographic examination were performed and patients were treated by surgical periodontal therapy along with PRF and nanocrystalline hydroxyapatite. Post-surgical, clinical and radiographic re-evaluation were carried out at regular intervals to assess healing and radiographic changes. Surgical Periodontal debridement along with the placement of PRF in combination with nanocrystalline hydroxyapatite in angular defects of the subjects showed decreased probing pocket depth, gain in attachment level and radiographic bone fill as compared with the baseline. Because of the more favourable bony morphology of subjects with AgP and the ideal characteristics of PRF, reconstructive therapy along with PRF and hydroxyapatite in angular defects of AgP patients can be an effective approach for periodontal regeneration.

Keywords: Aggressive periodontitis, bone defects, nanocrystalline hydroxyapatite, PRF, regeneration

Introduction

Aggressive periodontitis

Aggressive periodontitis is a form of periodontitis characterized by rapid progression of attachment and bone loss occurring in an otherwise healthy subjects with a familial pattern of occurrence being a consistent feature.

The 1999 Consensus Report published by the American Academy of Periodontology permitted the subdivision of aggressive periodontal disease into localized and generalized forms based on enough individually specific features, as follows:

Localized aggressive periodontitis

- Circumpubertal (around the time of puberty) onset
- Robust serum antibody response to infective agents: the dominant serotype antibody is IgG2.
- Localized first molar/incisor presentation
- Gingival inflammation, edematous, bleeding, pocketing

Generalized aggressive periodontitis

- Usually affects patients under 30 years of age
- Poor serum antibody response to infective agents
- Pronounced episodic nature of periodontal destruction
- Generalized presentation affecting at least 3 permanent teeth other than first molars and incisors.
- More bony destruction and more rapid than the LAP
- Bleeding, deep pocketing, Periodontal abscess. No gingival inflammation [1].

Regeneration is dependent upon 4 major biologic principles: primary wound closure, blood supply, space maintenance, and wound stability. Bone grafting is most successful when it occurs in a contained, well vascularized environment stressing the importance of primary closure and the promotion of angiogenesis [2].

Platelet-rich plasma (PRP) was proposed as a method of introducing concentrated growth factors PDGF, TGF- β , and IGF-1 (Platelet derived growth factor, Transforming growth factor β , Insulin like growth factor 1) to the surgical site, enriching the natural blood clot in order to expedite wound healing and to stimulate bone regeneration [3].

The PRF production was introduced which attempts to accumulate platelets and released cytokines in a fibrin clot. PRF was first developed in France by Choukroun *et al* in 2001 [4] It is a second generation platelet concentrate and is a strictly autologous fibrin matrix containing a large quantity of platelet and leukocyte cytokines. It is widely used to accelerate soft and hard tissue healing.

Advantages

The advantages of PRF includes, ease of preparation / application, minimal expense, and lack of biochemical modification.

Preparation of PRF

PRF preparation requires an adequate table centrifuge and collection kit including: a 24 gauge butterfly needle and 9 ml blood collection tubes. The protocol for PRF preparation is very simple, whole blood is drawn into the tubes without anticoagulant and is immediately centrifuged (3000rpm for 10 min). The result is a fibrin clot containing the platelets located in the middle of the tube, just between the red blood cell layer at the bottom and acellular plasma at the top.

This clot is removed from the tube and the attached red blood cells are scraped off and discarded. The PRF clot is then placed on the grid in the PRF Box.

The PRF Box was devised to produce membranes of constant thickness that remain hydrated for several hours and to recover the serum exudate expressed from the fibrin clots which is rich in the proteins, vitronectin and fibronectin.⁽⁵⁾

The exudate collected at the bottom of the box may be used to hydrate graft materials, rinse the surgical site, and store autologous grafts.

Nanocrystalline hydroxyapatite (NcHA)

Hydroxyapatites (HAs) represent a family of bone grafting materials with a high degree of biocompatibility that is largely attributable to its presence in natural calcified tissue. Preliminary experimental studies have shown that nano sized ceramics may represent a promising class of bone graft substitutes due to their improved osseointegrative properties. Accordingly, a synthetic nanocrystalline hydroxyapatite (NcHA) bone graft has been introduced for augmentation procedures in intrabony defects. Advantages of NcHA material are osteoconductivity, bioresorbability, and close contact. A special feature of nanostructured materials is an extremely high number of molecules on the surface of the material.

When the NcHA was used as a bone graft substitute, rapid healing of critical size defects was observed in animal experiments and in human applications. NcHA binds to bone and stimulate bone healing by stimulation of osteoblast activity. NcHA has been used for the treatment of metaphyseal fractures in orthopedic surgery, ridge

augmentation, and periimplantitis lesions [6].

