

# Qualitative and Quantitative assessment of bone formation following socket preservation with Deproteinized Bovine Bone Mineral, Advanced platelet-rich fibrin and a combination of both: A Randomised Controlled Clinical Trial



## Abstract

### Objective

Although several biomaterials have been tried for alveolar ridge preservation post tooth extraction. There is still no ideal biomaterial suggested for socket preservation. Among the various bone grafts used, xenografts such as De-proteinized bovine bone mineral (DBBM) have been widely used. It has a slow substitution rate and takes several months to years to resorb which could be detrimental to bone-to-implant contact (BIC). In this scenario, platelet concentrates, especially third generation such as advanced platelet-rich fibrin (A-PRF) have been used for regenerative therapy.

It has a unique advantage of sustained release of growth factors, apart from the presence of elevated levels of leucocytes which not only reduces the inflammatory reaction but also helps in the recruitment of osteoprogenitor cells, thereby facilitating new bone formation.

The present study aimed to evaluate the effectiveness of the combination of DBBM and A-PRF in alveolar ridge preservation.

### Materials and Methods

Thirty-nine patients requiring extraction of teeth and replacement with dental implants were randomized into one of the three ridge preservation approaches: DBBM, A-PRF, and DBBM+A-PRF. Four months post socket preservation, bone cores were harvested before implant placement. The bone samples were then subjected to histological and histomorphometric analysis.

### Results

Significantly more vital bone was present in the DBBM+A-PRF group compared to the A-PRF group and DBBM group ( $P = 0.00$ ). However, the connective tissue formed was more in the DBBM group and A-PRF group compared to the DBBM+A-PRF group ( $P = 0.00$ ), which was statistically significant.

### Conclusion

New bone formation was qualitatively superior in the DBBM+A-PRF group compared to when DBBM and A-PRF were individually used which could be detrimental to BIC thereby providing better osseointegration.

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Alveolar ridge preservation, De-proteinized bovine bone mineral, Advanced platelet-rich fibrin, Tooth extraction, Randomised controlled clinical trial.

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## INTRODUCTION

The extraction sockets with no adjunctive grafting at the time of extraction undergo volumetric changes of the hard tissues of the alveolar process that occur 3 months following tooth extraction, resulting in a horizontal bone loss of 29–63% and vertical bone loss of 11–22% (1). While considering the soft tissue thickness, in thick phenotypes the soft tissue dimensions remain unchanged during healing (2). However, by nature the phenotype in most of the patients particularly in the anterior maxilla is thin ranging between 0.5 - 1 mm (3,4). In such cases, the soft tissue dimensions reveal a sevenfold spontaneous soft tissue thickening along with rapidly resorbing thin facial bone wall which favours facial soft tissue ingrowth due to its high proliferative rate.

To prevent the loss of hard and soft tissues post-tooth extraction, alveolar ridge preservation (ARP) (socket grafting with socket seal) has been used (5). A variety of bone graft materials including autografts, alloplasts, allografts, xenografts and platelet concentrates have been used for socket preservation (SP) (6). Among these xenografts are widely used because they are osteoconductive in nature and have a slow substitution rate. Apart from the catabolic activity and loss of periodontal ligament, there is also inflammation that occurs after tooth extraction. To reduce the osteoclastic activity and inflammatory burden following a tooth extraction, platelet concentrates such as platelet-rich fibrin (PRF), introduced by Choukroun et al (7) have been used.

Platelet concentrates, especially third generation such as the Advanced platelet-rich fibrin, (A-PRF) have been used for regenerative therapy. They have a unique advantage of sustained release of growth factors, apart from the presence of elevated levels of leucocytes which not only reduce the inflammatory reaction but also help in the recruitment of osteoprogenitor cells, thereby facilitating new bone formation (8–10). It maintains dimensional stability and facilitates healing (11).

Following 8 – 12 weeks of tooth extraction, there is an increase in osteoblastic activity following the initial osteoclastic activity which occurs 8 weeks post-tooth extraction. Hence there is a need for a biomaterial that lasts longer than 12 weeks for the sustained action of the osteoblastic activity. The xenograft, De-proteinized bovine bone mineral, (DBBM) is preferred as it is a slowly resorbing graft material which is beneficial by providing good space maintenance throughout the entire time course of healing (12). The slow substitution rate of DBBM coincides with the peak of osteoblastic activity after extraction i.e., after 8 weeks. However, the drawback usually associated with DBBM is the remnant graft material which could be detrimental to the bone-to-implant contact. Among

the platelet concentrates, A-PRF has a more sustained release of growth factors (10). Therefore, by combining both DBBM and A-PRF, the osteogenic potential of DBBM could be enhanced by the release of growth factors which act as signalling molecules for bone formation. Hence, the present study aimed to evaluate the effectiveness of the combination of DBBM and A-PRF in alveolar ridge preservation. The quality and quantity of bone were assessed by the amount of bone formed using histological and histomorphometric analysis respectively.

## MATERIALS AND METHODS

This study was a prospective double-blinded, parallel arm, randomized controlled clinical trial performed according to the CONSORT guidelines (Figure 1).

