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RESEARCH ARTICLE

CLINICAL AND RADIOGRAPHIC EVALUATION OF PERIODONTAL REGENERATION USING BASIC FIBROBLAST GROWTH FACTOR IN COMBINATION WITH AN ALLOPLAST OR A COLLAGEN SPONGE IN PERIODONTAL INTRAOSSEOUS DEFECTS

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ABSTRACT

Basic fibroblast growth factor (bFGF) have been discovered to induce strong angiogenic activity and proliferative capacity in undifferentiated mesenchymal cells. The purpose of this study was to evaluate the regenerative effect of bFGF with and without synthetic alloplastic bone graft (bioactive glass) in periodontal infrabony defects. **Methods and Material:** Following initial periodontal therapy, 16 systemically healthy patients in the age group 20-65 yrs., having bilateral intrabony defects, were randomly assigned to two treatment groups using a split mouth design i.e. Group A treated with bFGF and bone graft and Group B treated with bFGF with absorbable collagen sponge (ACS). All the clinical parameters and radiographic measurements were performed at baseline and at different time intervals following surgical therapy. **Results:** In both thegroups, all clinical and radiographic parameters statistically improved from baseline. There were statistically insignificant differences in the values of plaque and gingival indices of both groups, but reduction in probing pocket depth and attachment gain were statistically significantly higher in group A than in group B at 24th week from baseline. Gain in height of alveolar bone was statistically significantly higher in group A than in group B at all the time intervals **Conclusions:** Although, both interventions resulted in greater clinical and radiographic improvement at various follow up visits., but impregnation of bFGF onto a bioactive glass improved the efficacy of FGF-2, this combination has potential for clinical applications.

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INTRODUCTION

Periodontitis is an inflammatory disease characterized by destruction of the alveolar bone, root cementum, PDL, and gingiva as a response to insults elicited by microbial accumulations on tooth surfaces (Lee *et al*., 2010). Loss of alveolar bone is generally considered to represent the anatomical sequelae to apical spread of periodontitis. These alterations if left untreated will eventually lead to tooth loss (Cochran, 2008). The ultimate goal of periodontal therapy is the regeneration of periodontal tissues, which consists of stimulating new cementum formation, new alveolar bone apposition and a functionally-oriented periodontal ligament (PDL) reconstruction (Bashutski, 2011).

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Various methods of regenerative periodontal therapy, including the use of barrier membranes, bone replacement grafts (auto grafts, allografts, xenografts, alloplasts which further included Plaster of Paris, Polymers, Calcium carbonate, Ceramics, Resorbable,' I'ricalcium phosphate, Resorbable hydroxyapatite, Dense hydroxyapatite, Porous hydroxyapatite, Bioalass), growth factors and the combination of these procedures have been investigated. Growth factors also aid in regeneration by augmenting the wound healing process through anabolic bone formation, angiogenesis, cementogenesis, osteoblast differentiation, mitosis, chemotaxis, and other processes that improve the healing environment (Bashutski, 2011; Saygin *et al*., 2000). These factors include insulin-like growth factors (IGF-1), fibroblast growth factors (FGF), epidermal growth factor (EGF), platelet-derived growth factors (PDGF), vascular endothelial growth factor (VEGF), parathyroid hormone (PTH), transforming

growth factor-β (TGF- β) and bone morphogenetic proteins (BMP) (Caffess, 2000). Fibroblast growth factors (FGFs) are a family of structurally related polypeptides characterized by a high affinity to heparin, that signal through FGF receptors (FGFRs), regulate a broad spectrum of biological functions e.g. angiogenesis, wound healing and embryonic development (Beenken, 2009; Gupta, 2013; Shirakata *et al*., 2010). Attempts have been made to accelerate the regeneration of periodontal tissue by direct local application of combinations of various factors, and use of growth factors with various bone grafts have resulted in periodontal regeneration and it has been proven clinically (Canalis, 1988; Jansen *et al*., 2005; Bartold *et al*., 2000; Kaigler *et al*., 2011; Lee *et al*., 2010). Many studies have been performed to assess the regenerative abilities of different growth factors, but a few in-vivo studies involving basic fibroblast growth factor have been conducted. The aim of this study is to evaluate the regenerative effect of human recombinant basic fibroblast growth factor (bFGF) with and without synthetic alloplastic bone graft in periodontal infrabon

MATERIALS AND METHODS

A comparative clinical and radiographic study was carried out to assess the efficacy of b-FGF with bone graft (Group A) and b-FGF without bone graft (Group B) in the treatment of human intrabony defects. Patients were selected from Out Patient Department of Periodontics. A total of 16 patients between 20 to 65 years with moderate to severe periodontitis with clinical and radiographic evidence of bilateral angular bony defects were recruited for the study. Thus, a total of 32 angular defects were selected for the study.