Case report 1

A 29 year old male patient reported to the Department of Periodontics with the chief complaint of generalized hypersensitivity, pain and mobility in relation to the upper and lower anteriors, posteriors for the past 6 months. His medical and family history was not contributory.

On clinical examination his oral hygiene status was good. The periodontal parameters including probing pocket depth, clinical attachment loss and tooth mobility were measured.

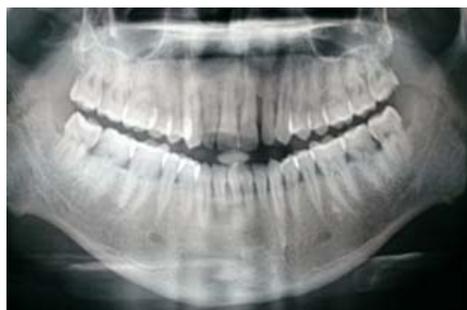
The probing pocket depth was 9mm in relation to 11. Pathological migration was evident in relation to 11 with grade II mobility. Routine haematological investigations, intraoral periapical radiographs and orthopantomogram were carried out for the patient. The investigations showed normal hemogram.

Radiographic findings

Angular bony defect present in relation to the mesial and distal aspect of 11 was noted. Arc shaped bone loss was found in relation to 46. Bony defect angle was 20° in relation to 11, 21.



Case I



Case II

Case report 2

A 31 years old male patient reported to the department of periodontics with the labial migration of upper front tooth for the past 3 months. History revealed no systemic illnesses and his family history was not contributory. His oral hygiene was good. The periodontal parameters were recorded. The probing pocket depth was 9mm in relation to 21. There was a pathological migration with grade I mobility in relation to 21. Routine haematological investigations, intraoral periapical radiographs and orthopantomogram was advised for the patient. Tooth vitality was checked in relation to 21 and was found to be nonvital.

Radiographic findings

OPG revealed angular bony defect in relation to mesial and distal aspect of 21. The defect angle was 31°.

Case report 3

A 22 year old female patient reported to the department of periodontics with the chief complaint of labial migration of upper front teeth for the past 6 months. Her medical and family history were not contributory. Her oral hygiene status was good. Periodontal parameters including probing pocket depth, clinical attachment level and tooth mobility were measured. Pathological migration along with a probing pocket depth of 8, 7mm was present in relation to 11, 21 with grade II mobility.

Routine haematological investigations, intraoral periapical radiographs and orthopantomaogram were advised for the patient. Tooth vitality was checked in relation to 11, 21. Both the teeth were vital.

Radiographic findings

There was an angular defect in relation to the mesial aspect of 11, 21. The defect angle was 20°.

Pre surgical procedures

After completing the initial phase 1 therapy recall visits were made. On re –evaluation probing pocket depth was found to be persistent. For case 1, 11 was checked for vitality, which was non vital. Root canal therapy was done in relation to 11 and Extracoronary splinting was performed as a part of presurgical procedure from 13 to 23. Open flap debridement with regenerative therapy using PRF and nanocrystalline hydroxyapatite was planned for the patient in relation to 11 and 21. For case 2, Periodontal splinting (provisional) was done from 13 to 23 and Root canal therapy was performed in relation to 21 as a part of presurgical procedures. For case 3, Periodontal splinting with incisal loops (in relation to 11, 21) was performed from 13 to 23 as a part of presurgical procedure.



Splinting with incisal loops

Surgical therapy

For cases 1 & 2, after administration of local anesthesia from 13 to 23, sulcular incisions were made on the buccal and palatal sides and the mucoperiosteal flaps were reflected. Thorough surgical debridement was carried out using Gracey’s area specific curettes with betadine saline as the irrigant.

Simultaneously the patient’s venous blood was collected, and PRF preparation was started. Then Nano crystalline hydroxyapatite granules with particle sizes of 100µm were mixed with PRF at a proportion of 1:1.

For case 1, at the surgical site the minced PRF with Hydroxyapatite mixture was delivered on the labial aspect of 11, 21 & 11, 12. Then a compressed PRF membrane is trimmed and placed over the defect.

