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Buccal bone reconstruction using injectable platelet rich fibrin (i-prf) combined with xenograft (sticky bone) covered with a-prf+ (advanced injectable platelet rich fibrin+) membrane in periodontally hopeless tooth socket: radiographic analysis- A case series

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Abstract

Following the tooth extraction, buccal bone subjects to a higher degree of resorption than the lingual or palatal bony plates. This alteration in the alveolar ridge dimension hinders implant placement in the ideal prosthetic position. This study aimed to assess clinically and radiographically the efficacy of sticky bone Deprotinized bovine bone mineral (DBBM)+ injectable platelet-rich fibrin (i- PRF)] with Advanced platelet-rich fibrin plus (A-PRF+) membrane for socket augmentation of periodontally hopeless tooth socket. This prospective clinical pilot study was conducted in twelve systemically healthy patients with 16 sites having at least one periodontally hopeless tooth indicated for extraction with adjacent periodontally healthy teeth. Atraumatic extraction was performed using periotome following which the sockets were augmented with sticky bone[DBBM+i- PRF] and covered with A-PRF+ membrane. Clinical ridge height and radiographic ridge width were recorded at baseline and 6 months post-operatively. Clinically, an increase in mean vertical height buccally with an average difference of 4.38±1.86mm ($p < .05$) was observed. Mean radiographic buccolingual/Bucco-palatal width at 2mm was 7.71±2.04mm at baseline and six months it was 7.97±1.95mm ($P > .05$) and at 4 mm mean buccolingual/Bucco-palatal width at baseline was 8.39±1.84mm and at six months it was amplified to 9.21±2.05mm($P < .05$) below the crest respectively, demonstrating a significant increase in width of the alveolar ridge at middle and the apical areas. This was followed by implant placement and delivery of the

prosthesis. It can be concluded that the combination of sticky bone with A-PRF+ membrane was found to be beneficial in buccal bone reconstruction in the periodontally hopeless tooth socket.

Keywords: Alveolar ridge augmentation; Bio-Oss®; Bone regeneration; PRF; Tooth extraction.

Introduction

Tooth extraction triggers a sequence of biologic events that typically result in volumetric resorption leading to changes in the dimension and contour of the alveolar ridge. The esthetic and functional success of fixed prosthesis largely depends on the thickness of residual buccal bone[1]. Bone quality and quantity are critical in regulating long term function and stability of implants and peri-implant tissues. In the case of the periodontally hopeless tooth socket, bone dimensions are compromised, with pronounced bone loss, which if left untreated, may lead to severe destruction of alveolar bone. Such a site will be unsuitable for implant placement unless additional two-stage bone augmentation procedures are performed [2]. This kind of approach not only increases the overall treatment time but also adds a financial burden to the patient. Efforts to limit bone resorption reduces the overall treatment time and maximize therapeutic liability. This has led to the use of bone grafts in combination with membranes to enhance the architecture of residual alveolar ridge producing better results at clinical and radiographic level [3].

Platelet-rich fibrin (PRF), a second-generation platelet concentrate, was first developed in France by Choukroun et al in 2001[4]. Injectable platelet-rich fibrin (i-PRF), a modification of PRF is rich in platelets and enables slow and sustained release of growth factors that may contribute to wound-healing processes with increased vascularization [5]. Another modification is advanced platelet-rich fibrin plus (A-PRF+) [6] which promotes gingival fibroblast migration, proliferation, and collagen1 m-RNA production along with collagen synthesis from osteoblast-like cells. These events are the key factors during the remodeling of a socket [7].

Geistlich Bio-Oss® (Geistlich Pharma AG, Wolhusen, Switzerland) is a deproteinized, slowly resorbable, sterilized bovine-derived xenograft (DBBM) consisting of a mineral osseous matrix identical to the human bone which has demonstrated excellent properties as an osteoconductive bone graft [8].

Scare literature available for regenerating buccal bone in the periodontally hopeless tooth socket.