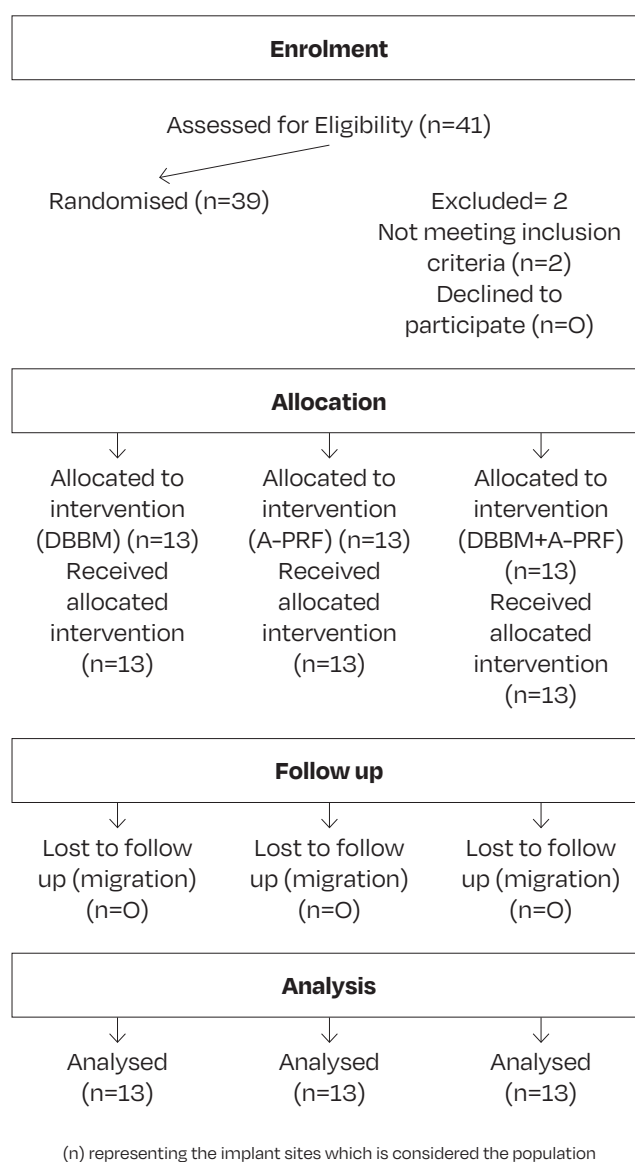


Fig. 1 Consort flow diagram.



Fig. 2A



Fig. 2B



Fig. 2C

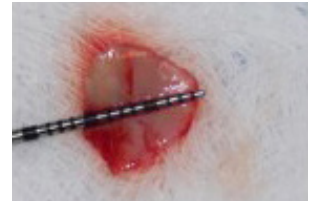


Fig. 2D



Fig. 2E



Fig. 2F



Fig. 2G



Fig. 2H

**Fig. 2A-2H** Socket preservation – DBBM Group **2A** Atraumatic extraction of the tooth using periosteal in relation to **2B** Socket after tooth extraction **2C** Outline of free gingival graft **2D** and **2E** Dimensions of procured free gingival graft **2F** Extraction socket filled with bone graft **2G** Socket seal achieved using a free gingival graft **2H** Horizontal cross sling sutures and Ab-gel to stabilize the palatal donor site.

The sample size calculation was done based on the summary statistics with an observed difference of 0.92, the standard deviation of 1.83 from a previous study by Scheyer et al in 2016 (11) with a power of 80% and alpha error of 5 % using a G-power software, V.3.1. This resulted in 13 patients per group. The randomization was achieved by computer-generated tables. The allocation concealment was done in a 1:1 ratio.

A total of 39 patients who had reported to the Department of Periodontology, Sri Ramachandra Dental College were recruited into the study, based on specific inclusion and exclusion criteria. The study was approved by the Institutional Ethics Committee (REF: IEC/19/APR/150/20). A written informed consent was obtained from all the participants before the clinical procedure.

#### Inclusion criteria

- Patients in the age group of 25–50 years.
- Patients with type I extraction sockets in the maxillary and mandibular anterior and posterior teeth.

#### Exclusion criteria:

- Presence of pathological lesions around the surgical area.
- Patients with any systemic diseases known to interfere with periodontal treatment
- Patients on regular medications affecting periodontal healing or anticoagulant therapy.
- Uncooperative patients and patients who were not willing to participate in the study and report for follow-up.
- Pregnant / Lactating women.

#### Treatment approaches:

- Group 1: Socket preservation with De-proteinized bovine bone mineral (DBBM).
- Group 2: Socket preservation with Advanced platelet-rich fibrin (A-PRF).
- Group 3: Socket preservation with a combination of A-PRF and DBBM.

