

Evaluation of Demineralised Freeze-dried Bone Allograft and Autologous Dentin Graft with Advanced Platelet-rich Fibrin in Socket Preservation: A Research Protocol

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ABSTRACT

Introduction: Alveolar ridge resorption is initiated rapidly within weeks following tooth extraction, therefore successful implant outcomes depend upon preservation of alveolar ridge. Techniques like atraumatic extraction and socket grafting helps in reduction of bone loss. Demineralised Freeze-dried Bone Allograft (DFDBA) is a commonly used due to its osteo-inductive properties which are enhanced when combined with Advanced Platelet Rich Fibrin (A-PRF). A-PRF enhances healing and regeneration of both soft and hard-tissues. Autologous Dentin Graft (ADG), derived from extracted teeth, mimics natural bone and offers osteoinductive and osteoconductive benefits. Radiographic and histological analysis along with clinical evaluation are reliable methods to assess outcomes.

Need of the study: The ADG eliminates the need for a second surgical site and reduces patient morbidity. Furthermore, the combined effect of ADG with A-PRF has not yet been evaluated, highlighting the need for this study.

Aim: To assess and compare the effectiveness of DFDBA with A-PRF and ADG with A-PRF in socket preservation using clinical, radiographical, and histological analysis.

Materials and Methods: This randomised controlled clinical trial compares DFDBA with A-PRF and ADG with A-PRF in socket preservation. The trial will be conducted at the Department of Periodontics, Sharad Pawar Dental College, Sawangi (Meghe), Wardha, from May 2025 to June 2026, which will involve 10 participants in each group. Participants will be randomly assigned to one of two groups: (a) Socket preservation performed using DFDBA with A-PRF; (b) Socket preservation performed using ADG with A-PRF. Cone Beam Computed Tomography (CBCT) will be performed postoperatively and at four months, along with clinical evaluation and implant placement. Bone biopsies obtained during implant placement will be analysed histomorphometrically in a blinded manner. Statistical analysis will be performed using paired and unpaired Student's t-tests, with a p-value <0.05 will be considered statistically significant.

Keywords: Alveolar ridge resorption, Autograft, Bone regeneration, Implant

INTRODUCTION

Loss of teeth initiates a cascade of biological responses leading to notable structural and local anatomic changes in periodontal tissues [1]. These changes includes resorption in bone height and width leading to alveolar ridge atrophy due to the natural process of bone remodelling and healing [2,3]. Alveolar Ridge Preservation (ARP) is an essential procedure for maintenance of structural integrity and prevention of bone loss. Although, atraumatic extraction is an essential procedure to minimise trauma and socket expansion during tooth extraction, ARP is a key therapeutic technique aimed at minimising alveolar ridge resorption and maintaining both soft and hard-tissue contours of the ridge post-extraction [4].

Dental implants are a widely used solution for restoration of fully or partially edentulous arches, with a growing emphasis on safe reconstruction and optimal timing after extraction. Successful and aesthetic implant outcomes depend on adequate vertical and horizontal bone volume. Socket preservation after tooth extraction is crucial for successful future implant placement [1,3]. While bone and soft-tissue augmentation can address alveolar ridge atrophy, their success and predictability vary. As reported in the article by Song SJ et al., (2022), ARP via socket grafting may prevent 1.0-2.5 mm of mid-buccal, 0.8-1.5 mm of mid-lingual vertical bone resorption, and 1.5-2.4 mm of horizontal bone resorption compared to extraction alone. When these values are interpreted in percentage terms based on the average post-extraction ridge resorption reported in the literature, this corresponds to approximately 25-70% reduction in vertical resorption and 30-60% reduction in horizontal resorption,

indicating that overall ridge volume reduction can be limited to about 15-30% with socket preservation techniques [5]. Using graft materials for socket grafting, helps in reduction of dimensional shrinkage of the alveolar ridge after tooth extraction. Though socket grafting does not prevent bundle bone resorption, it remains an effective method to limit the dimensional changes typically occurring post-extraction in an alveolar ridge [6,7].

For optimal outcomes, a bone graft should possess osteoinductive, osteoconductive and osteogenic properties [8]. Several biomaterials and surgical techniques have been developed to facilitate socket preservation, using both natural and synthetic graft materials. Among these, DFDBA is clinically accepted, and the most commonly used bone allograft material for periodontal repair. DFDBA promotes new bone formation by reducing remodelling of socket and maintaining the fixture-socket gap. The osteoinductive properties of DFDBA are due to Bone Morphogenetic Proteins (BMPs 2, 4, and 7), which accelerate bone regeneration during healing [9].