Inclusion Criteria

- Patients in good systemic health.
- Patients with atleast one infrabony defect ≥ 3 mm apical to the remaining alveolar bone crest on each side.
- Patients with probing depth 6 mm or greater at baseline.
- Patients with radiographic evidence of bone loss
- Patients in age group of 20-65 yrs.

Exclusion Criteria

- Patients with history of periodontal surgery on the study teeth.
- Clinical or radiographic signs of untreated acute infection at the surgical site, apical pathology, root fracture, severe root irregularities, cemental pearls, cemento-enamel projections not easily removed by odontoplasty.
- Patients who are either pregnant or lactating.

The patients selected were explained about the treatment procedure, the associated risks and benefits and their written informed consent was obtained. The study was approved by the institutional ethical committee.

Using a split-mouth design, 16 patients with chronic periodontitis having probing pocket depths (PPDs) of ≥6 mm following initial periodontal therapy (scaling, root planning and oral hygiene instructions) were randomly assigned to two treatments groups in contralateral areas of the dentition. (Group A) bFGF with bone graft: 16 intrabony defects were subjected to open flap debridement. After topical application with bFGF at the defect site, the bone graft (bioactive glass) impregnated with bFGF was placed in the defects. (Group B) bFGF without bone graft: 16 intrabony defects were subjected to open flap debridement. After topical application with bFGF at the defect site, acellular collagen sponge (ACS) impregnated with bFGF was placed in the defects

Method of preparation of 0.3% FGF-2: 10 µg of recombinant human bFGF (lyophilized) was reconstituted in 100 µl of 10mM tris with a ph of 7.6 and a 0.1 mg /ml of stock solution was prepared in laminar flow cabinet (Figure 1.A). It was further diluted with 0.1% BSA (bovine serum albumin) containing PBS (phosphate buffer saline) to obtain 0.3 % FGF-2. Then from the prepared solution vials each containing 200 µl of FGF-2 were obtained (Kitamura *et al*., 2008). Hence, a 200 µl of 0.3% FGF-2 was used in each defect with absorbable collagen sponge or synthetic alloplastic bone graft.FGF-2 was incorporated in absorbable collagen sponge or synthetic alloplastic bone graft by soaking in it.

Surgical procedure: Sulcular incisons were given to raise a full thickness flap. All the tissue tags and granulation tissue were removed with a curette. The root surfaces were examined, scaled and planed. After complete debridement of the surgical area, the area was isolated with cotton rolls and a clean, dry field was obtained. FGF-2 was topically applied to the defect site and then novabone dental putty impregnated with FGF-2 in Group A (as depicted in Figure), and collagen sponge impregnated with FGF-2 in Group B was placed in defect (as depicted in figure). Pre-suturing was done prior to the placement of graft material to prevent its dislodgment.

A periodontal dressing was placed and post-operative instructions were given. oral antibiotics –combination of amoxicillin 500mg and clavulanic acid 125 mg 8 hourly for 5 days and anti-inflammatory analgesic combination of ibuprofen (325 mg) and paracetamol (500mg) was given 8 hourly for 5 days. The rationale of using antibiotics with regenerative procedures is to increase the predictability of results by controlling the sub gingival microflora in order to reduce the risk of postoperative infection. A 10 ml of 0.2% chlorhexidine gluconate mouth rinse was also prescribed. Post-operative appointments were scheduled at 3 weeks, 6 weeks, 12 weeks and 24 weeks. Each postoperative appointment consisted of evaluation of the healing tissues and reinforcement of oral hygiene instructions.

Following were parameters recorded at different time intervals were

At baseline: Loe H and Silness P gingival index 1963 (GI), Silness P and Loe H Plaque index 1964(PI), Probing pocket depth (PPD) (mm), measured by University of North Carolina□15, periodontal probe using gingival margin as a reference Clinical attachment level (CAL) (mm) was recorded using acrylic stent with grooves to ensure reproducible placement of the probe and radiographic evaluation (IOPA, RVG). Full mouth IOPAs, OPG and RVG were taken. IOPA and RVG were taken using XCP system with an occlusal stent and a radiographic grid (Duckworth *et al*., 1983).
The area of the defect was calculated using AUTOCAD software before surgery and at subsequent visits.