#### Surgical procedure

All surgical procedures (Figures 2, 3 and 4) were performed by an expert surgeon (MS). All clinical

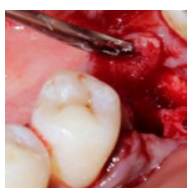


Fig. 3A



Fig. 3B



Fig. 3C

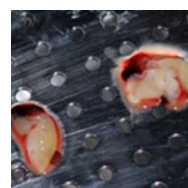


Fig. 3D



Fig. 3E



Fig. 3F

**Fig. 3A-3F** Socket preservation – A-PRF group **3A** Root stumps in relation to **3B** Outline for procurement of free gingival graft **3C** Free gingival graft procured from hard palate **3D** A-PRF clots prepared **3E** A-PRF placed in the socket **3F** Socket seal achieved using free gingival graft and sutured



Fig. 4A



Fig. 4B



Fig. 4C



Fig. 4D

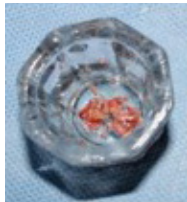


Fig. 4E



Fig. 4F



Fig. 4G



Fig. 4H



Fig. 4I

**Fig. 4A-4I** Socket preservation – DBBM+A-PRF group **4A** Root stumps evident in relation to 25 **4B** Incisions given **4C** Full-thickness mucoperiosteal flap raised **4D** Curettage done **4E** DBBM+A-PRF **4F** DBBM+A-PRF placed in the socket **4G** and **4H** Free gingival graft procured from the hard palate **4I** Socket seal achieved using free gingival graft and sutured



Fig. 5A

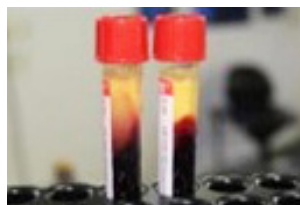


Fig. 5B



Fig. 5C



Fig. 5D

**Fig. 5A-5D** Collection and preparation of A- PRF **5A** Venous blood collected from the patient **5B** A-PRF prepared in tubes **5C** A-PRF gel formed **5D** A-PRF clots are prepared using the PRF box.

parameters and radiographs were analysed by a trained examiner. Under local anaesthesia (using 2% lignocaine in 1:100,000 adrenaline) an atraumatic extraction was performed using periostomes to preserve the available alveolar bone, following which the socket was debrided gently using curettes and irrigated with saline solution. The socket was filled with bone graft, De-proteinized bovine bone mineral (DBBM; Bio-Oss, Geistlich) in Group 1. In patients belonging to the other two groups, venous blood was collected from the patient via venepuncture of the forearm in the antecubital vein into a 10 ml sterile glass vacuum tube by a trained phlebotomist. The blood sample was immediately centrifuged at 1500 rpm (208 g force) for 14 minutes to obtain A-PRF (Figure 5). The PRF clot was then separated from the three distinct layers that formed within the tube cut into small pieces and filled up to the bony crest with light compression in the A-PRF group. A free gingival graft was then harvested from the hard palate which was carefully inserted into the socket using a guiding suture and placed over the socket orifice in all the groups.

In Group 3, the A-PRF clot was cut into small pieces and DBBM was added to achieve a volume with a 1:1 ratio of graft particulate (DBBM; Bio-Oss, Geistlich) to A-PRF.

The socket was filled with this mixture up to the bony crest with light compression following which the free gingival graft was used to achieve the socket seal.

In all the groups, the flaps were sutured using 3-0 Black braided silk (BBS) sutures and hemostasis was achieved. The patients were then followed up and assessed at periodic intervals. Oral hygiene instructions were reinforced throughout the study period.

At 4 months post-surgery, (Figures 6, 7 and 8) the test sites were re-entered for implant placement. After local anaesthesia, flaps were elevated, and a trephine with a 2 mm internal diameter was used to obtain a core sample of the bone. Harvested bone cores were immediately placed in 10% neutral buffered formalin. The osteotomy was then widened for implant placement. The implant sites were prepared without cleaning the preserved socket, and implants (Dio implants, Busan, Korea) of appropriate diameter were placed and immediately loaded. Three months later, implants were assessed for stability. Impressions were taken at the implant level, using impression coping and individualized trays, following which permanent crowns were fabricated and cemented on the titanium abutments.

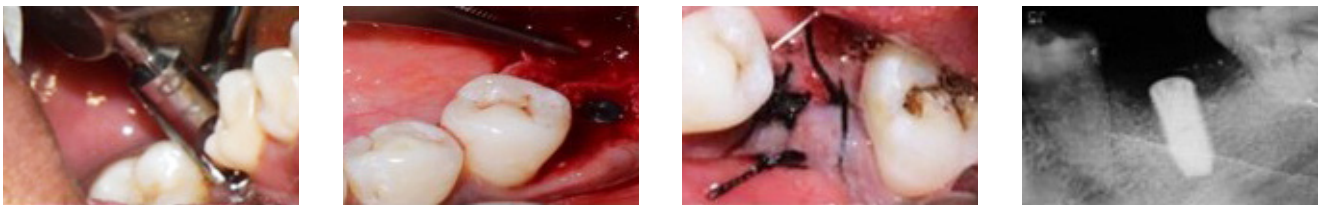


**Fig. 6A** **Fig. 6B** **Fig. 6C** **Fig. 6D** **Fig. 6E**

**Fig. 6A-6E** Four months post socket preservation – DBBM group **6A** Subcrestal incision placed for flap elevation **6B** Bone sample procurement using trephines **6C** Implant placed with cover screw **6D** Flaps approximated and sutured **6E** Intraoral periapical radiograph showing implant in position