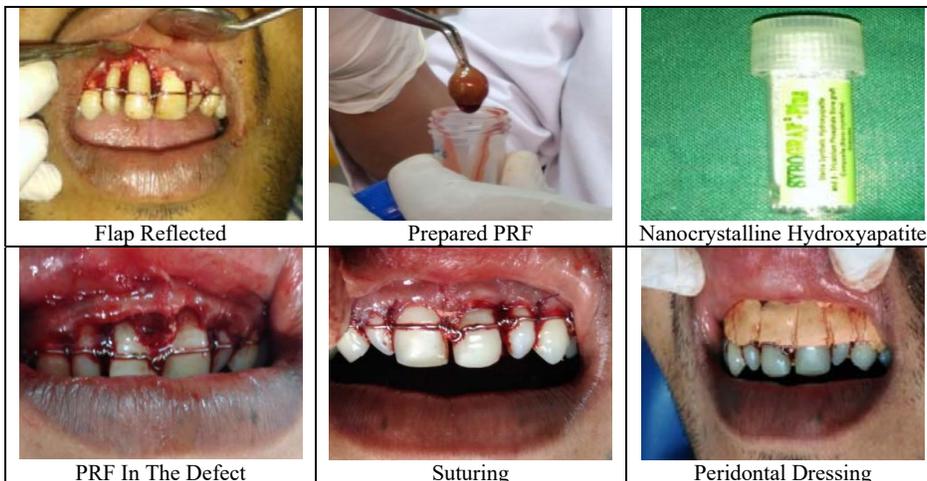
Following the placement of PRF, defect was closed with independent sling suture in relation to 11 and simple interrupted sutures in relation to other teeth, using 4 0 vicryl suture.

For case 2, Presuturing was done by vertical mattress suture using 40 vicryl. In the meanwhile PRF is prepared from collecting patient’s venous blood. Nano crystalline Hydroxyapatite crystal along with PRF mixture in the ratio of 1:1 is placed on the palatal side between 11, 21 and 21, 22 (since the defect was on the palatal side for this patient). Then a compressed PRF membrane is placed over the defect. Then the suturing was completed.

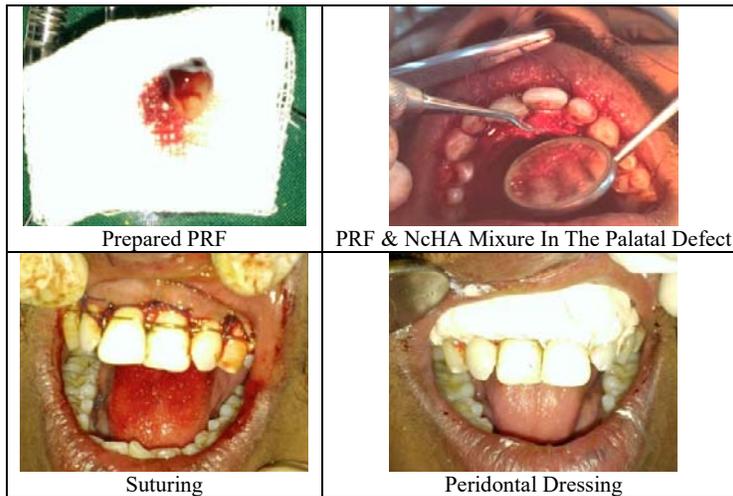
For case 3, Presuturing was done using a horizontal mattress suture using 40 vicryl. Nanocrystalline Hydroxyapatite crystal along with PRF mixture in the ratio of 1:1 is placed on the palatal side between 11, 21 and 21, 22. Then a compressed PRF membrane is placed over the defect. Then the suturing was completed. Periodontal dressing was given. Post surgical instructions were given.