This study aimed to assess clinically and radiographically the efficacy of sticky bone [DBBM)+i- PRF] with A-PRF+ membrane for socket augmentation of periodontally hopeless tooth socket at baseline and 6 months post-operatively.

Materials and method

This prospective clinical study was conducted in twelve systemically healthy patients with 16 sites from the outpatient Department of Periodontics in accordance with the Declaration of Helinski. Ethical approval for the same was obtained from the Institutional Ethical Committee Each patient was given a detailed verbal & written description of the risks & benefits of the proposed treatment & signed consent was obtained before the commencement of the study.

Male and female subjects within the age range of 25-55 years with good oral hygiene (Plaque index<1.9) [9] and tooth indicated for extraction with loss of buccal bone from central incisors to first pre-molar with adjacent healthy teeth were included. Subjects who were chronic smokers, pregnant women or lactating mothers, subjects on drug therapy (like biphosphonates, antiplatelets, anticoagulants) were excluded.

The sample size was calculated using α value as 1.960 and β value as 0.842, the estimated sample size was 16 sites.

Patients who satisfied inclusion criteria were selected and phase 1 periodontal therapy was performed for them. Surgery was carried out only after oral hygiene was found to be satisfactory (PI <1.9) [9]. On the day of the surgery, extraoral scrubbing with 2% povidine iodine and pre-procedural mouth rinse with 0.2% chlorhexidine was performed. Following this, the site of interest was anaesthetized using local anesthesia (2% Lignocaine hydrochloride with 1:80,000 epinephrine). A sulcular incision was given with blade no.12 circumferentially around the isnvolved tooth to release supracrestal fibers. Atraumatic extraction was carried out using periotome to preserve as much socket bone as possible. A vertical releasing incision was given unilaterally on the line angle of the adjacent tooth to reflect mucoperiosteal flap on the facial aspect in corono-apical direction. This was extended until the defect margin was visualized. Thorough debridement of the socket was done using standard Gracey curettes and was flushed with sterile saline.

I-PRF & A-PRF+ were prepared with DUO® Centrifuge (Process Nice, France) according to the manufacturer protocol [5,6]. To procure an A-PRF+ membrane, 10 ml of venous blood was collected without anticoagulants in an A-PRF tube (Lifecare devices, Mumbai) and immediately centrifuged at 1300 rpm for 8 minutes[6]. The obtained membrane was collected with a tweezer and compressed in Choukroun PRF BOX.

For i-PRF, 10 ml of venous blood was collected without anticoagulant in an i-PRF tube and immediately centrifuged at 700 rpm for 3 minutes. After centrifugation, the blood is separated into a yellow-orange upper phase (i-PRF) and a red lower phase (red cell fraction). I-PRF was collected using a syringe by controlled aspiration of the upper fluid phase just above the red blood corpuscles (RBC) layer. The obtained i-PRF was then mixed with DBBM (particle size 0.25–1 mm, 0.5cc) in a bone well to obtain sticky bone[5]. This was then slightly overfilled into the socket and finally covered with an A-PRF+ membrane. Primary closure was achieved with horizontal mattress and interrupted sutures using black monofilament polyamide non-absorbable suture [4-0 Ethilon®, USA]. Medications prescribed were tab. Augmentin 625 mg [500 mg Amoxycillin and potassium clavulanate equivalent to 125 mg clavulanic acid], Tab. Ketorol DT 10 mg [Ketorolac] twice daily for 5 days. The patients were instructed to avoid chewing or applying any kind of pressure and avoid brushing at the operated site for 10 days and advised mouth rinse with 0.12% chlorhexidine gluconate for 2 weeks [10].

After flap reflection, clinically, measurement of the vertical height of ridge was carried out by keeping UNC-15 periodontal probe horizontally to CEJ of adjacent teeth on either side as the reference point, other probe was placed vertically on the deepest point of the buccal alveolar crest and recorded at baseline and 6 months post-operatively.

