

Demineralized Freeze Dried Bone Matrix With Prf And Hydroxyapatite Graftwithprf, Asridgepreservativesfollowingextractions– Acomparativeclinicalandradiologicalstudy

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ABSTRACT

BackgroundandObjectives: The purpose of this study was to compare the efficacy of Demineralized Freeze-Dried Bone Allograft (DFDBA) with Platelet Rich Fibrin (PRF), and Hydroxyapatite bone graft (HA) with Platelet Rich Fibrin (PRF) as ridge preservatives in extraction sockets of maxillary teeth.

Materials and Methodology: 60 extraction sites were selected and divided into Group A and Group B. Each group comprising of thirty extraction sites were subjected to one of the two modalities of treatment (DFDBA with PRF and HA with PRF) for maxillary extraction socket. Post-operatively the patients were evaluated clinically for pain and wound healing and radiographically for bone height, width and relative bone density using radiograph taken at interval of 1st month, 3rd month and 6th month.

Results: Results did not show statistical differences ($p > 0.05$) for parameters like bone height and density with Group A, when compared with Group B. Radiographically Bone height and Bone density was significantly higher in DFDBA with PRF group when compared with HA and PRF group. Clinically there was no difference in terms of soft tissue healing with both the groups.

Interpretation and Conclusion: Our study suggested that even though both modalities used in this study were effective for grafting extraction sockets, DFDBA and PRF group proved to be better in terms of bone height and density postoperatively. We concluded that the overall summation of the results of the study showed that DFDBA with PRF seems to offer better significant results both clinically and radiographically than HA and PRF in ridge preservation of maxillary extraction sockets.

Keywords: Demineralized Freeze Dried Bone Allograft; Hydroxyapatite bone graft; Platelet Rich Fibrin; Maxillary Extraction Socket.

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I. INTRODUCTION

Tooth extraction often results in decrease in volume and morphological change of the alveolar socket. These changes can make replacement of teeth difficult. Tooth extraction normally results in significant resorption of the alveolar ridge. The bone resorption process is initiated immediately after extraction, leading to an average of 40–60% decrease in the horizontal and vertical dimensions of the alveolar ridge, especially during the first 2 years¹. The majority of post extraction bone loss is more evident on the buccal aspect of the ridge and occurs predominantly within the first 3 months².

Post extraction maintenance of the alveolar ridge minimizes residual ridge resorption (RRR) and, thus, allows replacement of teeth that satisfy esthetic and functional criteria. In order to preserve the original ridge dimensions following extraction, various bone grafts and substitutes have been suggested and utilized for grafting of the post extraction socket, such as autogenous bone, demineralized freeze-dried bone allograft, mineralized freeze-dried bone allograft, deproteinized bovine bone, alloplastic polymers and bioactive glasses alone or in combination with absorbable or non-absorbable membrane are widely tested.

Bone loss in grafted sockets is seen to be less than 2mm and 0.5mm in width and height respectively as compared to non-grafted sockets where the resorption was ranging upto about 2-6mm and 1mm in width and height respectively^{2,3}.

Demineralized freeze dried bone graft (DFDBA) is an allograft. It is an osteoconductive and osteoinductive product, but has no osteogenic capacity because of its processing. Decalcification process of DFDBA, exposes on its surface, the bone morphogenetic proteins (BMPs) which are osteo-inductive⁴, that is, they induce differentiation of mesenchymal cells into cartilage and bone. In addition, the freeze-drying process at

-196°C destroys the antigenicity of the DFDBA⁵. The osteoinductive capacity of DFDBA can be affected by storage, demineralization process, washing procedure, sterilization method and vary from donor to donor resulting in differences between and within products. DFDBA has no immunological rejection as the antigenic surface structure of the bone is destroyed during freeze drying at -196°C⁵. Since DFDBA was found to be effective and safe as an option of bone grafting, it has been widely used to induce bone formation in various procedures. When it is used in osseous defects, it bypasses the phase of obligatory resorption and shows early evidence of new bone formation.

Calcium hydroxyapatite (HA) is a biocompatible osteo-conductive material, HA has low-density ultra-porous structure, which allows migration of osteoblasts, fibroblasts and osteoclasts, providing a scaffold for bone to grow⁵. Calcium HA can be obtained from natural sources as well as from a synthetic process. Hydroxyapatite is an apatite of calcium phosphate, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, a ceramic naturally found in vertebrate tooth and bone. The compound has a Ca/P mole ratio of 1.67, and is formed by precipitation of calcium nitrate and ammonium dihydrogen phosphate. Each pore is 100-140µm with constant interporous distance. Hydroxyapatite alone has been found to be insufficient for formation of bone in numerous studies. Hydroxyapatite has only osteoconductive properties. Mixing it with autologous bone marrow or graft would provide an osteoinductive stimulus.

Platelet rich fibrin (PRF) is a fibrin matrix in which platelet, cytokines, growth factors and cells are trapped and may be released after a certain time and that can serve as a resorbable membrane⁶. Platelet rich fibrin (PRF) was first introduced in France by Choukroun and colleagues⁶, and has been most widely used in cardiothoracic surgery, vascular surgery, general surgery, plastic surgery, to reduce postoperative hematoma, and in sinus lift procedures and implantation. PRF is simply centrifuged blood without any addition. PRF consists of a fibrin matrix polymerized in a tetra molecular structure, with incorporation of platelets, leucocytes, cytokines, and circulating stem cells.

