

How to Cite:

Raj, A., Prasad, R., Gupta, B., Swahney, A., Tiwari, V., & Mistry, L. (2022). Comparative evaluation demineralized freeze dried bone allograft with and without platelet rich fibrin in intrabony defects: A clinical and radiographical study. *International Journal of Health Sciences*, 6(S1), 872-886. <https://doi.org/10.53730/ijhs.v6nS1.4836>

Comparative Evaluation Demineralized Freeze Dried Bone Allograft with and Without Platelet Rich Fibrin in Intrabony Defects: A Clinical and Radiographical Study

Akshatha Raj

Assistant professor, Faculty of dental sciences, Ramaiah university of applied sciences, Bengaluru, India

Rohit Prasad

Assistant professor, Faculty of dental sciences, Ramaiah university of applied sciences, Bengaluru, India

Bharat Gupta

Associate professor, Department of Periodontics, MGM dental college, Navi Mumbai, India

Anshul Swahney

Assistant professor, department of dentistry (periodontics), Maharaja suhel dev autonomous state Medical college, bahraich, Uttar Pradesh, India

Vaibhav Tiwari

Associate Professor, Department of Periodontology and Implantology, Government Dental College, Raipur, India

Laresh Mistry

Associate professor, department of pedodontics, BVP dental college, Navi Mumbai, India

Abstract---Background and Objectives: The study was designed to evaluate the clinical and radiological effectiveness of demineralized freeze dried bone allograft with and without platelet rich fibrin in intrabony defects. Materials and Methods: twelve patients with 2 intrabony defects were recruited for the study. The two defects in each patient were randomly assigned to receive either DFDBA (site A) or DFDBA with PRF (site B). Soft tissue measurements and radiographs were taken at baseline, 3, 6 and 9 months. Results: The results

indicated that probing depths were reduced by 3.08 ± 1.16 mm at site A and 3.33 ± 1.72 mm at site B. Site A resulted in 2.42 ± 1.24 mm attachment gain, while site B had a 3.17 ± 1.40 mm gain in attachment Site A. Defects displayed 2.00 mm bone fill. Site B defects showed 3.00 mm bone fill. Both treatments provided soft and hard tissue improvement when compared to baseline. No statistical significance was noted between the two groups. Conclusion: The Clinical evaluation is suggestive of the fact that both DFDBA and PRF are biocompatible with the tissues.

Keywords---comparative evaluation, DFDBA, intrabony defects, PRF.

Introduction

Periodontal disease is a chronic infection of the periodontium which affects the soft and mineralized tissue surrounding the teeth, along with subgingival bacterial colonization and biofilm formation that provokes chronic inflammation of the soft tissue, degradation of collagen fibers supporting the tooth to the gingiva and alveolar bone resulting in resorption of alveolar bone and formation of osseous defects.

The management of these osseous defects has always been a challenge. The initial objectives of periodontal therapy are infection management. To control the pathogenic microflora and to prevent further destruction, scaling and root planning and surgical therapies with antimicrobial therapy are used. Despite successful disease management, changes in the anatomy as a result of past disease activity must be corrected. Therapeutic approaches for correcting these anatomic defects include procedures such as flap debridement, resective procedures and periodontal regenerative therapy. Conventional surgical approaches, such as open flap debridement, provide critical access to and detoxify root surfaces and to establish improved periodontal form and architecture; but these surgical techniques has limited potential in restoring or reconstituting components of periodontal tissues ([Scannapieco, 2005](#)).

Today, attention appears to focus on two main approaches for periodontal regeneration. On the one hand there is the approach that requires a “filler” material which induces bone regeneration. On the other hand, techniques are being developed to guide and instruct the specialized cellular components of the periodontium to take part in the regenerative process ([Bartold et al., 2000](#)). Bone grafting materials function as structural scaffolds and matrices for attachment and proliferation of anchorage-dependent osteoblasts. A wide range of bone grafting materials, such as autografts, allografts, xenografts, and alloplasts are the most widely used in treating periodontal osseous defects.

Demineralized freeze-dried bone allograft (DFDBA) has been used extensively in periodontal therapy for 2 decades. They have been successfully used to reconstruct intraosseous periodontal defects and furcation defects. Regeneration of periodontal ligament, cementum, and new bone has also been documented. The widespread use of DFDBA is based on the osteoinductive ability of bone graft.

Demineralization of the graft exposes the bone inductive proteins located in the bone matrix, and may activate them. Many studies have found that protein extracts from DFDBA contain immunoreactive bone morphogenetic protein (BMP) as well as other biologically active molecules which aids in regeneration of lost bone (Schwartz et al., 1996).

The hormones and growth factors on the other hand play important roles in regeneration. Many studies have examined the effects of systemic hormones and growth factors on bone and soft-tissue metabolism. These growth factors regulate cellular events in wound healing, such as proliferation, differentiation, chemotaxis and morphogenesis of tissues and organs. Especially platelet-derived growth factor (PDGF) and transforming growth factor- β (TGF- β), have been shown to promote periodontal regeneration in vitro, in animals and in human. A recently developed procedure platelet-rich plasma (PRP) by De Obarrio et al., is widely used in periodontal regeneration.

