



Comparative Evaluation Of Concentrated Growth Factor With Bioactive Glass And Collagen Membrane In The Treatment Of Horizontal Osseous Defects With Open Flap Debridement

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Abstract

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Introduction

Periodontitis is defined as an infectious disease resulting in inflammation within the supporting tissues that is characterized by pocket formation and/or gingival recession or both.¹ In accordance with the morphology of bone loss, the osseous defects in periodontitis can be of horizontal, vertical, combined (horizontal and vertical) and furcation defects. Among these, the horizontal defect has a high prevalence rate of 92.2% but is receiving treatment as less as 3.2% only. This refocuses our attention to the area of management of horizontal defect-*the* periodontal orphan.

Periodontal regeneration is defined as the reproduction /reconstruction of lost or injured part in such a way that the architecture and function of lost or injured tissue is completely restored. The prevention of epithelial and connective tissue cells migration from the wound lead to the development and application of GTR membranes. Ossix plus is a resorbable, natural barrier membrane, composed of porcine type1 collagen with ribose sugar crosslinking, with resorption period of 4-6 months.

The alloplastic bone graft material - bioactive glass comes into contact with the tissue fluids, a double layer of silica gel and calcium phosphate is formed through a surface reaction. This layer incorporates

organic ground proteins such as chondroitin sulphate and glycosaminoglycan, which acts as a chemotaxis factor for osteoblast cells and thus leads bone formation. Growth factors are biologic mediators that can regulate the proliferation, differentiation and chemotaxis of the locally derived progenitor cells to the defect site. Concentrated Growth Factor (CGF) an advanced second-generation platelet concentrate, developed by Sacco, has higher adhesive strength, tensile strength, and higher viscosity.

The utilization of bone grafts, barrier membranes, and the biologic mediators are helpful in attaining a good success rate in the regenerative surgeries. The present study evaluated the clinical, radiographic efficacy of Concentrated Growth Factor and bioactive glass nova bone putty with Ossix plus collagen membrane in the treatment of horizontal osseous defects.

Materials And Methods:

The study population were selected from the outpatient sections of the Department of Periodontics, Tamilnadu Government Dental College and Hospital, Chennai, India Ethical clearance was obtained from Institutional Ethical Committee and principles were meticulously followed throughout the course of the

study. In this clinical study the participants were recruited prospectively and selected randomly if they satisfy the inclusion criteria, with no discrimination on the basis of sex, caste, religion or socioeconomic status. After explaining the study procedure, written informed consent obtained from all the subjects selected for the study. All the subjects underwent full mouth periodontal probing and charting and radiographic evaluation.

Patients within the age group 20 - 50 years of either gender, willing for voluntary participation & have signed informed consent, patients with stage III periodontitis with probing depth ≥ 5 mm following Phase-I therapy, radiographic evidence of horizontal bone loss up to or less than the middle third of the root, systemically healthy patients are included. Patients showing poor oral hygiene maintenance after Phase-I therapy, patients with known systemic diseases/ metabolic disorders, under any medication, patients using tobacco or tobacco related products, pregnant / lactating women, and patients with known allergies were excluded.

A total of 20 sites diagnosed as stage III periodontitis with clinical and radiographic evidence of horizontal bone loss up to or less than middle third of the root, indicated for regenerative periodontal surgery were randomly assigned into group A and group B Group A with patients receiving open flap debridement (OFD) and intra-marrow penetration (IMP) only, and Group B with patients receiving open flap debridement and intra marrow penetration and, Novabone putty with concentrated growth factor and bioresorbable porcine based collagen membrane to support the graft.

Clinical parameters like Plaque index, Gingival bleeding index, Probing pocket depth in mm (PPD) and Clinical attachment level in mm (CAL) were evaluated at baseline, 3 months and 6 months post surgically. Acrylic occlusal stents were fabricated over the study models. Self-cure acrylic was used for this purpose. The stent covered the occlusal and coronal 1/3rd of the labial and lingual surfaces of the teeth. Vertical grooves were made to guide the placement of the probe in the same plane and direction repeatedly during measurements to avoid any variation. The recordings were made using a UNC 15 periodontal probe.

