COMPARATIVE EVALUATION OF CHORION MEMBRANE IN COMBINATION WITH DEMINERALIZED FREEZE DRIED BONE ALLOGRAFT AND DEMINERALIZED FREEZE DRIED BONE ALLOGRAFT ALONE IN TREATMENT OF GRADE II FURCATION DEFECTS: A CLINICORADIOGRAPHIC STUDY

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LIST OF ABBREVIATIONS

Sr. No.	Short Form	Full Form
1	GTR	Guided tissue regeneration
2	CM	Chorion membrane
3	DFDBA	Demineralized freeze dried bone allograft
4	BMP	Bone morphogenetic protein
5	IOPA	Intraoral periapical
6	2D	Two dimensional
7	3D	Three dimensional
8	CT	Computed tomography
9	CBCT	Cone beam computed tomography
10	DICOM	Digital imaging and communications in medicine
11	FOV	Field of view
12	ROI	Region of interest
13	CAL	Clinical attachment level
14	PPD	Probing pocket depth
15	PDL	Periodontal ligament
16	OFD	Open flap debridement
17	CPF	Coronally positioned flap
18	PI	Plaque index
19	GI	Gingival index
20	PAL	Probing attachment level
21	BOP	Bleeding on probing
22	PD	Probing depth
23	GR	Gingival recession
24	ePTFE	Expanded polytetrafluoroethylene
25	CAL-V	Vertical clinical attachment loss
26	CAL-H	Horizontal clinical attachment loss
27	RAL	Relative attachment level
28	RVG	Radiovisiography
29	BG	Bone gain

Sr. No.	Short Form	Full Form
30	ACM	Amnion-chorion membranes
31	dPTFE	Dense polytetrafluoroethylene membranes
32	VAS	Visual analog scale
33	DBM	Demineralised bone matrix
34	HPO	Human periosteal cells
35	ALP	Alkaline Phosphatase
36	EMD	Enamel matrix derivative
37	REC	Recession
38	FDBA	Freeze dried bone allograft
39	BDX	Bovine derived xenograft
40	DD	Defect depth
41	PDGF	Platelet derived growth factor
42	MSCs	Mesenchymal stem cells
43	AM	Amnion membrane
44	PRF	Platelet‑rich fibrin
45	RVCAL	Relative vertical clinical attachment level
46	GML	Gingival margin level
47	PMMA	Poly-methyl-methacrylate
48	PHEMA	Poly-hydroxyl-ethyl-methacrylate
49	AOC	Autogenous osseous coagulum
50	PLACA	Polylactic acid membrane softened with citric acid esters
51	CCD	Charge-coupled device
52	FI	Furcation involvement
53	DSR	Digital subtraction radiography
54	HIV	Human immunodeficiency virus
55	SD	Standard deviation
56	CEJ	Cementoenamel junction
57	FGM	Free gingival margin
58	BP	Base of the pocket
59	ABM/P-15	Anorganic bone matrix/cell binding peptide 15

INTRODUCTION

Chronic periodontitis is a complex bacterially induced chronic inflammatory disease which involves intricate interactions of the biofilm with the host immunoinflammatory response leading to subsequent alterations in bone and connective tissue homeostasis that results in destruction of the connective tissue and alveolar bone supporting the teeth.[1]

Mid 1960's marked the beginning of modern era, that speculated the evolution in knowledge and revolution of the conceptual models in understanding of pathogenesis of periodontal diseases. These models demonstrated the critical role of specific gram-negative, anaerobic or microaerophilic bacteria in the initiation of disease process[2], the

protective and destructive role of host immunoinflammatory response[3] and also implied that the genetic and environmental factors modified a range of host factors and clinical expression of the disease.

Periodontitis leads to loss of attachment and if untreated in initial stage may present with alveolar bone destruction. This pattern of alveolar bone loss is challenging in the furcation areas of the molars. The furcation area represents a unique periodontal site with anatomic and pathogenic characteristics suitable for colonization of the subgingival biofilm in the posterior teeth which may affect the bifurcation or trifurcation of multirooted teeth.

The American Academy of Periodontology has defined furcation involvement as the pathologic resorption of inter-radicular alveolar bone in the bifurcated or trifurcated area of a multirooted tooth where the roots diverge.[4] The complex anatomic morphology of the furcation area, such as root trunk length, root concavities, diameter of furcation entrance, cervical enamel projections, makes it difficult to debride this area properly during routine periodontal instrumentation. Furcation involvement is therefore an important risk factor for progression of further attachment loss, which at the same time, reduces the efficacy of periodontal therapy.