After 3 weeks: -GI index, PI index

After 6 weeks: GI index, PI index, PPD, CAL

After 12 Weeks: GI index, PI index, PPD and CAL and radiographic evaluation

After 24 weeks: GI index, PI index, PPD and CAL and radiographic evaluation

All data was compiled and put to statistical analysis.

STATISTICAL ANALYSIS

A frequency distribution was performed on 32 hard and soft tissue measurements on 16 patients by an examiner in an effort to ensure examiner calibration. The results obtained in this study were statistically analyzed using mean of continuous variables and standard deviation. Paired t tests were used to compare clinical and radiographical measurement at baseline and at different time intervals for intragroup analysis. Differences between sites treated with a combination of bfgf and bone graft and sites treated with a combination of bfgf and absorbable collagen sponge were assessed using unpaired Student t tests. Probabilities <0.05 were considered statistically significant. In an effort to compare the relative benefits of sites treated with bfgf and bone graft and sites treated with a combination of bfgf and absorbable collagen sponge a number of calculations were performed, including plaque index, gingival index, PD reduction, CAL gain, percentage of CAL gain, gain in height of alveolar bone, defect fill and percentage of bone gain.

RESULTS

A total of 16 patients having bilateral, moderate to severe (≥6 mm) periodontal osseous defects completed the 6 months follow□up period. In all cases, postoperative healing was without any complications and infections. Table 1 summarize the Intra group comparison of the clinical parameters of the two groups**.** Analysis of frequency distribution was used to determine whether the examiner was statistically in quantitative agreement. At baseline, mean values of plaque index score of group A and group B were 1.05± 0.033 and 1.10±.08, and at 24^{th} week were 0.908±0.31 and 1.033±0.041 respectively. The difference between the mean values of plaque index at baseline and at 24^{th} week in group A was non-significant (P value 0.0736) but that of group B were significant (P value 0.0121).

At baseline, mean values of gingival Index score of group A and group B were 0.696± 0.39 and 0.8± 0.36, at 24^{th} week were 0.166± 0.09 and 0.28 ± 0.245 respectively. The difference between the mean values of gingival index at baseline and at 24^{th} week in both groups (P values 0.0001) were statistically significant. However, in the intergroup analysis, for both the indices, no statistically significant difference was found at any of the time intervals**.** (as depicted in Table 2). The present study demonstrated a decrease in probing depth (as depicted in Table 1 and figures) at different time intervals from baseline, with the mean difference of 2.437±0.727 mm at 6^{th} week (P value≤0.001), 3.312± 0.793 mm at 12^{th} week ((P value≤0.001, 3.75 ± 0.93mm (P value≤0.001) at 24^{th} week in group A, and 2.062 ± 0.573mm at 6^{th} week (P value≤0.001), 2.875 ± 0.619 mm at 12^{th} week(P value≤0.001) and 3.062± 0.573mm at 24^{th} week (P value≤0.001) in group B. The comparison of the score between baseline and at all the intervals in both groups (as depicted in table 2) was highly significant.

However, in the intergroup analysis, group A showed statistically significant results than group B at 24^{th} week only (P value 0.017). Similar observations were seen for CAL (as depicted in table 1 and figure 1) where the difference of CAL for group A at 6^{th} week from baseline was 1.437± 0.512 mm (P value≤0.001), at 12 week was 2.187±0.655 mm (P value≤0.001), and at 24^{th} week was 2.312 ± 0.478 mm (P value≤0.001), and that for group B at 6^{th} week from baseline was 1.375± 0.619 mm (P value≤0.001), at 12^{th}week was 2.062 ± 0.573mm (P value≤0.001) and at 24^{th} week was 1.937 ± 0.573mm(P value≤0.001). The comparison of the score between baseline and at different intervals in both groups was statistically highly significant. However, in the intergroup analysis (as depicted in table 2) group A showed statistically significant results than group B at 24^{th} week only (P value 0.053) Percentage of CAL gain at 12^{th} week in group A was 22.4%, in group B was 22.5% and at 24^{th} week were 23.7% and 21.2% respectively.

For radiographic analysis (as depicted in radiographic images and table 1), IOPA and RVG were taken at baseline, 12^{th} week, 24^{th} week. Mean difference of increase in alveolar bone height (IOPA) in group A from baseline to 12th week was 0.645 ±0.095 (P value 0.000) and to 24^{th} week was 1.053±0.111 (P value 0.000). This difference in group B from baseline to 12^{th} week was 0.335± 0.145 (P value 0.000) and to 24^{th} week was 0.858±0.115mm (P value<0.001) Percentage of bone gain in group A at 12^{th} week was 11.5% and at 24^{th} week was 19% which are statistically significant. Percentage of bone gain in group B at 12^{th} week was 6.3% and at 24^{th} week was 16 % which are statistically significant.