Platelet-rich fibrin (PRF) represents a new step in the platelet gel therapeutic concept with simplified processing without artificial biochemical modification. Unlike PRP this technique requires neither anticoagulants nor bovine thrombin (nor any other gelifying agent), making it no more than centrifuged natural blood without additives. Developed in France by Choukroun et al in 2001, the PRF production protocol attempts to accumulate platelets and releases cytokines in a fibrin clot. A physiologic fibrin matrix (such as PRF) will have very different effects than a fibrin glue enriched with cytokines (such as PRP), which will have a massively uncontrollable and short-term effect (Toffler et al., 2009). In this study an attempt has been made to compare the effectiveness of demineralized freeze dried bone allograft with and without platelet rich fibrin in a periodontal vertical bone defects.

Materials and Methods

The randomized clinical trial was conducted in a department of periodontics. The study was performed according to Helsinki declaration ethical clearance was obtained from institutional ethical committee. Patients were informed verbally and written informed consent was taken before the surgery. 12 patients with 2 vertical defects each were selected in the age group between 25- 58 years comprising of both sexes. Patients were followed up for a period of 9 months. The pre and postoperative assessment was done by a single examiner.

- **INCLUSION CRITERIA:** Patients with clinical and radiological evidence of moderate to severe periodontitis, systemically healthy, who has not received any kind of periodontal therapy for last six months, with two or more vertical osseous defects with residual probing pocket depth of > 5 mm.
- **EXCLUSION CRITERIA:** Patients who have received antibiotic therapy in the past six months, Teeth with furcation involvement, gingival recession and grade II & grade III mobility.

Radiological Procedure

Intra-oral periapical radiographs standardized by means of long cone paralleling technique to assess the defects at baseline and 9 months following surgery. A 1mm marking grid was used for recording the distance from CEJ to alveolar crest, distance from CEJ to base of the defect.

The following calculations were made from the clinical measurements recorded:

- Pocket depth: Fixed reference point to Base of the pocket (FRP to BOP) – Fixed reference point to gingival margin (FRP to GM).
- Clinical attachment level: Fixed reference point to Base of the pocket (FRP to BOP) – Fixed reference point to Cementoenamel junction (FRP to CEJ).
- Gingival recession : Fixed reference point to Cementoenamel junction (FRP to CEJ) – Fixed reference to Gingival margin (FRP to GM).
- Selected sites were then randomly distributed to site A and site B. Site A was treated with demineralised freeze dried bone allograft with open flap debridement and site B with platelet rich fibrin with demineralised freeze dried bone allograft with open flap debridement.

Clinical parameters

Plaque and gingival index were recorded at baseline, 1,3,6,9 months. Probing pocket depth and Clinical attachment loss (CAL) were recorded at baseline and at 9 months. Clinical parameter such as probing pocket depth, clinical attachment level, gingival recession were recorded to the nearest millimetre with the help of a University of North Carolina (UNC-15) probe by a single investigator at each surgical site at baseline and 9 months post operatively. An occlusal stent was prepared by cold cure acrylic resin and groove was prepared on the stent so as to guide the position of the probe

Surgical procedure

Under local anaesthesia Crevicular incisions were given and full thickness flaps were elevated by means of Lining pocket epithelium was removed so that a fresh connective tissue bed was in contact with the graft material. 10ml of blood was drawn from the patient by using a 24 gauge disposable syringe. The blood was drawn into a test tube without adding any anticoagulants and is immediately centrifuged for 3000 rpm for 10min. The fibrin clot containing the platelet is located. This clot is removed from the tube and the attached red blood cells scraped off ([Choukroun et al., 2006](#)).

In the test site the Platelet Rich Fibrin obtained was mixed with demineralised freeze dried bone allograft and placed in the defect. In a control site demineralised freeze dried bone allograft was mixed with a few drops of saline and fill the defect. Buccal and lingual flaps were approximated using 3-0 non resorbable silk sutures by interrupted suture technique and periodontal dressing was placed.

Follow up care

- Patient's were given routine post surgical instructions. Antibiotics and analgesics were prescribed followed by Chlorhexidine gluconate 0.2% mouthrinse twice daily for 2 weeks.
- Periodontal dressing and sutures were removed one week after surgery and they were instructed to gently brush the area with a soft bristles toothbrush using charters techniques.
- Patients follow up was done 3, 6, 9 months. plaque index and gingival index was recorded at 3 ,6, 9 months and probing pocket depth, clinical attachment loss and the radiograph was taken at 9 months.

Results

All patients showed good compliance and the healing period was uneventful for both the treated groups, without showing any signs of inflammation, infection, and swelling indicating the biocompatibility of the material. The handling characteristics of both these graft materials demonstrated cohesiveness of the material, which facilitated accuracy of placement (Meadows et al., 1993; Holtzclaw et al., 2008; Zhang et al., 1997; Nevins et al., 2003; Dohan Ehrenfest et al., 2010; He et al., 2009; Lucarelli et al., 2010; Yukna, 1994; Persson et al., 2000; Zamet et al., 1997).