Clinical studies reveal that this biomaterial would be a favourable matrix for the development of a coherent healing, without any inflammatory excess. PRF in the form of a platelet gel can be used in conjunction with bone grafts, which has several advantages, such as promoting wound healing, bone growth and maturation, wound sealing and haemostasis, and imparting better handling properties to graft materials. It can also be used as a membrane. Many clinical trials suggest the combination of bone grafts and PRF to enhance bone density.

The aim of this study was to evaluate clinically and radiographically the efficacy of osteo-inductive decalcified freeze dried bone allograft with PRF and osteo-conductive hydroxyapatite allograft with PRF on bone healing after extraction of maxillary teeth with bone grafting objectives to clinically evaluate pain and wound healing following extraction and bone grafting and to evaluate the height, width and bone density radiographically at 1st month, 3rd month and 6th month post-operatively.

II. MATERIALS AND METHODOLOGY

The study included patients reporting to the Department of Oral and Maxillofacial Surgery, Dayananda Sagar College of Dental Sciences and Hospital Bangalore, requiring extraction of maxillary teeth. A clinical and radiographic study was planned after the due approval from Ethical Committee. The study involved both male and female patients. Inclusion criteria:

1. Patients of age 18 years and above.
2. Maxillary teeth which otherwise cannot be restored or rehabilitated

Exclusion criteria:

1. Patients with Uncontrolled or severe systemic diseases.
2. Patients with bleeding disorders or on medication associated with compromised bone healing
3. Patients with deleterious habits such as smoking.
4. Patients unwilling to be part of the study.

A custom made case history proforma was designed for the study to record the case history. After obtaining the complete history, patients were examined clinically and were explained about the procedure, its complications and the follow up period involved in the study. A Written Informed consent was obtained from all the patients. A total of 60 extraction sites of maxillary teeth indicated for extraction was included in the study. They were randomly divided into Group A and Group B of 30 in each group.

For Group A, which included 30 extraction sites, demineralized freeze dried bone graft with platelet rich fibrin was filled into the socket and in Group B, which included 30 extraction sites, hydroxyapatite bone graft with platelet rich fibrin was filled into the socket.

Demineralized freeze dried bone graft - Demineralized freeze dried bone graft (Figure 1) for study was procured from TATA MEMORIAL HOSPITAL, MUMBAI.

Hydroxyapatite bone graft - Hydroxyapatite bone graft (Figure 2) for study was procured from SURGIWEAR COMPANY

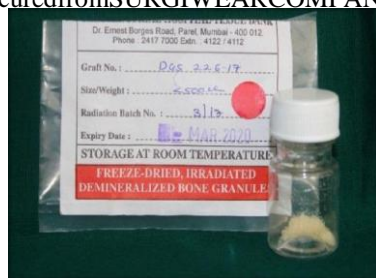


Figure 1: Demineralised Freeze Dried Bone Allograft

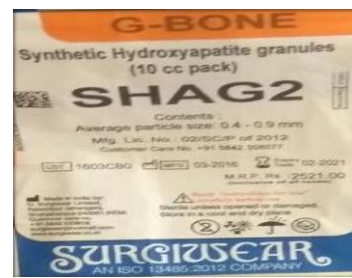


Figure 2: Hydroxyapatite Graft

Preparation of Platelet Rich Fibrin (PRF)

Routine hematological investigation and informed consent were taken before withdrawal of blood for platelet rich fibrin preparation. A tourniquet was placed on the hand from which blood was to be drawn. In all patients, brachial vein in the ante-cubital fossa was used for blood withdrawal. An 18-gauge needle was used for drawing blood. 5 ml of blood was drawn from the patient and placed in a test tube with no anticoagulant. The tube was then placed in a centrifugal machine and centrifuged at 3,000 revolutions per minute (RPM) for 10 min, after which it settles into the following three layers (Figure 3): (a) Upper fraction - straw-colored acellular plasma (b) Middle fraction - containing the fibrin clot. (c) Lower colored fraction - containing red blood cells (RBCs)



Figure 3: Centrifugation of Blood



Figure 4: PRF Membrane

The upper straw-colored layer is then removed and middle fraction is collected, 2 mm below the lower dividing line, which is the PRF. The mechanism involved in this is; the fibrinogen concentrated in upper part of the tube, combines with circulating thrombin due to centrifugation to form fibrin. A fibrin clot is then formed in the middle between the red corpuscles at bottom and acellular plasma at the top. The clot, is platelet strapped massively in fibrin meshes. The success of this technique entirely depends on time gap between the blood collection and its transfer to the centrifuge and it should be done in least time. Owing to the absence of an anticoagulant, blood begins to coagulate as soon as it comes in contact with the glass surface. Therefore, for successful preparation of PRF, speedy blood collection and immediate centrifugation, before the clotting cascade is initiated, is absolutely essential. PRF can be made into a membrane by squeezing out the fluids in the fibrin clot (**Figure 4**).