Similarly, mean difference of increase in alveolar bone height (RVG) in group A from baseline to 12^{th} week was 0.643 ± 0.097 (P value 0.000) and to 24^{th} week was 1.112±0.394 mm P value <0.001), and this difference in group B from baseline to 12^{th} week was 0.381± 0.250 mm (P value 0.000) and to 24^{th} week was 0.821 ±0.223mm (P value<0.001). Increase in height of alveolar bone was significant in both groups at all intervals (table1) and intergroup analysis (Table 2) for increase in alveolar bone height was statistically significant at all the time intervals in group A.

DISCUSSION

The use of basic fibroblast growth factor -2 associated with biomaterials is an alternative treatment approach that may favor the process of bone tissue regeneration.[16,17,18] FGFs are multifunctional regulatory peptides with a great impact on studies of tumorigenesis, cardiovascular disease, and repair of tissue injury, neurobiology and embryonic development.

They are responsible for critical functions in wound healing, tissue repair, angiogenesis, and homeostatic regulation (Murakami *et al*., 1999; Rossa, 2000; McCauley, 1998; Ishii, 2013). But a very few studies have been done on FGF-2 as well as on its various combinations. Therefore the present study was conducted to compare the clinico-radiographic efficacy of FGF2 with and without alloplastic bone graft. A 200 µl solution of 0.3% FGF-2 was used in the defect sites. Kitamura *et al* 2008 and Kitamura 2011 cited in their human clinical trials that concentration range of 0.03–0.3% was effective for periodontal regeneration.

Table 1. Intra group comparison of the clinical parameters of the two groups

	PI		GI		PPD		CAL		RM			
	GP A GP B		GP A GP B		GP A GP B		GP A GP B		GP A		GP B	
BASELINE	1.05± .033	1.10± .08	.696± .39	.8± .36	7.62± 1.2	7.06± .85	9.75 ± 1.8	9.12± 1.89	IOPA	RVG	IOPA	RVG
3RD WEEKS	1.03±.085	1.088 ± .068 1.06± .04	.475±.22	.660± .477 .40± .23	-	-	-	-	5.348±1.034	5.142±1.168	5.331±1.16	5.025±1.102
6TH WEEKS	1.03± .015	1.04± .043	.266± .11	.32± .246	5.18± .75	5±.89	8.31± 1.66		-	-		
12TH WEEK	1.03± .017	1.033± .041	.227± .12	.28 ± .245			7.56± 1.75	7.75± 1.77				
24TH WEEK	.908± .31		.166± .09		4.31± .79	4.18± .655	7.43± 1.67					
					3.87± .80	4± .816		7.06± 1.73				
								7.18± 1.72	4.738±1.00	4.498±1.135	4.995±1.195	4.643±1.17
									4.330±0.968	4.03±0.937	4.472±1.142	4.203±1.118

Table 2. Inter group comparison of the clinical parameters of the two groups

Mean difference Baseline to	PI		GI		PPD		CAL		RM			
3RD week	GP A GP B		GP A GP B		GP A GP B		GP A GP B					
Baseline to 6TH Week	0.014 ± 0.072	0.014± 0.058	0.22±0.195	0.139±0.294	-	-	-	-	IOPA		RVG	
Baseline to 12TH week									Gp A	Gp B	Gp A	GP B
									-	-	-	-
	0.019 ±0.035	0.041 ±.0.068	0.429 ± 0.344	0.398± 0.389	2.437±0.727	2.062 ± 0.573	1.437± 0.512	1.375± 0.619	-	-	-	-
Baseline to 24TH week	0.021 ± 0.038	0.061 ± 0.088	0.468 ±0.371	0.48± 0.398	3.312± 0.793	2.875 ± 0.619	2.187±0.655	2.062 ± 0.573	0.645 ±0.095	0.335± 0.145	0.643 ± 0.097	0.381± 0.250
	0.143 ±0.298	0.068 ±0.096.	0.53 ± 0.383	0.52± 0.393	3.75 ± 0.93	3.062± 0.573	2.312 ± 0.478	1.937 ± 0.573	1.053±0.111	0.858±0.115	1.112±0.394	0.821 ±0.223

The delivery and presentation of a growth factor at the defect site requires an appropriate scaffold. Scaffold serves as a framework, which maintains the shape of the defect (Dabra *et al*., 2012; Giannobile *et al*., 2011; Rios *et al*., 2011). The main purpose of the delivery system is to ensure adequate protein concentrations at the defect site for as long as it takes to enable the regenerative cells to migrate, proliferate and differentiate (Rios *et al.,* 2011). It has been established that growth factors in combination with alloplastic materials are more effective with enhanced osteogenic properties (Ivanovski, 2009; Nevins, 2003).