For radiographic measurements, CBCT was taken (Planmeca 3 D classic, FINLAND) at baseline before tooth extraction and 6 months postoperatively. The analysis was carried out as previously described by Hani et al [11]. In brief, the scans (standardized as FOV 5x5, 90 KVP, 10 mA, 17 seconds) were analyzed using ROMIXES software (version 3.6.1). After the imaged volume was reconstructed, the images were viewed under Optimal viewing conditions. The images were oriented along the long axis of the tooth plane which was displayed in all 3 orthogonal planes (axial, coronal, and

sagittal). The sagittal section was considered to analyze the buccolingual or Bucco-palatal bone thickness at 2mm and 4mm below the ridge pre-operatively and 6 months postoperatively. The horizontal plane was oriented at different levels in all three planes. The measurements were carried out using a measuring tool by drawing a line from the coronal most point on the ridge as a reference point. From the above procedure, dimensional alternations could be well appreciated.

All patients returned for CBCT evaluation six months post-operatively and bone augmentation. All the clinical & radiographic parameters were recorded and further procedure of implant placement was initiated. After administration of anesthesia, full-thickness flaps were reflected. Complete healing of the ridge was evident in all cases with no detectable loose graft particles. The osteotomy was prepared and an implant was placed with primary stability at 70-75Ncm, the healing cap was positioned and in two cases additional grafting was done. Subsequently, impressions were created and the final prosthesis was delivered.

Statistical Analysis

All the clinical and radiographic values were entered in a standard proforma & subjected to appropriate statistical analysis using Statistical package for social sciences (SPSS 20) software. Wilcoxon signed-rank test was used to analyze the changes in clinical and radiographic parameters. 'P' value of < 0.05 was considered to be significant.

Results

All patients completed stipulated follow up time without any dropouts. No adverse events such as signs of inflammation, redness, pus discharge, or any other complication were reported.

The mean defect depth from a fixed reference point (CEJ of adjacent teeth) till the deepest point of the defect was 9.63±2.92mm at baseline and 6 months after ridge augmentation, the mean value decreased to 5.25±1.06mm The mean difference in pre-operative and post-operative defect depth 4.38±1.86mm which was statistically highly significant ($p<0.0001$, Table 1)

In radiographic parameters, the mean buccolingual/Bucco-palatal width at 2mm below the crest was 7.71±2.04mm at baseline and at 6 months it was 7.97±1.95mm. The mean difference between them was 0.26±0.09 which was statistically non-significant ($p=0.127$)

At 4mm below the crest, mean buccolingual/Bucco-palatal width was 8.39±1.84mm at baseline and at 6 months it was amplified to 9.21±2.05mm with a mean difference of 0.82±0.21mm ($p=0.001$) which was found to be statistically significant. (Table 2)

Discussion

Bone regeneration is marvellous biology and biomechanics enfolded in distinct sequential events developed by a dense vascular cascade in the re-establishment of its form and function [12]. Pro-inflammatory cytokines, proteins belonging to the transforming growth factor-β (TGF-β) superfamily, and angiogenic factors play a central role in the regeneration of bone. Lack of interproximal bone and buccal bone poses a crucial task in bone augmentation procedure and further implant placement. Literature studies have exhibited, survival rates of dental implants inserted at augmented sites are similar to the survival rates of implants placed in natural bone [13]. Particular attention needs to be given to the buccal bone because of its extensive remodeling ability as well as its role in supporting the esthetic buccal mucosa [14]. Different strategies of ridge preservation and socket management have been employed to minimize bone resorption and to optimize the availability of bone volume [14]. In case of

the periodontal hopeless tooth causes further alveolar bone loss to pose a questionable prognosis [15]. Bone regeneration in such defects is very technique-sensitive [16].

A study by Jana et al [17], demonstrated the effectiveness of using tooth root as a block graft for ridge augmentation in the periodontally hopeless extraction site that makes it more suitable for future implant placement. Also, Desai et al [18] demonstrated predictable immediate ridge augmentation with autogenous chin block graft and observed perfect 3-Dimensional prosthetic positioning of the implant in severely deficient ridges.