Surgical procedure

The surgical area was anesthetized. Teeth were extracted with minimum trauma to the investing tissues. Forceps and elevators were used with great care to preserve the buccal bone and surrounding soft and hard tissues. The socket was curetted with soft tissue curettes and irrigated with normal saline to remove any granulation tissue if present

- In Group A patients – Demineralized Freeze Dried Bone Graft (DFDBG) was condensed into extraction sockets until the crestal level, and platelet rich fibrin (PRF) was used as a barrier to cover the graft material as a membrane. (**Figure 5**)
- In Group B patients – Hydroxyapatite graft (HA) was condensed into extraction socket until crestal level and platelet rich fibrin (PRF) was used as a barrier to cover the graft material as a membrane. (**Figure 6**)

In both the groups, the flaps were sutured with horizontal mattress suture technique, to cover as much as possible of the biomaterials. Postoperative instructions were given, and the patients were recalled at the intervals of 7 days, 1st month, 3rd month and 6th month and clinical and radiographic measurements were recorded.

Figure 5: Group A- Demineralised Freeze Dried Bone Allograft with PRF

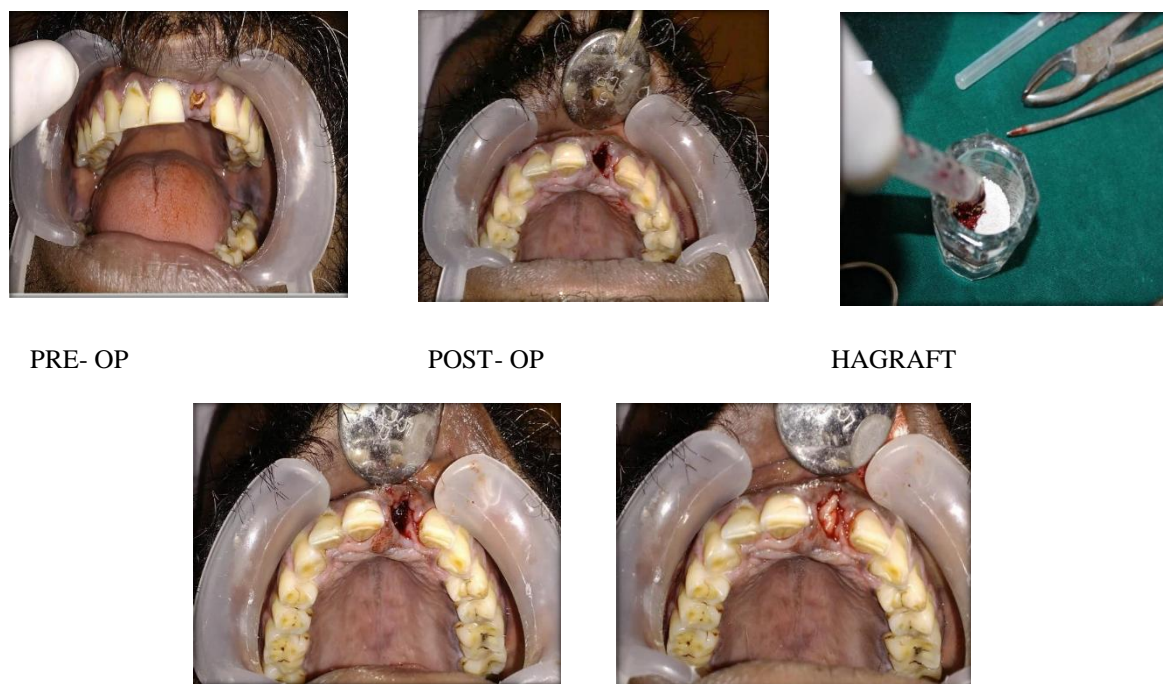


PRE- OPPOST- OPDFDBAGRAFT



EXTRACTION SOCKET FILLED WITH GRAFT FOLLOWED BY PRF PLACEMENT

Figure 6: GroupB-HydroxyapatiteGraftwithPRF



EXTRACTIONSOCKETFILLEDWITHGRAFT FOLLOWED BY PRF PLACEMENT

ClinicalEvaluation

Patientof both thegroupswere assessed clinicallyon7th dayforpresenceof

- **PAIN** which was evaluated using a 10-point Visual Analog Scale, with a score of “0”equal to “no pain”and “10”equal to “veryseverepain”, and
- **WOUND HEALING** which was evaluated as uneventful or eventful. In case ofeventful healing, it was evaluated based on sloughing i.e. presence/ absence ofslough tissue over the socket and wound dehiscence based on the wound gappingortissueloss from theregion of thesocket.

RadiographicEvaluation

Radiographic assessment for bone healing was done using radiovisiography (RVG) at 1stmonthand3rd monthand6th month post-operatively (**Figure 7 & 8**).Radiographswere takenby thesamepersonneloneveryfollowandwerestandardizedbykeepingtheexposureparameters constant, using position indication devices (PID). Parameters were assessedwere height, width and density of the bone. The extraction sockets were measured usingcomputer graphicsoftwareprogram-ImageJ.

The radiographic images were transferred to ImageJ software. Linear measurement toooption available in a software was used to measure height and width of extraction socket.Then tracing of the size of the residual cavity using freehand selections tool was done foreach defect. The gray value of the residual cavity which is denoted as ‘mean’wascalculated.Thegray valueoftheresidualcavitywascalculatedonallRVGs.This indicated the density of defect. The increasing mean value of the surgical defect overtimegaveus therelative bone fillingin theareaof thesocket.