Seeherman & Wozney 2005, Ginebra *et al* 2006 Jansen *et al* 2005 reported that growth factors like BMP-2&7, TGF-β, bFGF, IGF-1&2 and VEGF are commonly introduced into these scaffolds due to their osteoinductive properties and vascularization. Bergeron *et al*. in 2007 stated that a delivery system consisting of collagen Type I gel, recombinant human BMP-2 (rhBMP-2) and 45S5 bioglass microspheres seems to be a promising system for bone regeneration An absorbable collagen sponge (ACS) is regarded as the benchmark as it has been used in several studies and approved for clinical application (Kim *et al*., 2013) thus ACS was used as one of the carrier in the present study.

In this study, values of plaque indices and gingival indices decreased with time following periodontal surgical treatment in the both the groups. This was achieved by the reinforcement of plaque control measures and oral hygiene maintenance instructions at various recall periods. However, no statistically significant differences were noted between the two groups at any time interval.

Pocket depth decreased highly significantly from baseline in both the groups at all visits and but the difference in pocket depth reduction was significantly higher in group A than in group B at 24th week only. The decrease in pocket depth was initially due to shrinkage of gingival tissue and later it was due to resolution of defect as confirmed by radiographs. One possible explanation of the decrease in pocket depth can be that when roentgenograms are taken under conditions of controlled angulationpost-treatment of infrabony pockets by surgical therapy, which is accompanied by resolution of pocket, reliable clinical results are obtained due to the bone fill in the defect (Patur, 1962). Attachment gain was achieved and was highly significant in both groups from baseline at all the visits. The similar results of FGF2 were obtained by multicentric trials done by Kitamura at al 2008 and Kitamura *et al* 2011.