Intraoral periapical radiographs were taken with radiographic grid for each site using long cone paralleling technique and XCP holders at baseline and post surgically at 6 months. All radiographs were digitalized using digital camera² and transferred to the computer as JPEG image. To measure the linear radiographic defect depth (DD) in millimetre (mm), **Image J** software designed for image analysis by **National Institute of Health (NIH)**^{3,4,5} was used. The following linear measurements such as Defect Depth (CEJ to bottom of the defect) , CEJ to root apex^{6,7} Correction factor to estimate distortion between the consequent radiographs {CEJ to RA (baseline) \div CEJ to RA (post-op) = Correction Factor }⁸, Defect fill (DF) [CEJ to BD (baseline)] - [CEJ to BD (post op) x CF] and Defect fill percentage (DF %) {[defect fill/defect depth (baseline)] x 100 } were recorded and subjected to statistical analysis.

Presurgical procedures comprises of clinical case history record and clinical photographs of the site of interest, clinical probing pocket depth assessed with customized acrylic stents to help reproducibility of probe placement, intraoral periapical radiographs using long cone paralleling technique and radiographic grid, phase I therapy which includes oral hygiene instructions, scaling and root planing using hand and ultrasonic instruments, were performed. Adjunctive chemical plaque control in the form of chlorhexidine mouthwash 0.12% twice daily was advised and after 3-4 weeks of phase 1 therapy, re-evaluation was performed. The subjects showing acceptable oral hygiene were selected for the study and informed consents were obtained from the patients. After correlating with radiographic findings surgical procedure was planned. The patients were randomly assigned into group A or B with routine haematological investigations.

Intra-oral and extra-oral antiseptics were performed with 0.2% chlorhexidine digluconate rinse and 5% povidone iodine solution respectively. The surgical site was anaesthetized with 2% Lignocaine HCl with adrenaline (1:80,000) using block or infiltration techniques. Then crevicular incisions were made using Bard Parker blade No.15/15C on the facial and lingual/palatal surfaces, extending to one tooth on either side of the defect. A full thickness mucoperiosteal flap was reflected using the periosteal elevator. After flap reflection and exposure of the osseous defect, a thorough debridement of soft and

hard tissue was done using the area specific Gracey curettes. Debridement was followed by copious irrigation with 0.9% normal saline. In addition, Intra Marrow Penetration (IMP) was performed with micro motor hand piece and round bur with copious irrigation in both groups. In Group B following the debridement and IMP, Nova bone Putty bone graft in combination with CGF. CGF was prepared when 9 ml of blood was drawn from the cubital fossa of the patient in sterile test tubes without anticoagulant solutions^{9,10,11}. These tubes were immediately centrifuged using a program with the following characteristics: 30 seconds acceleration, 2400 - 2700 rpm for 12 minutes and 36 seconds deceleration and stop. At the end of the process, three blood fractions were created in which the fibrin block (interim phase) and RBC layer (lower phase) beneath it was cut into pieces of 1~2 mm and mixed with Nova Bone Putty at a relative volume of 1:1 and homogenized. The mixture was then placed into the osseous defect with light pressure in the decorticated area as well as at the level of defect. A biodegradable porcine based collagen membrane (Ossix Plus) was placed to support the graft from displacement. The mucoperiosteal flaps repositioned and secured using 3-0 black braided silk sutures. Periodontal dressing (Coe-pac™) was placed. Post-operative instructions were given and patients were prescribed systemic antibiotics (Amoxicillin 500mg thrice daily, Metronidazole 400mg twice daily) and analgesics (Ibuprofen 400mg thrice daily) for 1 week. Next day, the patients were re-evaluated for any acute signs of inflammation or infection. Periodontal dressings and sutures were removed 14 days after surgery. Post-surgical evaluation and review was done at 3rd and 6th month in which Plaque Index, Gingival Bleeding Index, Probing Pocket Depth, Clinical Attachment Levels were reassessed. Intraoral periapical radiographs were taken using long cone paralleling technique, with grid in position at 6 months to evaluate the post treatment bone fill.

