

**EFFECTIVENESS OF BIOACTIVE GLASS IN PUTTY AND  
PARTICULATE FORM FOR THE TREATMENT OF GRADE II  
FURCATION DEFECTS – A COMPARATIVE STUDY.**

**Dissertation**

**Submitted to**

**BABU BANARASI DAS UNIVERSITY,  
LUCKNOW, UTTAR PRADESH**

*In the partial fulfilment of the requirements for the degree*

*Of*

**MASTER OF DENTAL SURGERY**

**In**

**PERIODONTICS**

**By**

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*Under the guidance of*

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**BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES  
LUCKNOW**

**(Faculty of Babu Banarasi Das University)**

**BATCH: 2015-2018**

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I hereby declare that this dissertation entitled “**EFFECTIVENESS OF BIOACTIVE GLASS IN PUTTY AND PARTICULATE FORM FOR THE TREATMENT OF GRADE II FURCATION DEFECTS – A COMPARATIVE STUDY.**” is a bonafied and genuine research work carried out by me under the guidance of **Dr. Mona Sharma**, Reader, Department of Periodontics, Babu Banarasi Das College Of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

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***Dr. Vandana Gupta***

***Enrolment Number:***



# *CONTENTS*

<b>S.No.</b>	<b>Particulars</b>	<b>Page No.</b>
1.	List of Tables	
2.	List of Graphs	
3.	List of Figures	
4.	List of Appendices	
4.	Abbreviations	
5.	Abstract	
6.	Introduction	
7.	Aim and Objectives	
8.	Review of Literature	
9.	Materials and Methods	
10.	Observations and Results	
11.	Discussion	
12.	Conclusion	
13.	Summary	
14.	Bibliography	
15.	Appendices	

## *LIST OF TABLES*

<b>Table No.</b>	<b>Title</b>	<b>Page No.</b>
Table 1	Comparison of PI between the groups across the time periods	23
Table 2	Comparison of mean change in PI from baseline to subsequent time periods in group A and group B	25
Table 3	Comparison of GI between the groups across the time periods	27
Table 4	Comparison of mean change in PI from baseline to subsequent time periods in group A and group B	29
Table 5	Comparison of clinical HPD between the groups across the time periods	31

<b>Table No.</b>	<b>Title</b>	<b>Page No.</b>
Table 6	Comparison of mean change in clinical HPD from baseline to subsequent time periods in Group A and Group B.	32
Table 7	Comparison of RDD between the groups across the time periods	34
Table 8	Comparison of mean change in clinical RDD from baseline to subsequent time periods in Group A and Group B.	35

## *LIST OF GRAPHS*

<b>Graph No.</b>	<b>Title</b>	<b>Page No.</b>
1.	Comparison of PI between the groups across the time periods	
2.	Comparison of mean change in PI from baseline to subsequent time periods in group A and group B	
3.	Comparison of GI between the groups across the time periods	
4.	Comparison of mean change in PI from baseline to subsequent time periods in group A and group B	
5.	Comparison of clinical HPD between the groups across the time periods	

<b>Graph No.</b>	<b>Title</b>	<b>Page No.</b>
6	Comparison of mean change in clinical HPD from baseline to subsequent time periods in Group A and Group B.	
7	Comparison of RDD between the groups across the time periods	
8	Comparison of mean change in clinical RDD from baseline to subsequent time periods in Group A and Group B.	

## *LIST OF FIGURES*

S.No.	Title	Plate No.
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		

## *LIST OF APPENDICES*

<b>Appendix No.</b>	<b>Title</b>	<b>Page No.</b>
Appendix – I	Ethical Committee Approval Form	
Appendix – II	Case sheet	
Appendix– III	Consent Form (English)	
Appendix– IV	Tables Of Clinical & Radiographic Parameters	
Appendix– V	Statistical Tools Used In The Study	

# *ABBREVIATIONS*

BG	Bioactive glass
BCP	Biphasic calcium phosphate
BMP	Bone morphogenetic protein
GI	Gingival index
HDD	Horizontal defect depth
HCA	Hydroxycarbonated apatite
IGF	Insulin like growth factor
PDGF	Platelet derived growth factor
PDGF-AB	Platelet derived growth factor- AB
PI	Plaque index
PRF	Platelet rich fibrin
TGF- $\beta$	Transforming growth factor beta
VEGF	Vascular endothelial growth factor



The goal of any periodontal therapy is the control of active inflammation, the arrest of disease progression and the reconstruction of structures lost to disease, where appropriate. Different types of bony deformities such as horizontal, vertical, craters, and furcation result from periodontal disease, of which vertical and Grade II furcation defects are more amenable to regenerative periodontal as they are contained defects.

Autograft is considered as gold standard graft material, but carrier a disadvantage of second surgical site, patient discomfort and morbidity. The effort to find a means to regenerate the periodontium has created a renaissance of research in the utilization of alloplasts. Bioactive glass was invented by Larry Hench in 1971 as a four-component glass system, containing  $\text{SiO}_2$ ,  $\text{CaO}$ ,  $\text{Na}_2\text{O}$ , and  $\text{P}_2\text{O}_5$ . Bioactive glass has an osteostimulatory effect in addition to its osteoconductive properties. So we decided to carry out a study to compare two forms of bioactive glass (particulate and putty) in the treatment of grade II furcation defects.

30 sites fulfilling the inclusion and exclusion criteria were selected and divided randomly into Group A and Group B. After phase I therapy was done in both the groups, Group A was further treated with Novabone Putty and Group B with Novabone morsels (particulate). PRF was used as a membrane in both the groups. Clinical parameters recorded were gingival index (GI), plaque index (PI) and horizontal defect depth (HDD). Radiographic parameter was radiographic defect depth (RDD) which was calculated using CBCT. Clinical parameters were recorded at baseline, 3 months and six months. Radiographic parameter was recorded at baseline and 6 months. After statistical analysis it was found that both the group showed significant improvement in soft and hard tissue parameters. Inter group comparison of HDD and RDD was statistically significant with a p value of 0.01 and 0.02 respectively, showing that putty was superior to morsels in treating grade II furcation defects.

Periodontitis is a chronic inflammatory condition initiated by microbial infection that leads to gingival tissue destruction and alveolar bone resorption<sup>1</sup>. This progressive inflammation leads to various types of bone destructive patterns or bony defects and furcation defect is one of them.

The progress of inflammatory periodontal disease, if unabated, ultimately results in attachment loss sufficient enough to affect the bifurcation and trifurcation of multirooted teeth. The furcation is an area of complex anatomic morphology<sup>2-4</sup> that may be difficult or impossible to maintain by routine home care methods.<sup>5-6</sup> Also, this area is difficult to debride by routine periodontal instrumentation.<sup>7-8</sup> The presence of furcation involvement is one clinical finding that can lead to a diagnosis of advanced periodontitis and potentially to a less favorable prognosis for the affected tooth or teeth. Furcation involvement therefore presents both diagnostic and therapeutic dilemmas.

Regeneration of lost structure has become the primary therapeutic goal in periodontics and there are numerous therapeutic modalities for restoring periodontal osseous defects that have been investigated.<sup>9</sup> The periodontal literature has well documented therapeutic efforts designed to induce new attachment and/or regeneration on molars with furcation defects. Many surgical procedures using a variety of grafting materials (bone & non-bone)<sup>10-12</sup> have been tested on teeth with different classes of furcation involvement. Furcation defects with deep two-walled or significant three-walled components may however be candidates for regeneration procedures.

Several types of bone graft have been studied over the years. Alloplastic biomaterials are biocompatible inorganic synthetic bone grafting materials; types include nonporous and porous hydroxyapatite, beta tricalcium phosphate, polymethylmethacrylate, hydroxyethylmethacrylate polymers, and bioactive glasses. The outcome of alloplastic bone

grafting materials is dependent primarily on their chemical composition, structure, and physical properties.<sup>13-14</sup>

Recently, putty formulations of bioactive glass with glycerin and polyethylene glycol as an additive have received significant attention due to the combination of their osteostimulative and osteoconductive properties with superior handling characteristics and ease of use in grafting osseous defects.<sup>15</sup> This putty form of bioactive glass enhances the handling characteristics of the graft.<sup>16-17</sup> It is a premixed composite of bioactive calcium phosphosilicate particulate and a synthetic, absorbable binder. The bioactive particulate is composed solely of elements that exist naturally in native bone (Ca, P, Na, Si, O)

Bioactive glass in particulate form Crystalline composite is composed of oxides of silicon, calcium, sodium, and phosphorous in a silica base. The particle size ranges from 500 $\mu$  – 1000 $\mu$  with a mean particle size of 750 $\mu$ . It has a combination of physical characteristics (macroporosity) and ionic release (osteostimulation).

Platelet rich fibrin (PRF) is an autologous biomaterial containing leukocytes, platelets, and a wide range of key healing proteins within a dense fibrin matrix. PRF holds promise as a regenerative material as it releases high amounts of growth factors (FGF, BMP, PDEGF, PDGF-AB, TGF- $\beta$ , VEGF, IGF) and matrix glycoproteins. Thus it may enhance proliferation of different cell types, including fibroblasts, osteoblasts, adipocytes, and keratinocytes.<sup>18</sup>

Hence, in the present study, we aimed to compare these two different form of bioactive glass bone grafts along with PRF as a membrane in treating grade II furcation defects.

**AIM** – To evaluate the clinical and radiographic outcome observed in treatment of grade II furcation (Glickman classification ,1953) defect with two forms of bioactive glass (putty and particulate) with PRF.

**OBJECTIVES:-**

1. To assess the efficacy of bioactive glass putty for treating grade II furcation defect.
2. To determine the efficacy of particulate form of bioactive glass for treating grade II furcation defects.
3. To compare the difference in bone gain between the two graft materials.

**Froum SJ et al (1998)<sup>19</sup>** conducted a study to compare bioactive glass synthetic bone graft material with open debridement in the treatment of periodontal defects. Fifty-nine defects in 16 healthy adults were selected. Each patient had at least 2 sites with attachment loss of at least 6 mm with clinical and radiographic evidence of intrabony or furcation defects. One to 3 months after cause related therapy (oral hygiene instructions, scaling and root planing), the following measurements were recorded prior to surgery: probing depths, clinical attachment level, and gingival recession. Each defect was surgically exposed and measurements made of the alveolar crest height and base of osseous defect. The test defects were implanted with bioactive glass. The other sites served as unimplanted controls. Flaps were sutured at or close to the presurgical level. After 6 months defect depth reduction was significantly greater in the bioactive glass sites (4.36 mm) compared to the control sites (3.15 mm). In conclusion, bioactive glass showed significant improvement in clinical parameters compared to open flap debridement.

**Lovelace TB et al (1998)<sup>20</sup>** conducted a study to compare the use of bioactive glass with demineralized freeze-dried bone allograft (DFDBA) in the treatment of human periodontal osseous defects. Fifteen systemically healthy patients with moderate to advanced adult Periodontitis were selected for the study. All patients underwent initial therapy, which included scaling and root planing, oral hygiene instruction, and an occlusal adjustment when indicated. Paired osseous defects in each subject were randomly selected to receive grafts of bioactive glass or DFDBA. Both soft and hard tissue measurements were taken the day of surgery (baseline) and at the 6-month re-entry surgery. No statistical difference was found when comparing bioactive glass to DFDBA. This study suggests that bioactive glass is capable of producing results in the short term (6 months) similar to that of DFDBA when used in moderate to deep intrabony periodontal defects.

**Anderegg CR et al (1999)**<sup>21</sup> conducted a study to evaluate bioactive glass in the treatment of molar furcation. 15 patients with moderate to advanced periodontitis were included in this study. Each patient received surgical therapy consisting of regenerative therapy using bioactive glass compared to open flap debridement alone in mandibular furcation defect. The result of this study was statistically significant in the defect treated with bioactive glass.