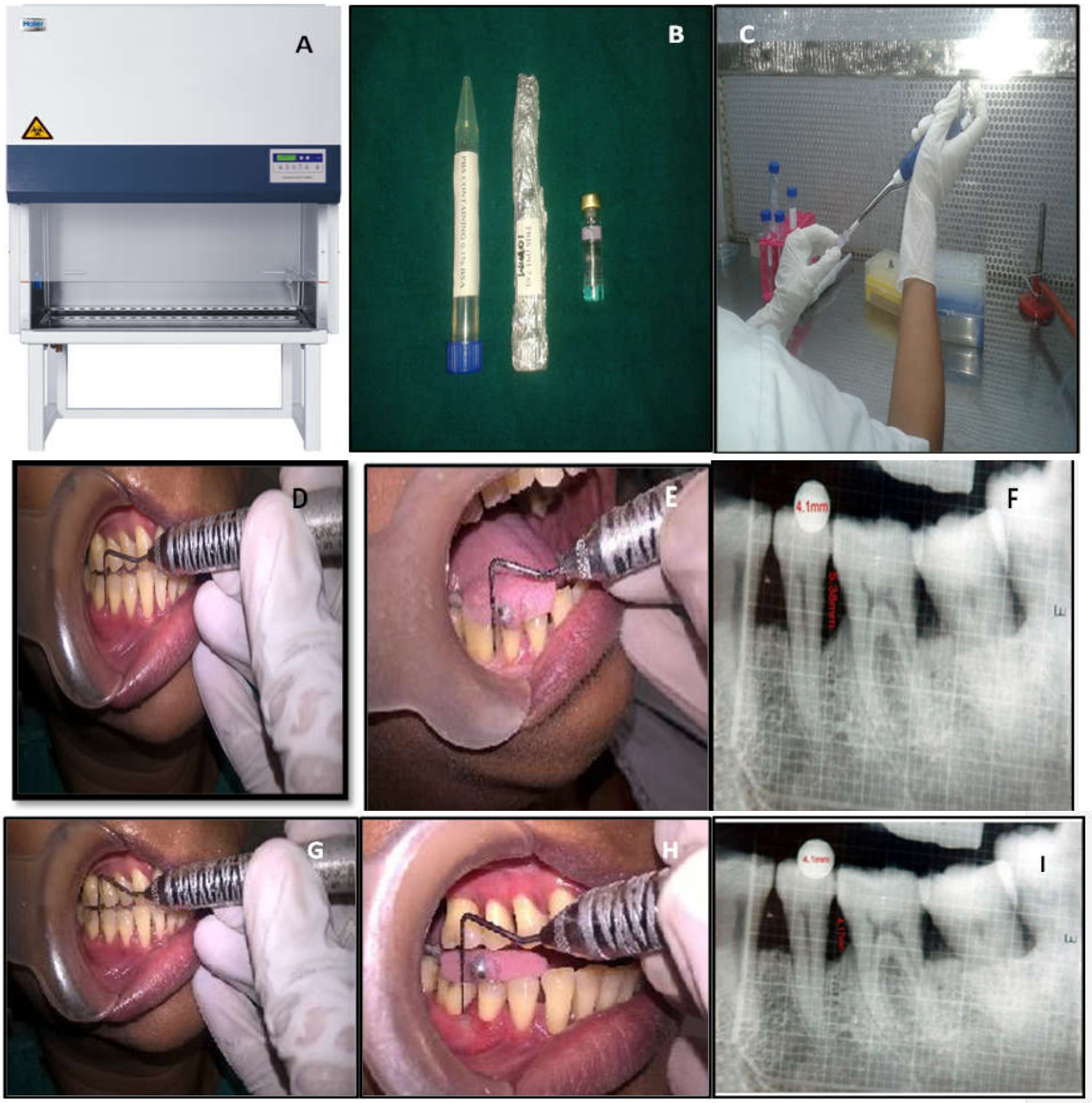

Figure 1(a) GROUP A (A) biosafety cabinet(B) and (C) reconstitution of Bfgf (D)probing pocket depth before surgery (at baseline)(E) Clinical attachment level before surgery (at baseline) (F) standardized IOPA before surgery (G) probing pocket depth at 24th week following surgery (H) Clinical attachment level at 24th week following surgery (I) standardized IOPA 24th week following surgery

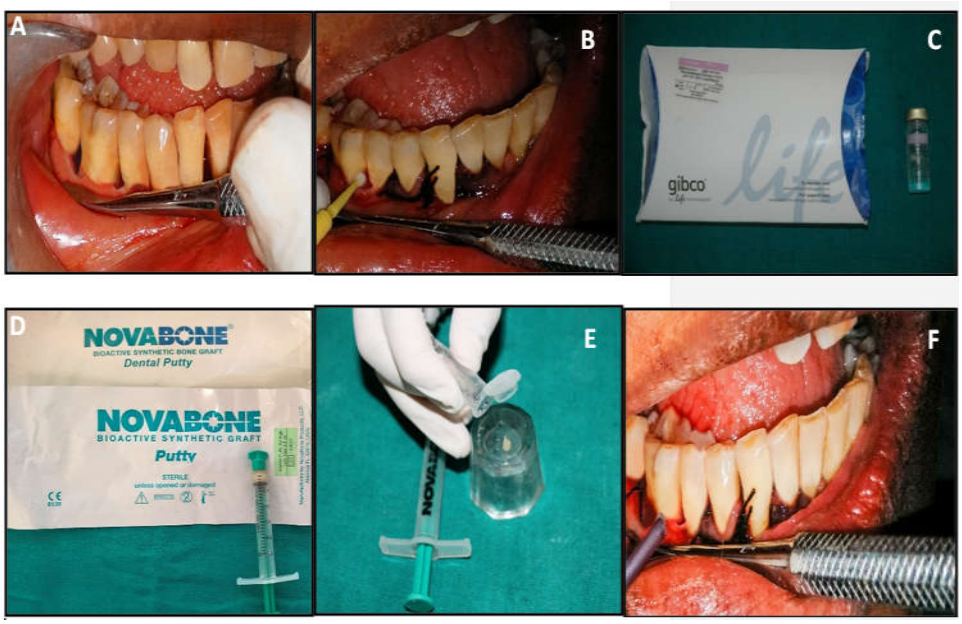

Figure 1 (b) SURGICAL PROCEDURE OF GROUP A (A)Image depicting intrabony defect after flap reflection (B) Application of 0.3 % bFGF with applicator tip (C) bFGF(Lypholized form) (D) Bioactive glass (Novabone dental putty) (E) Mixing of 0.3 % bFGF (reconstituted form) with bioactive glass(Novabone dental putty) (F) Application of graft impregnated with bFGF