Data analysis:

Data were analysed by IBM SPSS version 20.0. Quantitative data was represented as Mean and SD (PI, GI, PPD, CAL, DD, DF). **Student's t test/Independent samples t test** were used for inter-group comparison (Difference in clinical parameters between 2 study groups). **Repeated Measures ANOVA** was used for Intra-group comparison (for

assessment of significant change in clinical parameters over study period). Throughout the study a P value of <0.05 was considered as statistically significant difference

Results:

The present study was conducted to evaluate clinically and radiographically the effectiveness of bioactive glass Novabone putty combined with autologous concentrated growth factor in the treatment of horizontal bony defect. A total of 20 sites with radiographic horizontal bone loss from patients of aged 20-50 years were enrolled for the study. The final results and statistical analysis were done for 20 sites, 10 sites in each of the groups. Plaque index, Gingival bleeding index and Probing pocket depth showed no difference in mean scores ($p=0.82$, $p=0.61$, $p=0.56$), whereas Clinical attachment level was statistically significant ($p=0.033$, $p=0.034$) at baseline, 3rd and 6th month when compared between the groups.

Intragroup comparison in group B shows substantial decrease in plaque score, which was statistically significant ($p=0.001$). In group A, the reduction in gingival bleeding index score from baseline to 3rd month was 46.09 ± 3.37 and from 3rd month to 6 months was 26.3 ± 2.10 and in group B the reduction from baseline to 3rd month was 44.17 ± 2.59 and from 3rd month to 6th month was 29.14 ± 2.10 and which were statistically significant ($p=0.001$).

The probing pocket depth reduction from baseline to 3rd month was 2.5 ± 0.29 and from 3rd month to 6 months was 1 ± 0.26 in group A and was 3.72 ± 0.31 from baseline to 3rd month and from 3rd month to 6 months was 0.98 ± 0.32 in group B. The reduction in score from baseline to 6 months was statistically significant ($p=.001$) when compared within the groups. The comparison of PPD scores between group A and group B at baseline showed no difference ($p=0.47$) while at 3rd month and 6th month there was a statistically significant difference among the groups. ($p=0.041$, $p=0.047$)

The clinical attachment levels in Group A was 4.60 ± 0.69 , 2.40 ± 0.99 and 1.57 ± 0.31 at baseline, 3rd month and 6th month respectively. The mean reduction in clinical attachment levels from baseline to 3rd month was 2.2 ± 0.29 and from 3rd month to 6 months was 0.83 ± 0.26 . In group B mean CAL at

baseline was 6.14 ± 2.9 and at 3 months was 1.72 ± 0.63 and at 6 months was 0.97 ± 0.41 . The reduction in clinical attachment levels from baseline to 3rd month was 4.41 ± 0.31 and from 3rd month to 6 months was 0.32 ± 0.35 . The reduction in score from baseline to 6 months was statistically significant ($p=0.001$). The comparison of CAL scores between group A and group B at baseline demonstrated no difference ($p=0.13$) while at 3rd month and 6th month there

Radiographic parameters showed the mean defect depth reduction from baseline to 6th month was 0.33 ± 0.13 in group A and a statistically significant reduction in defect depth from baseline to 6th month which was 2.42 ± 0.20 ($p=0.001$) between the groups. The mean defect depth comparison at baseline between group A and B showed no statistical difference in values but the 6th month evaluation showed statistically significant reduction in defect depth in group B compared to group A ($p=0.032$). The mean DF (defect fill) within the groups was 0.34 ± 0.14 in group A at 6th month and in Group B: at 6th month the defect fill was 1.40 ± 0.17 . The mean defect fill compared between group A and B at 6th month exhibited statistically significant ($p=0.001$) defect fill in group B than in group A. The mean DF % (defect fill percentage) at 6th month was 10.42 ± 4.48 in group A and was 33.97 ± 11.32 in group B. The mean defect fill percentage was higher in group B when compared to group A and it was statistically significant ($p=0.001$).

Discussion

The treatment modalities for each of the patients will differ according to the nature of the disease and defects present, and the therapeutic objectives which the clinician aimed at. Generally, the sequel of management follows from nonsurgical to surgical therapy, of which scaling and root planing being the gold standard of treatment. The objectives of bone grafting techniques gave by **Schallhorn 1977**¹², includes - Probing Pocket depth reduction/elimination, reinstatement of lost alveolar bone and restoration of a functional attachment apparatus.

Bioactive synthetic bone grafts (alloplastic) are one among the bone graft material available for regenerative surgeries. Nova Bone Dental Putty is an osteoconductive, bioactive bone graft material

composed of a calcium-phosphorus sodium-silicate (active component) particulate combined with poly ethyl glycol which performs the function of temporary binding agent for the particulate. On grafting, tissue infiltration take place between the Bioglass particles by the absorption property of the binder. In the healing process the particles of the graft are replaced by new bone tissue by a slow absorption process.