Height,widthandboneregenerationresultsoftheparticipantsinGroupAandGroupB at 1st month, 3rd month and 6th months were assessed and compared and statisticallyanalyzed.



Figure 7: Post- Operative Radiographic Assessment of Group A; Pre-op, 7th day, 1st month, 3rd month, 6th month

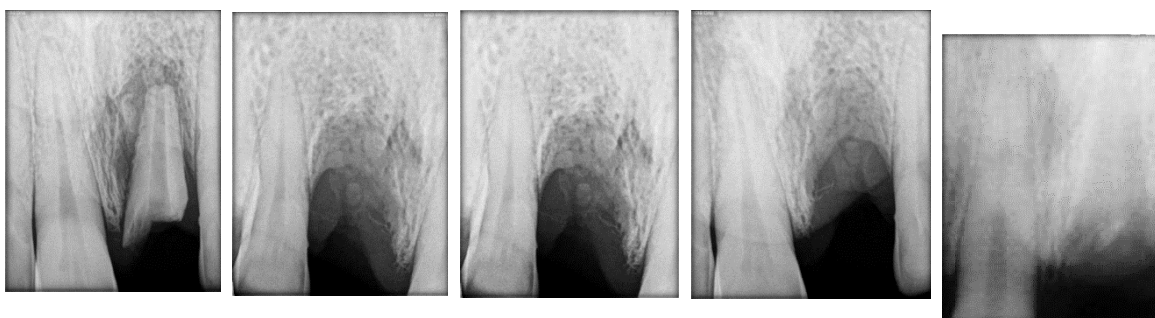


Figure 8: Post- Operative Radiographic Assessment of Group B; Pre-op, 7th day, 1st month, 3rd month, 6th month

- Antibiotics (Cap Amox 500mg t.i.d for five days) and analgesic drugs (Tab Divon plus t.i.d for three days) were prescribed along with oral hygiene maintenance instructions.
- Patients were checked for any pain/swelling/infection/ wound break down in the grafted region on theseventh dayand onemonthpostoperatively.
- Suture removal was done on the seventh day following the procedure.

Statistical Analysis:

The study data was analyzed using SPSS [Statistical Package for Social Sciences] software V.22, IBM. Corp. The frequency distribution was expressed in terms of number & percentage for categorical variables [each study parameter] to be compared between the two groups. Chi Square test was used to compare the distribution / association of the study variables between the two groups at each time interval. The mean & SD was obtained and was compared between the groups using one-way ANOVA test followed by Tukey's HSD test as the Post hoc analysis. The level of significance [P-Value] was set at $P < 0.05$.

III. RESULTS

A total of 60 extraction sites requiring extraction of teeth in the maxillary anterior and posterior region reporting to Department of Oral and Maxillofacial Surgery were reconsidered for the study. Both male and female patients above the age group of 18 years were included in the study. Non-restorable teeth with chronic irreversible pulpitis indicated for extraction were considered.

Patients were divided into two groups, GROUP A and GROUP B, of 30 extraction sites each. In GROUP A patients, Demineralized Freeze Dried Bone Allograft with PRF was placed in the extraction sockets and in GROUP B Hydroxyapatite Bone Graft with PRF was placed as socket preservation technique.

Patients with uncontrolled or severe systemic diseases, bleeding disorders or on medication associated with compromise of bone healing, deleterious habits such as smoking etc. were excluded from the study.

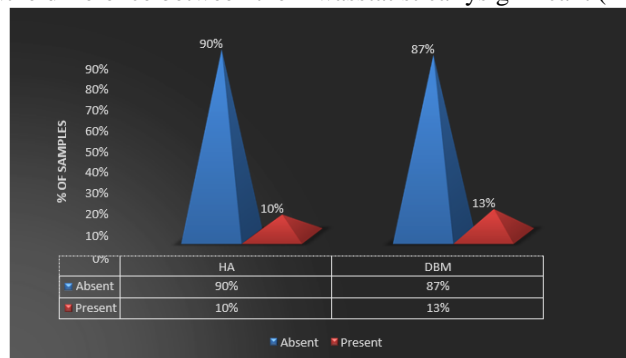
Patient of both the groups were assessed clinically on 7th day for soft-tissue healing and pain. Radiographic assessment for bone healing was done at 1st month, 3rd month and 6th month after extraction. The parameters assessed were bone height, width and relative density.

In HA group, pain was present in 10% of cases whereas in DFDBA group pain was present in 13% of cases (**Graph 1**).

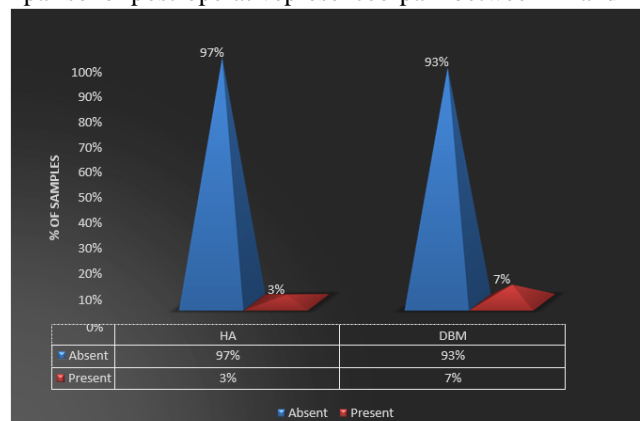
In HA group, wound healing was uneventful in 97% of cases whereas in DFDBA group wound healing was uneventful in 93% of cases (**Graph 2**).