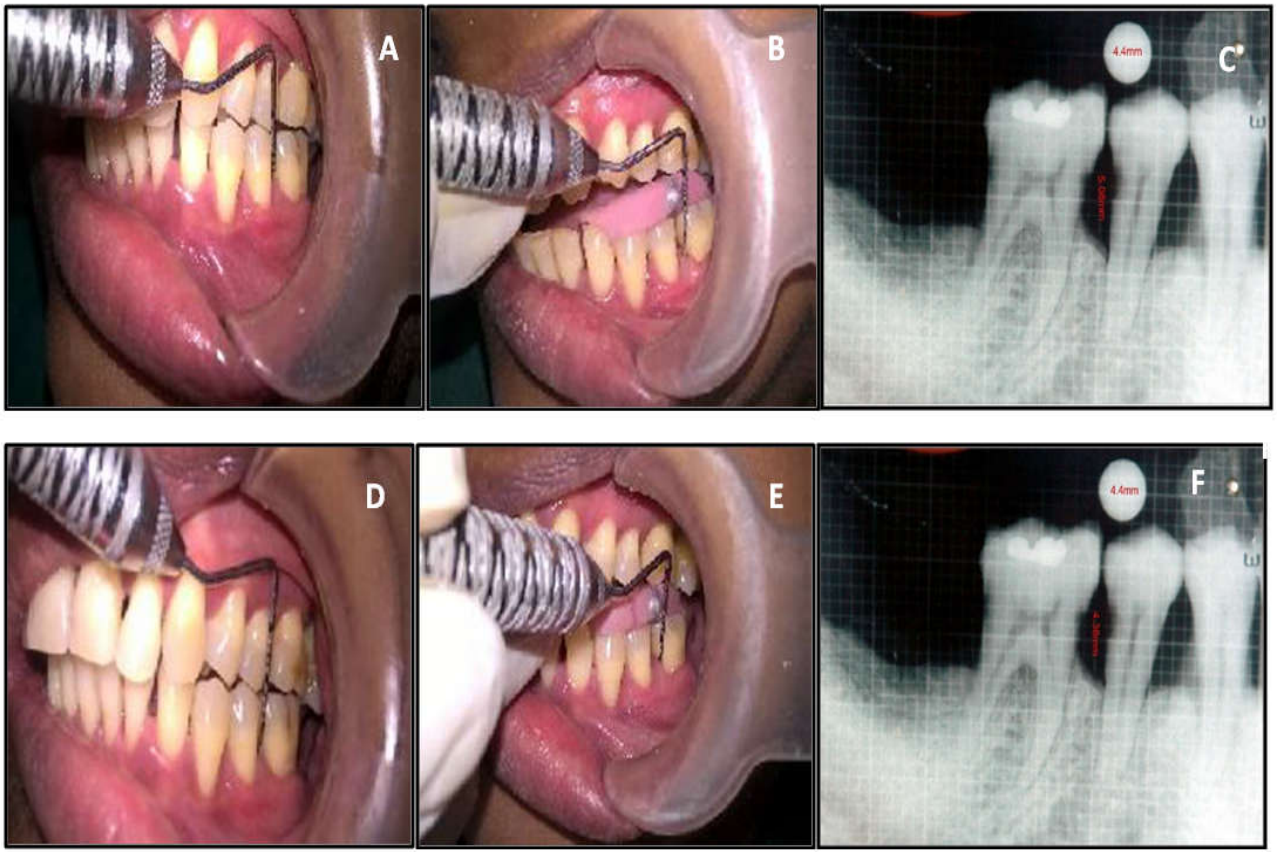

Figure 2 (a) GROUP B (A) probing pocket depth before surgery (at baseline)(B) Clinical attachment level before surgery (at baseline) (C) standardized IOPA before surgery (D) probing pocket depth at 24th week following surgery (E) Clinical attachment level at 24th week following surgery (F) standardized IOPA 24th week following surgery