However, on a thorough literature search, we did not come across any study using a combination of DBBM, i-PRF, and A-PRF+ in the reconstruction of buccal bone in the periodontally hopeless tooth socket. Thus, the present study was aimed to assess clinically and radiographically the efficacy of sticky bone [DBBM)+i- PRF] with A-PRF+ membrane for socket augmentation of periodontally hopeless tooth socket at baseline and 6 months post-operatively. It was demonstrated a significant gain in vertical height of buccal bone ($p < 0.05$) as compared from baseline to 6 months postoperatively. Also, radiographically, mean pre-operative buccolingual/Bucco-palatal thickness at 2mm below the crest demonstrated increase in the dimension of alveolar ridge (P-value 0.127) and at 4mm below the crest, the measurements were amplified at 6 months (P value 0.001)

A xenograft is a slowly resorbing material that contributes to space maintenance for a prolonged period, ensuring sufficient time for the host cells to repopulate in the wound [19]. Nart et al [20] suggested Bio-Oss® (DBBM) seems to be a suitable biomaterial for ridge preservation procedures. The osteoconductive potential of xenogenic bone graft was enhanced by the addition of i-PRF that is "sticky bone", which on completion of the coagulation process formed gel/putty-like consistency with the graft particles. The graft thus formed had good workable consistency, was conducive for grafting, and could be easily manipulated in the defect. The advantage of converting particulate graft into sticky bone is multi-fold. It gets encapsulated in the fibrin matrix of i-PRF which polymerizes and stabilizes around the graft particles and reduces micro-motion, thereby, accelerates tissue healing allowing for new vital bone formation[21].

Further, the use of i-PRF led to an enrichment of graft with platelets and leukocytes. I-PRF is a safe and reliable material, which can effectively shorten the healing time and enhance the effect of osteogenesis [22] which is in agreement with the results of our study. This also had an additional benefit of bio-activating the xenograft by the release of a large number of growth factors at the site. In a recent study, sticky bone (xenograft with i-PRF) used for sinus augmentation, demonstrated normal osseous healing promotion with new bone formation by interacting with the surrounding tissues[23]. In effect, in the present study, the osteoconductive graft was converted to osteopromotive which may have contributed to providing early and superior bone reconstruction and an implant was placed in all the cases. Two cases needed additional grafting at the time of implant placement. Thus, the combination of DBBM + i- PRF with A-PRF+ membrane indicates a predictable approach in the reconstruction of lost buccal bone in a periodontally hopeless extraction socket.

Modification of the preparation protocol by reducing the applied relevant centrifugation force (RCF) resulted in an improved preparation protocol for advanced PRF+ (A-PRF+). In an in-vitro study, over 10 days, A-PRF+ matrices demonstrated significantly enhanced

accumulated growth factors especially vascular endothelial growth factor (VEGF) compared to conventional PRF [24]. The low-speed centrifugation concept (LSCC) indicates that, by RCF, the regeneration capacity of PRF matrices can be enhanced [25]. Further, the use of PRF membrane in the present study acted as "biologic connexion" that yields in the migration of osteoprogenitor cells to the center of the graft [26]. i-PRF matrix with enhanced monocyte concentrations could serve as an autologous source of regenerative cells to support guided bone and tissue regeneration. This was the rationale for use of A-PRF + membrane in the present study. The usage of PRF membrane was sufficient to maintain xenogenic graft in a consolidated matrix for the bone regeneration to continue as it disintegrates in around 7-11 days [27]. This was in accordance with the study by Mariano Sanz etal [28]. Also, an in-vitro study by L. Pitzurra et al [29] to compare the effectiveness in migration and proliferation of A-PRF+ induced higher cell proliferation. The results observed in the present study can be attributed to sufficient interdental bone for the containment of graft contributed to mechanical stability and native cell transformation. Also, the usage of PRF (i-PRF and A-PRF+) provided an intimate assembly of cytokines inducing angiogenesis which supports proliferation and differentiation of bone cells during wound healing and remodeling [30].