For enhanced bone regeneration bone graft materials can be combined with autologous biologic mediators such as CGF, PRF etc. Compared to PRF, CGF has more strength because of the presence of fibrin network and also characterized by enriched release of growth factors^{13,14}, cytokines and antimicrobial properties. It also hastens wound healing and promotes osteogenesis¹⁵. Barrier membranes are used in regenerative surgeries to prevent the epithelial migration and to provide space for the graft materials¹⁶. Ossix plus is a resorbable collagen membrane with natural sugars cross linking and improved properties and an average resorption rate of 4-6 months. Ossix plus derived from type I porcine collagen introduced in 2006 by Datum Biotech.

Intramarrow penetration (IMP) or intentional drilling of holes through the cortical bone into the cancellous bone or the removal of cortical bone (decortication) to expose cancellous bone has a very significant role in periodontal regenerative surgical procedures. The enhanced bone regeneration in Intramarrow penetration can be attributed to (1) improved angiogenesis^{17,18} (2) local increase in growth factors like bone morphogenetic proteins, cytokines from the injured cortical area, endosteal surface and wounded vessels¹⁹ (3) Regional acceleratory phenomenon from trauma to the cortical area^{20,21,22,23}.

The present study was conducted to evaluate the efficacy of CGF, Nova bone putty bone graft with a resorbable collagen membrane Ossix plus in decorticated horizontal osseous defects compared with open flap debridement and Intramarrow penetration. The study period was uneventful with no adverse reactions suggesting the biocompatibility of CGF, Novabone putty, and Ossix plus membrane. Even though the occurrence of horizontal osseous defects are very high, due to the difficulties for regeneration, clinical studies in these defects are very minimal. The study showed significant defect fill and

defect depth reduction, clinical attachment gains and pocket depth reduction in test groups compared to control groups.

In the present study, on evaluation of clinical parameters, plaque index and gingival index at base line, 3 months and 6 months showed similar results in both the groups without significant changes and intragroup comparison of two groups demonstrated considerable reduction in values indicating good oral hygiene throughout the course of the study. At baseline, the mean pocket depths were 6.60 ± 0.70 and 7 ± 1.53 for group A and B respectively. This shows the cases included in the study were of equal severity. At 3rd month evaluation the mean values were 4.10 ± 0.70 and 3.29 ± 1.1 for group A and B correspondingly. The mean values at 6th month evaluation for group A was 3.10 ± 0.32 and for group B was 2.30 ± 0.01 . There was a significant reduction in probing pockets depths from base line to 3rd and to 6th month in both groups but intergroup comparison reveals a statistically significant reduction in group B at 3rd and 6th month evaluation.

The clinical attachment loss for group A at base line, 3rd month and 6th month were 4.60 ± 0.70 , 2.40 ± 1.0 , 1.57 ± 0.32 respectively. For Group B 6.14 at baseline, 1.73 ± 0.64 for 3rd month and for 6th month 0.97 ± 0.41 . The gain in clinical attachment levels were significant in both group A and B at the end of 3rd and 6th months. Nevertheless, intergroup assessment exhibited a significant gain in clinical attachment levels at 3rd and 6th months in group B patients. The radiographic evaluation at baseline and 6th month unveiled a noteworthy reduction in Defect Depth and improvement in Defect Fill (DF) and DF% in test group (group B). The mean Defect Depth (DD) at baseline was 3.44 ± 1.07 and 4.68 ± 2.11 for group A and B respectively. At 6th month evaluation mean Defect Depth for group A was 3.11 ± 1.01 and for group B was 2.25 ± 1.12 . The mean Defect Fill (DF) at the end of 6th month for group A was 0.34 ± 1.04 and in group B, 1.40 ± 1.07 . For group A, the mean Defect Fill Percentage (DF%) was 10.42 ± 4.48 and 33.97 ± 11.32 in group B.

The bioactive glass induces the proliferation and osteogenic differentiation of human osteoblasts and stimulates osteogenesis. Bioactive glass dissolution produces ionic products that have a direct influence on the gene-expression profile of human osteoblasts.

They induce genes which have known roles in processes pertinent to metabolism of osteoblast and bone homeostasis²⁴. The effectiveness of IMP histologically revealed significant increase in bone regeneration and higher percent area of mineralized bone which enforces the results acquired in group A and B in terms of DD, DF & DF% with the addition of IMP to this present study.

