Original Research

A randomized comparative study of platelet-rich fibrin along with hydroxyapatite graft for the treatment of 3-walled defects in chronic periodontitis

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Abstract

Background. Platelet Rich Fibrin (PRF) is a concentrate of leucocyte and platelets that contains various polypeptide growth factors. It has immense potential for use as a periodontal regenerative material in periodontal defects. Porous hydroxyapatite (HA) has long been used as bone grafting material. Recently it has been reported that when PRF is used in combination with HA, itgives a synergistic effect and results in a better periodontal regeneration. The present study aims to explore the clinical and radiographic effectiveness of autologous PRF versus PRF+HA in the regenerative treatment of intrabony defects (IBD) in patients with chronic periodontitis.

Methods. Sixty patients with IBDs were divided into test and control groups. The test group patients were treated with autologous PRF, whereas the control group patients were treated with PRF+HA. Clinical parameters were recorded at baseline, three months, six months and nine months' time interval. Radiographic measurements were recorded at baseline and nine months.

Results. A reduction in PI, MGI, PPD, RAL, and IBD was observed in both the groups at nine months. Mean IBD reduction in the control group was 3.70 ± 1.16 mm, whereas, the mean reduction in the test group was 4.80 ± 1.03 mm, showing a significant reduction in IBDs. Similarly, the percentage of bone fill in the test group was 65% ± 3.67%, whereas bone fill in controls was 56.7% ± 3.56%, showing a significant bone fill in the study group.

Conclusion. Treatment of IBDs with PRF+HA showed a significant improvement in all the clinical and radiographic parameters. When HA was added to PRF, it increased the regenerative effect in the treatment of 3 wall IBDs.

Keywords: guided tissue regeneration, hydroxyapatite graft, periodontitis, platelet rich fibrin

Introduction

The periodontium consists of the tooth-supporting structures that are composed of the gingiva, cementum, periodontal ligament and alveolar bone [1]. Periodontitis is an inflammatory disease that leads to the degradation of periodontal tissues, causing tooth movement and eventually tooth loss [2]. Regeneration is defined as the reproduction or reconstitution of a lost or injured part of the body in such a way that the architecture and function of the lost or injured tissues are completely restored [3]. There is a broad range of treatment options available for regeneration of alveolar bone, but only a few are truly regenerative. For regenerative materials to be considered as a gold standard, it should demonstrate the formation of bone, cementum, periodontal ligament in histological sections [4]. A recent paper stated that the current regenerative periodontal therapy could only restore a

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fraction of lost tissue [1]. Hence, a complete regeneration still remains a dream for the periodontal surgeon. Based on the various endogenous regenerative techniques, various biomaterials have been used for regeneration in addition to allogenic [5,6] and autogenic [7,8] bone grafts, but still, no perfect materials have been discovered as a gold standard in the treatment of intrabony defects (IBDs).

For a long time various research has been conducted to use biologically active molecules like extracellular matrix proteins [9], growth factors [10], and mediators of cell metabolism and activity [11], to attain periodontal regeneration. Various growth factors such as fibroblast growth factor, epidermal growth factor, platelet-derived growth factors (PDGF), insulin-like growth factors, vascular endothelial growth factor (VEGF), parathyroid hormone, transforming growth factor-β (TGF- β), and bone morphogenic proteins have been tested alone or in combination to obtain periodontal regeneration [12].

Platelet Rich Fibrin (PRF) is an autogenic biomaterial which was developed by Choukroun in France [13]. It is a second-generation platelet concentrate. It is thought to accelerate soft and hard tissue healing. The major advantage of PRF is its ease of preparation and minimal expense, and lack of biochemical modification. It is also more homogenous, stable, and easy to handle [14]. PRF release important growth factors such as TNF-β, and PDGF, which help carry cells that are vital for the tissue regeneration [15,16]. A current study showed that PRF membrane has a very slow sustained release of vital growth hormones [17]. This results in a PRF membrane to stimulate its surgical environment for a significant time during healing. PRF is beneficial in several other fields like plastic surgery, coronally advanced flap, and sinus lift procedures [18-20]. PRF has also been demonstrated to act as a scaffold for breeding human periosteal cells which in future may be suitable for bone tissue engineering [21].