The results of the clinical parameters recorded are as follows

- Plaque Index (Silness & Loe, 1964)
Mean plaque index score of 12 patients at baseline was 1.96 which is considered as fair. The mean plaque index was 1.19 at 9 months. During the study period plaque index remained good. There was statistically significant change noted in the plaque index score during the study period compared to baseline.
- Gingival Index (Loe & Silness, 1964)
The mean gingival index was 0.79 at 9 months. There was statistically significant changes noted in gingival index

Probing pocket depth

The post surgical (9months) probing pocket depths for site A ranged between 3-6 mm with a mean of 4.33 ± 1.07 , whereas for site B, it ranged between 3-7 mm with a mean of 4.58 ± 1.44 . Post operatively there was reduction in probing pocket depth at both site A and site B. At site A it reduced to 1-5 mm with a mean of 3.08 ± 1.16 . The P value was 0.008, which was statistically very highly significant. At site B, it reduced to 1-7mm with a mean of 3.33 ± 1.72 . The P value was 0.003, which was also statistically highly significant. Decrease I n probing pocket depth in site B (3.33 ± 1.72) was more when compared to Site A (3.08 ± 1.16). The P value was 0.68 which was statistically not significant.

Clinical attachment level

The post operative (9months) clinical attachment level for Site A ranged between 3-8 mm with a mean of 4.67 ± 1.50 , whereas for Site B, it ranged between 3-7 mm with a mean of 4.67 ± 1.23 . Post operatively there was gain in clinical attachment level at both site A and site B. At Site A it increased to 1 – 4 mm with a mean of 2.42 ± 1.24 . The P value was 0.0082, which was statistically significant. At Site B it increased to 1 – 5 mm with a mean of 3.17 ± 1.40 . The P value was 0.002, which was statistically highly significant. The gain in clinical attachment level in Site B was slightly more when compared to Site A. The P value was 0.176 which was statistically not significant (Quintero et al., 1982; Lovelace et al., 1998; Lang, 2000; Rosen et al., 2000; Mellonig, 1991; Brunsvold & Mellonig, 1993; Shigeyama et al., 1995; Masters et al., 1996).

Radiographic changes

At baseline the distance from CEJ to crest of the alveolar bone at site A ranged between 1-6mm at a mean of 3.83 ± 1.59 and in site B it ranged from 3-7 mm at a mean of 4.50 ± 1.24 . Post operatively (9 months), the distance from CEJ to crest of the alveolar bone at site A ranged between 1-6 mm with a mean of 3.50 ± 1.78 and at site B it ranged from 2-6 mm with a mean of 3.67 ± 1.37 . Postoperatively there was decrease in the distance from CEJ to crest of the alveolar bone measurements at both Site A and site B. At site A it reduced 0-2 mm with a mean of 0.42 ± 0.67 . The P value was 0.635, which was statistically not significant. At site B it reduced to 0-3 mm with a mean of 0.83 ± 1.03 . The P value was 0.131, which was also statistically not significant.

The distance from CEJ to crest of the alveolar bone at Site A was less when compared to Site B. The P value was 0.25 which was statistically not significant. At baseline distance between CEJ to depth of the defect at Site A ranged between 5 -11mm with a mean of 7.33 ± 1.87 and for site B it ranged between 5 - 15 mm with a mean of 9.00 ± 3.13 . Postoperatively (9 months), distance between CEJ to depth of the defect at site A ranged between 2-9mm with mean of 5.33 ± 2.31 , whereas for site B, it ranged between 3- 9mm with a mean of 6.00 ± 1.86 . At site A it reduced 1-5 mm with a mean of 2.00 ± 1.21 . The P value was 0.027, which was statistically significant. At site B it reduced to 1-9 mm with a mean of 3.00 ± 2.22 . The P value was 0.0084, which was also statistically significant. The distance from CEJ to base of the pocket at Site A was less when compared to Site B. The P value was 0.018 which was statistically significant (Zhang et al., 1997; Rummelhart et al., 1989; Masters et al., 1996; Gurinsky et al., 2004; Parashis et al., 1998; Bowen et al., 1989; Mellado et al., 1995).