**Sculean A et al (2002)**<sup>22</sup> conducted a study to compare the treatment of deep intrabony defects with a combination of an enamel matrix protein derivative (EMD) and a bioactive glass (BG) to BG alone. Twenty-eight patients with chronic periodontitis, each of whom displayed at least one intrabony defect, were randomly treated with a combination of EMD and BG or with BG alone. Soft tissue measurements were made at baseline and at 1 year following therapy. No statistically significant differences in any of the investigated parameters were observed between the test and control group. It was concluded that both therapies led to significant improvements of the investigated clinical parameters, and the combination of enamel matrix derivative and bioactive glass does not seem to additionally improve the clinical outcome of the therapy.

**Cetinkaya BO et al (2006)**<sup>23</sup> conducted a study to compare the proliferative activity in gingival epithelium after surgical treatments of intrabony defects with bioactive glass and bioabsorbable membrane. Proliferating cell nuclear antigen (PCNA) was used as a marker of cell proliferation after surgical treatments. 20 intrabony defects were randomly assigned treatments with bioactive glass (BG group) or bioabsorbable membrane (BM group). Gingival biopsies were taken at preoperative and postoperative 12 weeks. After histological processing, the number of the inflammatory cells was measured in hematoxylin and eosin-stained sections; PCNA expression was determined in immunohistochemically-stained sections. PCNA expression was significantly greater in BG group at postoperative 12 weeks. These results suggest that epithelial cell proliferation is more prominent after treatment of

intrabony defects with bioactive glass compared to the treatment with bioabsorbable membrane.

**Hench LL (2006)**<sup>24</sup> published “the story of bioglass”. He invented bioglass and here he discussed the discovery, property, mechanism of action, generations, advantages, limitation and future of it.

**Mengel R et al (2006)**<sup>25</sup> conducted a clinical and radiological prospective 5-year study to compare the long term effectiveness of a bioabsorbable membrane and a bioactive glass in the treatment of intrabony defects in patients with generalized aggressive periodontitis. Sixteen patients with generalized aggressive periodontitis were enrolled in the study. Twenty-two of the defects were treated with the membrane (RXT group) and 20 with the bioactive glass (PG group). The clinical parameters plaque index (PI), gingival index (GI), PD, bleeding on probing (BOP), gingival recession (GR), clinical attachment level (CAL), and tooth mobility were recorded before surgery and at 6 months and every year for 5 years after surgery. Intraoral radiographs were taken using a standardized paralleling technique at baseline and every year for 5 years. Highly significant improvements in the parameters PD and CAL were recorded after 5 years with both regenerative materials. Radiographically, the defects were found to be filled significantly more in the bioactive glass group.

**Felipe MC et al (2009)**<sup>26</sup> conducted a study to find out the potential of bioactive glass particles of different size ranges to affect bone formation in periodontal defects, using the guided tissue regeneration model in dogs. In six dogs, 2-wall intrabony periodontal defects were surgically created and chronified on the mesial surfaces of mandibular third premolars and first molars bilaterally. After 1 month, each defect was randomly assigned to treatment with bioabsorbable membrane in association with bioactive glass with particle sizes between 300 and 355  $\mu$ m (group 1) or between 90 and 710  $\mu$ m (group 2), membrane alone (group 3),

or negative control (group 4). The dogs were sacrificed 12 weeks after surgeries, and histomorphometric measurements were made of the areas of newly formed bone, new mineralized bone, and bioactive glass particle remnants. There was a statistically significant difference between groups 1 and 2, favoring group 1. There were greater areas of mineralized bone in groups 1 and 2 compared to groups 3 and 4. The bioactive glass particles of small size range underwent faster resorption and substitution by new bone than the larger particles.

**Aroca S et al (2009)**<sup>27</sup> conducted a study to evaluate the additional effect of PRF in coronally advanced flap for the treatment of gingival recession. Twenty subjects, presenting three adjacent Miller Class I or II multiple gingival recessions of similar extent on both sides of the mouth, were enrolled in the study. Each patient was treated on both sides by an MCAF technique; the combination treatment (with a PRF membrane) was applied on the test side. Probing depth (PD), recession width, clinical attachment level (CAL), keratinized gingival width, and gingival/ mucosal thickness (GTH) were measured at baseline and at 6 months post-surgery. Gingival recession was measured at baseline and at 1, 3, and 6 months post-surgery. They concluded that the addition of a PRF membrane positioned under the MCAF provided inferior root coverage but an additional gain in GTH at 6 months compared to conventional therapy.

**Kaur M et al (2010)**<sup>28</sup> conducted a study to compare the efficacy of platelet rich plasma (PRP) associated with bioactive glass (BG) and BG alone in the treatment of periodontal intrabony defects. Ten patients participated in the study. Using a split-mouth design, interproximal bony defects were surgically treated with either PRP+BG or BG alone. There was statistically significant greater PPD reduction at 3 months and CAL gain at 6 months for PRP+BG compared to BG alone, but no significant difference was observed in defect fill. The association of PRP with a BG graft material seemed to add some benefits to the improvement of the clinical parameters in the treatment of intrabony defects.



**Pradeep AR et al (2012)**<sup>29</sup> conducted a randomized controlled clinical trial to evaluate the efficacy of PRF combined with porous hydroxyapatite graft for the treatment of three walled defects in chronic periodontitis. 90 intrabony defects were treated either with autologous PRF with open flap debridement (OFD) or PRF+HA with OFD or OFD alone. Clinical and radiological parameters such as probing depth (PD), clinical attachment level (CAL), intrabony defect depth and % defect fill were recorded at baseline and 9 months postoperatively. They reported that treatment of intrabony defects with PRF results in significant improvements of Clinical parameters compared with baseline. HA when added to PRF increases the regenerative effects observed with PRF in the treatment of human three wall intrabony defects.

**Tatullo M et al (2012)**<sup>30</sup> described the clinical and histological evaluations of PRF in reconstructive surgery of atrophied maxillary bones. 60 patients with maxillary atrophy of residual ridge <5mm were selected, 72 sinus lifts were performed with subsequent implant insertions. They found that the use of PRF and piezo surgery reduced the healing time, compared to the 150 days described in literature, favouring optimal bone regeneration. At 106 days it is already possible to achieve good primary stability of endo-osseous implants.

**Grover V et al (2012)**<sup>31</sup> conducted a study to evaluate the efficacy of a bioactive synthetic graft material in the treatment of intrabony periodontal defects. Fourteen intrabony defects in twelve systemically healthy subjects having moderate to severe chronic periodontitis were evaluated after bone grafting with bioactive ceramic filler for a period of 6 months. Mean radiographic defect fill of 64.76% ( $2.49 \pm 0.5$  mm) was observed in 6 months, which was statistically significant. Which suggest that bioactive glass is an efficacious treatment option for the reconstruction of intrabony periodontal defects.

**Bolukbasi N et al (2013)**<sup>32</sup> described the use of PRF in combination with biphasic calcium phosphate in the treatment of bone defects. Defects 5mm in diameter were created in both

tibias of 6 sheep. The defects were left empty or grafted with BCP, PRF or BCP+PRF. After histologic and histomorphometric analysis, they concluded increasing bone formation with the addition of PRF to BCP.

**Panda S. et al (2013)**<sup>33</sup> presented a case report to evaluate the combined effect of PRF and alloplast for treating intrabony defect. The patient presented with an intrabony defect extending up to apical third of the mesial root of left mandibular first molar with a probing depth of 8 mm. Intrabony defect was treated with autologous platelet rich fibrin (PRF) along with use of alloplastic bone mineral. A decrease in probing pocket depth, gain in clinical attachment level and significant bone fill was observed at end of 6 months.

**Kovacs A et al (2013)**<sup>34</sup> conducted a study to report the clinical outcome of GBR with a calcium phosphosilicate alloplastic putty bone substitute performed simultaneously with implant placement. Twelve patients presenting with Class I Seibert defects in 14 edentulous sites were treated with GBR using a CPS putty with a collagen membrane or titanium mesh following implant placement. In order to be included in the study, at least one implant thread had to be exposed on the facial aspect of the implant following implant placement. During 1st stage surgery, the distance from the most apical level of the bone crest on the facial aspect of the implant to the platform of the implant was estimated. The same measurement was retaken during second stage surgery.

**Drago L et al (2013)**<sup>35</sup> conducted a study on Bioactive glass BAG-S53P4 for the adjunctive treatment of chronic osteomyelitis of the long bones: an in vitro and prospective clinical study. He concluded that antibacterial activity of BAG S53P4 showed a marked bactericidal activity after 24 hrs against all the tested species. This activity continued in the subsequent 24 hrs and no growth was observed for all strains after 72 hrs. Results of the clinical study evidenced no signs of infection in 24 patients (88.9%) at the follow-up.

**Shivashankar VY et al (2013)**<sup>36</sup> presented a case report of treatment of large inflammatory periapical lesion using Combination of platelet rich fibrin, hydroxyapatite and PRF membrane. The surgical defect was filled with a combination of PRF and HA bone graft crystals. The defect was covered by PRF membrane and sutured. Clinical examination revealed uneventful wound healing. Radiographically the HA crystals have been completely replaced by new bone at the end of 2 years.

**Coraca-Huber D et al (2014)**<sup>37</sup> evaluated the effectiveness of different sizes of bioactive glass S53P4 against *Staphylococcus aureus* biofilms grown on metal discs in vitro. *S. aureus* biofilms were cultivated on titanium discs. BAG-S53P4 (0.5–0.8 mm and <45 µm) were placed in contact with the discs containing biofilms. Glass beads (0.5 mm) were used as a control. After each interval, the pH from each sample was measured. Colony forming units were counted for the biofilm recovery verification. They tested the activity of bioactive glass against *S. aureus* planktonic cells. They found that BAG-S53P4 can suppress *S. aureus* biofilm formation on titanium discs in vitro. The suppression rate of biofilm cells by BAG-S53P4 <45 µm was significantly higher than by BAG-S53P4 0.5–0.8 mm.

**Tunali M et al (2014)**<sup>38</sup> developed a new product called titanium-prepared platelet-rich fibrin (T-PRF). The T-PRF method is based on the hypothesis that titanium may be more effective in activating platelets than the silica activators used with glass tubes in Chouckroun's leukocyte- and platelet-rich fibrin (L-PRF) method. Scanning electron microscope (SEM) revealed that the platelet activation by titanium seems to offer some high characteristics to T-PRF.

**Nguyen T. et al (2014)**<sup>39</sup> conducted a study to evaluate the clinical outcomes following placement of implants simultaneously with lateral window sinus augmentation with a calcium phosphosilicate (CPS) putty bone substitute in ridges with minimum residual bone height.

Seventeen healthy, adult patients with less than 5mm of vertical bone height in at least one posterior maxillary site, underwent sinus floor elevations according to a modification of the out fracture osteotomy technique. 30 implants were placed. In all cases (100%) at least 20N/cm<sup>2</sup> of MIT were achieved. At the second stage surgery all implants successfully osseointegrated.

**Kumar T. et al (2014)<sup>40</sup>** conducted a study to evaluate horizontal augmentation utilizing ridge-split technique and calcium phosphosilicate. 15 patients with thin residual alveolar ridge were included in this study. A full thickness flap was reflected. Using the piezo surgery, the sagittal bone cuts were made initially at the crest leaving at least 1 mm of margin at the palatal bone. The mesial and distal vertical cuts were made at a distance of 1 mm from the adjacent teeth. Ridge split and implant placement was done along with CPS and covered with either collagen membrane or titanium mesh over the split, and the flaps were released to achieve primary closure. The mean pre-operative ridge width was 2.9mm (range 2-4mm) and the postoperative width was 7.1mm (range 5.5-8mm).