A study by Gil et al [22] evaluated the clinical and histological changes in the healing of the extraction socket treated with i- PRF mixed with a xenograft Bio-Oss® and a Leukocyte PRF (L- PRF) membrane and proposed that i-PRF significantly enhance the quality of both soft and hard tissue healing. The results are consistent with our study. Also, a study by Lee et al on beagle dogs [31] where extraction socket was grafted with deproteinized porcine bone mineral (DPBM) and concluded that ridge augmentation to a damaged extraction socket with a buccal-bone deficiency can have a greater clinical impact. These results were also in agreement with our study.

Thus, the combination of A-PRF+ and i-PRF along with xenograft allowed good bone reconstruction and successful placement of the implant in all the cases. Hence, it can be concluded that the use of sticky bone with A-PRF+ membrane resulted in a definitive increase in alveolar ridge height and width radiographically at six months in periodontally hopeless teeth socket.

Conclusion

Although bone resorption is an unavoidable sequel regardless of the usage of any biomaterials, using Bio-Oss® mixed with i-PRF (sticky bone) and A-PRF+ membrane seemed to positively influence the dimensional changes of the ridge in periodontally hopeless teeth socket and allow for the placement of implants at the end of 6 months. However present study was a pilot study, studies with a larger sample size with long-term follow-up are deemed necessary.

List of abbreviations

DBBM = Deprotinized bovine bone mineral

i- PRF = injectable platelet-rich fibrin

A-PRF= Advanced platelet-rich fibrin plus

VEGF = vascular endothelial growth factor

LSCC =low-speed centrifugation concept

RCF =relevant centrifugation force

TGF= Transforming growth factor

CBCT= Cone beam computed tomography

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Legend Figure and Table

Table1: descriptive statistics of clinical parameters

Vertical height measurements of defect					
	Baseline	6 Months	Mean Difference	Mean Change	P Value
Buccal	9.63±2.92mm	5.25±1.06mm	4.38±1.86mm	45.48%	0.000*
Lingual/palatal	6.00±2.63mm	3.63±1.15mm	2.37±1.48mm	39.5%	0.000*

*Statistical significant ('P' value of < 0.05 was considered to be significant)

Table 2: Descriptive Statistics of Radiographic Parameters

	Baseline	6 Months	Mean Difference	Mean Change	P Value
1 mm below	7.22±2.10mm	6.70±2.21mm	0.52±0.11mm	7.2%	0.007
2 mm below	7.22±2.10mm	7.97±1.95mm	0.26±0.09mm	3.37 %	0.127*
4 mm below	8.39±1.84mm	9.21±2.05mm	0.82±0.21mm	9.77%	0.001

*Statistical non-significant ('P' value of < 0.05 was considered to be significant)