A peculiar feature of furcation lesion is the horizontal progression of periodontal attachment loss which implies that the pocket has a lateral extension toward the interior of the furcation, as well as a vertical extension along the root. This horizontal component of the pocket is considered as groundwork for the classification of the furcation lesions and is diagnosed by periodontal probing.[4]

Different classifications have used the extent of horizontal attachment loss to define the different degrees or categories.

A few of the widely used classifications for furcation are as follows:

1. Glickman (1953)[5]

Grade I - There is pocket formation into the furcation, but inter-radicular bone is intact.

Grade II - Consists of loss of inter-radicular bone and pocket formation but not extending through to the opposite side.

Grade III - Consists of through-and-through lesion.

Grade IV - Consists of through-and-through furcation involvement with gingival recession, leading to a clearly visible furcation area.

2. Hamp et al. (1975)[6]

Degree 0 - Furcation defect is not accessible with a periodontal probe.

Degree I - The horizontal loss of periodontal tissue support is up to 3mm.

Degree II - The horizontal loss of support exceeds 3 mm, but is not more than 6mm.

Degree III - Consists of through and through horizontal destruction of the periodontal tissue support in furcation area.

3. Tarnow & Fletcher (1984)[7]

Sub-divided each grade of horizontal involvement into three subgroups, based on the degree of vertical involvement

Subclass A - 0–3 mm

Subclass B - 4–6 mm

Subclass C - >7 mm

The treatment of furcation lesions is the core component of periodontal therapy. The treatment of furcation lesion facilitate elimination of the subgingival biofilm and calculus and establish an anatomy that facilitates proper plaque control and thus prevents further attachment loss.[4]

In the course of periodontal history, several techniques have been proposed and promoted to treat these furcated molars based on their degree of involvement and thus improve their prognosis. Various regenerative procedures have been tried with the aim of closing Grade II furcation, such as open flap debridement, bone replacement grafts, coronally repositioned flaps and guided tissue regeneration barriers.

Periodontal regeneration has been established as a viable therapeutic option for the treatment of various furcation defects, among which Class II defects represent a highly predictable scenario and the application of a combined therapeutic approach (i.e., barrier, bone replacement graft with or without biologics) appears to offer an advantage over monotherapeutic algorithms.[8]

The potential to regenerate furcation lesions in a predictable manner emerged with the development of the concept of guided tissue regeneration (GTR), which suggested placement of a barrier membrane between the flap and root surface to prevent both gingival connective tissue and gingival epithelial cells from repopulating the denuded root surface during healing and allowed cells from the periodontal ligament or the alveolar bone marrow to repopulate the furcation area, thus inducing the formation of new cementum and a new connective tissue attachment. The need to exclude both the epithelial and connective tissue cells of the gingiva from the wound area led to development and application of GTR membranes.

One of the newest membranes which has been also been used recently include placental membranes. The membranes being immunoprivileged possess antimicrobial and antigenic properties.[9] The membrane used in this study is chorion membrane (CM) with the size of membrane being 2 × 259 cm. The membrane has numerous growth factors, proteins and stem cell reserves that help in faster wound healing with regeneration of the lost tissues. The preserved human chorion membrane is a novel tissue engineered biomaterial that is recently applied in the field of medicine and dentistry to test its ability to regenerate the lost tissues and accelerate repair.

Bone replacement grafts are a group of materials used for periodontal regeneration. One of the properties of an ideal bone replacement graft is it should have potential to trigger osteogenesis. However, most of them have been shown to work on the principles of osteoinduction and/or osteoconduction. Pioneering work by Urist and Strates in 1960's demonstrated that demineralized bone has osteoinductive potential by stimulating bone formation in extraskeletal sites, and that, the osteoinductive potential of Demineralised freeze dried bone allograft (DFDBA) is related to the amount of bone morphogenetic proteins (BMPs) that remain after demineralization processing has been completed.[10] In this study DFDBA is used. The use of DFDBA has been the focus of much attention throughout the past 30 years as one such material which may be capable of promoting regeneration of the attachment apparatus.

There are various methods for evaluation of new attachment and periodontal reconstruction. Radiographic evaluation plays a decisive role in confirming and establishing diagnoses and also provides information on the type and severity of alveolar bone damage. Evidence of reconstruction of marginal periodontium can be obtained by clinical, radiographic, surgical re-entry or histologic procedures.