**Mahesh L., Ven N (2014)<sup>41</sup>** conducted a study to evaluate periotest value of implants placed in sockets augmented with CPS putty graft and compared it with implants placed in naturally healed sockets. Patients were divided into 2 groups. Group A (control group) consisted of 22 patients with naturally healed sockets, where 26 implants were placed and PTV was recorded. Group B (test group) consisted of 22 patients where CPS Putty was placed for socket augmentation in single extraction sockets. Six months after grafting, Implants were placed and PTV measured. There was moderately significant difference in the implant stability between the two groups, with Group B exhibiting higher stability.

**Chandran P, Sivdas A (2014)<sup>42</sup>** reviewed role of PRF in periodontal regeneration and concluded that PRF is a powerful healing biomaterial with inherent regenerative capacity and

can be used in various procedures such as for the treatment of periodontal intrabony defects ,treatment of furcation, sinus lift procedures and as a scaffold for human periosteal cells in vitro, which finds application in tissue engineering.

**Baslarli O et al (2015)<sup>43</sup>** conducted a study for the evaluation of osteoblastic activity in extraction sockets treated with platelet-rich fibrin. A total of 20 patients with bilateral soft tissue impacted mandibular third molars were included in this study. The left and right third molars were extracted during the same session. Subsequently, the PRF membrane was randomly administered to one of the extraction sockets. After 30 and 90 days post-op evaluation they concluded that PRF might not lead to enhanced bone healing in impacted mandibular third molar extraction sockets.

**Nishimoto S et al (2015)<sup>44</sup>** piloted a study for growth factor measurement and histological analysis in PRF. PRF and PRP were obtained from the same sample of peripheral blood. Extraction of proteins was done with lysis buffer, accompanied by freeze and thaw procedures. Concentration of two representative growth factors in platelets: platelet derived growth factor (PDGF) and transforming growth factor beta (TGF- $\beta$ ), were measured with enzyme-linked immunosorbent assay (ELISA). The growth factor level in PRF was higher than in peripheral blood and comparable to those in PRP. Growth factor levels in bottom part of PRF were much higher than in top and middle part.

**Chandrashekhar RM et al (2015)<sup>45</sup>** conducted a study to evaluate antimicrobial property of bioactive glass used in regenerative periodontal therapy. *S. salivarius* (ATCC strain 13419) was procured from HIMEDIA laboratories. The revived strain was inoculated on 5% sheep blood agar plates and incubated in a 5% CO<sub>2</sub> incubator at 37°C overnight. The microbial cultures were harvested and suspended in brain-heart infusion (BHI) broth at a concentration of 10<sup>5</sup> colony-forming units per milliliter (CFU/mL). different concentration of bioactive

glass added to it. There was direct co-relation between concentration of BG and its antibacterial activity.

**Keceli HG et al (2015)**<sup>46</sup> conducted a randomized controlled trial to evaluate the adjunctive effect of platelet rich fibrin to connective tissue graft in the treatment of buccal recession defects. 40 patients were surgically treated either with CAF+CTG+PRF (test group) or CAF+CTG (control group). Clinical parameters of plaque index, gingival index, vertical recession, probing depth, clinical attachment level, keratinized tissue width, horizontal recession, MGJ localization, tissue thickness were recorded at baseline, 3 months and 6 months post-surgery. Root coverage, complete RC, attachment gain, and keratinized tissue change were also calculated. According to the results, PRF did not develop the outcomes of CAF+CTG treatment except increasing the tissue thickness.

**Chadwick et al (2016)**<sup>47</sup> conducted a study to compare PRF and DFDBA for the treatment of periodontal infrabony defect. Thirty-six patients completed the study protocol. Each patient contributed a single intrabony defect, which was randomized to receive either DFDBA or PRF. Clinical and standardized radiographic data were collected at baseline and 6 months after treatment. Primary outcomes measures included radiographic bone fill as measured from the CEJ to base of bony defect, and change in clinical attachment level. They concluded that treatment of intrabony defects with either DFDBA or PRF resulted in a significant gain in CAL as well as bone fill after 6 months of healing, with no significant difference between materials.

**Martande SS et al (2016)**<sup>48</sup> conducted a controlled clinical trial to evaluate the effect of PRF and PRF+ 1.2% atrovastatin for the treatment of intrabony defect in chronic periodontitis. Ninety six individuals with single defects were categorized into three groups: OFD with PRF, OFD with PRF+1.2% ATV and OFD alone. Plaque index, modified sulcus bleeding index, probing depth, relative attachment level and gingival marginal level were recorded at baseline

before surgery and 9 months post-operatively. Percentage radiographic intra-bony defect depth reduction was evaluated at baseline and 9 months. It was reported that PRF+1.2% ATV showed similar improvements in clinical parameters with greater percentage radiographic defect depth reduction as compared to PRF alone in treatment of intrabony defects in CP individuals.

**Hehn J et al (2016)<sup>49</sup>** conducted a randomized controlled clinical trial to evaluate the effect of PRF on soft tissue thickening and bone loss around implants. 31 implants were placed in 31 patient in the mandible using a split-flap technique. In the test group (10 patients), mucosa was treated with a PRF membrane. In the control group (21 patients), implantation was realized without soft tissue augmentation. This study concludes that soft tissue augmentation with PRF performed with a split-flap technique cannot be recommended for thickening thin mucosa.

**Sachdeva S et al (2016)<sup>50</sup>** presented a case report on management of buccal dehiscence of implant using PRF. This case report presents the successful management of buccal dehiscence of single-staged implant for replacement of left central incisor with the platelet-rich fibrin (PRF) and autogenous bone graft material. The initial mobility was completely resolved after 12 weeks.

**Rastogi S. et al (2016)<sup>51</sup>** conducted a clinical and prospective study to evaluate the versatility of PRF in the management of alveolar osteitis. 100 adult patients with age group ranging from 18 to 40 years along with established dry socket after maxillary and mandibular molar extractions who have not received any treatment for the same were included in the study. PRF was placed in the maxillary and mandibular molar extraction sockets after adequate irrigation of the socket. There was significant reduction in pain associated with AO at the 3rd and 7th postoperative day along with better wound healing by the end of 2nd week.

**Biswas S et al (2016)**<sup>52</sup> conducted a study to compare bioactive glass and PRF in treating furcation defect. The 20 mandibular molar furcation defects with grade II furcation defect according to Glickman's classification were randomly allocated as follows: Group I, 10 furcation defects were treated using bioactive glass bone graft putty material; Group II, 10 furcation defects were treated using platelet rich fibrin (PRF). They concluded that use of bioactive glass osteostimulative biomaterial yields superior clinical results, including increased pocket depth reduction of class II furcation defects as compared to an autologous platelet concentrate.

**Bembi NN (2016)**<sup>53</sup> conducted a study To compare and evaluate clinically and radiographically the bone regeneration and the amount of bone fill in intrabony component of periodontal osseous defects through the osteoconductive and osteostimulative effect of bioactive glass. Twenty-two sites in 11 patients, within the age range of 25 to 60 years, showing intrabony defects were selected according to split mouth design and divided into group I and group II. All the selected sites were assessed with the clinical and radiographic parameters. At the end of study it was found that bioactive glass improve healing outcomes and lead to a reduction of probing depth, a resolution of osseous defects, and a gain in clinical attachment.



The following clinical, experimental prospective study was carried out in the Department of Periodontology, Babu Banarasi Das College of Dental Sciences (BBDCODS), Lucknow. Patients were selected based upon the following inclusion and exclusion criteria.

### **Inclusion criteria -**

- Chronic periodontitis Patients with grade II (Glickman classification, 1953) furcation defects.
- Patients who have not undergone any periodontal therapy in the last 6 months.

### **Exclusion criteria –**

- Patients with any systemic diseases that will affect the periodontal treatment outcome.
- Patients who have used antibiotics in the last 3 months.
- Smokers, tobacco and/or pan masala chewers.
- Subjects with a known allergy to the material being used.
- Pregnant and lactating women.

### **Materials:-**

1. Local anaesthetic agent 2% Lignocaine (Xicaine).
2. Bioactive glass putty (Novabone Putty, Osteogenic Biomedical).
3. Bioactive glass particulate (Novabone Dental Morsels).
4. Syringe 3ml and 5ml.
5. Bone graft carrier.
6. Bone graft condenser.
7. Adams tissue holding forceps.

8. A set of surgical curettes.
9. Castroviejo scissors.
10. Castroviejo needle holder.
11. Sutures (4-0) non-resorbable braided silk.
12. Nabers probe.
13. PRF centrifuge (Remi centrifuge R303).
14. Coe-pack dressing.

**Study design:**

Patients fulfilling the above mentioned inclusion and exclusion criteria were selected from the O.P.D of the department. The treatment procedure was fully explained to the patient and a duly signed consent form was taken from each patient before initiating the procedure. 30 sites fulfilling the criteria were evaluated and then randomly distributed into two groups viz. Group A and Group B.

- Group A – grade II furcation defect treated with bioactive glass (Novabone putty, osteogenic biomedical) and PRF.
- Group B - grade II furcation defect treated with crystalline composite (composed of oxides of silicon, calcium, sodium & phosphorous) in a silica base (Novabone morsel) and PRF.

At Baseline following parameters were recorded:

**Clinical parameter:**

- Gingival Index ( Loe and Silness, 1963).
- Plaque Index (Silness and Loe, 1964).
- Horizontal defect depth (HDD) of furcation (using Nabers probe).

### Radiographic parameter

- Radiographic defect depth (RDD) of furcation (using CBCT).

The patients selected were subjected to Cone Beam Computed Tomography (CBCT) that was done at Raydent i-CAT Dental and Maxillofacial Imaging Center, Gomtinagar, Lucknow. Data were captured at a resolution of 0.4 mm voxel size and exposure time of 20 s (110 kVp, 2.6 mA and 13.6 mA s). Images were then obtained in sagittal and coronal sections at constant slice thickness of 1 mm. CBCT was preferred as it provided multiple sections of dental anatomy with considerably lower radiation exposure. It provides near to accurate changes in the bone morphology with multiple fields of view and higher resolution and help authenticate the study.

Radiographic defect depth was measured on the CBCT in coronal section. A vertical line drawn on the buccal aspect of the crown was taken as reference on the CBCT and all measurements was done from this line. Horizontal distance from this line to the deepest point in the furcation defect was termed RDD.

### Surgical procedure

All the subjects underwent Phase I therapy. They were recalled after one month, those who fulfilled all inclusion and exclusion criteria were included in the study.

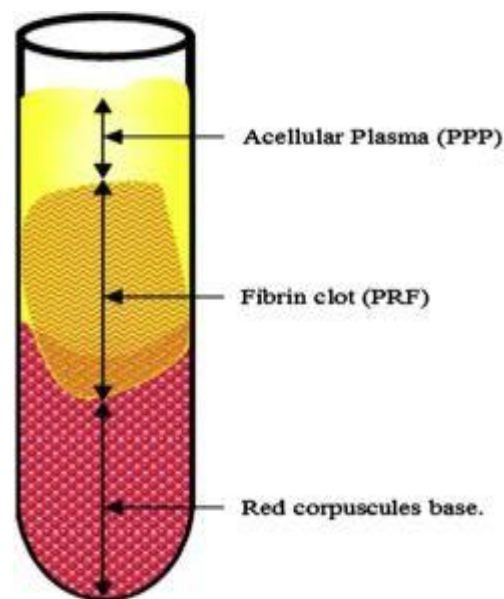
All clinical and radiographic parameters were recorded as Baseline readings. The patients were recalled for surgical procedures. They were asked for a pre-procedural rinse with 10 ml of 0.2% chlorhexidine gluconate solution for 1 minute. The surgical procedure was performed under aseptic conditions.