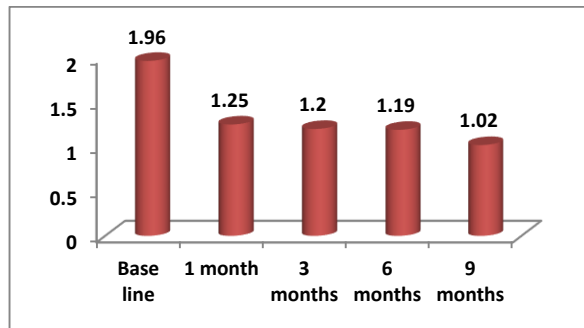


Figure 1. Comparison of mean plaque index

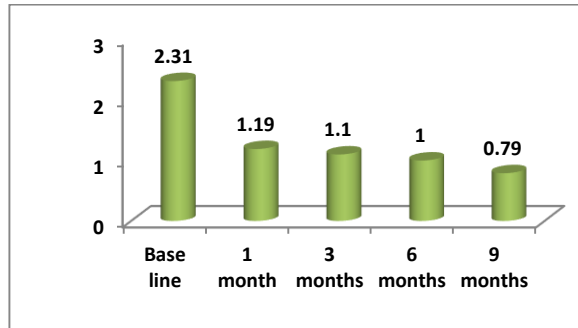


Figure 2. Comparison of mean gingival index

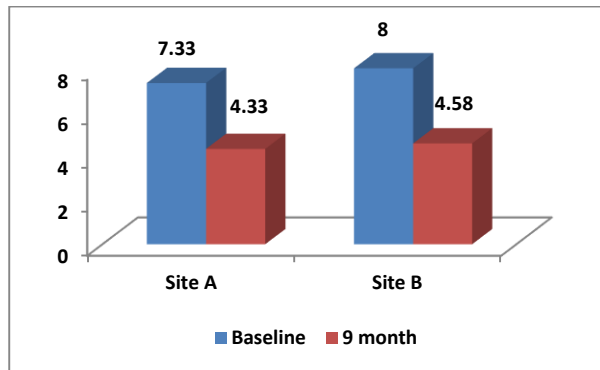


Figure 3. Comparison of mean probing pocket depth

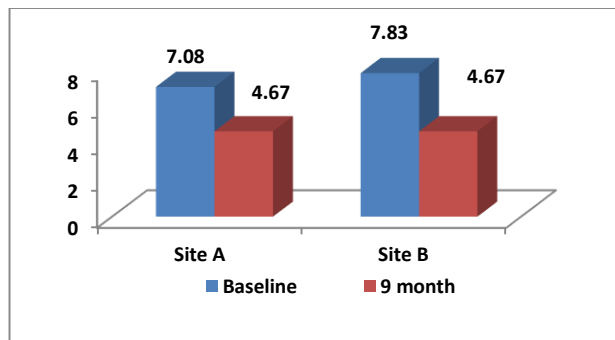


Figure 4. Comparison of mean clinical attachment level

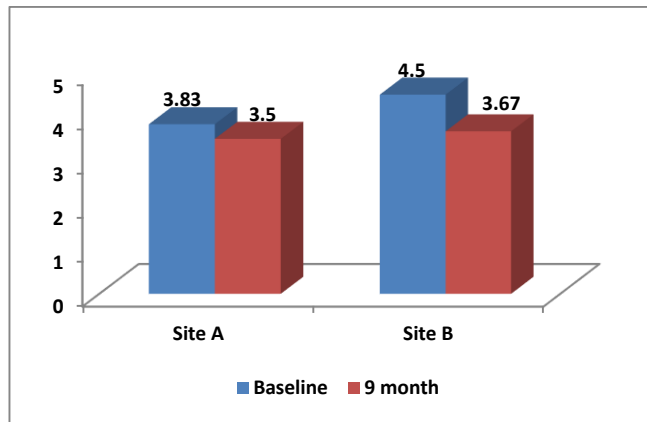


Figure 5. Comparison of mean CEJ to alveolar crest distance

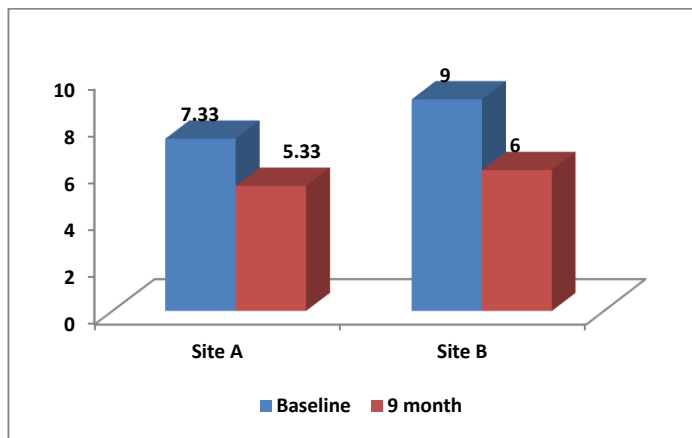


Figure 6. Comparison of Mean CEJ to Base of the defect distance

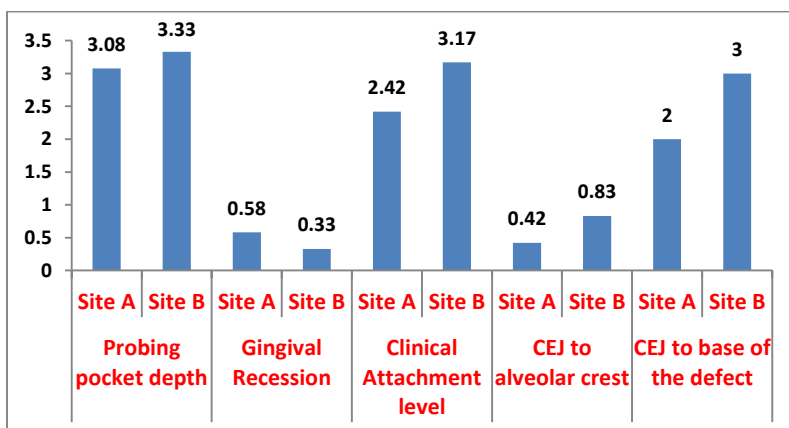


Figure 7. Comparison of mean change (Baseline -9 months) between the sites

Discussion

The results of the study showed that the mean probing depth in the control site at baseline was 7.33 ± 1.56 mm, which was reduced to 4.33 ± 1.07 mm at the end of 9 months. At the experimental site, the mean probing depth at baseline was 8.00 ± 1.41 mm which was reduced to 4.58 ± 1.44 mm at the end of 9 months, indicating that there was a marked reduction in probing pocket depth in both control and experimental sites from baseline to 9 months. The results of our study at control sites compare favourably with earlier studies of DFDBA (Bowen et al., 1989; Reynolds et al., 2003). At the experimental site comparison is made to other studies of PRF combined with bovine porous bone mineral or other alloplasts (Döri et al., 2008; Aroca et al., 2009), as there are no studies on combination of DFDBA and PRF.