Hydroxyapatite (HA) bone grafts are porous material that has been used to fill IBDs, resulting in clinically recognizable responses. HA is formed by conversion of the porites goniopora coral exoskeleton that has pores of approximately 600 micrometres and pore interconnection averaging 260 micrometres in diameter [22]. It has been shown that porous HA grafts have wonderful bone conductive properties that enhance the outgrowth of osteogenic cells from existing bone surfaces into the adjacent bone material [23]. HA does not induce any allergic reaction due to the absence of any organic component in it; hence it is very well tolerated.

PRF and HA, when used in periodontal therapy, offer excellent results in treating osseous defects [24]. However, there is very few research literature showing the combined effect of HA and PRF. It is not clearly known whether the above combination of these materials can give a better result. Hence the purpose of this study is to find out the efficacy of PRF and HA in combination with PRF with open flap debridement in the treatment of three wall bony defects.

Method

Subject selection

Sixty systemically healthy patients were selected for this study. Among these, 33 were males, and the remaining were females. They were undergoing periodontal treatment in the Department of Periodontology. They were enrolled in this study for a time period of nine months. This study was conducted from January 2019 to February 2020. Patients were made aware of the study protocol, and written consents were obtained at the beginning of the study. Ethical clearance was obtained from the research and review board committee (KSD/2018/290). This study was conducted in accordance with the declaration of Helsinki.

The chronic periodontitis was diagnosed based on the 1999 classification of periodontal disease [25]. The subjects were included with the presence of three wall IBDs ≥ 3mm (distance in relation to alveolar crest to the base of the defect on intraoral periapical radiograph [IOPA]), and an interproximal pocket probing depth of ≥ 5mm after phase 1 therapy. Patients excluded were: with one wall or two wall defects, presence of interdental craters, aggressive periodontitis, known systemic illness, any medication that may affect the outcome in regeneration, any bleeding disorders, pregnant patients, and tobacco user in any form. In addition to this, teeth with furcation defects, non-vital, having grade II mobility were also excluded from this study.

Each patient was given clear instructions regarding the maintenance of proper oral hygiene. Full mouth scaling and root planing (SRP) was performed under local anesthesia. Eight weeks after the first phase therapy, the periodontal evaluation was performed to confirm patients qualified for the study. The subjects were divided randomly in each group by coin toss method. The control group consisted of patients who were treated with PRF, while test group patients were treated by PRF in combination with HA.

A single operator performed all the surgeries in both the group to eliminate the inter-operator bias and the second operator recorded all the radiographic and clinical measurements without the knowledge of particular treatment.

Clinical and radiographic measurements

The clinical parameters recorded were Plaque Index (PI), Modified Gingival Index (MGI), Probing Pocket Depth (PPD), Relative Attachment Level (RAL), and Bone fill. A customized acrylic stent was used in the measurements of PPD and RAL to ensure a reproducible placement of the periodontal probe. All IBDs were measured at baseline and nine months' time interval postoperatively. In order to measure the bone defect, distance from the base of the defect to the crest of the alveolar bone was considered. Individual customized bite

blocks and paralleling angle technique were considered to obtain standardized radiographs. Grid radiograph was obtained by the use of a digital radiovisiography (Carestream, Vita CR, USA). This same software was used to the IBD depth and total area of defect.

PRF Preparation

The PRF was prepared using the criteria laid down by Choukroun et al [15]. Just prior to surgery, intravenous blood was withdrawn by venipuncture of the antecubital vein in two 10 ml sterile vacuum tubes without anticoagulant and immediately centrifuged (R 4C, REMI, Mumbai, India) at 3000 rpm for 10 minutes. Once the centrifugation was done, PRF was easily separated from red corpuscles using sterile tweezers and scissors just after removal of PPP (Platelet Poor Plasma) and then transferred to a sterile compress. A stable membrane of fibrin was obtained by squeezing serum out of the PRF clot.

Surgical procedure

Intraoral asepsis was performed by using betadine mouthwash whereas extraoral asepsis was performed by using iodine solution. Local anaesthesia was administered, buccal and lingual sulcular incisions were made, and mucoperiosteal flaps were reflected. Utmost care was taken to preserve as much interproximal soft tissue as possible. Complete defect debridement and root planing were performed using ultrasonic scalers (Setelec, Acteon, Norwich, United Kingdom).