On 7th day, higher mean height was recorded in HA group compared to DFDBA group but the difference between them

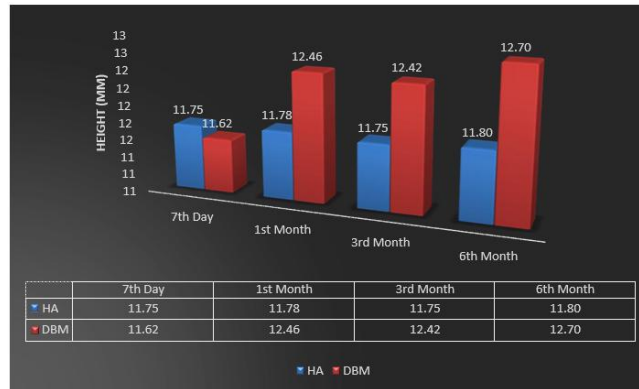
was not statistically significant ($P > 0.05$).
 At 1st month, higher mean height was recorded in DFDBA group compared to HA group and the difference between them was statistically significant ($P < 0.05$).
 At 3rd month, higher mean height was recorded in DFDBA group compared to HA group and the difference between them was statistically significant ($P < 0.05$).
 At 6th month, higher mean height was recorded in DFDBA group compared to HA group and the difference between them was statistically significant ($P < 0.01$) (**Graph 3**).
 On 7th day, higher mean width was recorded in HA group compared to DFDBA group but the difference between them was not statistically significant ($P > 0.05$).
 At 1st month, higher mean width was recorded in HA group compared to DFDBA group but the difference between them was not statistically significant ($P > 0.05$).
 At 3rd month, higher mean width was recorded in HA group compared to DFDBA group but the difference between them was not statistically significant ($P > 0.05$).
 At 6th month, higher mean width was recorded in HA group compared to DFDBA group but the difference between them was not statistically significant ($P > 0.05$) (**Graph 4**).
 On 7th day, higher mean density was recorded in DFDBA group compared to HA group but the difference between them was not statistically significant ($P > 0.05$).
 At 1st month, higher mean density was recorded in DFDBA group compared to HA group and the difference between them was statistically significant ($P < 0.05$).
 At 3rd month, higher mean density was recorded in DFDBA group compared to HA group and the difference between them was statistically significant ($P < 0.05$).
 At 6th month, higher mean density was recorded in DFDBA group compared to HA group and the difference between them was statistically significant ($P < 0.05$) (**Graph 5**).



Graph 1: Comparison of post-operative presence of pain between HA and DFDBA group



Graph 2: Comparison of post-operative wound healing between HA and DFDBA group



Graph3: Comparison of the mean bone height between HA & DFDBA group using Student t test



Graph4: comparison of the mean bone width between HA and DFDBA group using student t test



Graph5: Comparison of the mean relative bone density between HA and DFDBA group

In HA group, the change in mean height was not statistically significant between 7th day & 1st month ($P>0.05$), 7th day & 3rd month ($P>0.05$), 7th day & 6th month ($P>0.05$), 1st & 3rd month ($P>0.05$), 1st & 6th month ($P>0.05$) as well as 3rd & 6th month ($P>0.05$). The change in mean width was not statistically significant between 7th day & 1st month ($P>0.05$). It was found to be statistically significant between 7th day & 3rd month ($P<0.05$), 7th day & 6th month ($P<0.05$), 1st & 3rd month ($P<0.05$), 1st & 6th month ($P<0.01$) as well as 3rd & 6th month ($P<0.05$). The change in mean density was found to be statistically significant between 7th day & 1st month ($P<0.001$), 7th day & 3rd month ($P<0.001$), 7th day & 6th month ($P<0.001$), 1st & 3rd month ($P<0.001$), 1st & 6th month ($P<0.001$) as well as 3rd & 6th month ($P<0.001$).

In DFDBA group the change in mean height was found to be statistically significant between 7th day & 1st month ($P<0.001$), 7th day & 3rd month ($P<0.001$), 7th day & 6th month ($P<0.001$), 1st & 6th month ($P<0.001$) as well as 3rd & 6th month ($P<0.001$). It was not found to be statistically significant between 1st & 3rd month ($P>0.05$). The change in mean DFDBA was found to be statistically significant between 7th day & 1st month ($P<0.001$), 7th day & 3rd month ($P<0.001$), 7th day & 6th month ($P<0.001$), 1st & 3rd month ($P<0.01$), 1st & 6th month ($P<0.001$) as well as 3rd & 6th month ($P<0.01$). The change in mean density was found to be statistically significant between 7th day & 1st month ($P<0.001$), 7th day & 3rd month ($P<0.001$), 7th day & 6th month ($P<0.001$), 1st & 3rd month ($P<0.001$), 1st & 6th month ($P<0.001$) as well as 3rd & 6th month ($P<0.001$).