When we compared the decrease in probing pocket depth between Site A and Site B, it was statistically not significant. The results of our study indicate that the mean clinical attachment level in experimental group at baseline 7.83 ± 1.34 mm, which reduced to 4.67 ± 1.23 mm respectively at 9 months. In a study they have shown the gain of 3.3 – 3.4 mm post surgery (Sharma & Pradeep, 2011; Okuda et al., 2005). At the control site, the mean clinical attachment level at baseline was 7.08 ± 1.62 mm which was reduced to 4.67 ± 1.50 mm at 9 months. In a study they have shown a gain of 2 – 3 mm (Parashis et al., 1998). This suggests that there is a statistically significant attachment gain from baseline to 9 months in both control and experimental sites.

The gain in attachment could be due to gain in new attachment, healing by long junctional epithelium or change in soft tissues post operatively. The comparison between the groups was found to be statistically insignificant, indicating that all the treatment modalities showed increased gain in clinical attachment level. Evaluation of soft tissue parameters at the end of 9 months indicated that addition of PRF did not significantly improve the clinical outcome. There were no statistically significant difference between the two groups as regards to periodontal probing pocket depth and clinical attachment gain, though test sites appeared to show greater improvement over time. This suggests that had the sample size been larger, the difference could well have been demonstrably significant (Cochran & Wozney, 1999; Tozum & Demiralp, 2003; Laurell et al., 1998; Raja & Naidu, 2008; Sharma & Pradeep, 2011; Mazor et al., 2009; Camargo et al., 2009; Hanna et al., 2004).

A 1mm marking grid was used to study the radiographs. Crestal bone resorption is a characteristic feature after the flap procedure. However, alloplasts are reported to have decreased the amount of resorption though not totally prevented or regenerated the crestal bone (Reynolds et al., 2003). Most of the alveolar bone changes following regenerative therapy of intrabony defects occur in the intrabony component while crestal resorption may be minimal or may not occur at all (Machtei, 1997). Our study showed no significant changes in the level of the crest in both experimental

The mean radiographic defect fill in experimental group at baseline is 9.00 ± 3.13 at the end of 9 months was 3.00 ± 2.22 which was highly statistically significant.

The mean radiographic defect fill in control group at baseline was 7.33 ± 1.87 at the end of 9 months was 5.33 ± 2.31 this was in accordance with few studies (Bowen et al., 1989). A marked reduction in the radiographic defect depth was noted at both the experimental and control sites and the difference were statistically significant from baseline to 9 months. Comparison across the groups also showed the results to be statistically significant at the end of 9 months.

Significant improvement in clinical and radiological parameters both at control and experimental sites may be attributed to the physical characteristic of the materials used. Combination of PRF and demineralised freeze dried bone allograft demonstrated more favourable radiographic results compared to demineralised freeze dried bone allograft. The precise role played by PRF in the defect fill is difficult to determine but may be explained on the basis of tissue engineering. Tissue engineering combines three key elements for regeneration i) scaffolds or matrix ii) signaling molecules iii) cells. By combining these elements under appropriate biological and environmental conditions tissue regeneration will become more predictable. The PDGF and TGF's in PRF may work in promoting the growth and differentiation of periodontal and alveolar bone cells rapidly in the experimental sites. In an vitro study it was suggested PDGF acts mostly on osteoblastic cell proliferation exerting most of its effect during early phases of wound healing, whereas TGF- β plays a role in osteoblast and cementoblast differentiation (Strayhorn, 1999).

Within the limits of the present study, it can be concluded that the combination of demineralised freeze dried bone allograft and platelet-rich fibrin though effective in improving the radiologic parameters did not enhance the clinical outcome of the therapy compared to the demineralised freeze dried bone allograft alone. However, long-term clinical trials with large samples along with histological examination are needed to evaluate the regenerative potential of this combination. Further studies using PRF are necessary to examine the individual role played by PRF in achieving such results (Lekovic et al., 2012; Lekovic et al., 2002; Li et al., 2000; Froum et al., 2002; Hall et al., 1999; Barnett et al., 1989).

Conclusion

- Clinical evaluation is suggestive of the fact that both DFDBA and PRF are biocompatible with the tissues. There was a definite improvement in the clinical parameters (Plaque index , gingival index, probing pocket depth, clinical attachment level,) radiographic parameters (alveolar crest resorption and defect fills) in the two groups, from the baseline.
- Though each group showed improvement in clinical parameters, while comparing the results between the two groups there were no statistically significant difference between them.
- Histologic examination would be the best method to draw conclusive evidence on regeneration with the bone graft materials.