**Fig. 7A** **Fig. 7B** **Fig. 7C**

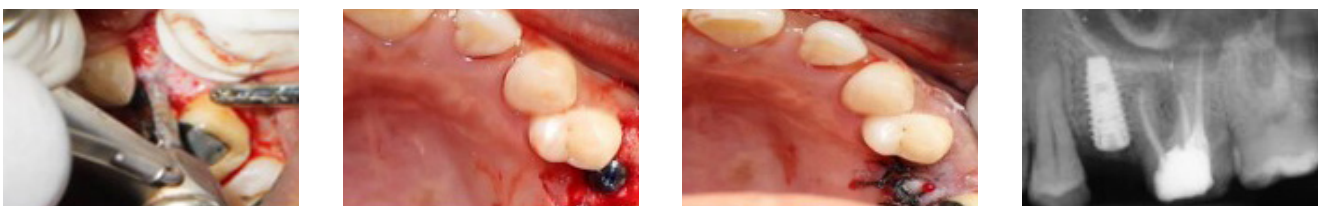


**Fig. 7D** **Fig. 7E** **Fig. 7F** **Fig. 7G**

**Fig. 7A-7G** Four months post socket preservation – A-PRF group **7A** Pre-operative site in relation to 36 **7B** Mid-crestal incision given **7C** Full-thickness mucoperiosteal flap raised **7D** Bone sample procured using trephine **7E** Implant with the cover screw placed in relation to 36 **7F** Flaps approximated and sutured **7G** Intra-oral periapical radiograph showing implant in position



**Fig. 8A** **Fig. 8B** **Fig. 8C**



**Fig. 8D** **Fig. 8E** **Fig. 8F** **Fig. 8G**

**Fig. 8A-8G** Four months post socket preservation – DBBM+A-PRF group **8A** Pre-operative site in relation to 25 **8B** Mid-crestal incision given **8C** Full-thickness mucoperiosteal flap raised **8D** Bone sample procured using trephine **8E** Implant with the cover screw placed in relation to 25 **8F** Flaps approximated and sutured **8G** Intra-oral periapical radiograph showing implant in position

### Histological and histomorphometric analysis

The bone samples were fixed in 10% neutral buffered formalin immediately after retrieval and later decalcified with 10 % formic acid for one week. The histological analysis of the bone samples was done by a pathologist using the projection microscope (Visopan, Reichert, Leica) at 20x magnification (Figures 9, 10 and 11). Newly formed bone was identified by the presence of osteocytes with lacunae and intense eosinophilic staining (13). The residual graft was identified by the presence of osseous tissue

fragments with pale eosinophilic staining and without lacunae. The residual bone graft was seen integrated into the new bone formed. The remaining connective tissue was fibrous connective tissue which comprised of fibroblasts, collagen fibers and small capillaries. The percentage of bone formed, connective tissue and residual graft present was calculated by an experienced pathologist. The newly formed bone and vascular structures were more in the apical region compared to the middle and coronal regions in all the groups. Whereas the connective tissue was more

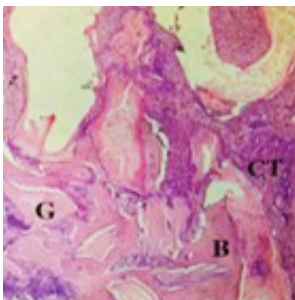


Fig. 9A

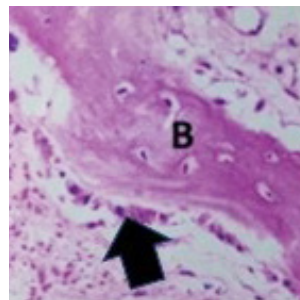


Fig. 9B

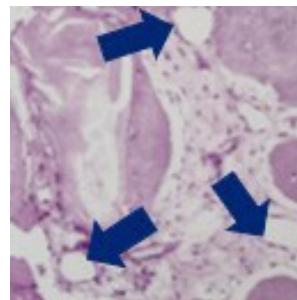


Fig. 9C

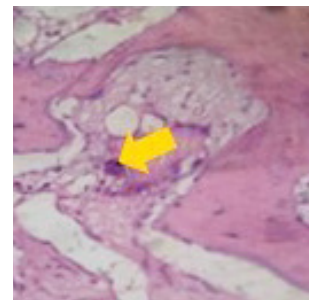


Fig. 9D

**Fig. 9A-9D** Histological analysis of the DBBM group sample **9A** New bone (B), connective tissue (CT) and residual graft (G)(10x) **9B** Black arrow depicting osteoblast rimming the newly formed bone (10x) **9C** Blue arrows depicting blood vessels in Bio Oss Sample (10x) **9D** Yellow arrow depicting multinucleated cells in close proximity resorbing the graft particle (10x)