Fig 1: pre-op cbct scan of 31

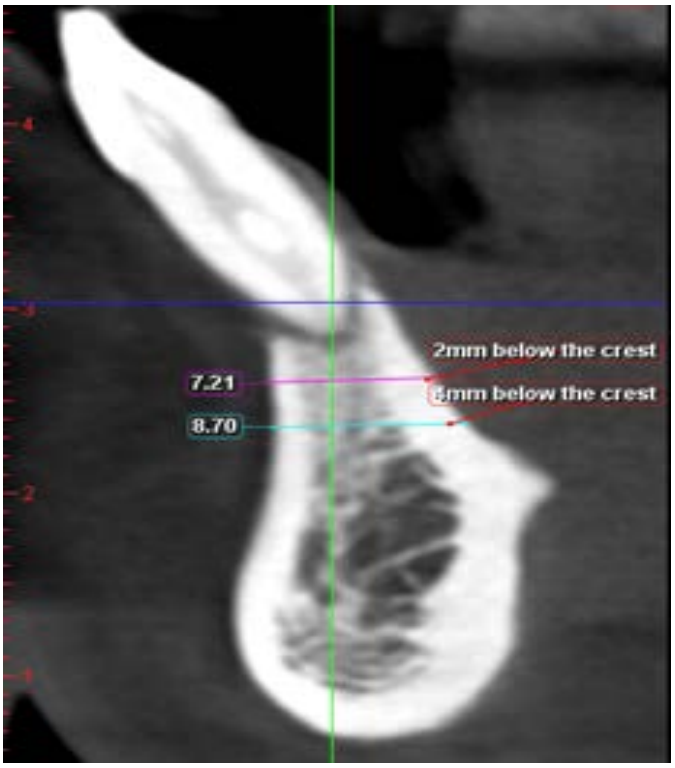

Fig 2: pre –op clinical view after reflection

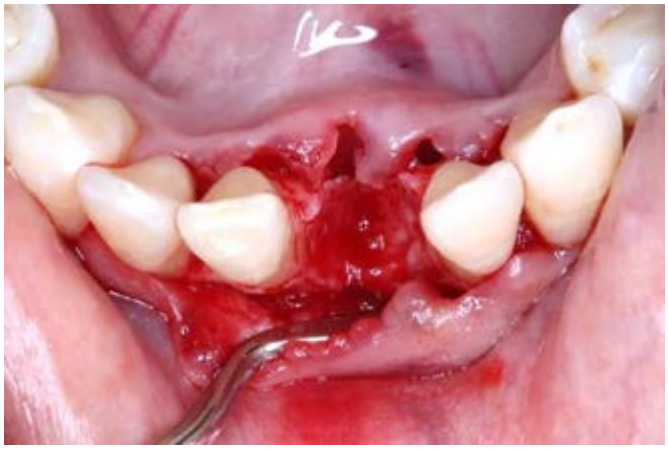

Fig 3: i-prf mixed with bio-oss®(sticky bone) grafted in the socket in surgically created buccal pouch

Fig 4: surgical site protected with a-prf+ membrane

Fig 5: surgical site secured with 5-0 silk sutures

Fig 6: post operative cbct scan at 6 months

Fig 7: post-operative view at 6 months

Fig 8: full thickness mucoperiostealflap reflected

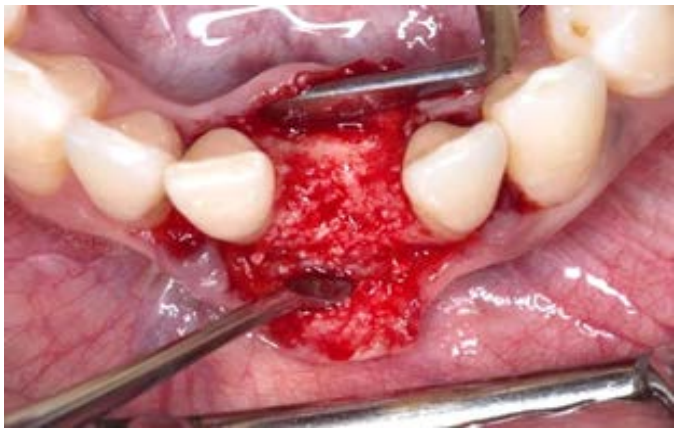

Fig 9: implant placement done

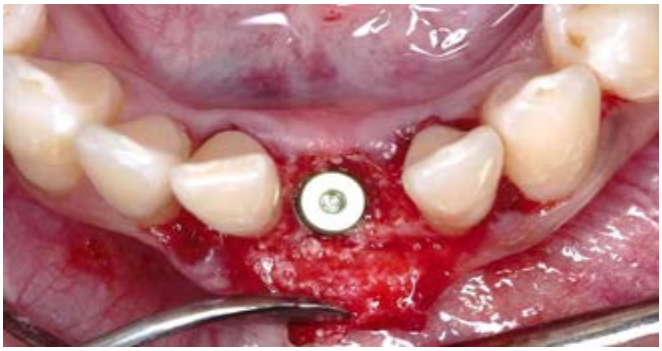

Fig 10: final prosthesis delivered