The operative sites were anesthetized with a solution of 2% lignocaine with 1:80,000 adrenaline. Sulcular incisions were given and full thickness flap was reflected. If required vertical

incision were given to reposition the flap coronally. The surgical area was then irrigated with sterile saline and was carefully inspected to ensure complete debridement.

To obtain PRF, 10 ml blood was drawn from the median cubital vein from the cubital fossa and was placed in sterilized test tubes without anticoagulant and centrifuged immediately at 3300 rpm for 10 min using the centrifuge (Remi centrifuge R303). The resultant product consisted of the following three layers

1. Topmost layer – a cellular platelet poor plasma
2. Middle – Platelet Rich Fibrin (PRF)
3. Bottom layer – Red blood corpuscles



The acellular plasma layer was discarded and the PRF clot was retrieved along with the associated RBC layer with tweezers from the test tube. The RBC Layer just below PRF-RBC junction was cut using scissors. The PRF clot was then placed on a glass slab over a gauge piece and gently compressed using another glass slab to remove excess serum.

The defect sites in Group A were grafted with Novabone Putty and condensed into the furcation defect. Similar surgical procedure was done for Group B. The sites were grafted with Novabone morsel. Care was taken to avoid overfilling the defect so as to ensure adequate closure

of flap. PRF was placed over the graft, which also served the purpose of membrane. The flap was sutured in close approximation using interrupted sutures and tension free primary closure of the flaps was achieved. Surgical site was protected by applying periodontal dressing.

Antibiotics and analgesics were prescribed for both the groups. Patients were recalled after 10 days for suture removal, dressing removal and examination. Plaque control was reinforced at the time of suture removal.

Patients were recalled for clinical re-evaluation at 3 months and 6 months post operatively. Radiographic evaluation was done 6 months post-operatively. At each visit, plaque control measures were reinforced and supra-gingival scaling was done if required.

At the end of the study, the entire data thus collected was subjected to suitable statistical analysis and interpretation for final results.

**Statistical analysis**

The results are presented in mean $\pm$ SD. The Unpaired t-test was used to compare the continuous variables between the groups. The Paired t-test was used to compare change in the continuous variables from baseline to subsequent time periods. The mixed linear was applied to find the quarterly change in GI and PI. The p-value <0.05 was considered significant. All the analysis was carried out on SPSS 16.0 version (Chicago, Inc., USA).

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**Clinical parameters****1. Comparison of PI between Group A and Group B at Baseline, 3 months and 6 months**  
**(post- operatively)****Inter-Group:-**

PI score were recorded at three time intervals (baseline, 3 month, 6 month) in both the groups as given in table 1.

At baseline, the mean PI reading for Group A was  $1.98 \pm 0.66$  and Group B was  $2.00 \pm 0.69$ . The p-value for this was 0.95, which is statistically non-significant.

3 months post-operatively, the mean PI reading for Group A was  $1.93 \pm 0.64$  and Group B was  $1.78 \pm 0.66$ . The p-value for this was 0.54 which is statistically non-significant.

At 6 months post-operatively the mean PI reading for Group A was  $1.88 \pm 0.65$  and Group B was  $1.63 \pm 0.65$ . The p-value for this was 0.30 which is statistically non-significant.

<b>Time periods</b>	<b>Group A</b>	<b>Group B</b>	<b>p-value<sup>1</sup></b>
Baseline	$1.98 \pm 0.66$	$2.00 \pm 0.69$	0.95
3 months	$1.93 \pm 0.64$	$1.78 \pm 0.66$	0.54
6 months	$1.88 \pm 0.65$	$1.63 \pm 0.65$	0.30

<sup>1</sup>Unpaired t-test

**Table-1: Comparison of PI between the groups across the time periods**

**Intra-Group**

In Group A, the mean PI at baseline was  $1.98 \pm 0.66$  that reduced to  $1.93 \pm 0.64$  after 3 months, showing a reduction of  $0.05 \pm 0.06$ . This change was found to be statistically significant.

In Group A, the mean PI at baseline was  $1.98 \pm 0.66$  that reduced to  $1.88 \pm 0.65$  after 6 months, showing a reduction of  $0.10 \pm 0.07$ . This change was found to be statistically significant.

In Group A, the mean PI at 3 months was  $1.93 \pm 0.64$  that reduced to  $1.88 \pm 0.65$  after 6 months, showing a reduction of  $0.04 \pm 0.003$ . This change was found to be statistically significant.

In Group B, the mean PI at baseline was  $2.00 \pm 0.69$  that reduced to  $1.78 \pm 0.66$  after 3 months, showing a reduction of  $0.21 \pm 0.12$ . This change was found to be statistically significant.

In Group B, the mean PI at baseline was  $2.00 \pm 0.69$  that reduced to  $1.63 \pm 0.65$  after 6 months, showing a reduction of  $0.36 \pm 0.18$ . This change was found to be statistically significant.

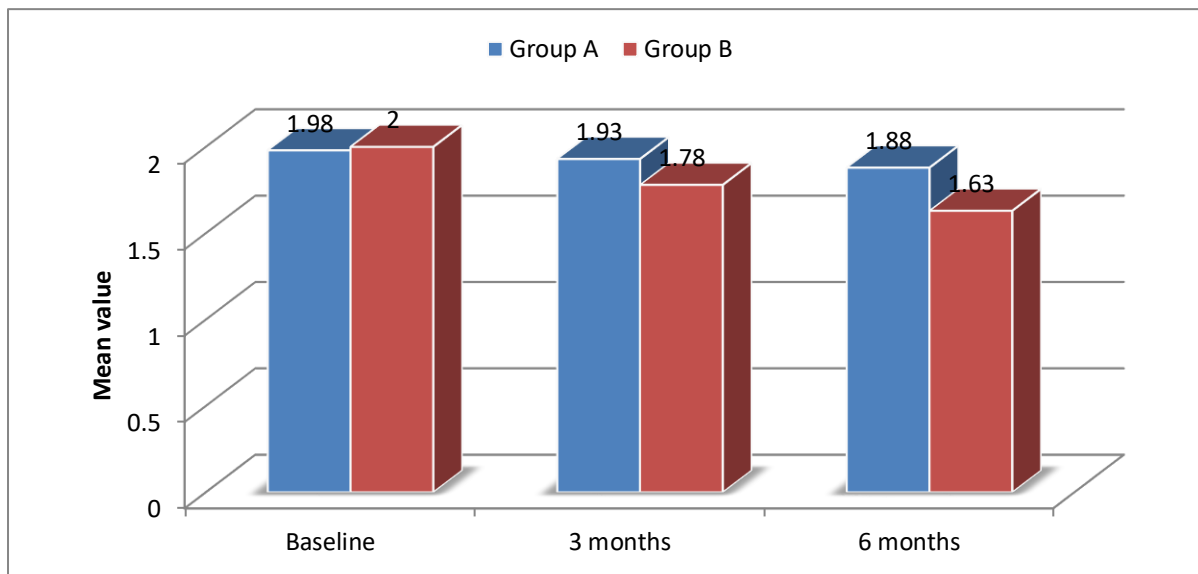
In Group B, the mean PI at 3 months was  $1.78 \pm 0.66$  that reduced to  $1.63 \pm 0.65$  after 6 months, showing a reduction of  $0.14 \pm 0.10$ . This change was found to be statistically significant.



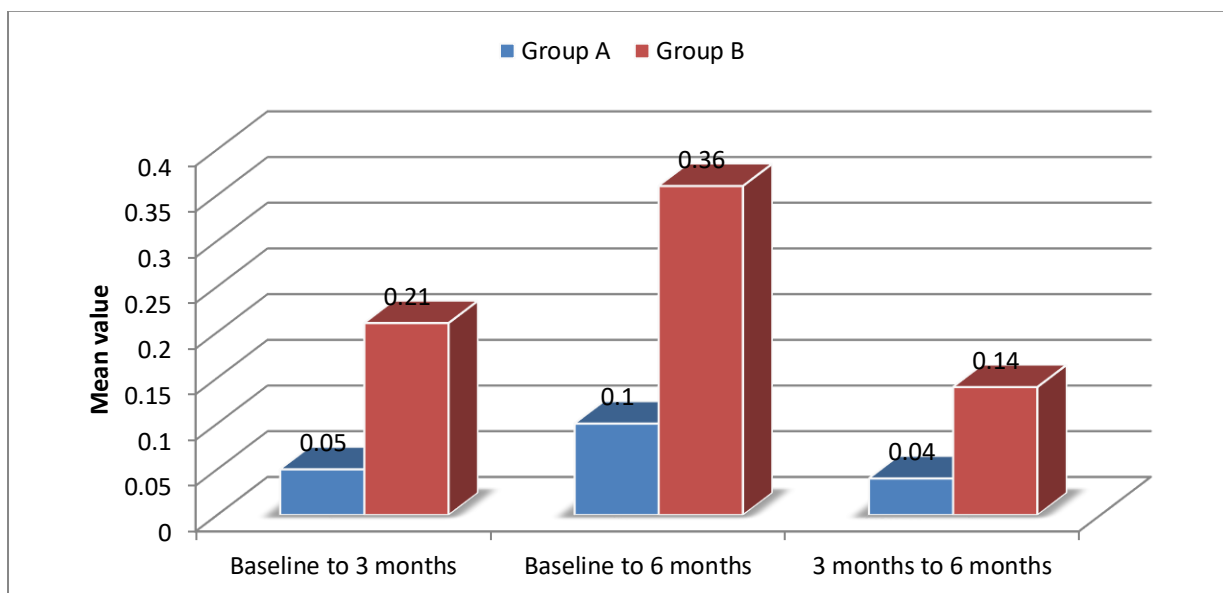
Time periods	Group A		Group B	
	Mean change	p-value <sup>1</sup>	Mean change	p-value <sup>1</sup>
Baseline to 3 months	0.05±0.06	0.004*	0.21±0.12	0.0001*
Baseline to 6 months	0.10±0.07	0.001*	0.36±0.18	0.0001*
3 months to 6 months	0.04±0.003	0.001*	0.14±0.10	0.0001*

<sup>1</sup>Paired t-test, \*Significant

**Table-2: Comparison of mean change in PI from baseline to subsequent time periods in Group A and Group B**



**Fig. 1: Comparison of PI between the groups across the time periods**



**Fig. 2: Comparison of mean change in PI from baseline to subsequent time periods in Group A and Group B**

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**Comparison of GI between Group A and Group B at Baseline, 3 months and 6 months**  
**(post- operatively)**

**Inter-Group:**

GI score were recorded at these time intervals in both the groups.

At baseline, the mean GI reading for Group A was  $2.01 \pm 0.62$  and Group B was  $2.07 \pm 0.75$ . The p-value for this was 0.80 which is statistically non-significant.

3 months post-operatively, the mean GI reading for Group A was  $1.96 \pm 0.62$  and Group B was  $1.84 \pm 0.71$ . The p-value for this was 0.62, it was statistically non-significant.

At 6 months post-operatively the mean GI readings for Group A was  $1.92 \pm 0.61$  and Group B was  $1.65 \pm 0.65$ . The p-value for this was 0.25 that was statistically non-significant.

<b>Time periods</b>	<b>Group A</b>	<b>Group B</b>	<b>p-value<sup>1</sup></b>
Baseline	$2.01 \pm 0.62$	$2.07 \pm 0.75$	0.80
3 months	$1.96 \pm 0.62$	$1.84 \pm 0.71$	0.62
6 months	$1.92 \pm 0.61$	$1.65 \pm 0.65$	0.25

<sup>1</sup>Unpaired t-test

**Table-3: Comparison of GI between the groups across the time periods**

**Intra-Group**

In Group A, the mean GI at baseline was  $2.01 \pm 0.62$  that reduced to  $1.96 \pm 0.62$  after 3 months, showing a reduction of  $0.04 \pm 0.01$ . This change was found to be statistically significant.