In the test group (PRF + HA), HA (SyboGraf, Eucare Pharmaceuticals, Chennai, India) granules with a particle size of 600 to 700 µm were mixed with PRF at a proportion of 1:1 by volume. The PRF + HA mixture was placed in the defect without overfilling it. The PRF membrane was trimmed and adapted over the defect.

In the control group (PRF), PRF was placed in the IBD, and another portion was used to prepare the membrane by which the defect was covered. The periosteal flap was secured in its original position and sutured using 3-0 non-resorbable silk suture (Ethicon, Johnson & Johnson, NJ, USA). Interrupted sutures were placed, and the periodontal pack was placed (COE-PAK, GC America, IL, USA).

Postoperative care

Amoxicillin, 500 mg, four times daily for five days and Ibuprofen, 800 mg, three times daily for three days were prescribed post-surgery. Patients were also prescribed chlorhexidine digluconate rinses (0.12%) twice daily for two weeks. Periodontal dressing and suture removal was done two weeks postoperatively. Patients were reinstructed to maintain utmost oral hygiene for the next eight weeks postoperatively. Patients recalls were scheduled at three months, six months, and nine months' time.

Post-surgical measurements

Soft tissue evaluation was performed on every recall visit, whereas hard tissue evaluation was performed only after nine months post-surgery. Soft tissue measurements were repeated with previously used customized acrylic stents. A second grid IOPA was taken at the end of nine-month for revaluation of hard tissue.

Statistical analyses

The data were analyzed using SPSS (V11 for Windows, SPSS, Chicago, IL, USA). Power calculations were performed at 90% before the initiation of the study. The results were averaged for each clinical parameter at three months, six months and nine months whereas, radiographic parameter at baseline and nine months. An unpaired t-test was performed to assess the statistical significance among the data.

Results

All the sixty patients (60 sites) completed the study. Out of these sixty sites, 38 were mandibular sites 22 were maxillary sites. All the patients showed uneventful healing. A statistically significant reduction in PI, MGI, PPD, RAL, and IBD was observed in both the groups at nine months (Table I).

Table I. Clinical and radiographic parameters in test and control group at baseline and 9 month.

		PRF		PRF + HA	
Plaque Index	Baseline	0.70 ± 0.18	0.002*	0.75 ± 0.14	0.0001*
	9 Months	0.45 ± 0.12		0.45 ± 0.12	
MGI	Baseline	1.48 ± 0.21	0.0001*	1.51 ± 0.19	0.0001*
	9 Months	0.51 ± 0.12		0.52 ± 0.11	
PPD	Baseline	7.00 ± 1.05	0.0001*	6.90 ± 0.88	0.0001*
	9 Months	3.10 ± 0.74		2.40 ± 0.52	
RAL	Baseline	8.10 ± 1.20	0.0001*	7.70 ± 0.95	0.0001*
	9 Months	4.00 ± 0.67		3.20 ± 0.42	
IBD	Baseline	5.55 ± 1.38	0.0001*	5.05 ± 0.68	0.0001*
	9 Months	1.85 ± 0.53		1.95 ± 1.14	

* Statistically Significant

All the mean values are reported in table I, the mean changes in PPD, RAL, IBD, and Bone defect fill are reported in table II. PRF + HA group showed more reduction in PPD, RAL gain, but the change was not significant (Table II). Similarly, gain in RAL was more in the test group, but the gain was non-significant (p-value = 0.506, Table II). Mean IBD reduction in the control group was 3.70 ± 1.16 mm whereas, the mean reduction in the test group was 4.80 ± 1.03 mm, showing a significant reduction in IBDs (p-value = 0.03, Table II). Similarly, percentage of bone fill at test was 65% ± 3.67% whereas, bone fill at control site was 56.7% ± 3.56%, showing a significant bone fill at the test site (p-value = 0.0001, Table II).

Table II. Mean changes in clinical and radiographic parameters among groups nine months post-surgery.