Platelet concentrates are known to accelerate post-extraction healing by promoting tissue regeneration through the sustained release of Platelet-Derived Growth Factors (PDGF) and release of intracellular proteins. A-PRF, a second-generation platelet concentrate provides improved platelet and leukocyte distribution along with thicker fibrin network and offers advantages like enhanced soft and hard-tissue regeneration, epithelial healing, increases bone density, and aids in reducing postoperative pain and swelling when used alone or with graft material. Compared to earlier platelet concentrates, A-PRF offers improved cellular migration, more homogenous distribution of regenerative elements, and an extended release of bioactive

molecules, making it beneficial for socket preservation and bone regeneration procedures [1,6,10].

Autologous materials provide numerous advantages like excellent biocompatibility and reduced risk of immune reactions. ADG provides similar advantages in terms of socket grafting material. ADG, derived from extracted teeth, is emerging as a viable option for ARP due to its similar properties to human cortical bone in terms of density, roughness, and biochemical composition. Dentin is composed of 70% inorganic material, primarily calcium phosphate having low crystal concentration, making it easily resorbed by osteoclasts. Its 20% organic matrix includes 90% type I collagen, 10% non-collagenous proteins that aid in calcification, and growth factors like BMPs that contribute to its osteoinductive potential, with the remaining 10% being water. The osteoconductive properties of human dentin is from four calcium phosphate forms: amorphous calcium phosphate, tricalcium phosphate, octacalcium phosphate, and hydroxyapatite. Thus, ADG exhibits both osteoinductive and osteoconductive properties along with an advantage of being biocompatible [11].

While clinical and radiographic evaluations are most commonly used to evaluate outcomes, histological analysis remains the gold standard, as it provides detailed insight into composition of tissue, including the inorganic and organic components such as hydroxyapatite, BMPs, and type I collagen [12].

Therefore, the present study aims to evaluate healing outcomes while maintaining sufficient alveolar bone dimensions to support subsequent implant placement.

Primary Objectives

1. To evaluate the outcomes of DFDBA with A-PRF using clinical, radiographical, and histological analysis in socket preservation.
2. To evaluate the outcomes of ADG with A-PRF using clinical, radiographical, and histological analysis in socket preservation.

Secondary Objectives

1. To compare the outcomes of DFDBA with A-PRF versus ADG with A-PRF using clinical, radiographical, and histological analysis in socket preservation.

Null hypothesis: There is no statistically significant difference between DFDBA with A-PRF and ADG with A-PRF in socket preservation with respect to clinical, radiographic, and histological outcomes.

Alternate hypothesis: There is statistically significant difference between DFDBA with A-PRF and ADG with A-PRF in socket preservation with respect to clinical, radiographic, and histological outcomes.

REVIEW OF LITERATURE

Socket preservation is a technique specifically developed to minimise post-extraction bone resorption, thereby maintaining the volume and contour of alveolar ridge. This approach is essential for facilitating implant placement in future, in a prosthetically guided position, ultimately contributing to functionally stable and aesthetically enhanced outcomes [13]. In the assessment of the outcomes of socket preservation, radiographic evaluation plays a pivotal role. CBCT now a cornerstone in implant dentistry, offers superior three-dimensional imaging with enhanced resolution and lower radiation exposure compared to conventional CT scans or Two-dimensional (2D) imaging [14]. Histological analysis is the method known for its high sensitivity and precision, for evaluating the quality and composition of regenerated bone, offering insights into both inorganic (e.g., hydroxyapatite) and organic (e.g., BMPs and collagen) components [15].

On the basis of histological outcomes, Minetti E et al., demonstrated the capability of demineralised autologous tooth material as a viable

grafting option in promoting significant formation of new vital bone, with low residual graft content, supporting their osteoinductive and osteoconductive potential. Histologically, new bone formation in direct continuity with the dentin granules, and osteoclast-mediated resorption indicating effective integration and remodelling was appreciable. Autologous dentin in comparison with traditional xenografts or alloplasts, which often show delayed resorption, offers a biocompatible, minimally invasive, and efficient alternative. The concept of "proximity homologation," where the graft mimics native bone behaviour, is well supported by the study [16].

The ADG, being autologous material, offers both osteoinductive and osteoconductive properties reducing the risk of disease transmission and immunological rejection, unlike allografts. Hussain AA et al., reported the favourable outcomes by using ADG in socket preservation. The observations were made that ADG leads to significantly better preservation of ridge dimensions when compared to spontaneous healing. Reduced dimensional changes in the ADG-treated group was confirmed by CBCT scans. Histological evaluations demonstrated effective remodelling of the dentin graft, with denser trabecular patterns and higher new bone formation than in controls [17].