In the current study we made an attempt in the regeneration of the horizontal alveolar bone defect which has always been neglected because of the inadequate containment of the defect. The test group of the study (group B) treated with OFD, IMP and CGF with Bioactive glass bone graft and a resorbable collagen membrane (Ossix plus) for space provision. The study exhibited better outcomes in test group in relation to both clinical and radiographic parameters although intra group comparison demonstrated enhanced results from base line to 6th month of evaluation in both groups. The results may be accredited to the synergistic effects of CGF, Novabone putty bone graft material and IMP. However further long term studies with histological analysis and large sample sizes are required to confirm the regenerative potential of horizontal defect with the help of emerging technologies and efficient materials such as CGF, which can be a real solution for the long term headache of the clinicians.

Conclusion

The present study was carried out to evaluate the efficacy of CGF combined with Novabone putty bone graft with a resorbable collagen membrane in horizontal osseous defects which were decorticated. There are multitude of factors such as patient related factors, defect characteristics, treatment modalities, materials used and follow up which will influence the final outcome of any therapy. A total of 20 sites were selected for the study. The patients were randomly divided into Group A and Group B, 10 sites in each Group. Group A received OFD & IMP, whereas Group B received OFD, IMP, CGF, Nova Bone Putty bone graft with a resorbable collagen membrane. The clinical parameters evaluated at base line, 3rd month and 6th month and radiographic evaluation done at baseline and 6th months. During the period of the study CGF, Nova Bone Putty, and Ossix plus membrane were well borne by the periodontium without any foreign body reactions. The results

showed a definite improvement from baseline to 6th month in clinical and radiographic parameters in both the groups, radiographic parameters evaluated (DF&DF% and defect depth) have a statistically significant improvement at the end of 6th month in both the groups and finally, group B catered better results in CAL, PPD, DF&DF% which indicates the beneficial effect of combination of CGF and Nova Bone Putty in addition to OFD and IMP.

Hence, it is very crucial to be cautious at every step right from the patient selection till the maintenance phase for the success of the treatment. By this study, it can be concluded that the regeneration in horizontal defect is possible with the combined use of bioactive mediators like CGF and Nova bone graft. The addition of IMP to OFD is advantageous in regenerative procedures rather than performing OFD alone. Henceforth the results obtained from the present study can pave light into further research in the arena of horizontal defect restoration with development of new innovative materials and technologies.