In Group A, the mean GI at baseline was  $2.01 \pm 0.62$  that reduced to  $1.92 \pm 0.61$  after 6 months, showing a reduction of  $0.08 \pm 0.02$ . This change was found to be statistically significant.

In Group A, the mean GI at 3 month was  $1.96 \pm 0.62$  that reduced to  $1.92 \pm 0.61$  after 6 months, showing a reduction of  $0.03 \pm 0.01$ . This change was found to be statistically significant.

In Group B, the mean GI at baseline was  $2.07 \pm 0.75$  that reduced to  $1.84 \pm 0.71$  after 3 months, showing a reduction of  $0.22 \pm 0.12$ . This change was found to be statistically significant.

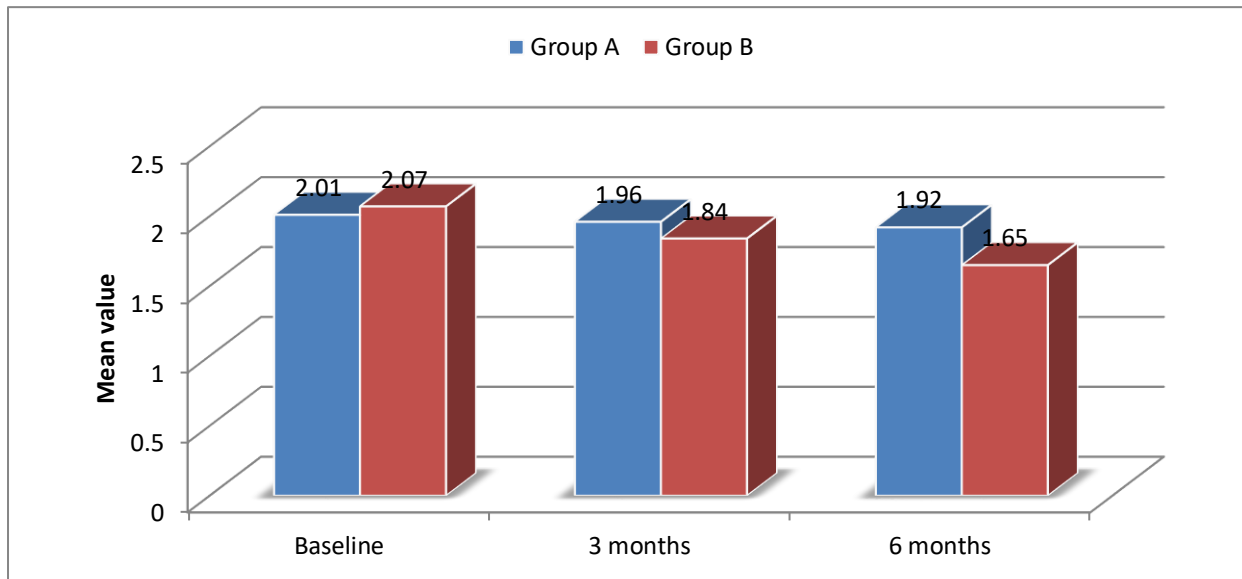
In Group B, the mean GI at baseline was  $2.07 \pm 0.75$  that reduced to  $1.65 \pm 0.65$  after 6 months, showing a reduction of  $0.42 \pm 0.22$ . This change was found to be statistically significant.

In Group B, the mean GI at 3 months was  $1.84 \pm 0.71$  that reduced to  $1.65 \pm 0.65$  after 6 months, showing a reduction of  $0.19 \pm 0.14$ . This change was found to be statistically significant.

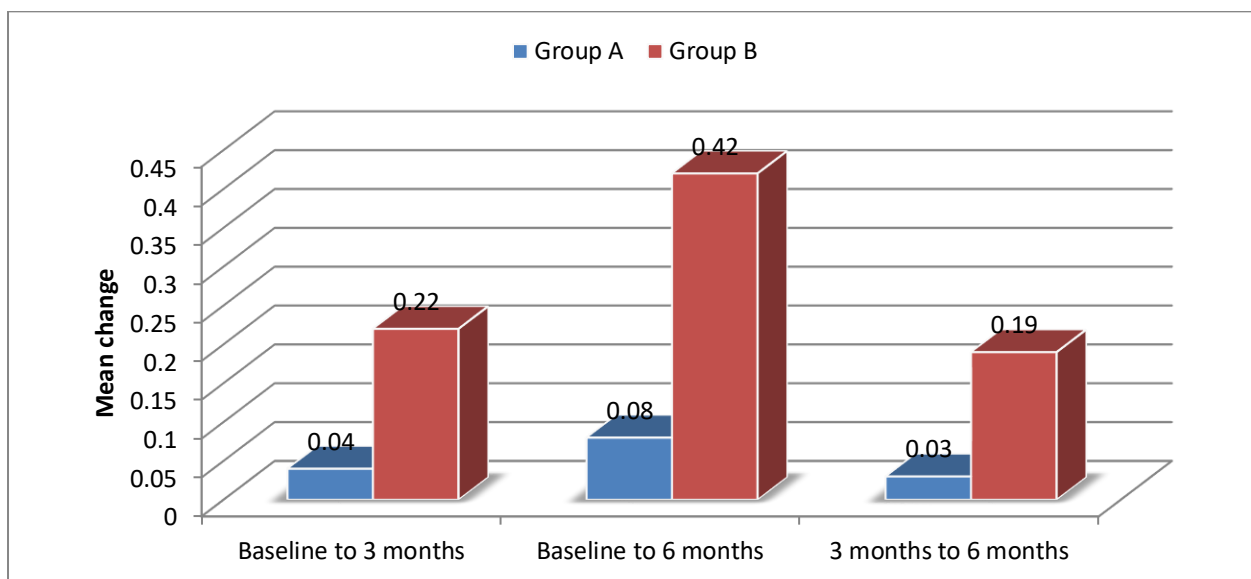
Time periods	Group A		Group B	
	Mean change	p-value <sup>1</sup>	Mean change	p-value <sup>1</sup>
Baseline to 3 months	0.04±0.01	0.001*	0.22±0.12	0.0001*
Baseline to 6 months	0.08±0.02	0.001*	0.42±0.22	0.0001*
3 months to 6 months	0.03±0.01	0.001*	0.19±0.14	0.0001*

<sup>1</sup>Paired t-test, \*Significant

**Table-4: Comparison of mean change in GI from baseline to subsequent time periods in Group A and Group B.**



**Fig. 3: Comparison of GI between the groups across the time periods**



**Fig. 4: Comparison of mean change in GI from baseline to subsequent time periods in Group A and Group B**

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**2. Comparison of Clinical Horizontal Probing Depth(HPD) between Group A and Group B at Baseline and 6 months (post- operatively)**

**Inter-Group:**

At baseline the mean HPD reading for Group A was  $4.20 \pm 0.77$  and Group B was  $4.13 \pm 0.99$ , the p-value for both the groups was 0.83 that was statistically non- significant.

6 months post-operatively, the mean HPD reading for Group A was  $2.13 \pm 0.97$  and Group B was  $3.00 \pm 0.75$ , the p-value for both the groups was 0.01 that was statistically significant.

It was also observed that HPD reduction was higher in Group A as compared to Group B.

<b>Time periods</b>	<b>Group A</b>	<b>Group B</b>	<b>p-value<sup>1</sup></b>
Baseline	$4.20 \pm 0.77$	$4.13 \pm 0.99$	0.83
6 months	$2.13 \pm 0.97$	$3.00 \pm 0.75$	0.01*

<sup>1</sup>Unpaired t-test, \*Significant

**. Table-5: Comparison of clinical HPD between the groups across the time periods**

**Intra-Group:**

In Group A, the mean HPD at baseline was  $4.20 \pm 0.77$  that reduced to  $2.13 \pm 0.97$  after 6 months showing a reduction of  $2.06 \pm 1.01$ . This change was found to be statistically significant.

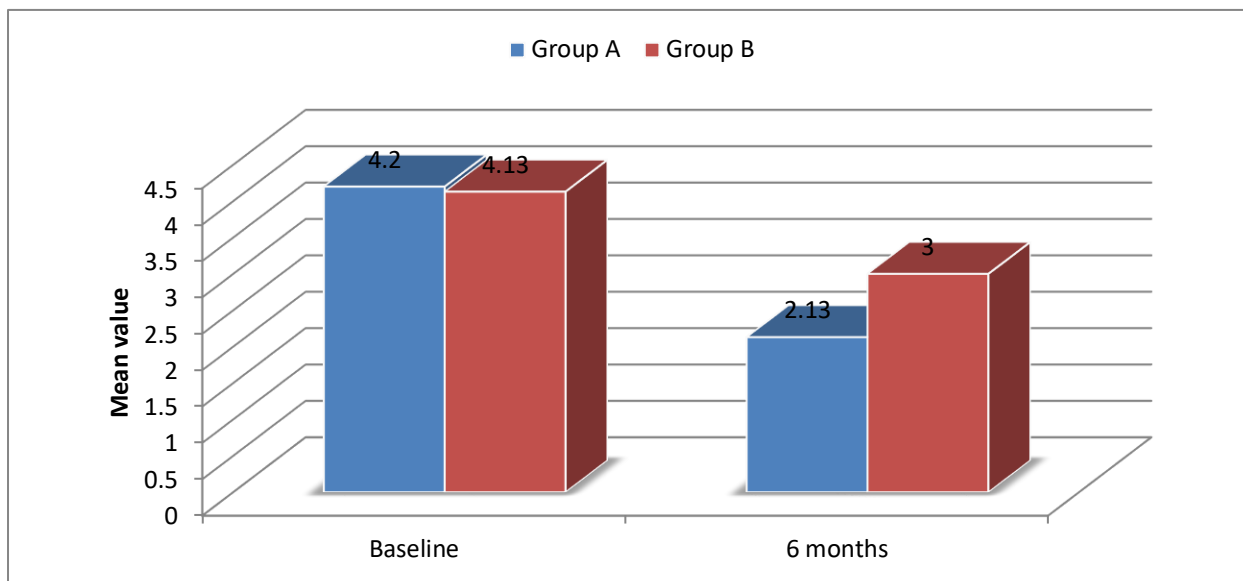
In Group B, the mean HPD at baseline was  $4.13 \pm 0.99$  that reduced to  $3.00 \pm 0.75$  after 6 months showing a reduction of  $1.13 \pm 0.74$ . This change was found to be statistically significant.