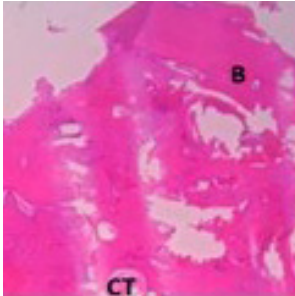


Fig. 10A

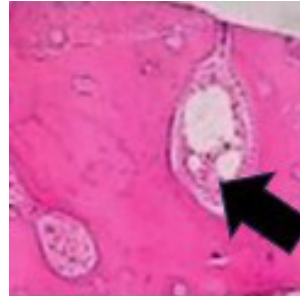


Fig. 10B

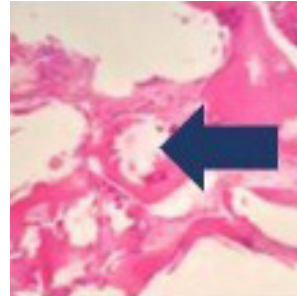


Fig. 10C



Fig. 10D

**Fig. 10A-10D** Histological analysis of A-PRF group sample **10A** New bone formed (B) & Connective tissue (CT) (10x) **10B** Thick blood vessel formation as depicted by the black arrow (20x) **10C** Blue Arrow depicting adipocytes (20x) **10D** Yellow arrow depicting inflammatory cells (20x)

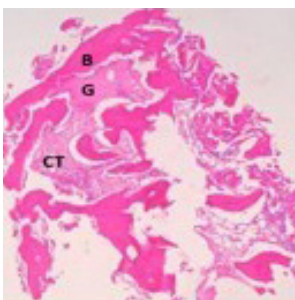


Fig. 11A



Fig. 11B

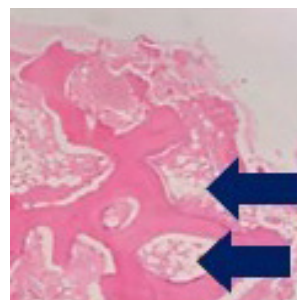


Fig. 11C



Fig. 11D

**Fig. 11A-11D** Histological analysis of DBBM+A-PRF group sample **11A** a) New bone (B), connective tissue (CT) and residual graft (G)(20x). **11B** Residual graft (G) seen integrating into the new bone (B) formed with the presence of osteocytes as depicted by the black arrow (20x). **11C** Blood vessels in the sample as depicted by the blue arrows (20x) **11D** Osteoblasts rimming in the newly formed bone as depicted by the yellow arrow.

|              | DBBM      | A-PRF      | DBBM + A -PRF |
|--------------|-----------|------------|---------------|
| Mean age     | 34.38±9.9 | 30.15±4.39 | 34.61±8.61    |
| Gender       |           |            |               |
| Males (22)   | 8         | 7          | 7             |
| Females (17) | 5         | 6          | 6             |

**Tab. 1** Demographic data of patients in the groups

in the coronal region compared to the apical region. The histomorphometric analysis for both groups was done by a trained examiner using the Image J analysis software.

### Statistical analysis

Statistical analysis was performed using the SPSS software package version (version 21.0, SPSS Inc.). The normality of the data was tested using the Shapiro-Wilk test and the Kolmogorov-Smirnov test. Since the p-value was >0.5, the data was normally distributed. Hence independent t-test was used to compare the histological and histomorphometry values between the groups. Bonferroni post hoc analysis was done for comparison of histologic and histomorphometric aspects of the groups. The demographic variables and age were tested using the one-way ANOVA test and gender was evaluated using the chi-square test. The level of significance was set at p-value <0.05. Pearson correlation analysis was done for the correlation between histological and

histomorphometric interpretations between the groups.

## RESULTS

A total of 39 extraction sites were treated and implants were placed, four months post-socket preservation. The results were computed and analysed for statistical analysis. The one-way ANOVA test was used to test the age distribution between the groups. The results revealed that age was equally distributed within the groups and had no effect on the results at p=0.43. The chi-square test was used to test the distribution of gender within the groups (Table 1).

### Histological Analysis

The amount of bone, connective tissue and residual graft were examined histologically and interpreted by an experienced pathologist. Histological analysis revealed a statistically significant difference between vital bone and fibrous connective tissue between the groups at p= 0.00 respectively. The amount of residual graft was 17.14±11.93% and 14.07±10.96% in the DBBM and DBBM+A-PRF groups respectively(p=0.50). (Table 2).