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Figures

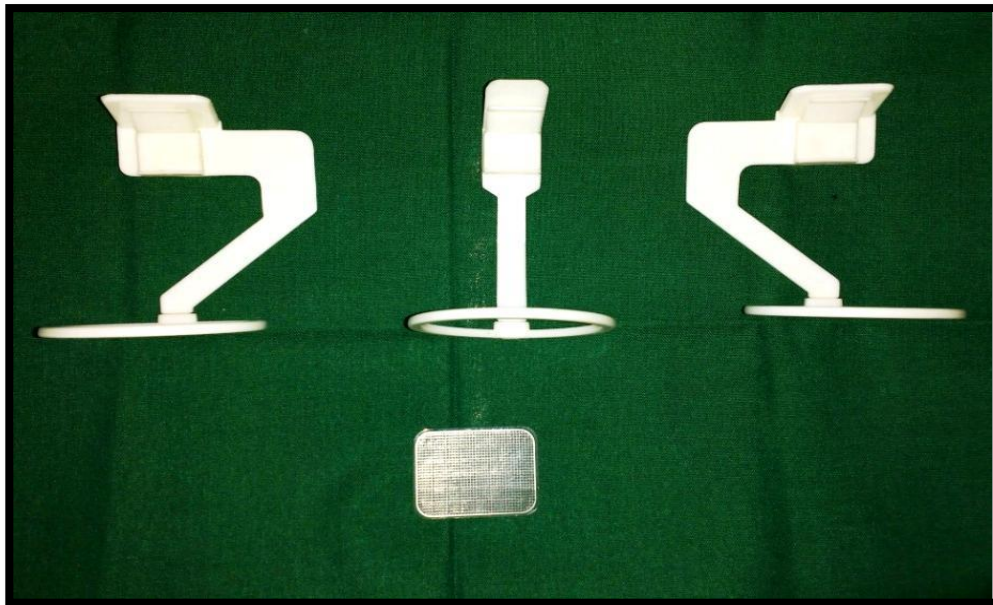


Figure 1: XCP Holders with Radiographic grid

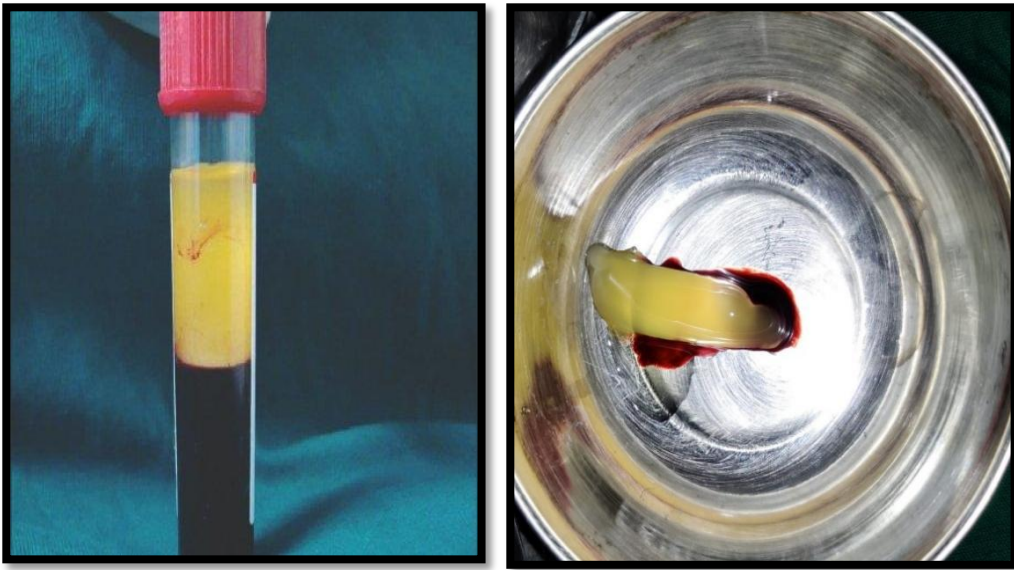


Figure 2 : CGF After Centrifugation

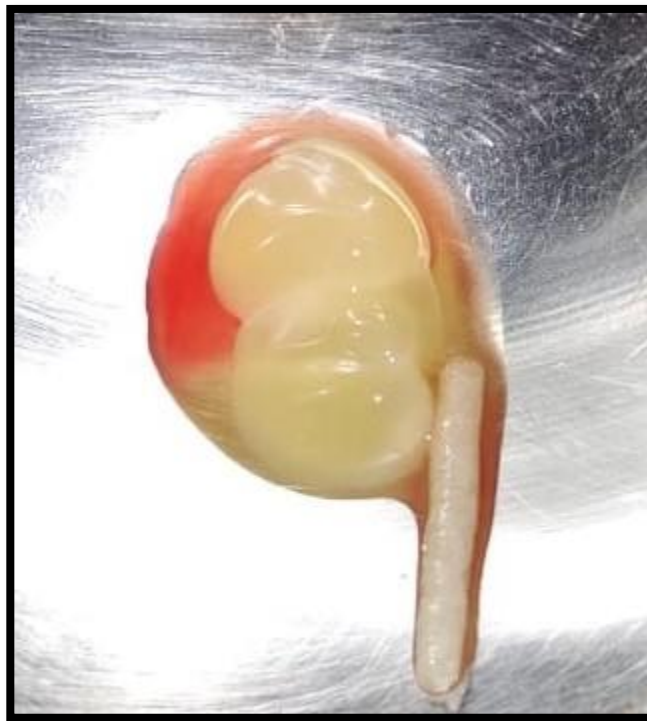


Figure 3 : CGF with Nova Bone Putty Bone Graft



Figure 4 : Group A - Pre-operative view



Figure 5 : Group A - Intra-operative view with Intra Marrow Penetration



Figure 5 - Group A- Suturing





Figure 6: Post-operative view after 3 and 6 months

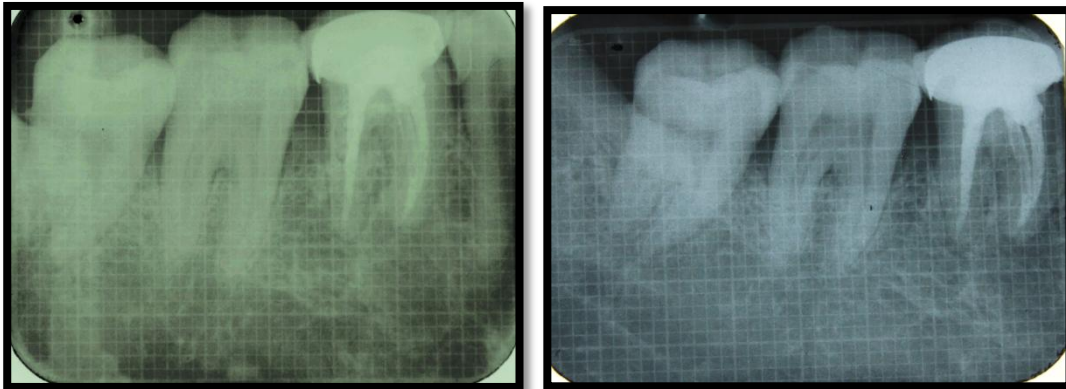


Figure 7: Radiographic view At Baseline and at 6 months

Tabular Columns

TABLE 1: INTRAGROUP COMPARISON OF CLINICAL PARAMETERS FOR GROUP A

GROUP A				
PARAMETERS	BSAELINE	3 RD MONTH	6 TH MONTH	P VALUE
	(MEAN±SD)	(MEAN±SD)	(MEAN±SD)	
PI	1.66±0.16	1.09±0.20	0.45±0.10	0.001*
GBI	85.01±7.04	38.92±6.63	12.62±2.80	0.001*
PPD	6.60±0.70	4.10± 0.70	3.10±0.32	0.001*
CAL	4.60±0.70	2.40±1.00	1.57±0.32	0.001*

TABLE 2: INTRAGROUP COMPARISON OF CLINICAL PARAMETERS FOR GROUP B

GROUP B				
PARAMETERS	BSAELINE	3 RD MONTH	6 TH MONTH	P VALUE
	(MEAN±SD)	(MEAN±SD)	(MEAN±SD)	
PI	1.63±0.28	1.03±0.28	0.50±0.24	0.006*