Groups	Baseline to 6 months	
	Mean change	p-value <sup>1</sup>
Group A	$2.06 \pm 1.01$	0.0001*
Group B	$1.13 \pm 0.74$	0.0001*

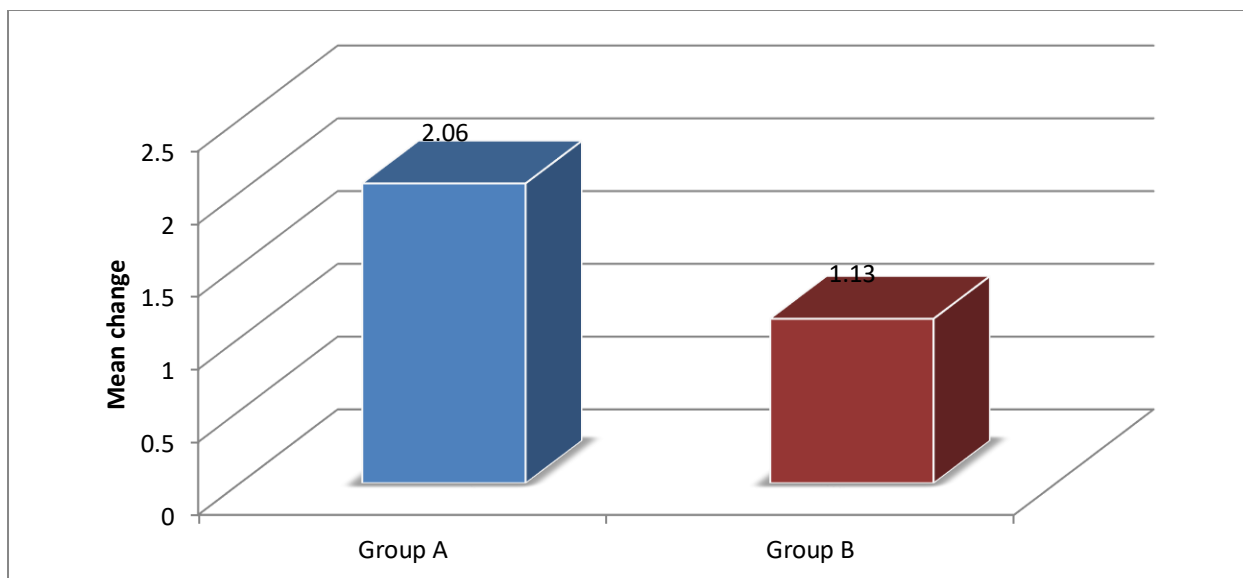
<sup>1</sup>Paired t-test, \*Significant

**Table-6: Comparison of mean change in clinical HPD from baseline to subsequent time periods in Group A and Group B**





**Fig. 5: Comparison of clinical HPD between the groups across the time periods**



**Fig. 6: Comparison of mean change in clinical HPD from baseline to subsequent time periods in Group A and Group B**

### 3. Comparison of Radiographic defect depth(RDD) between Group A and Group B at Baseline and 6 months (post- operatively) using CBCT

#### Inter-Group:

At baseline the mean RDD reading for Group A was  $4.26 \pm 0.67$  and Group B was  $4.16 \pm 0.99$ , the p-value for both the groups was 0.75 that was statistically non- significant.

6 months post-operatively, the mean RDD reading for Group A was  $2.26 \pm 0.77$  and Group B was  $2.93 \pm 0.78$ , the p-value for both the groups was 0.02 that was statistically significant.

It was also observed that RDD reduction was higher in Group A as compared to Group B.

Time periods	Group A	Group B	p-value <sup>1</sup>
Baseline	$4.26 \pm 0.67$	$4.16 \pm 0.99$	0.75
6 months	$2.26 \pm 0.77$	$2.93 \pm 0.78$	0.02*

<sup>1</sup>Unpaired t-test, \*Significant

**Table-7: Comparison of RDD between the groups across the time periods**

**Intra-Group:**

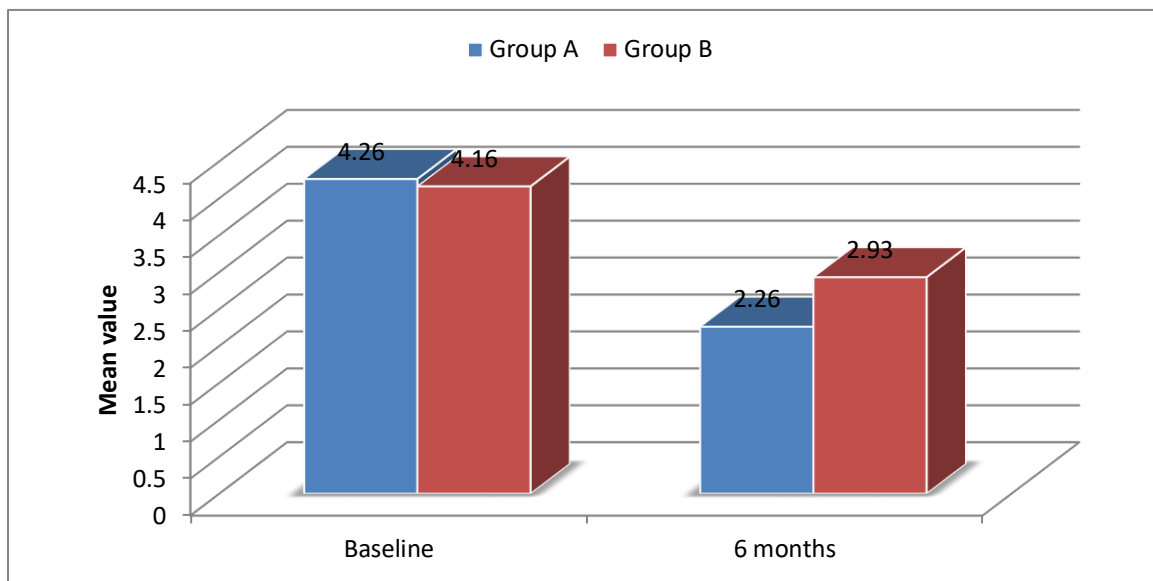
In Group A, the mean RDD at baseline was  $4.26 \pm 0.67$  that reduced to  $2.26 \pm 0.77$  after 6 months showing a reduction of  $2.00 \pm 0.88$ . This change was found to be statistically significant.

In Group B, the mean RDD at baseline was  $2.26 \pm 0.77$  that reduced to  $2.93 \pm 0.78$  after 6 months showing a reduction of  $1.23 \pm 0.70$ . This change was found to be statistically significant.

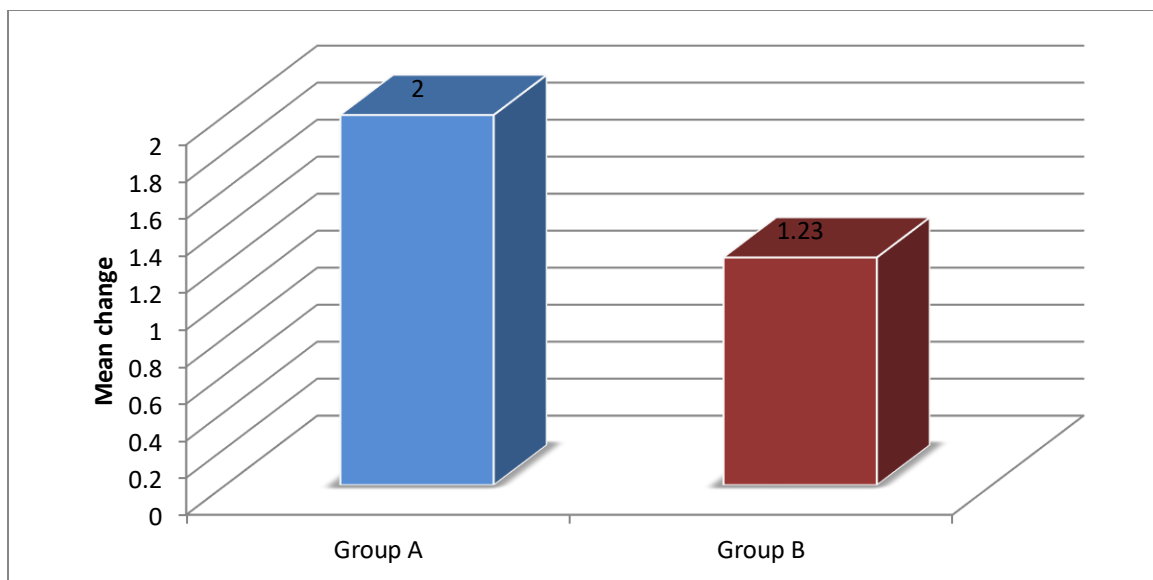
Groups	Baseline to 6 months	
	Mean change	p-value <sup>1</sup>
Group A	$2.00 \pm 0.88$	0.0001*
Group B	$1.23 \pm 0.70$	0.0001*

<sup>1</sup>Paired t-test, \*Significant

**Table-8: Comparison of mean change in RDD from baseline to subsequent time periods in Group A and Group B**



**Fig. 7: Comparison of RDD between the groups across the time periods**



**Fig. 8: Comparison of mean change in RDD from baseline to subsequent time periods in Group A and Group B**

Periodontal disease is an infectious, complex, multifactorial, chronic inflammatory disease of supporting periodontal tissues that alters the bone morphology. The goal of any periodontal therapy is the control of active inflammation, the arrest of disease progression and the reconstruction of structures lost to disease, where appropriate.<sup>54</sup> Different types of bony deformities such as horizontal, vertical, craters, and furcation result from periodontal disease, of which vertical and Grade II furcation defects are more amenable to regenerative periodontal therapy<sup>55</sup>. Periodontal regeneration is a multifactorial process and requires an orchestrated sequence of biological events including cell adhesion, migration, multiplication and differentiation which involves recruitment of locally derived progenitor cells to the site.<sup>56</sup>

Procedures for the treatment of molar furcation invasion defects range from open flap debridement, apically repositioned flap surgery, hemisection, tunneling or extraction to regenerative therapies using bone grafts or bone replacement grafts, guided tissue regeneration therapy or a combination of both.<sup>56-57</sup> There are many clinical studies assessing the value of regenerative therapies in the control and management of the molar furcation invasion.<sup>58-60</sup> Several clinical evaluations using regenerative techniques have reported the potential for osseous repair of treated furcation invasions.<sup>61</sup>

The effort to find a means to regenerate the periodontium has created a renaissance of research in the utilization of autogenous, allogenic, xenograft and alloplastic bone replacement materials in the treatment of periodontal defects.<sup>53</sup> Treatment modalities that use these graft materials come with their own set of advantages and inherent disadvantages, such as second surgical site for procurement of the graft and patients discomfort (autogenous graft), antigenic reactivity (xenograft), chance of transmission of diseases etc.<sup>62</sup>

The use of alloplastic grafts such as various types of (synthetic hydroxyapatite may result in clinically acceptable responses, but histologically the healing often occurs by

connective tissue encapsulation of the graft and formation of long junctional epithelium without periodontal regeneration.<sup>63-65</sup> Recent innovations have suggested a substantial role of a bioactive glass on bone regeneration in periodontal osseous defects.