IV. DISCUSSION

To achieve a predictable esthetic and functional restoration of the missing teeth, it is important to preserve the dimensions of alveolar ridge width and height after tooth extraction. Following extraction of tooth, various patterns of bone resorption occur especially on the buccal side, therefore socket preservation plays a very crucial role in maintaining adequate bone height, width and density as this may lead to esthetic and functional defects so severe that implant placement can be difficult or impossible without using augmentation procedures and also can interfere with the use of removable dentures.

The literature suggests various methods ridge preservation and augmentation techniques that are available to minimize and restore available bone. Numerous grafting materials, such as autografts, allografts, xenografts, and alloplasts, currently are used for ridge preservation. Other materials, such as growth factors, also can be used to enhance biologic outcome.

Alveolar ridge bone resorption is a biologic phenomenon of bone remodeling that occurs following tooth extraction and cannot be prevented. Araujo et al.²⁵ found that the coronal aspect of buccal bone was often comprised only of bundle bone and hypothesized that this bundle bone would resorb after tooth extraction. Other authors proposed that surgical trauma during extraction results in the separation of the periosteum from the underlying bone, causing vascular damage and an acute inflammatory response, which mediates bone resorption.

Leblebicioglu et al.²⁵ have shown that ridge height loss is greater in mandibular than maxillary sites, and ridge width loss is greater on the buccal plate in both the mandibular and maxillary sites. Thinner buccal plates also appear to be associated with more post-extraction resorption²⁵. Other studies have shown that elevating a full mucoperiosteal flap may be associated with bone loss following tooth extraction²⁵, resulting in approximately 0.6 mm of crestal bone loss²⁵. The vertical lineal extent of alveolar bone resorption occurs primarily during the first 3–6 months following extraction²⁵. The buccal plate of bone is the most affected because its crestal portion is comprised solely of bundle bone. It is also generally thinner than the lingual palatal plate, about 0.8 mm at the anterior teeth and 1.1 mm at the premolar teeth²⁵.

Aimetti²⁶ in his study of extraction sockets with no ridge preservation, after three months observed a mean vertical reduction of 1.2 ± 0.8 mm at the buccal aspects of the edentulous ridge, a 0.9 ± 1.1 mm loss at the palatal aspects, and a 0.5 ± 0.9 mm loss at the interproximal sites, and horizontal bone resorption of 3.6 ± 0.72 mm.

Wound healing in the extraction sockets occurs through a number of processes, including hematoma and clotting, formation of granulation tissue, re-epithelialization, replacement of granulation tissue with connective tissue, and bone formation. In the first few minutes after tooth extraction, a blood clot consisting of erythrocytes and platelets that are trapped in a fibrous matrix forms within the extraction socket. Granulation tissue, a new connective tissue that is highly vascularized, then starts to form after 48 hours and is completed by day seven. The granulation tissue is totally replaced by connective tissue in about 30 days. Concurrently, re-epithelialization starts after four days and is completed around six weeks, depending on the site of the extracted tooth. After six weeks, osteogenic cells from the apical aspects and the walls of the socket migrate into the developing granulation tissue, differentiate into mature osteoblasts, and initiate bone deposition that will be completed in 4–6 months.

Although studies have shown that ridge preservation does not completely prevent bone loss, it aids in reducing the extent of that loss. In a systematic review, Vittorini *et al.*²⁵ concluded that ridge preservation has a slight advantage over no treatment due to less horizontal and vertical bone loss. In their meta-analysis, they noted that following tooth extraction, it is preferable to perform ridge preservation at esthetic areas where the buccal bone thickness is less than 1.5 to 2 mm when several teeth are extracted or when anatomical structures such as the maxillary sinus and mandibular canal are located in immediate proximity.

In a clinical and histological study on maxillary and mandibular extraction sockets, Iasella *et al.*²⁵ found a significant difference in the horizontal alveolar ridge dimensional changes between extraction with no preservation (EXT) (decreased from 9.1 ± 1.0 mm to 6.4 ± 2.2 mm) and ridge preservation (RP) (decreased from 9.2 ± 1.2 mm to 8.0 ± 1.4 mm) using freeze-dried bone allograft and collagen membrane, favoring preservation (a difference of 1.6 mm). In addition, a significant difference was observed in the vertical dimension. For the RP group, there was a gain of 1.3 ± 2.0 mm vs. a loss of 0.9 ± 1.6 mm for the EXT group (a difference of 2.2 mm).

Barone *et al.*²⁵ found that an alveolar ridge preservation technique with collagenated porcine bone and a resorbable membrane was able to limit the vertical changes after tooth extraction. In his study, the control group showed vertical bone resorption of 1 ± 0.7 mm, 2.1 ± 0.6 mm, 1 ± 0.8 mm, and 2 ± 0.73 mm at the mesial, buccal, distal, and lingual sites, respectively, vs. 0.3 ± 0.76 mm, 1.1 ± 0.96 mm, 0.3 ± 0.85 , and 0.9 ± 0.98 mm at the mesial, buccal, distal, and lingual sites in the test group, respectively. Also, ridge preservation demonstrated better efficacy in the horizontal dimension (-3.6 ± 0.72 in control vs. -1.6 ± 0.55 mm in test sites). Aimetti *et al.*²⁵ also found less vertical and horizontal changes when ridge preservation was performed using calcium sulfate hemihydrate than extraction with no preservation. Ultimately, the indications for ridge preservation include maintenance of the existing hard and soft tissues of the alveolar ridge, and to simplify subsequent treatment (such as implant or denture replacement).