Parameter	PRF	PRF + HA	P-value
Mean PPD Change	3.90 ± 0.99	4.10 ± 0.85	0.164
Mean RAL Gain	4.10 ± 1.60	4.50 ± 0.97	0.506
Mean IBD Depth reduction	3.70 ± 1.16	4.80 ± 1.03	0.03*
Bone Defect fill percentage	56.70 ± 3.56	65 ± 3.67	0.0001*

* Statistically Significant

Discussion

The current study compares the effectiveness of autologous PRF and PRF + HA in the treatment of IBDs in patients suffering from chronic periodontitis. Patient teeth with osseous defects were similar at baseline. Each patient maintained good to excellent oral hygiene throughout the duration of the study. There was complete uneventful healing. This is in accordance with the study done by Pradeep et al [26,27]. Hence this shows that autologous PRF has the properties to enhance periodontal wound healing. This study excluded the subjects that were using tobacco in any form since tobacco use significantly influence the regenerative treatment outcome [28,29]. Since this study excluded the subjects with poor oral hygiene and tobacco users, it may be assumed that careful patient selection may be responsible for the positive outcome of this study.

Most vital clinical outcome of the current study is the reduction in PPD, IBD, and gain in RAL. This outcome determines the success of any periodontal treatment. In this study, a prominent PPD reduction and gain in RAL is found in both the groups at the end of 9 months compared to baseline. However, the reduction in PPD and gain in RAL was not statistically significant. In the present study, the percentage of IBD fill in PRF+HA group (65% ± 3.67) was significantly higher than the PRF group (56.70% ± 3.56). This supports the fact that there is a significant advantage of various growth factors present in PRF when combined with a porous bone graft, may accelerate the soft and hard tissue healing [24,30]. Lock and Liu et al in his study asserted that nanocrystalline HA loaded on a nanocomposite scaffold combined with PRF stimulate attachment and differentiation of undifferentiated mesenchymal cells which may be the probable reason for significantly more bone fill in PRF+HA Group [31]. Kanaya and co-workers observed that HA could stimulate differentiation of PDL cells, mediated by mechanosensitive signaling pathway and expression of BMP-2 [30]. Jain and co-workers showed that degradation of calcium sulphate particles which decreases local pH could result in demineralization of wall defects that in turn release the growth factors, such as BMP-2, BMP-7, TGF-ß, and PDGF-BB [32]. The PPD, IBD depth reduction and increased percentage of IBD bone fill in this study is similar to study done by Sharma [33] and Thorat et al [34]. The findings of this study are also in accordance to the study done by Lekovic et al [35].

PRF consists of an autologous leukocyte-platelet-rich fibrin matrix [15], composed of a tetra molecular structure, with cytokines, platelets, cytokines, and stem cells within it [36,37]. Clinically observed healing properties of PRF can be attributed to the ability of PRF to progressively release cytokines during fibrin matrix remodelling [36]. Dohan et al. reported that PRF organized as a dense fibrin scaffold with a higher number of leukocytes concentrated in one part of the clot with a specific slow release growth factors [38]. Leukocytes appear to have a vital influence on growth factors release [39], anti-infectious activities, immune regulation [40], and matrix remodeling during healing. Kornsuthisopon et al. [41] in his research study hypothesized that PRF promoted wound healing by reducing inflammatory reaction that resulted from the various anti-inflammatory cytokines.

Several reports have shown that the combination of mineralized graft material, with a semifluid, non-rigid agent, such as enamel matrix protein, expressively augments the clinical results of IBDs treated without the addition of rigid graft material [42,43]. PRF+HA group had an enhanced bone formation when compared to autologous PRF group. This can be attributed to PRF whose effects are enhanced in IBD area by HA which is thought to preserve the space for tissue regeneration and also exercise osteoconductive effect [44].

In the current research, test and control sites were filled with PRF and/or HA, and they were covered by PRF membrane. The important function of the PRF membrane was to secure the HA or PRF in IBD during initial healing. The primary outcome of this study is the bony defect fill since the radiographic evaluation is a non-invasive examination for bony defect gain. There may be possible errors in the calculation of IBD fill, which can be attributed to exposure time and the angulation of the sensor. In order to calculate the accurate bone fill, various studies have to be done using the surgical re-entry method. This will help in acquiring accurate data of bone fill. Surgical re-entry will substantiate the current data for more accuracy.

Conclusion

The analysis of the data from this study suggests that the treatment of IBDs with PRF+HA resulted in a significant reduction in PPD, RAL, and an increase in the percentage of bone fill when compared to the baseline. When PRF is used in conjugation HA, it improves the clinical outcome when compared with autologous PRF alone in the treatment of 3 wall IBDs. However, long term studies are required to determine the clinical and radiographic evaluation. Along with the clinical and radiographic evaluation, it is important to evaluate the histologic nature of newly formed hard and soft tissue.

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