Thus it can be concluded that the two modalities of treatment were efficient in improving the clinical parameters as well as brought about comparable regenerative effects when used in the treatment of human periodontal infrabony defects. However, a long-term, multicenter randomized controlled clinical trial is

needed to determine the clinical and radiographic effects of PRF on bone regeneration.

References

- Aroca, S., Keglevich, T., Barbieri, B., Gera, I., & Etienne, D. (2009). Clinical evaluation of a modified coronally advanced flap alone or in combination with a platelet-rich fibrin membrane for the treatment of adjacent multiple gingival recessions: A 6-month study. *Journal of periodontology*, *80*(2), 244-252.
- Barnett, J. D., Mellonig, J. T., Gray, J. L., & Towle, H. J. (1989). Comparison of Freeze-Dried Bone Allograft and Porous Hydroxylapatite in Human Periodontal Defects. *Journal of Periodontology*, *60*(5), 231-237.
- Bartold, P. M., Mcculloch, C. A., Narayanan, A. S., & Pitaru, S. (2000). Tissue engineering: a new paradigm for periodontal regeneration based on molecular and cell biology. *Periodontology 2000*, *24*(1), 253-269.
- Bowen, J. A., Mellonig, J. T., Gray, J. L., & Towle, H. T. (1989). Comparison of decalcified freeze-dried bone allograft and porous particulate hydroxyapatite in human periodontal osseous defects. *Journal of periodontology*, *60*(12), 647-654.
- Bowen, J. A., Mellonig, J. T., Gray, J. L., & Towle, H. T. (1989). Comparison of decalcified freeze-dried bone allograft and porous particulate hydroxyapatite in human periodontal osseous defects. *Journal of periodontology*, *60*(12), 647-654.
- Brunsvold, M. A., & Mellonig, J. T. (1993). Bone grafts and periodontal regeneration. *Periodontology 2000*, *1*(1), 80-91.
- Camargo, P. M., Lekovic, V., Weinlaender, M., Divnic-Resnik, T., Pavlovic, M., & Kenney, E. B. (2009). A surgical reentry study on the influence of platelet-rich plasma in enhancing the regenerative effects of bovine porous bone mineral and guided tissue regeneration in the treatment of intrabony defects in humans. *Journal of periodontology*, *80*(6), 915-923.
- Choukroun, J., Diss, A., Simonpieri, A., Girard, M. O., Schoeffler, C., Dohan, S. L., ... & Dohan, D. M. (2006). Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part V: histologic evaluations of PRF effects on bone allograft maturation in sinus lift. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, *101*(3), 299-303.
- Cochran, D. L., & Wozney, J. M. (1999). Biological mediators for periodontal regeneration. *Periodontology 2000*, *19*, 40-58.
- Dohan Ehrenfest, D. M., Del Corso, M., Diss, A., Mouhyi, J., & Charrier, J. B. (2010). Three-dimensional architecture and cell composition of a Choukroun's platelet-rich fibrin clot and membrane. *Journal of periodontology*, *81*(4), 546-555.
- Döri, F., Huszar, T., Nikolidakis, D., Tihanyi, D., Horvath, A., Arweiler, N. B., ... & Sculean, A. (2008). Effect of platelet-rich plasma on the healing of intrabony defects treated with beta tricalcium phosphate and expanded polytetrafluoroethylene membranes. *Journal of Periodontology*, *79*(4), 660-669.
- Froum, S., Cho, S. C., Rosenberg, E., Rohrer, M., & Tarnow, D. (2002). Histological comparison of healing extraction sockets implanted with bioactive glass or demineralized freeze-dried bone allograft: A pilot study. *Journal of periodontology*, *73*(1), 94-102.