Allografts can be fresh-frozen, freeze-dried, or demineralized freeze-dried. The use of freeze-dried bone allografts (FDBA) and demineralized freeze-dried bone allografts (DFDBA) has reduced the problem of immunogenicity that was associated with fresh-frozen bone. They are the most common allografts used currently for ridge preservation²⁸. FDBA revascularization occurs through integration/replacement (creeping substitution) at the recipient site and the formation of connective tissue areas. Small particles of the allograft may remain for several months to a year before they are completely resorbed^{27,28}. Al-Ghamdi *et al.*²⁵ suggested that FDBA is only osteoconductive, while DFDBA can be both osteoconductive and osteoinductive.

DFDBA showed more vital bone and less residual grafting material compared to FDBA when placed in extraction sockets 19 weeks after extraction. The extent of allograft osteoinductivity depends on the donor age and the amount of bone morphogenetic proteins (BMPs) present in the graft. Grafts obtained from younger donors generally have more BMPs and are more osteoinductive²⁵. FDBA and DFDBA have been widely used for regenerative therapy and ridge preservation. In a histological study, Yukna and Vastardis²⁵ compared bone regeneration with FDBA or DFDBA and noted more regeneration with DFDBA. Dahlin²⁵ also showed that the reconstruction of atrophic maxillae with DFDBA, combined with guided bone regeneration (GBR technique), could be performed with similar treatment outcomes to autologous bone obtained from the iliac crest. To avoid disease transmission from allografts, several chemical and physical processing techniques have been used. Chemical treatment with agents, such as 5% peracetic acid, 0.1% ethylene-diamine-tetraacetic acid, or 0.1% sodium dodecyl sulfate, can alter the bone structure but may not sufficiently inactivate pathogens. Physical treatment, such as ultrasonication, may alter the microcrystal structure of bone mineral and denature organic components. With FDBA and DFDBA, more satisfactory results have been obtained through lyophilization, but cellular debris might remain after this treatment that could interfere with healing. Tutoplast™ processing uses a multi-step preservation and sterilization process to remove tissue antigenic properties and is reported to inactivate pathogens without changing the structure, biomechanics, and convertibility of the tissues²⁵.

Platelet-rich fibrin (PRF) a natural fibrin matrix, is an immune and platelet concentrate collecting on a single fibrin membrane, containing all the constituents of a blood sample which are favourable to healing and immunity.^[6] PRF first described by Choukroun *et al.*²⁷ is a new second generation of platelet concentrate. It is simply centrifuged blood without any addition. PRF consists of a fibrin matrix polymerized in a tetra molecular structure, with incorporation of platelets, leucocytes, cytokines, and circulating stem cells²⁷. Clinical studies reveal that this biomaterial would be a favourable matrix for the development of a coherent healing, without any inflammatory excess. PRF in the form of a platelet gel can be used in conjunction with bone grafts, which has several advantages, such as promoting wound healing, bone growth and maturation, wound sealing and haemostasis, and imparting better handling properties to graft materials²⁷. It can also be used as a membrane. Many clinical trials suggest the combination of bone grafts and PRF to enhance bone density.

PRF has several advantages. It eliminates the redundant process of adding bovine thrombin to promote conversion of fibrinogen to fibrin, which is necessary in PRP²⁷. The use of anticoagulants is also avoided. Conversion of fibrinogen to fibrin takes place slowly with small quantities of physiologically available thrombin present in the blood sample itself. Thus a physiologic architecture, which is very favourable to the healing process, is obtained due to slow polymerization. The fibrin network generated here is very similar to a natural one, and leads to a more efficient cell migration and proliferation, and thus cicatrization. Slow polymerization during PRF processing leads to the intrinsic incorporation of platelet cytokines

and organic chains in the fibrin meshes. This result would imply that PRF, unlike the other platelet concentrates would be able to release cytokines during the fibrin matrix remodelling. Such a mechanism might explain the clinically observed healing properties of PRF. And PRF has a supportive effect on the immune system.

The nature of extraction socket is such that it can cause the loss of the majority of bone graft⁷. Therefore, to avoid the loss of graft material, PRF was used in both the groups. PRF not only avoids the loss of graft material but also induces, stabilizes wound and promote blood clot formation. Among all the available membrane PRF was preferred due to its easy procurement, decreased immune reaction as it is prepared from patient's own blood and hemostatic activity that can facilitate clot formation and would stabilize. It also promotes cell migration, and primary wound coverage. Maximum efforts were made to achieve complete coverage of membrane, but complete coverage was not obtained in all cases. In a study done by Nam and Park⁷ in 2009 showed that if membrane exposure occurs during the healing phase, it does not affect the outcome of ridge preservation. In the present study there was uneventful healing noted in all the cases except in two, where sloughing of tissue was seen⁷.

Calcium hydroxyapatite (HA) is a biocompatible osteo-conductive material, HA has low-density ultra-porous structure, which allows migration of osteoblasts, fibroblasts and osteoclasts, providing a scaffold for bone to grow⁵. Calcium HA can be obtained from natural sources as well as from a synthetic process. Hydroxyapatite is an apatite of calcium phosphate, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, a ceramic naturally found in vertebrate tooth and bone. The compound has a Ca/P mole ratio of 1.67, and is formed by precipitation of calcium nitrate and ammonium dihydrogen phosphate. Each pore is 100-140 μm with constant interporous distance. Hydroxyapatite alone has been found to be insufficient for formation of bone in numerous studies. Hydroxyapatite has only osteoconductive properties. Mixing it with autologous bone marrow or graft would provide an osteoinductive stimulus.