Gupta PS et al., demonstrated the clinical effectiveness of ADG in comparison with Autogenous Bone Grafts (ABG) in socket preservation. In their split mouth trial, they demonstrated higher bone density and slightly better vertical height preservation after six months in the sockets preserved with ADG by radiographic analysis. While soft-tissue healing parameters were similar in both groups, supporting the use of ADG as a viable, easy to prepare, biocompatible, and osteoconductive graft material for socket preservation [18].

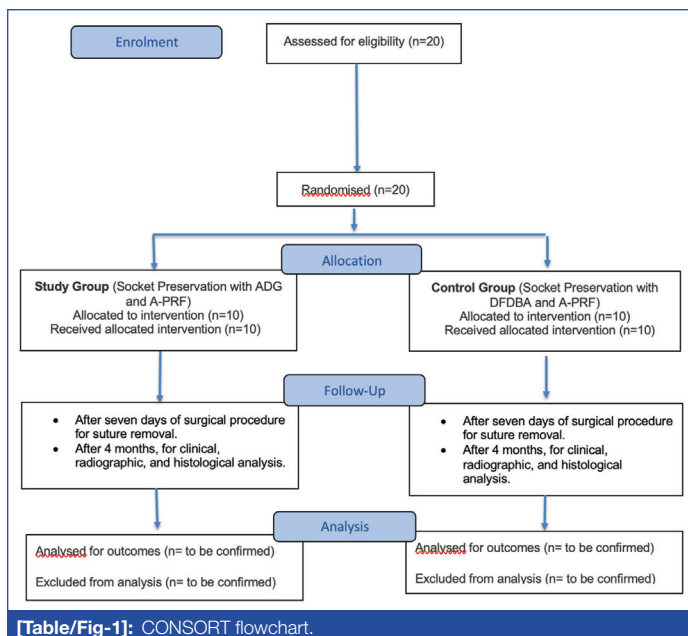
In a recent systematic review and meta-analysis by Kalburgi V et al., the clinical and radiographic outcomes of DFDBA in dental implant procedures were evaluated. The findings demonstrated stable peri-implant health, reduced postoperative pain, active bone remodelling, and successful osseointegration along with significant increase in mesial bone height [19].

Several studies have demonstrated A-PRF as an effective autologous material due to its prolonged release of growth factors leading to enhanced regenerative properties, angiogenesis and healing outcomes [6,10]. Makki AZ et al., reported the similar outcomes in their study, where they compared the effects of A-PRF and Leukocyte Platelet-rich Fibrin (L-PRF) on post-extraction pain and early soft-tissue healing. The results showed that A-PRF significantly reduced postoperative pain, particularly on the first and second days of post extraction. Additionally, A-PRF demonstrated superior soft-tissue healing compared to L-PRF and control groups at both one and two weeks [20].

Thus, this study aims to assess and compare the efficacy of DFDBA and ADG in combination with A-PRF in socket preservation, using clinical, radiological, and histological analysis.

MATERIALS AND METHODS

This study is a randomised controlled prospective parallel-arm clinical trial. The patients will be recruited from the Department of Periodontics, Sharad Pawar Dental College, Sawangi (Meghe), Wardha, Maharashtra, India, from May 2025 to June 2026. The study is registered with the Clinical Trials Registry of India (CTRI/2025/03/082589). Ethical approval was obtained from the Institutional Ethics Committee of Datta Meghe Institute of Higher Education & Research (Deemed to be University) (Ref No. DMIHER(DU)/IEC/2025/559). Written informed consent will be obtained from all participants. A simplified outline of the study protocol is explained in Consolidated Standards of Reporting Trials (CONSORT) flowchart [Table/Fig-1].



Inclusion criteria

- Patients indicated for tooth extraction and subsequent implant placement due to conditions such as failed endodontic treatment, root fracture, internal or external resorption, non-restorable carious lesions, residual roots, or over-retained primary teeth;
- Good oral hygiene;
- Thick gingival biotype;
- Presence of antagonist tooth to the extraction site;
- A minimum of 4 mm of bone seen radiographically at the root apex;
- Intact alveolar bone walls, confirmed with clinical and radiographical examination.
- D-1, D-2, or D-3 bone quality.