- Gurinsky, B. S., Mills, M. P., & Mellonig, J. T. (2004). Clinical evaluation of demineralized freeze-dried bone allograft and enamel matrix derivative versus enamel matrix derivative alone for the treatment of periodontal osseous defects in humans. *Journal of periodontology*, 75(10), 1309-1318.
- Hall, E. E., Meffert, R. M., Hermann, J. S., Mellonig, J. T., & Cochran, D. L. (1999). Comparison of bioactive glass to demineralized freeze-dried bone allograft in the treatment of intrabony defects around implants in the canine mandible. *Journal of Periodontology*, 70(5), 526-535.
- Hanna, R., Trejo, P. M., & Weltman, R. L. (2004). Treatment of intrabony defects with bovine-derived xenograft alone and in combination with platelet-rich plasma: A randomized clinical trial. *Journal of periodontology*, 75(12), 1668-1677.
- He, L., Lin, Y., Hu, X., Zhang, Y., & Wu, H. (2009). A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblasts in vitro. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, 108(5), 707-713.
- Hoidal, M. J., Grimard, B. A., Mills, M. P., Schoolfield, J. D., Mellonig, J. T., & Mealey, B. L. (2008). Clinical evaluation of demineralized freeze-dried bone allograft with and without enamel matrix derivative for the treatment of periodontal osseous defects in humans. *Journal of periodontology*, 79(12), 2273-2280.
- Holtzclaw, D., Toscano, N., Eisenlohr, L., & Callan, D. (2008). The safety of bone allografts used in dentistry: a review. *The Journal of the American Dental Association*, 139(9), 1192-1199.
<https://doi.org/10.14219/jada.archive.2008.0334>
- Lang, N. P. (2000). Focus on intrabony defects—conservative therapy. *Periodontology 2000*, 22(1), 51-58.
- Laurell, L., Gottlow, J., Zybutz, M., & Persson, R. (1998). Treatment of intrabony defects by different surgical procedures. A literature review. *Journal of periodontology*, 69(3), 303-313.
- Lekovic, V., Camargo, P. M., Weinlaender, M., Vasilic, N., & Kenney, E. B. (2002). Comparison of platelet-rich plasma, bovine porous bone mineral, and guided tissue regeneration versus platelet-rich plasma and bovine porous bone mineral in the treatment of intrabony defects: A reentry study. *Journal of periodontology*, 73(2), 198-205.
- Lekovic, V., Milinkovic, I., Aleksic, Z., Jankovic, S., Stankovic, P., Kenney, E. B., & Camargo, P. M. (2012). Platelet-rich fibrin and bovine porous bone mineral vs. platelet-rich fibrin in the treatment of intrabony periodontal defects. *Journal of periodontal research*, 47(4), 409-417.
- Li, H., Pujic, Z., Xiao, Y., & Artold, P. M. (2000). Identification of bone morphogenetic proteins 2 and 4 in commercial demineralized freeze-dried bone allograft preparations: pilot study. *Clinical Implant Dentistry and Related Research*, 2(2), 110-117.
- Loe, H. A. R. A. L. D., & Silness, J. O. H. N. (1964). Periodontal disease in pregnancy. I. *Prevalence and severity Acta Odontologica Scandinavica* 1963; 21: 533, 551.
- Lovell, T. B., Mellonig, J. T., Meffert, R. M., Jones, A. A., Nummikoski, P. V., & Cochran, D. L. (1998). Clinical evaluation of bioactive glass in the treatment of periodontal osseous defects in humans. *Journal of periodontology*, 69(9), 1027-1035.

- Lucarelli, E., Beretta, R., Dozza, B., Tazzari, P. L., O'Connell, S. M., Ricci, F., ... & Donati, D. (2010). A recently developed bifacial platelet-rich fibrin matrix. *Eur Cell Mater*, 20(1), 13-23.
- Machtei, E. E. (1997). Outcome variables for the study of periodontal regeneration. *Annals of Periodontology*, 2(1), 229-239.
- Masters, L. B., Mellonig, J. T., Brunsvold, M. A., & Nummikoski, P. V. (1996). A clinical evaluation of demineralized freeze-dried bone allograft in combination with tetracycline in the treatment of periodontal osseous defects. *Journal of periodontology*, 67(8), 770-781.
- Masters, L. B., Mellonig, J. T., Brunsvold, M. A., & Nummikoski, P. V. (1996). A clinical evaluation of demineralized freeze-dried bone allograft in combination with tetracycline in the treatment of periodontal osseous defects. *Journal of periodontology*, 67(8), 770-781.
- Mazor, Z., Horowitz, R. A., Del Corso, M., Prasad, H. S., Rohrer, M. D., & Dohan Ehrenfest, D. M. (2009). Sinus floor augmentation with simultaneous implant placement using Choukroun's platelet-rich fibrin as the sole grafting material: a radiologic and histologic study at 6 months. *Journal of periodontology*, 80(12), 2056-2064.
- Meadows, C. L., Gher, M. E., Quintero, G., & Lafferty, T. A. (1993). A comparison of polylactic acid granules and decalcified freeze-dried bone allograft in human periodontal osseous defects. *Journal of periodontology*, 64(2), 103-109.
- Mellado, J. R., Salkin, L. M., Freedman, A. L., & Stein, M. D. (1995). A comparative study of ePTFE periodontal membranes with and without decalcified freeze-dried bone allografts for the regeneration of interproximal intraosseous defects. *Journal of Periodontology*, 66(9), 751-755.
- Mellonig, J. T. (1991). Freeze-dried bone allografts in periodontal reconstructive surgery. *Dental Clinics of North America*, 35(3), 505-520.
- Nevins, M., Camelo, M., Nevins, M. L., Schenk, R. K., & Lynch, S. E. (2003). Periodontal regeneration in humans using recombinant human platelet-derived growth factor-BB (rhPDGF-BB) and allogenic bone. *Journal of periodontology*, 74(9), 1282-1292.
- Okuda, K., Tai, H., Tanabe, K., Suzuki, H., Sato, T., Kawase, T., ... & Yoshiex, H. (2005). Platelet-rich plasma combined with a porous hydroxyapatite graft for the treatment of intrabony periodontal defects in humans: A comparative controlled clinical study. *Journal of periodontology*, 76(6), 890-898.
- Papapanou, P. N., & Tonetti, M. S. (2000). Diagnosis and epidemiology of periodontal osseous lesions. *Periodontology 2000*, 22(1), 8-21.
- Parashis, A., Andronikaki-Faldami, A., & Tsiklakis, K. (1998). Comparison of 2 Regenerative Procedures—Guided Tissue Regeneration and Demineralized Freeze-Dried Bone Allograft—in the Treatment of Intrabony Defects: A Clinical and Radiographic Study. *Journal of periodontology*, 69(7), 751-758.
- Parashis, A., Andronikaki-Faldami, A., & Tsiklakis, K. (1998). Comparison of 2 Regenerative Procedures—Guided Tissue Regeneration and Demineralized Freeze-Dried Bone Allograft—in the Treatment of Intrabony Defects: A Clinical and Radiographic Study. *Journal of periodontology*, 69(7), 751-758.
- Persson, G. R., Falk, H., & Laurell, L. (2000). A retrospective radiographic outcome assessment study of intra-bony defects treated by osseous surgery or by bone graft procedures. *Journal of Clinical Periodontology*, 27(2), 104-108.