Bioactive glass was invented by Larry Hench in 1971 as a four-component glass system, containing SiO<sub>2</sub>, CaO, Na<sub>2</sub>O, and P<sub>2</sub>O<sub>5</sub>. Bioactive glass has an osteostimulatory effect in addition to its osteoconductive properties.<sup>66</sup>

A material is said to be bioactive, if it gives an appropriate biological response and results in the formation of a bond between material and the tissue.<sup>67</sup> Bioactive materials used for either tissue replacement or for tissue regeneration must possess controlled chemical release kinetics that synchronise with the sequence of cellular changes occurring in wound repair. If dissolution rates are too rapid the ionic concentrations are too high to be effective. If the rates are too slow the concentrations are too low to stimulate cellular proliferation and differentiation.<sup>68</sup> Release of ions changes the local osmolarity and p<sup>H</sup> thus influencing the physiological condition surrounding the grafted site. This accounts for the additional antimicrobial property and faster healing of defect using bioactive glass.<sup>69</sup>

Bioactive glasses can be produced by both melting and a sol–gel process. Sol–gel-derived bioactive glasses can be tailored to have a controlled pore size with an improved biodegradation rate. Increasing the pore size and surface area leads to higher bioactivity.<sup>70-71</sup>

When a bioactive glass is present in an aqueous solution, it leads to formation of hydrated silica and polycrystalline hydroxycarbonate apatite (HCA) bi-layer on the glass surface. These reaction layers enhance adsorption and desorption of growth factors and influence the length of time macrophages are required to prepare the site for tissue repair. This is followed by attachment, synchronized proliferation and differentiation of osteoblasts. Mineralization of the matrix follows soon thereafter and mature osteocytes, encased in a collagen-HCA matrix, are the final product by 6–12 days.<sup>72-73</sup>

In our study bioglass was used in putty and particulate form. Putty consistency of bioactive glass consists of two particle phases: 1st Phase is 90–710  $\mu$  bioactive glass particles and 2nd Phase is 32–125  $\mu$  calcium phosphosilicate. Phase 2 particles enhance the physical characteristics and improve handling. Putty consistency makes it easy to manipulate and adapts well to defects. Spaces between particles permit rapid vascularization and bone ingrowth. Bone forms in several areas in the defect simultaneously, thus enhancing the regeneration.<sup>53</sup> Shapoff et al. reported that this material was quick to prepare and easy to mix and place. They also reported that the material remained where placed in the bony defect, even with suctioning adjacent to the surgical site.<sup>74</sup>

Platelet-rich fibrin (PRF) described by Choukroun et al. is a second-generation platelet concentrate which contains platelets and growth factors (FGF, BMP, PDEGF, PDGF-AB, TGF- $\beta$ , VEGF, IGF) in the form of fibrin membranes prepared from the patient's own blood free of any anticoagulant or other artificial biochemical modifications. The PRF clot forms a strong natural fibrin matrix, which concentrates almost 97% platelets and growth factors of the blood harvest.<sup>75-76</sup>

Platelets play a key role in wound healing and hence wound healing after periodontal treatment can be accelerated by the use of platelet concentrates. Growth factors from platelets that promote inflammation, angiogenesis, immune response and tissue repair. PRF not only release growth factors but also stabilizes the graft material. <sup>obarrio et al</sup><sup>77</sup>

A major advantage of PRF is its simplicity of preparation. The centrifugation process activates the coagulation process and as a result the clot is formed. This clot consists of a 3-dimensional fibrin network in which the platelets and other blood cells are entrapped. The release of growth factors from the PRF clot commences 5 to 10 minutes after clotting and continues for at least 60 to 300 minutes.<sup>78-79</sup> In another study Release of GF was found to continues for up to 28 days.<sup>80</sup> Several studies with Choukroun's PRF have shown the tissue

regenerative potential of this cell-loaded three-dimensional scaffold. Interestingly, this scaffold is also a carrier for mesenchymal cells (B cell, T cell, monocytes, stem cells etc) which have additional advantage in healing process like maintenance of antibacterial environment, debridement of wound, self-regulation of inflammation etc. PRF also contains cytokines that promotes homeostasis.

Dohan et al. reported that the interleukin (IL) 1 $\beta$ , IL 6, tumor necrosis factor- $\alpha$ , IL 4, vascular endothelial growth factor (VEGF) in the PRF clot play a crucial role in balancing the tissue homeostasis, whereas the healing cytokines IL 4 and VEGF inhibit inflammatory signal pathways thereby support and coordinate the neovascularization which may be the reason for uneventful healing in present case.<sup>81</sup>

The cone beam computed tomography (CBCT) is a digital and mathematical imaging technique that quantifies the bony defects in 3D. The reformed CBCT images using NewTom NNT display multiple panoramic and cross-sectional image that assess 3D changes in periodontal osseous defects accurately to the nearest of 0.01mm. Also CBCT has superiority in evaluating underlying bony changes. Hence in this study, CBCT was used to measure accurately the bone gain in grade II furcation defects.

This study was designed to investigate the treatment of grade II furcation defect (Glickman classification, 1953). In group A periodontal flap surgery was done using bioactive glass in putty form along with PRF and in group B periodontal surgery was done using particulate bioactive glass along with PRF. Results revealed that both treatment modalities resulted in significant improvement in hard and soft tissues.



**Clinical parameters**

The clinical parameters of group A and group B at baseline, 3 months and 6 months are discussed as follows:

PI in both group A and group B decreased from baseline to 6 month. The decrease was statistically significant for both the groups at all-time intervals. Similarly GI also decreased for both the group from baseline to 6 month and it was statistically significant at all-time intervals. This change can be explained by the antimicrobial property of bioactive glass<sup>37</sup>, oral hygiene instruction and periodic recall of the patients. The intrinsic antimicrobial property of bioactive glass is due to the ion dissolution process that starts immediately after the bone substitute has been implanted in the body.<sup>82</sup> These ions may damage the cytoplasmic membrane, denature the proteins or damage bacterial DNA. These ions increase the extracellular  $p^H$  which alters integrity of cytoplasmic membrane. Bacteria may adapt to compensate the increased  $p^H$  by adjusting intracellular  $p^H$ , leading to decreased enzymatic activity and metabolism<sup>83</sup>. The antimicrobial activity is higher against gram negative bacteria.<sup>84</sup>

Similar result of decreased GI and PI was found in a study by Kaur M et al (2010) while evaluating effect of platelet-rich plasma and bioactive glass in the treatment of intrabony defects.<sup>28</sup>

Chandrasekar SR (2015) evaluated the antimicrobial effect of bioactive glass. He concluded that bioactive glass has antimicrobial activity against early colonizers and this effect may be advantageous for a predictable regenerative periodontal therapy as bacterial colonization can hamper therapeutic success.<sup>45</sup>

Inter-group comparison of GI and PI in both the groups was statistically non-significant, affirming that both the groups were almost equally good.

Upon intragroup evaluation of HPD, it was observed that in group A there was a mean reduction of  $2.06 \pm 1.01$  mm from baseline to 6 months, which was statistically significant (p value 0.0001). Similar results were seen in group B as well that was statistically significant (p value 0.0001). Thus showing that both materials facilitated reduction of HPD in involved grade II furcations, which proves the efficacy of both the materials.

Inter-group comparison of HPD between group A and group B it was seen that group A showed higher reduction of HPD in furcation areas which was statistically significant at 6 months (p value = 0.01). So treatment modality for group A was found superior to group B after 6 months.

Outcome of any type of regenerative procedure is strongly dependent upon the available space under the mucoperiosteal flap.<sup>45,46</sup> In the present study, flap collapse was probably hindered by the use of BG. The success of using BG is also supported by the fact that BG may also have contributed to an increase in wound stability, which is a crucial factor for obtaining periodontal regeneration.<sup>46</sup>

### **Radiographic parameter**

In group A RDD at baseline was  $4.26 \pm 0.67$  mm which decreased to  $2.26 \pm 0.77$  mm after 6 months, showing a mean decrease in RDD of  $2.00 \pm 0.88$  mm and the P value for this was 0.0001, similarly in group B the RDD at baseline was  $4.16 \pm 0.99$  mm which decreased to  $2.93 \pm 0.78$  mm after 6 months with a mean reduction of  $1.23 \pm 0.70$  mm and P value of 0.0001. Statistically significant result was found in both the groups. So both treatment modalities were efficient in treating grade II furcation defect.

Inter-group comparison of RDD between group A and group B was found statistically significant with a P value 0.02 at 6 months. So treatment modality for group A was found superior to group B after 6 months.

The enhanced bone formation of the putty in this study is likely a reflection of their osteoconductive and bioactive potential. It is composed of a calcium-phosphorus-sodium-silicate (bioglass) particulate mixed with a synthetic binder that acts as a temporary binding agent for the particulate. On implantation, the binder is absorbed to permit tissue infiltration between the bioglass particles. The particles are then slowly absorbed and replaced by new bone tissue during the healing process<sup>80</sup>.

The negatively charged surface of the HCA layer attracts proteins such as growth factors and fibrin which act like an "organic glue" attracting osteoblastic stem cells to the layer which differentiate into osteoblasts and produce bone. Collagen attaches to the surface and embeds into the HCA layer. Apical migration of the junctional epithelium is indirectly inhibited by the extension of the collagen up to the junctional epithelium.<sup>48</sup>

Our results were similar to a study done by Kotsaki A (2013) in which he concluded that Calcium phosphosilicate putty can be a successful scaffold for new bone growth in GBR procedures and better bone formation is achieved <sup>81</sup>.

In another study done by Bandar A. Almaghrabi (2014), he stated that synthetic putty bone graft performs as well as human demineralized putty bone graft material in socket preservation procedures. They found that synthetic putty bone graft seemed to favor soft tissue healing. Histologically, the percentage of residual graft material for the human demineralized putty material was higher than the synthetic material tested in this study<sup>82</sup>.

Putty is a third generation synthetic bone graft substitute. The material is cohesive and provides adequate retention at the defect site. Various studies indicate a capability possessed by CPS particles to stimulate differentiation towards cell lineage with therapeutic potential in tissue engineering.<sup>1,2</sup> This unique phenomenon (osteostimulation) occurs exclusively with CPS based substitutes and has been shown to be superior to conventional osteoconduction<sup>83</sup>.

Limitation of this study was 6 months follow up time, which could be regarded as rather shorter especially for the evaluation of osseous changes. Histology is the only valid method to visualize the investment of new periodontal ligament fibers to the root. Because of ethical consideration, neither reentry procedures permitting direct observation of bone fill nor histology were performed, so it is not possible for us to make any comment regarding the regeneration of a functional apparatus.

The findings of this study reveal that both forms of bioactive glass (Putty and particulate) are biocompatible, safe to use without causing any inadvertent tissue response and yield good results for the treatment of grade II furcation defects. 6 months after the surgical intervention, there was no significant difference in GI and PI between both the groups. There was highly significant reduction in HPD and RDD in both the groups and putty was found superior to morsels when intergroup comparison was done. Limitation to this study was follow up duration of 6 months, which could have been increased. Further study with greater follow up period may reveal additional informations.

# 1. Ubiquitination in Periodontal Disease: A Review.

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# Appendices

## APPENDIX – I

### ETHICAL COMMITTEE APPROVAL FORM

**Babu Banarasi Das University**  
**Babu Banarasi Das College of Dental Sciences,**  
**BBD City, Faizabad Road, Lucknow – 226028 (INDIA)**

**Dr. Lakshmi Bala**

Professor and Head Biochemistry and  
Member-Secretary, Institutional Ethics Committee

**Communication of the Decision of the IIIrd Institutional Ethics Sub - Committee**

**IEC Code: 05**

**BBDCODS/05/2016**

**Title of the Project** Effectiveness of bioactive glass in putty and particulate form for the treatment of grade II furcation defects - A comparative study

**Principal Investigator:** Dr. Rajeev Kumar

**Department:** Periodontology

**Name and Address of the Institution:** BBD College of Dental Sciences Lucknow.

**Type of Submission:** New, MDS Project Protocol

Dear Dr. Rajeev Kumar,

The Institutional Ethics Sub- Committee meeting comprising following four members was held on 03<sup>rd</sup> May, 2016.

- |    |                                       |   |
|----|---------------------------------------|---|
| 1. | Dr. Lakshmi Bala<br>Member Secretary  | Prof. and Head, Department of Biochemistry, BBDCODS,<br>Lucknow   |
| 2. | Dr. Narendra Kumar<br>Gupta<br>Member | Prof., Department of Prosthodontics, BBDCODS,<br>Lucknow          |
| 3. | Dr. Smita Govila<br>Member            | Reader, Department of Conservative Dentistry,<br>BBDCODS, Lucknow |
| 4. | Dr. Subhash Singh                     | Reader, Department of Pedodontics, BBDCODS, Lucknow               |

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.

The proposal was reviewed, comments were communicated to PI thereafter it was revised.