### Histomorphometric analysis

The mean percentage of the vital bone, and connective tissue examined using histomorphometric analysis was statistically significant between the groups at p=0.00. (Table 3). Pearson correlation analysis revealed a

|                      | Mean          | Standard. Deviation | p-value  |         |
|----------------------|---------------|---------------------|----------|---------|
| Histology Vital bone | DBBM          | 33.8154             | 15.74361 | 0.00000 |
|                      | A-PRF         | 55.3846             | 8.77058  |         |
|                      | DBBM + A -PRF | 68.0769             | 10.51556 |         |
|                      | Total         | 51.2094             | 17.56793 |         |
| Histology CT         | DBBM          | 51.1462             | 12.76046 | 0.00000 |
|                      | A-PRF         | 44.6154             | 8.77058  |         |
|                      | DBBM + A -PRF | 19.7692             | 11.54090 |         |
|                      | Total         | 42.1057             | 17.71452 |         |

**Tab. 2** Histologic analysis of vital bone and connective tissue in the groups

|                              | N             | Mean | Standard. Deviation | p-value  |         |
|------------------------------|---------------|------|---------------------|----------|---------|
| Histomorphometric Vital bone | DBBM          | 13   | 36.4462             | 14.65501 | 0.00000 |
|                              | A-PRF         | 13   | 54.0769             | 8.54850  |         |
|                              | DBBM + A -PRF | 13   | 67.2308             | 9.95953  |         |
|                              | Total         | 53   | 51.7547             | 16.19470 |         |
| Histomorphometric CT         | DBBM          | 13   | 50.3615             | 14.53378 | 0.00000 |
|                              | A-PRF         | 13   | 45.9231             | 8.54850  |         |
|                              | DBBM + A -PRF | 13   | 19.5385             | 10.50092 |         |
|                              | Total         | 53   | 41.7377             | 17.59583 |         |

**Tab. 3** Histomorphometric analysis of vital bone and connective tissue in the groups.

|            | Vital bone | Connective tissue |
|------------|------------|-------------------|
| DBBM       | 0.856**    | 0.83              |
| A-PRF      | 0.894**    | 0.894**           |
| DBBM+A-PRF | 0.928**    | 0.920**           |

\*\* Correlation is significant at the 0.01 level (2-tailed)

**Tab. 4** Pearson correlation analysis between histological and histomorphometric analysis for the groups.

significant correlation between both the histological and histomorphometric interpretations for both vital bone and connective tissue in the three groups (Table 4).

## DISCUSSION

The present study was conducted as a randomized controlled clinical trial to compare two socket preservation biomaterials, DBBM, A-PRF and a combination of both. A total of 39 patients were treated in three groups with 13 patients in each group. Bone biopsies were retrieved using trephine and dental implants (DIO implants, Busan, Korea) were placed in socket preservation treated sites after a 4-month healing period. Only patients with type I extraction sockets were included in this study for standardisation to evaluate the impact of biomaterials in socket preservation. To achieve primary wound closure following socket preservation, the socket seal surgery technique using free gingival graft was used in all the groups as it helps in achieving a primary closure at the site of treatment, which could be favourable in the integration of the DBBM used for ARP. Apart from obtaining primary wound closure that may preserve the bone graft from bacterial contamination, the placement of a free gingival graft to cover the augmented alveolar socket minimizes soft tissue shrinkage, optimizes esthetic results of implant restoration, and prevents secondary graft failures (14).

Literature evidence suggests that a majority of the remodelling occurs in the socket within the first 3 months post-extraction (15). Hence, a follow-up period of 4 months was selected in the present study as it would give a definitive indication of the effectiveness of the biomaterials used in ARP.

Four months following socket preservation, before implant placement, the bone core samples for histological evaluation in the test and control groups were collected using trephines of 2 mm internal diameter to obtain a bone sample to the measured depth of the original socket. Histological analysis was done to analyse the percentage of bone, connective tissue and remnant graft particles in all the samples. The histological method was chosen in this study as it is the gold standard and it helps in accurate differentiation of the vital bone and residual graft particulate (16) which is an important criterion for assessing the efficacy of a biomaterial in grafting approaches. In the present study, histomorphometric

analysis was also done to have an objective evaluation of the percentage of bone, connective tissue and residual graft using Image-J analysis software (17). This software was preferred as it is the most commonly used software in many studies (14,18–20) which allows the calculation of parameters defined by the areas they occupy, their boundary perimeters, or their distances from other points of reference by image analysis (17).

Based on histological and histomorphometric analysis of the study, the bone formation in all groups was more in the apical region as it was predominated by the newly formed bone and due to the reduced dimensions of socket morphology and the higher marrow-to-graft ratio in the apical region (21). It was identified by the presence of osteocytes within the lacunae. However, the amount of connective tissue was more in the coronal than the apical region due to the superficial surface facing the soft tissue side during retrieval of biopsy with the trephine. It was predominated by the presence of collagen, fibroblasts, and blood vessels. The residual graft material was identified with the absence of osteocytes within the lacunae. This represents the area of resorption and was found to be integrating with bone, transforming into new bone. It indicates that the osteoconductive process has happened leading to vital bone formation.