In our study, 60 patients reporting to the Department of Oral and Maxillofacial Surgery, Dayananda Sagar College of Dental Sciences and Hospital Bangalore, requiring extraction of maxillary teeth, were randomly divided into two groups by coin toss method- Group A and Group B. Socket preservation following extraction was done using DFDBA with PRF in Group A patients and HA with PRF in Group B patients. Patient of both the groups were assessed clinically on 7th day for presence of **pain**, which was evaluated using a 10-point Visual Analog Scale, with a score of "0" equal to "no pain" and "10" equal to "very severe pain", and **wound healing** which was evaluated as uneventful or eventful. In case of eventful healing, it was evaluated based on sloughing i.e. presence/absence of slough tissue over the socket and wound dehiscence based on the wound gapping or tissue loss from the region of the socket.

Radiographic assessment for bone healing was done using radiovisugraphy (RVG) at 1st month and 3rd month and 6th month post-operatively. Parameters assessed were height, width and density of the bone. The extraction socket was measured using computer graphic software program-ImageJ.

The radiographic images were transferred to ImageJ software. Linear measurement tool option available in a software was used to measure height and width of extraction socket. Then tracing of the size of the residual cavity using freehand selection tool was done for each defect. The gray value of the residual cavity which is denoted as 'mean' was calculated. The gray value of the residual cavity was calculated on all RVGs. This indicated the density of defect. The increasing mean value of the surgical defect over time gave us the relative bone filling in the area of the socket. Height, width and bone regeneration results of the participants on Group A and Group B at 1st month, 3rd month and 6th months were compared and statistically analyzed.

While comparing the statistical analysis results of GROUP A and GROUP B obtained, it was seen that pain was present in 10% of cases, in HA group, whereas in DFDBA group pain was present in 13% of cases. Wound healing was uneventful in 97% of cases, in HA group, whereas in DFDBA group wound healing was uneventful in 93% of cases. Our study demonstrated that HA graft material is better than DFDBA in terms of pain and wound healing.

On radiographic assessment, on 7th day, though higher mean height was recorded in HA group compared to DFDBA group, the difference between them was not statistically significant ($P > 0.05$). Higher mean height was recorded in DFDBA group compared to HA group and the difference between them was statistically significant ($P < 0.05$) at 1st, 3rd and 6th month. This is consistent with the earlier studies done using DFDBA alone for the purpose of socket preservation. The use of PRF along with DFDBA has significant advantages over the use of DFDBA alone. Use of PRF aids in retaining of the bone graft material within the walls of the socket, as it is a fibrin clot, it aids in the arrest of bleeding as well. However,

statistically significant improvement was not noted with respect to height from baseline to 180 days in both the groups. It showed similar results in terms of height, post-operatively.

In addition, there was no statistical difference in bone width between GROUP A and GROUP B. These findings were contrary to the findings reported in the study by Simon et al²⁸ showing mean width socket resorption of 0.57mm with PRF after 4 months and confirmed a significant advantage in the preservation of post extraction alveolar ridge dimension with the use of PRF²⁸. Relative bone density evaluation demonstrated that, on 7th day, though higher mean density was recorded in DFDBA group compared to HA group, the difference between them was not statistically significant ($P > 0.05$). Higher mean density was recorded in DFDBA group compared to HA group and the difference between them was statistically significant ($P < 0.05$) at 1st, 3rd and 6th month post extraction.

However, despite all attempts being made to carry out a study which considers all the required parameters, following are some limitations that do exist in this study as well. In this study, intraoral radiographic technique was used to measure the bone width and height changes. However, the use of cone beam computed tomography could have been done to achieve more accurate results.

V. CONCLUSION

In our study, we compared efficacy of Demineralized Freeze Dried Bone Allograft (DFDBA) with Platelet Rich Fibrin (PRF), and Hydroxyapatite Bone Graft (HA) with Platelet Rich Fibrin (PRF) in healing of extraction socket of maxillary teeth. Both the modalities can be performed with relative ease and comfort for the patient undergoing extraction. DFDBA and PRF have exhibited significantly better radiographic parameters like bone height and density when compared with HA and PRF group, in the management of ridge preservation in maxillary extraction sockets, as evaluated during the follow-up period. However, there was no statistically significant difference in the bone width between the two groups. The results of this study showed significant upsurge in ridge height for both groups at 180 days. Our observations showed that the extent of bone density was found to be greater in DFDBA and PRF when compared to HA with PRF.

This procedure would benefit the patient by providing ridge form to meet functional and aesthetic needs and spare from future ridge augmentation procedure.

It can be concluded that the overall summation of the results of the study showed that DFDBA and PRF seem to offer better clinically and radiographically significant results than HA with PRF in the management of ridge preservation in maxillary extraction sockets. Moreover, DFDBA & PRF definitely promote better osseous regeneration over HA with PRF in terms of uniformity and density of regenerated bone, which is statistically significant.

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