Exclusion criteria

- Patients with systemic conditions, such as diabetes mellitus, osteoporosis, or blood disorders, that may impair bone healing;
- Significant discrepancies in maxillo-mandibular space;
- Presence of parafunctional habits, history of heavy smoking, drug abuse, or alcoholism;
- Proclination, spacing, rotation, or malalignment of anterior teeth;
- Presence of untreated dental diseases;
- History of chemotherapy/radiotherapy;
- Pregnant/lactating women;
- Debilitating disorders of temporomandibular joint;
- D-4 bone quality.

Sample size calculation: Sample size was calculated using the formula:

$$n \geq \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 \times (\sigma_1^2 + \sigma_2^2 / r)}{(\mu_1 - \mu_2)^2}$$

Significance Level (α): 0.01 ($Z_{1-\alpha/2} = 2.576$)

Power (1- β): 0.99 ($Z_{1-\beta} = 2.33$)

Mean in Group 1 (μ_1): 12.12

Standard Deviation (SD) in Group 1 (σ_1): 0.4

Mean in Group 2 (μ_2): 11.37

Standard Deviation (SD) in Group 2 (σ_2): 0.2 [21]

Ratio (r): 1 (equal group sizes)

Pooled Variance = $\sigma_1^2 + \sigma_2^2 = 0.16 + 0.04 = 0.20$

Calculate the Sample Size:

$$n \geq \frac{(2.576 + 2.33)^2 \times 0.20}{(12.12 - 11.37)^2}$$

$$n \geq \frac{(4.906)^2 \times 0.20}{0.75^2}$$

$$n \geq \frac{24.0656 \times 0.20}{0.5625}$$

$$n \geq \frac{4.81312}{0.5625} = 8.56$$

Rounding up, $n = 9$ per group.

Calculated sample size: Nine participants per group ($n = 9$).

Therefore, total sample size of 20 is considered with dropout rate of 10%

The patients who are eligible will be randomly assigned to either the study group (socket preservation with ADG and A-PRF) or the control group (socket preservation with DFDBA and A-PRF).

Study Procedure

All participants will undergo periodontal health assessment via clinical examination. Each patient will be required to provide written informed consent and complete a comprehensive medical history form after receiving a comprehensive explanation of the intervention to be conducted. Central randomisation for this procedure will be facilitated by a secure computerised system. A unique study code will be assigned to each patient. While presenting data to the operators and research evaluators, patient confidentiality will be maintained by presenting data in a coded format. During data analysis, both radiologist and the statistician will remain blinded to the treatment. Additionally, a blinded quantitative histological assessment will be conducted by a trained specialist.

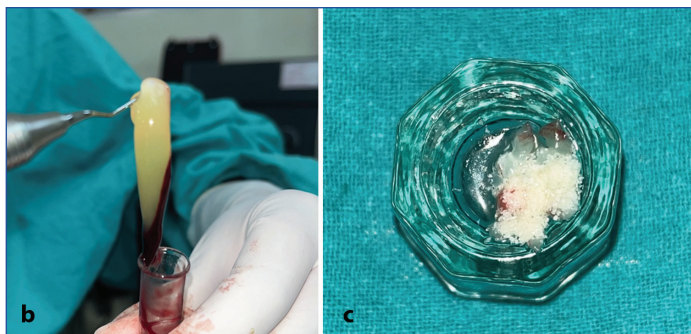
In presurgical phase, after thorough evaluation and diagnosis by clinical and radiographic techniques, all patients will undergo full-mouth ultrasonic scaling and will be guided for plaque control until their plaque scores reduce to below 25%. Diagnostic casts will be prepared before surgery for the assessment of maxillo-mandibular relationship, and a diagnostic wax-up of the replacement teeth or tooth will be created to guide implant placement.

For preparation of A-PRF, 10 mL of venous blood will be drawn immediately prior to surgery and transferred into a sterile glass tube. The blood will be centrifuged at 1500 rpm for 14 minutes to prepare A-PRF [Table/Fig-2a,b]. The resulting clot will be processed into a membrane by squeezing out serum, with part minced for grafting and part to cover the defect [Table/Fig-2c] [22].

In surgical phase for test group, patients will firstly be instructed to rinse with 0.12% chlorhexidine for a minute, followed by administration of local anaesthesia and flapless atraumatic tooth



[Table/Fig-2a]: A-PRF obtained after centrifugation.



[Table/Fig-2b]: Removing A-PRF obtained from the glass test tube.