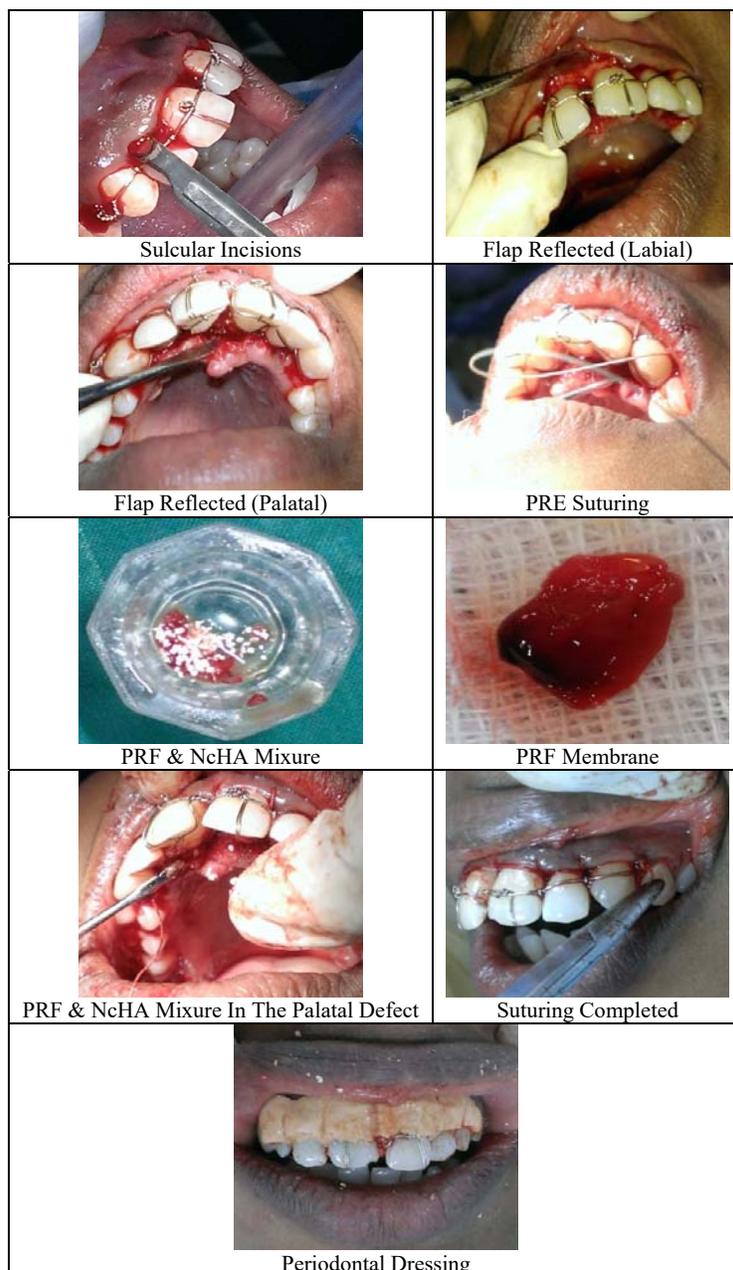
Periodontal splinting



Case I



Case II



Case III

Post surgical evaluation

Following surgery the patient was recalled once in a month for 3 months. For case 1, Post operative intraoral periapical radiograph after a month showed increased bone fill in relation to 11, 12, 11, 21. For cases 2 & 3, the intraoral periapical radiograph taken after one month showed increased bone fill 11, 21, 21, 22 which was measured using a standardized method. Periodontal parameters showed reduction in the probing pocket depth and gain in the clinical attachment level which were the secondary outcomes.



Post Operative Radiograph (1 month)

Case I



PRE Operative Radiograph

1 Month Post Operative Radiograph

Case II



PRE and Post Operative Radiograph (One Month)

Case - III

Discussion

The ultimate goal of periodontal therapy is to control the periodontal infection and to regenerate the lost periodontium. The recent innovation in periodontics is the placement of platelet rich fibrin.

The unique characteristics of PRF is that it does not require any clotting agent, when compared to other platelet concentrates including platelet-rich plasma.⁽⁷⁾ PRF is resorbed by the host very slowly, resulting in sustained release of platelet and leukocyte derived growth factors into the wound area.

Gradual extended release of autologous Growth factors such as PDGF and TGFβ and better induction of osteoblastic differentiation and proliferation by PRF was shown in experimental trials^[8].

Because of the release of leukocytes, PRF exerts antibacterial effect in the wound. PRF contains abundant vascular endothelial growth factor, which is an important factor for angiogenesis.

Hydroxyapatite bone grafting have excellent bone conductive properties which permit the outgrowth of osteogenic cells from already existing bone surfaces^[9]. Hydroxyapatite is a resorbable filling material, do not contain any organic material, and hence does not induce any allergic reaction^[10].

The PRF membrane was placed to contain the PRF and Nc HA mixture in the bony defect during its early healing phase. The PRF membrane have exerted guided tissue regeneration effect on the wound^[11]. In our study PRF membrane is placed to obtain similar results.

It is important to weigh the cost/Benefit ratio, the inexpensive nature of PRF, favourable molecular properties, less technique sensitive preparation makes PRF an ideal material for regenerative therapies.

Researchers have shown the effectiveness of PRF combined with bone grafts in the treatment of subjects with angular defects. AgP patients were selected in our study because of the complexities involved in the regenerative procedures of such cases.

Nikhil D chandradas *et al* evaluated the efficacy of Platelet Rich fibrin in the treatment of human intrabony defects with or without bone grafts. They concluded that PRF improves clinical and radiological parameters compared to open flap debridement alone in intrabony defects. In our present study also there were improvements in radiographic and clinical findings.

PRF helps in better healing and faster regeneration because of the biological modulators and helps in osteoinduction. Nano sized hydroxyapatite leads to increase in protein adsorption and osteoblastic adhesion. In this study this may explain why the PRF and Nanocrystalline HA, combination enhanced bone regeneration. Similar results were obtained in the study done by G. pecora *et al*.

Conclusion

Surgical Periodontal debridement along with the placement of PRF in combination with nanocrystalline hydroxyapatite in angular defects of the subjects showed decreased probing pocket depth, gain in attachment level and radiographic bone fill as compared with the baseline.

Because of the more favourable morphology of angular defects in subjects with AgP and the ideal characteristics of PRF reconstructive therapy along with PRF and nanocrystalline hydroxyapatite in angular defects of AgP patients can be an effective approach for periodontal regeneration.

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