**Decisions:** The committee approved the above protocol from ethics point of view.

Forwarded by:

*Lakshmi Bala*  
12/05/16  
(Dr. Lakshmi Bala)  
Member-Secretary  
IEC  
Institutional Ethic Committee  
BBD College of Dental Sciences  
BBD University  
Faizabad Road, Lucknow-226028

*Vivek Govila*  
(Dr. Vivek Govila)  
Principal  
BBDCODS  
PRINCIPAL  
Babu Banarasi Das College of Dental Sciences  
(Babu Banarasi Das University)  
BBD City, Faizabad Road, Lucknow-226028

**APPENDIX-2**

**CONSENT FORM**

Title of the Study .....

Study Number.....

Subject's Full Name.....

Date of Birth/Age .....

Address.....

Phone no. and e-mail address.....

1. I confirm that I have read and understood the Participant Information Document dated .....for the above study and have had the opportunity to ask questions. **OR** I have been explained the nature of the study by the Investigator and had the opportunity to ask questions.
2. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.
3. I understand that the sponsor of the project, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. However, I understand that my Identity will not be revealed in any information released to third parties or published.
4. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).
5. I permit the use of stored sample (tooth/tissue/blood) for future research. **Yes**  
[ ] **No** [ ] **Not Applicable** [ ]
6. I agree to participate in the above study. I have been explained about the complications and side effects, if any, and have fully understood them. I have also read and understood the participant/volunteer's Information document given to me.



Signature (or Thumb impression) of the Subject/Legally Acceptable Representative:.....

Signatory's Name..... Date .....

Signature of the Investigator..... Date.....

Study Investigator's Name..... Date.....

Signature of the witness..... Date.....

Name of the witness.....

Received a signed copy of the PID and consent form

Signature/thumb impression of the subject or legally acceptable representative Date.....

**APPENDIX-3**

**BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES  
(A Constituent Institution of Babu Banarasi Das University)  
BBD City, Faizabad Road, Lucknow – 227105 (INDIA)**

**Participant Information Document (PID)**

**1. Study Title**

Effectiveness of Bioactive glass in putty and particulate form for the treatment of grade II furcation defects - A comparative study.

**2. Invitation Paragraph**

You are being invited to take part in a research study, it therefore is important for you to understand why the study is being done and what it will involve. Please take time to read the following information carefully. Ask us for any clarifications or further information. Whether or not you wish to take part is your decision.

**3. What is the purpose of the study?**

The purpose of study is to find out which bone graft is better in treating furcation defect among Novabone putty and Novabone Dental Morsal.

**4. Why have I been chosen?**

You have been chosen for this study as you are fulfilling the required criteria for the diseased condition.

**5. Do I have to take part?**

Your participation in the research is entirely voluntary. If you do, you will be given this information sheet to keep and will be asked to sign a consent form. During the study you still are free to withdraw at any time and without giving a reason.

**6. What will happen to me if I take part?**

This study will last for 6 months and you will be recalled 3 times , first at the time of surgery and then 3 months and 6 months after surgery as a periodic recall. Procedure includes flap surgery along with placement of bone graft and a graft

like material prepared from your own blood. CBCT scan will be taken prior to surgery and 6 months post operatively.

**7. What do I have to do?**

You do not have to change your regular lifestyles for the study. You can drive, play sports, and take medicine etc. as usual.

**8. What is the procedure that is being tested?**

Formation of new bone by using synthetic bone graft material in the furcation defect is being tested.

**9. What are the interventions for the study?**

Flap surgery along with PRF and bone graft will be done.

**10. What are the side effects of taking part?**

There are no side effects on patients of this study.

**11. What are the possible disadvantages and risks of taking part?**

There are no possible disadvantages for the patients of this study.

**12. What are the possible benefits of taking part?**

Your diseased condition will be eliminated efficiently

**13. What if new information becomes available?**

If additional information becomes available during the course of the research you will be told about these and you are free to discuss it with your researcher, your researcher will tell you weather you want to continue in the study. If you decide to withdraw, your researcher will make arrangements for your withdrawal. If you decide to continue in the study, you may be asked to sign an updated consent form.

**14. What happens when the research study stops?**

If the study stops/finishes before the stipulated time, this will be explained to the patient/volunteer.

**15. What if something goes wrong?**

If any severe adverse event occurs, or something goes wrong during the study, the complaints will be handled by reporting to the institution (s), and IEC.

**16. Will my taking part in this study be kept confidential?**

Yes it will be kept confidential.

**17. What will happen to the results of the research study?**

The results of the study may determine to choose between different graft material for treating furcation defect.

**18. Who is organizing the research?**

This research study will be partially sponsored by the candidate and partially by you.

**19. Will the results of the study be made available after study is over?**

Yes.

**20. Who has reviewed the study?**

The study has been reviewed and approved by the Head of the Dept. and the IEC of the institution.

**21. Contact for further information**

Dr Rajeev Kumar  
Babu Banarasi College of Dental Sciences  
Lucknow  
[rajeev.10jan@live.com](mailto:rajeev.10jan@live.com)  
9956349827

OR Dr. Laxmi Bala,  
Member Secretary,  
Babu Banarasi College of Dental Sciences  
Lucknow  
[bbdcods\\_iec@gmail.com](mailto:bbdcods_iec@gmail.com)

**APPENDIX-4**  
**CASE HISTORY PROFORMA**

**Date:**

**OPD no:**

**Name:**

**Age:**

**Sex:**

**Address:**

**Mobile no.:**

**Occupation:**

**CHIEF COMPLAINT (S):**

**PAST MEDICAL AND DENTAL HISTORY**

**INDICES**

**PLAQUE INDEX:**

**At baseline**

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

**PI scoring**

**At 3 month**

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

**PI scoring**

**At 6 months**

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

**PI scoring**

**GINGIVAL INDEX: (At baseline)**

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

**GI scoring**

**At 3 months**

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

**GI scoring**

**At 6 months**

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

**GI scoring**

**HDD**

<b>Baseline</b>	<b>At 6 moonths</b>

**RDD**

<b>Baseline</b>	<b>At 6 moonths</b>

**ANNEXURE – 5**

Group Name	Group	SNO	Clinical								Radiographic	
			GI			PI			Horizontal probing depth (HPD) (mm)		Horizontal probing depth (HPD) (mm)	
			baseline	3 month	6 month	baseline	3 month	6 month	baseline	6 month	baseline	6 month
Putty	Group A	1	2.12	2.08	2.04	1.91	1.87	1.83	5	2.5	4.5	2.5
		2	1.75	1.7	1.66	1.66	1.62	1.58	5	2.5	5	2.5
		3	2.87	2.83	2.79	2.66	2.62	2.58	3	1	4.5	2.5
		4	2.16	2.12	2.08	1.95	1.91	1.87	4	2	4	2
		5	1.54	1.5	1.45	1.25	1.2	1.16	5	4	5.5	3.5
		6	1.54	1.5	1.45	2.95	2.91	2.87	4	2	4	2
		7	2.66	2.62	2.58	2.45	2.16	2.12	5	2	4.5	2
		8	1.54	1.5	1.45	2.91	2.87	2.83	4	1	4.5	1.5
		9	2.58	2.54	2.5	2.12	2.08	2.04	3	1	3	1
		10	1.37	1.33	1.29	1.25	1.2	1.16	5	2	5	2
		11	0.91	0.87	0.87	0.79	0.75	0.7	4	2	4	2
		12	2.91	2.87	2.83	2.79	2.7	2.66	4	1	4	1.5
		13	1.83	1.79	1.75	1.7	1.66	1.62	5	2	4.5	2
		14	1.58	1.54	1.5	1.45	1.41	1.37	4	4	4	4
		15	2.79	2.7	2.66	2	2	1.95	3	3	3	3
MORSELS	Group B	1	2.95	2.91	2.87	2.79	2.7	2.66	4	2	4	2
		2	2.62	2.16	2.04	1.54	1.41	1.25	4	2	4	2
		3	1.12	1	0.95	2.58	2.12	1.83	4	3	4	2
		4	2.95	2.7	2.54	1.5	1.2	1.08	6	4	6	4
		5	2.16	1.91	1.83	1.7	1.58	1.45	4	3	4.5	3
		6	2.79	2.54	2.12	1.87	1.75	1.58	4	3	4	3
		7	1.2	1.04	0.91	2.58	2.16	2.08	6	4	6	4
		8	1.66	1.45	1.29	1.12	1	0.91	5	4	5	4
		9	2.87	2.62	2.16	1.08	0.91	0.79	4	2	4	2.5
		10	2.83	2.54	2.04	1.79	1.58	1.49	3	3	3	3
		11	1.33	1.16	1.04	2.91	2.58	2.58	3	3	3	3
		12	1.83	1.7	1.58	1.37	1.08	0.95	3	2	3	2
		13	1.08	0.87	0.75	2.87	2.58	2.12	5	4	5	4
		14	2.54	2.04	1.79	1.41	1.29	1.12	4	3	4	3
		15	1.16	1.04	0.87	2.95	2.83	2.7	3	3	3	2.5



**ANNEXURE – 6****Formula used for the analysis****Mean and standard deviation (SD)**

The *sample mean* is the average and is computed as the sum of all the observed outcomes from the sample divided by the total number of events. We use  $\bar{x}$  as the symbol for the sample mean. In math terms,

$$\bar{x} = \frac{1}{n} \sum_{i=1}^n x$$

where  $n$  is the sample size and the  $x$  correspond to the observed values.

We define the *variance* to be

$$s^2 = \frac{1}{n-1} \sum_{i=1}^n (x - \bar{x})^2$$

and the *standard deviation* to be

$$s = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (x - \bar{x})^2}$$

**Unpaired t-test**

The unpaired t method tests the null hypothesis that the population means related to two independent, random samples from an approximately normal distribution are equal

$$t = \frac{x_1 - x_2}{\sqrt{1/n_1 + 1/n_2}}$$

$$s = \frac{\sum(x_j - \bar{x}_1)^2 + \sum(x_i - \bar{x}_2)^2}{(n_1 + n_2 - 2)}$$

where  $\bar{x}_1$  and  $\bar{x}_2$  are the sample means,  $s^2$  is the pooled sample variance,  $n_1$  and  $n_2$  are the sample sizes and  $t$  is a Student  $t$  quantile with  $n_1 + n_2 - 2$  degrees of freedom.

### **Paired t-test**

Paired sample t-test is a statistical technique that is used to compare two population means in the case of two samples that are correlated. Paired sample t-test is used in ‘before-after’ studies, or when the samples are the matched pairs, or when it is a case-control study. For example, if we give training to a company employee and we want to know whether or not the training had any impact on the efficiency of the employee, we could use the paired sample test. We collect data from the employee on a seven scale rating, before the training and after the training. By using the paired sample t-test, we can statistically conclude whether or not training has improved the efficiency of the employee. In medicine, by using the paired sample t-test, we can figure out whether or not a particular medicine will cure the illness.

$$t = \frac{d}{\sqrt{s^2/n}}$$

where  $d$  is the mean difference between two samples,  $s^2$  is the sample variance,  $n$  is the sample size and  $t$  is a paired sample t-test with  $n-1$  degrees of freedom.

The present study was conducted to evaluate the efficacy of putty and particulate form of bioactive glass for the treatment of grade II furcation defects and compare the same.

30 sites fulfilling the inclusion and exclusion criteria were selected from the OPD of the department of periodontics, BBDCODS Lucknow and were randomly divided into group A and group B. Sites in group A were treated with NOVABONE putty and PRF as membrane similarly sites in group B were treated with NOVABONE morsels and PRF as membrane. Clinical evaluation was done at baseline, 3 month and 6 month. Radiographic evaluation was done at baseline and 6 month.

After statistically analysis of the collected data we came to conclude that both treatment modalities were efficient for treating grade II furcation defects as there was decrease in GI, PI, HDD and RDD. Inter group comparison showed that putty was superior to morsels as HDD and RDD reduction was statistically significant.