- Quintero, G., Mellonig, J. T., Gambill, V. M., & Pelleu Jr, G. B. (1982). A six-month clinical evaluation of decalcified freeze-dried bone allografts in periodontal osseous defects. *Journal of Periodontology*, 53(12), 726-730.
- Raja, V. S., & Naidu, E. M. (2008). Platelet-rich fibrin: evolution of a second-generation platelet concentrate. *Indian Journal of Dental Research*, 19(1), 42.
- Reynolds, M. A., Aichelmann-Reidy, M. E., Branch-Mays, G. L., & Gunsolley, J. C. (2003). The efficacy of bone replacement grafts in the treatment of periodontal osseous defects. A systematic review. *Annals of periodontology*, 8(1), 227-265.
- Rosen, P. S., Reynolds, M. A., & Bowers, G. M. (2000). The treatment of intrabony defects with bone grafts. *Periodontology 2000*, 22, 88-103.
- Rummelhart, J. M., Mellonig, J. T., Gray, J. L., & Towle, H. J. (1989). A comparison of freeze-dried bone Allograft and demineralized freeze-dried bone allograft in human periodontal osseous defects. *Journal of periodontology*, 60(12), 655-663.
- Scannapieco, F. A. (2005). Systemic effects of periodontal diseases. *Dental Clinics*, 49(3), 533-550.
- Schwartz, Z., Mellonig, J. T., Carnes Jr, D. L., De La Fontaine, J., Cochran, D. L., Dean, D. D., & Boyan, B. D. (1996). Ability of commercial demineralized freeze-dried bone allograft to induce new bone formation. *Journal of periodontology*, 67(9), 918-926.
- Sharma, A., & Pradeep, A. R. (2011). Autologous platelet-rich fibrin in the treatment of mandibular degree II furcation defects: A randomized clinical trial. *Journal of periodontology*, 82(10), 1396-1403.
- Sharma, A., & Pradeep, A. R. (2011). Treatment of 3-wall intrabony defects in patients with chronic periodontitis with autologous platelet-rich fibrin: a randomized controlled clinical trial. *Journal of periodontology*, 82(12), 1705-1712.
- Shigeyama, Y., D'Errico, J. A., Stone, R., & Somerman, M. J. (1995). Commercially-prepared allograft material has biological activity in vitro. *Journal of periodontology*, 66(6), 478-487.
- Silness, J., & Løe, H. (1964). Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. *Acta odontologica scandinavica*, 22(1), 121-135.
- Strayhorn, C. L., Garrett, J. S., Dunn, R. L., Benedict, J. J., & Somerman, M. J. (1999). Growth factors regulate expression of osteoblast-associated genes. *Journal of periodontology*, 70(11), 1345-1354.
- Toffler, M., Toscano, N., Holtzclaw, D., Corso, M. D., & Ehrenfest, D. D. (2009). Introducing Choukroun's platelet rich fibrin (PRF) to the reconstructive surgery milieu. *J Implant Adv Clin Dent*, 1(6), 21-30.
- Tozum, T. F., & Demiralp, B. (2003). Platelet-rich plasma: a promising innovation in dentistry. *Journal-Canadian Dental Association*, 69(10), 664-665.
- Yukna, R. A. (1994). Clinical evaluation of coralline calcium carbonate as a bone replacement graft material in human periodontal osseous defects. *Journal of periodontology*, 65(2), 177-185.
- Zamet, J. S., Darbar, U. R., Griffiths, G. S., Bulman, J. S., Brägger, U., Bürgin, W., & Newman, H. N. (1997). Particulate bioglass® as a grafting material in the treatment of periodontal intrabony defects. *Journal of clinical periodontology*, 24(6), 410-418.

Zhang, M., Powers Jr, R. M., & Wolfinbarger Jr, L. (1997). Effect (s) of the demineralization process on the osteoinductivity of demineralized bone matrix. *Journal of periodontology*, 68(11), 1085-1092.

Zhang, M., Powers Jr, R. M., & Wolfinbarger Jr, L. (1997). Effect (s) of the demineralization process on the osteoinductivity of demineralized bone matrix. *Journal of periodontology*, 68(11), 1085-1092.

Queries

- This articles u mentioned was used to understand the concept of periodontal regeneration and about the growth factors
- With reference to your query a specific registration was not provided. However the ethics committee duly scrutinized the study protocol and issued ethics approval with the suggestions as mentioned in the approval letter. Iam here by adjoining the letter provided by the ethics committee
- Reference for the prf protocol has been added.
- L PRF used for the study
- At the time of study the use of paralleling cone techniques was more suitable and the cbct was not readily available