[Table/Fig-2c]: ADG mixed with A-PRF.

extraction using periostomes and forceps. Crevicular incision will be given to relieve Periodontal Ligament (PDL) fibres using surgical blade on mesial and distal sides. For luxation of the tooth, elevators will be used. Adjacent tissues will be taken care of, to prevent trauma. Debridement of the extraction socket will be done using bone curette. After careful socket debridement and inspection, extracted roots are cleaned to remove any debris, gutta-percha, artificial material, and then ground into particulate dentin using dentin grinder, which is processed using a 3-step protocol which includes: i) Cleaning, where particulate dentin is soaked for five minutes in dentin cleanser solution at room temperature, then dehydrated with sterile gauze; ii) Neutralisation where particulate dentin is rinsed twice with Phosphate-Buffered Saline (PBS), mixed using a sterile instrument, and dehydrated after each rinse to neutralise pH; and iii) Grafting, where the prepared ADG (particle size 300-1200 microns) is immediately combined with A-PRF and grafted into the extraction sockets. A collagen plug is used to seal the site and is secured with non-resorbable sutures.

In control group, following atraumatic extraction, DFDBA will be placed in an extraction socket along with A-PRF. In post-surgical care, participants will be instructed to avoid hot fomentation over surgical site, and to avoid vigorous brushing and rinsing. In addition, with antibiotics and analgesics for three days postsurgery, 0.2% chlorhexidine mouthwash will be prescribed to be used twice-daily for two weeks. Participants will be recalled after seven days for suture removal.

Outcomes

A. Clinical assessment:

- Oral hygiene status will be evaluated using the Full Mouth Plaque Index, based on the Turesky-Gilmore-Glickman modification of the Quigley Hein Index (1970) wherein plaque accumulation will be disclosed and scored on the buccal and lingual surfaces of all teeth on a scale from 0 to 5 based on the extent of plaque coverage [23].
- Measurement of gingival inflammation, the Full Mouth Papillary Bleeding Index by Muhlemann HR (1977) will be recorded by gently passing a periodontal probe along the gingival sulcus and recording the presence or absence of bleeding in the interdental papilla on a scale from 0 to 4, where 0 indicates no bleeding and 4 indicates profuse bleeding spreading beyond the papilla [24].
- Measurement of buccolingual dimensions of alveolar ridge at the extraction site, measurement of extracted root dimensions, and coronal buccolingual dimensions will be measured using UNC-15 probe. Recordings will be done by positioning the probe at standardised reference points to ensure reproducibility.

These measurements will help evaluate soft and hard-tissue healing.

B. Histological assessment:

- At the time of implant placement, the bone will be obtained using a trephine bur with a smaller diameter than the final drill to ensure primary stability. Profuse irrigation will be required while drilling.

Bone scoring will be done according to Misch guidelines, which classify bone density into D1 to D4 based on tactile resistance encountered during drilling [25] and biopsies will be taken for analysis, histomorphometrically in a blinded manner.

- For histological evaluation, samples will be fixed in 10% formalin followed by decalcification with 5% nitric acid, and finally processed into paraffin blocks. After sectioning into 5-micron slices, samples are stained with Haematoxylin & Eosin (H&E), and examined under light microscopy. Quantitative parameters, including percentage of new bone formation, number of osteocytes, trabecular pattern, and bone density in Hounsfield Units (HU) will be analysed using Planmeca Romexis® software (Planmeca Romexis version 6.2.0, Planmeca Oy, Helsinki, Finland).

Assessment will be done at four months after initial surgery at the time of implant placement.

C. Radiological assessment:

Radiographic outcomes will be evaluated using CBCT to assess changes in bone dimensions, including vertical alveolar bone height and buccolingual ridge width within the extraction socket. The bone density of the regenerated area will also be measured to evaluate the quality of the bone formed. These measurements will be taken at baseline and after four months following socket preservation. All the recordings will be performed by the same operator to ensure consistency across patients. The mean values of these indices will be calculated for outcome assessment.

STATISTICAL ANALYSIS

Using IBM Statistical Package for Social Sciences (SPSS) Statistics, SPSS v17, the mean±SD (Standard Deviation) values will be calculated for each periodontal parameter Marginal Bone Level (MBL) Plaque Index (PI) Papillary Bleeding Index (PBI) Clinical Attachment Level (CAL) Probing Pocket Depth (PPD) Width of Keratinized Gingiva (WKG). For equating the baseline findings to the four months values of each patient, a Student's paired t-test will be employed for the evaluation of differences in the intra-group data and Student's unpaired t-test will be utilised for the evaluation of differences in the inter-group data. To determine the importance of variations within and across groups, the data will be analysed using appropriate statistical methods, including the Wilcoxon signed-rank test. A p-value (probability value) greater than 0.05 will be considered statistically non significant, while less than 0.05 will indicate a statistically significant difference.

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