In the present study, significantly more vital bone formation was formed in the DBBM and A-PRF combination group when compared to the DBBM group and A-PRF group, this could be due to the synergistic effect of osteoconductive and osteoinductive properties of DBBM and A-PRF (22). The connective tissue component was enhanced in DBBM group and A-PRF group compared to DBBM and A-PRF combination group. This could be due to the ability to A-PRF to enhance soft tissue healing (9) and due to presence of increased remnant graft material in DBBM group, compared to DBBM and A-PRF combination group. The residual graft was 17.14±11.93% and 14.42±11.38% in groups 1 and 3 respectively, which could be due to longer resorption time associated with DBBM and non-interference in bone healing (23). Meanwhile, the percentage of connective tissue was 19.76±11.54 in the DBBM+A-PRF group which was the least among the groups, this reduction in the connective tissue could be due to the complementary effect of osteoconductive and osteoinductive property of DBBM and A-PRF which facilitates neovascularisation leading to new bone formation (24–26). Besides its jelly-like consistency favours the stability of the clot and graft material resulting in lesser fibrous tissue formation (27).

On the other hand, the amount of bone formation in the A-PRF was 55.38±8.77% though lesser than the DBBM+A-PRF group, it has shown a significant amount of bone formation, which could be attributed to the growth factors enriched in platelet-rich fibrin which stimulate bone formation and due to its tendency to enhance natural healing of the hard and soft tissues (28).

However, the percentage of connective tissue formed was  $44.61 \pm 8.77$  in the A-PRF group, significantly higher which could be because of the ability of the platelet-rich fibrin to enhance soft tissue proliferation. However, this group was not associated with residual grafts as only platelet-rich fibrin was used.

The amount of new bone formation measured histomorphometrically in the group DBBM+A-PRF was  $67.23 \pm 9.95\%$ , residual graft particles were  $13.53 \pm 9.96\%$  and connective tissue was  $19.53 \pm 10.50\%$ . The results of the present study concurred with that of other studies which demonstrated bone formation of  $65.92 \pm 10.91\%$  (29) and  $63.29 \pm 13.03\%$  (30), connective tissue of  $22.8 \pm 13.7$  (31) and residual graft of  $19.0 \pm 6.5\%$  (23) and  $20.62 \pm 9.91\%$  (32).

The results of the study differed with other studies which demonstrated bone formation of  $29 \pm 14\%$  (18),  $35.3 \pm 16.87$  (31),  $39.9 \pm 8.6\%$  (23), and  $27.35 \pm 12.39$  (32), connective tissue of  $60\%$  (18),  $32.4 \pm 9.2\%$  (23),  $28.27 \pm 13.22\%$  (29) and residual graft of  $3 \pm 3\%$  (18),  $8.69 \pm 5.99$  (29) and  $22.2 \pm 13.4$  (31). This could be attributed due to the difference in surgical technique and biomaterials used for socket preservation in the other studies.

In the present study, a significant correlation was observed between parameters measured by histomorphometry and histological analysis between the groups.

However, the present study also has certain limitations such as: volumetric changes were not assessed, Bone-to-implant contact was not evaluated, and long-term implant survival and success were not evaluated following socket preservation.

Within the limitations of this study, the conclusion drawn is that DBBM+A-PRF was effective for socket preservation as superior bone formation was evident in terms of qualitative and quantitative assessment. Future studies with volumetric changes and implant survival have to be conducted to evaluate if it can be considered as a material of choice for socket preservation.