GBI	84.51±7.76	40.34±5.41	11.20±2.39	0.001*
PPD	7.00±1.53	3.29±1.11	2.30±0.01	0.001*
CAL	6.14±2.97	1.73±0.64	0.97±0.41	0.001*

***Statistically significant**

TABLE 3: INTERGROUP COMPARISON OF CLINICAL PARAMETERS

PARAMETERS		MEAN±SD		P VALUE
		GROUP A	GROUP B	
PI	Baseline	1.66±0.16	1.63±0.28	0.82
	3 rd Month	1.09±0.20	1.03±0.28	0.61
	6 th Month	0.45±0.10	0.50±0.24	0.56
GBI	Baseline	85.01±7.04	84.51±7.76	0.89
	3 rd Month	38.92±6.63	40.34±5.41	0.64
	6 th Month	12.62±2.80	11.20±2.39	0.29
PPD	Baseline	6.60±0.70	7.00±1.53	0.47
	3 rd Month	4.10± 0.70	3.29±1.11	0.041*
	6 th Month	3.10±0.32	2.30±0.01	0.047*
CAL	Baseline	4.60±0.70	6.14±2.97	0.13
	3 rd Month	2.40±1.00	1.73±0.64	0.033*
	6 th Month	1.57±0.32	0.97±0.41	0.034*

TABLE 4: COMPARISON OF DEFECT DEPTH- INTRA and INTERGROUP COMPARISON- GROUP A&B

	MEAN ±SD		P VALUE
	GROUP A	GROUP B	
BASELINE	3.44±1.07	4.68±2.11	0.13
6 MONTHS	3.11±1.01	2.25±1.12	0.032
P VALUE	0.001	0.001	

TABLE 5: INTERGROUP COMPARISON OF DF&DF%

PARAMETERS	MEAN \pm SD		P VALUE
	GROUP A	GROUP B	
DF	0.34 \pm 0.14	1.40 \pm 0.17	0.001*
DF%	10.42 \pm 4.48	33.97 \pm 11.32	0.001*

***Statistically significant**