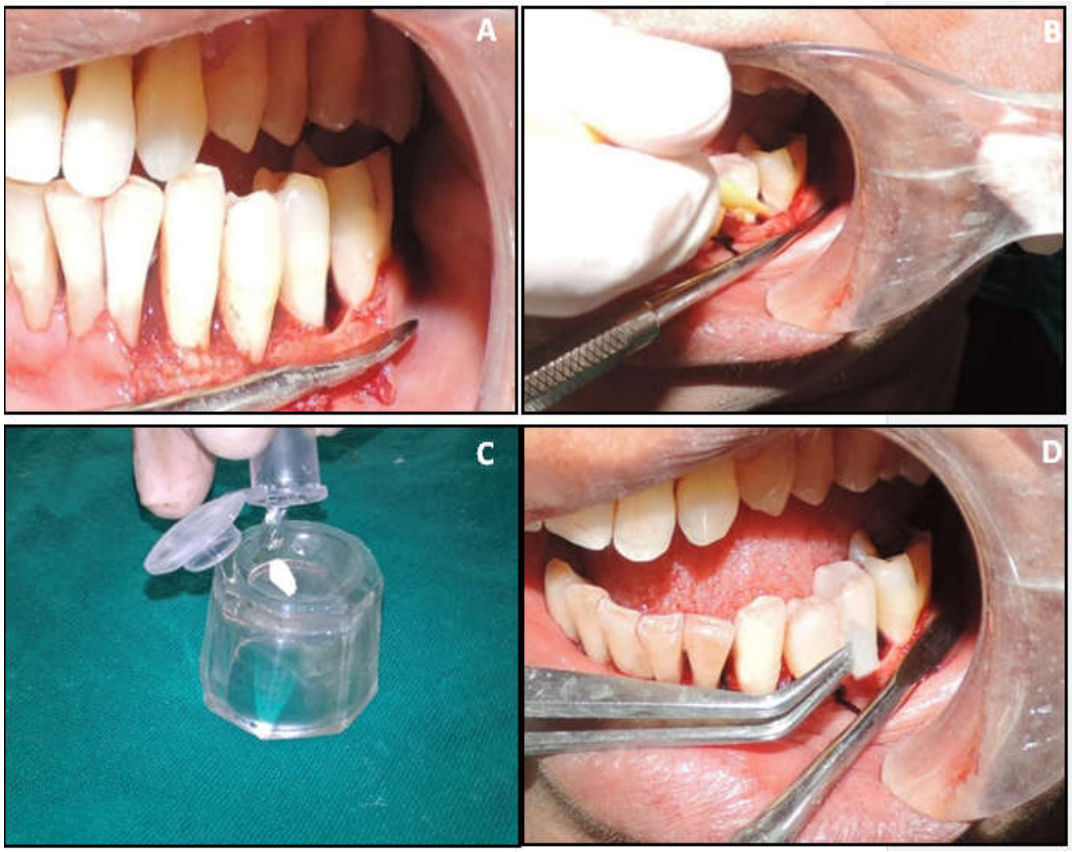

Figure 2 (b) SURGICAL PROCEDURE OF GROUP B (A)Image depicting intrabony defect after flap reflection (B) Application of 0.3 % bFGF with applicator tip (C) Mixing of 0.3 % bFGF with absorbable collagen sponge (D) Application of absorbable collagen sponge impregnated with bFGF

The gain in attachment was significantly higher in group A than in group B only at 24th week. Similar results were found in a study conducted by Nevins *et al* 2003, where they used a combination of allogenic bone graft and a growth factor (recombinant human platelet derived growth factor-BB) and allogenic bone in infrabony defects and class II furcation defects (Nevins *et al*., 2003). Gain in height of alveolar bone was statistically significantly higher in group A than in group B at all visits. These results coincide with results of studies done by Kim *et al*., 2013, Nevins *et al*., 2003, Bateman *et al*. 2005 and Jiang *et al* 1999. All these studies submit the evidence that growth factors in combination with alloplastic materials are more effective with enhanced osteogenic properties. Shirakata *et al* 2013 reported that when FGF2 was used in combination with β TCP, it resulted in more bone and cementum formation than bone graft alone or bone graft in combination with EMD. Moreover, Schepers *et al* 1991, Wilson and Low 1992 cited that bioactive glass has osteoconductive and osteopromotive abilities in the biocompatible interface for osseous migration, and a bioactive surface colonized by osteogenic cells free in the surgical wound. Their ability to bond to soft and osseous tissues seems to make a difference when compared to other materials available. One of the limitations of this study is that it did not involve any histological analysis, so, it cannot be infereneced that if true periodontal regeneration was achieved or not. Surgical re-entry evaluation was excluded from the study due to ethical reasons and patient concern. The population included in the study is relatively small and extrapolation of these results to a larger population would be inappropriate. It is recommended that future studies involve a greater number of patients as well as experimental studies be conducted to analyze the maximum potential of FGF-2.

Conclusion

This study provides evidence that both FGF-2 with bioactive glass, FGF-2 with absorbable collagen sponge (ACS) combination therapies result in greater clinical and radiographic improvement (soft and hard tissue) at various follow up visits which is presumably because of mitogenic activity of basic fibroblast growth factor. Although both interventions worked well in managing deep intrabony defects, but impregnation of basic fibroblast growth factor onto a bioactive glass improves efficacy of this growth factor in treatment of intrabony defects and this combination has potential for clinical applications

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