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## REFERENCES

- Tan WL, Wong TLT, Wong MCM, Lang NP. A systematic review of post-extraction alveolar hard and soft tissue dimensional changes in humans. *Clin Oral Implants Res.* 2012 Feb;23 Suppl 5:1–21.
- Chappuis V, Engel O, Shahim K, Reyes M, Katsaros C, Buser D. Soft tissue alterations in esthetic postextraction sites: a 3-dimensional analysis. *J Dent Res.* 2015;94(9\_suppl):187S–193S.
- Müller H, Schaller N, Eger T, Heinecke A. Thickness of masticatory mucosa. *J Clin Periodontol.* 2000;27(6):431–6.
- Fu J, Yeh C, Chan H, Tatarakis N, Leong DJM, Wang H. Tissue biotype and its relation to the underlying bone morphology. *J Periodontol.* 2010;81(4):569–74.
- Jung RE, Ioannidis A, Hämmerle CHF, Thoma DS. Alveolar ridge preservation in the esthetic zone. *Periodontol* 2000. 2018;77(1):165–75.
- Zhao R, Yang R, Cooper PR, Khurshid Z, Shavandi A, Ratnayake J. Bone Grafts and Substitutes in Dentistry: A Review of Current Trends and Developments. *Molecules.* 2021 May;26(10).
- Choukroun J. Advanced PRF, & i-PRF: platelet concentrates or blood concentrates. *J Periodontol Med Clin Pr.* 2014;1(1):3.
- Ghanaati S, Booms P, Orlowska A, Kubesch A, Lorenz J, Rutkowski J, et al. Advanced platelet-rich fibrin: a new concept for cell-based tissue engineering by means of inflammatory cells. *J Oral Implantol.* 2014;40(6):679–89.
- Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandalam U, Zhang Y, Choukroun J. Optimized platelet-rich fibrin with the low-speed concept: growth factor release, biocompatibility, and cellular response. *J Periodontol.* 2017;88(1):112–21.
- Ravi S, Santhanakrishnan M. Mechanical, chemical, structural analysis and comparative release of PDGF-AA from L-PRF, A-PRF and T-PRF—an in vitro study. *Biomater Res.* 2020;24(1):16.
- Scheyer ET, Heard R, Janakievski J, Mandelaris G, Nevins ML, Pickering SR, et al. A randomized, controlled, multicentre clinical trial of post-extraction alveolar ridge preservation. *J Clin Periodontol.* 2016;43(12):1188–99.
- Heinemann F, Hasan I, Schwahn C, Bouraueal C, Mundt T. Bone level change of extraction sockets with Bio-Oss collagen and implant placement: a clinical study. *Ann Anat = Anat Anzeiger Off organ Anat Gesellschaft.* 2012 Nov;194(6):508–12.
- Pietrokovski J, Massler M. Alveolar ridge resorption following tooth extraction. *J Prosthet Dent.* 1967;17(1):21–7.
- Landsberg CJ, Bichacho N. A modified surgical/prosthetic approach for optimal single implant supported crown. Part I—The socket seal surgery. *Pract Periodontics Aesthet Dent.* 1994 Mar;6(2):11–7; quiz 19.
- Schropp L, Wenzel A, Kostopoulos L, Karring T. Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. *Int J Periodontics Restorative Dent.* 2003;23(4).
- Heberer S, Al-Chawaf B, Hildebrand D, Nelson JJ, Nelson K. Histomorphometric analysis of extraction sockets augmented with Bio-Oss Collagen after a 6-week healing period: a prospective study. *Clin Oral Implants Res.* 2008 Dec;19(12):1219–25.
- Egan KP, Brennan TA, Pignolo RJ. Bone histomorphometry using free and commonly available software. *Histopathology.* 2012;61(6):1168–73.
- Clark D, Rajendran Y, Paydar S, Ho S, Cox D, Ryder M, et al. Advanced platelet-rich fibrin and freeze-dried bone allograft for ridge preservation: a randomized controlled clinical trial. *J Periodontol.* 2018;89(4):379–87.
- Kumar MS, Natta S, Shankar G, Reddy SHK, Visalakshi D, Seshiah G V. Comparison between silk sutures and cyanoacrylate adhesive in human mucosa—a clinical and histological study. *J Int oral Heal JIOH.* 2013;5(5):95.
- Kaplan M, Oral B, Rollas S, Sinan Kut M, Demirtas MM. Absorption of ethyl 2-cyanoacrylate tissue adhesive. *Eur J Drug Metab Pharmacokinet.* 2004;29:77–81.
- Lahey LA, Akella R, Ranieri JP. Angiogenesis: implications for tissue repair. *Bone Eng Toronto Em Squared Inc.* 2000;137–42.
- Indovina Jr A, Block MS. Comparison of 3 bone substitutes in canine extraction sites. *J Oral Maxillofac Surg.* 2002;60(1):53–8.
- Lindhe J, Cecchinato D, Donati M, Tomasi C, Liljenberg B. Ridge preservation with the use of deproteinized bovine bone mineral. *Clin Oral Implants Res.* 2014 Jul;25(7):786–90.
- Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. *Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology.* 2006;101(3):e37–44.
- Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. *Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology.* 2006;101(3):e45–50.
- Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part III: leucocyte activation: a new feature for platelet concentrates? *Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology.* 2006;101(3):e51–5.
- Tatullo M, Marrelli M, Cassetta M, Pacifici A, Stefanelli LV, Scacco S, et al. Platelet Rich Fibrin (PRF) in reconstructive surgery of atrophied maxillary bones: clinical and histological evaluations. *Int J Med Sci.* 2012;9(10):872.
- Banks RE, Forbes MA, Kinsey SE, Stanley A, Ingham E, Walters C, et al. Release of the angiogenic cytokine vascular endothelial growth factor (VEGF) from platelets: significance for VEGF measurements and cancer biology. *Br J Cancer.* 1998;77(6):956–64.
- Ivanova V, Chenchev I, Zlatev S, Iordanov G, Mijiritsky E. Comparative Study between a Novel In Vivo Method and CBCT for Assessment of Ridge Alterations after Socket Preservation—Pilot Study. *Int J Environ Res Public Health.* 2019;16(1):127.
- Ivanova V, Chenchev I, Zlatev S, Mijiritsky E. Comparison study of the histomorphometric results after socket preservation with PRF and allograft used for socket preservation—randomized controlled trials. *Int J Environ Res Public Health.* 2021;18(14):7451.
- Serrano Méndez CA, Lang NP, Caneva M, Ramírez Lemus G, Mora Solano G, Botticelli D. Comparison of allografts and xenografts used for alveolar ridge preservation. A clinical and histomorphometric RCT in humans. *Clin Implant Dent Relat Res.* 2017;19(4):808–15.
- Gholami GA, Najafi B, Mashhadiabbas F, Goetz W, Najafi S. Clinical, histologic and histomorphometric evaluation of socket preservation using synthetic nanocrystalline hydroxyapatite in comparison with a bovine xenograft: a randomized clinical trial. *Clin Oral Implants Res.* 2012;23(10):1198–204.