Traditionally intra-oral periapical (IOPA) radiograph were the mainstay to assess the periodontal bone loss, in addition to periodontal probing. But, periodontal probing has its own sets of limitation, and so do the IOPA radiographs as they represent the two dimensional (2D) image of three dimensional (3D) structure. The advent of digital imaging enabled the manipulation of contrast and density levels using specialized software and added considerable improvements to traditional IOPA radiographs. Nevertheless, they have their own sets of limitation including that they too represent 2D image of 3D structure and pose risk of overestimating or underestimating the amount of alveolar bone loss. The introduction of computed tomography (CT) has overcome the drawbacks associated with 2D images, however the application of CT imaging for periodontal diagnosis have unfavourable cost-benefit ratio, furthermore CT imaging exposes patient to high radiation dose. Recently cone beam computed tomography (CBCT) has been introduced in dentistry, which also offers 3D exploration and more accurate imaging than 2D imaging. The cost effectiveness and accuracy of CBCT led to its speedy ingress into dentistry and has proved useful in a wide range of application, especially related to implant site imaging.

CBCT uses a cone shaped X-ray beam and a reciprocating solid state flat panel detector, which rotates once around the patient's head, 180-369 degrees, scanning a defined anatomical volume. The single scan of a series of 360 exposures or projections, one for each degree of rotation, captures planed data which provides the raw digital data for reconstruction of the exposed volume by computer algorithm. In CBCT, voxel is used instead of pixel, since it is referring to volume and not to a 2D space. The image files are the DICOM (Digital Imaging and Communications in Medicine) format, which enables ease of telecommunication and usage with other third party imaging software.

The CBCT units can be classified into small, medium and large volume based on their size of field of view (FOV). Small-volume FOV offers higher image resolution because x-ray scattering is as the FOV decreases. The region of interest abbreviated as ROI, is the 3D volume to be evaluated. Smaller the ROI better the resolution. The radiation dose of CBCT is about 6-15 times lesser than conventional CT. The CBCT machine used in our study was the Orthophos® XG 3D/ Ceph manufactured by Sirona Dental Systems GmbH, Germany.[11]

Advantages of CBCT includes a rapid scan time ranging 5-40 seconds comparable to panoramic radiograph, less radiation dose when compared to conventional CT and comparable to full mouth IOPA, image accuracy as CBCT produces sub-millimeter resolution ranging from 0.4 mm to as low as 0.09 mm, less cost compared to conventional CT, availability of artifact suppression algorithms, allows multiplanar reformation and most important advantage of CBCT is that it provides unique 3D images demonstrating features that intraoral, panoramic, and cephalometric images cannot.

So far, the literature search did not reveal any clinical study that has been carried out solely to check the efficacy of DFDBA along with CM in Grade II furcation defects. The preliminary results with DFDBA and CM when used individually appear to be encouraging in terms of regeneration of the periodontal structures, and so, it was felt necessary to further study this material in the treatment of Grade II furcation defects. Also, CBCT is one of the latest methods of evaluation of periodontal regeneration and there are very few studies that have used CBCT for evaluation of regeneration using CM. So, the present study was planned to evaluate and compare the efficacy of DFDBA

alone and when used with CM in the treatment of Grade II furcation defects clinically and radiographically by CBCT.

AIM AND OBJECTIVES

The study was aimed to evaluate and compare demineralized freeze dried bone allograft (DFDBA) alone and in combination with chorion membrane (CM) in treatment of Grade II furcation defects, clinically and radiographically by CBCT.

Also, attached to this aim were certain objectives:

1. To clinically evaluate the efficacy of DFDBA alone in the treatment of Grade II furcation defects.
2. To clinically evaluate the efficacy of DFDBA in combination with CM in the treatment of Grade II furcation defects.

3. To clinically evaluate and compare the clinical attachment level (CAL) and probing pocket depth (PPD) in Grade II furcation defects treated with DFDBA alone and in combination with CM.
4. To evaluate and compare by CBCT, the amount of bone fill leading to reduction in bone defect volume in Grade II furcation defects treated DFDBA alone and in combination with CM, 6 months postoperatively.

REVIEW OF LITERATURE

Considering the complexity of current techniques for treatment of furcation involvement and in view of the long term results, predictable regeneration of the periodontium at furcation involved sites would represent a considerable progress. Regeneration can be achieved following a variety of surgical procedures involving root conditioning, often combined with coronally advanced flaps, bone grafts, organic or synthetic barrier membranes etc. The crucial element to tissue regeneration is to stimulate a coordinated cascade of healing events which can result in assimilation of periodontal tissues. Like other treatments, it is not a panacea for all patients affected with periodontitis, but research gives us enough evidence to support the use of regenerative therapies in

periodontology. However, assessment of regeneration also presents a challenge. A variety of outcomes of interest can be considered to assess the effectiveness of regenerative therapies in furcation defects: 1) clinical: closed measurements and open measurements 2) radiographic: furcation fill and changes in density; 3) histologic: evidence of periodontal regeneration and characteristics of different tissue compartments; 4) microbiologic 5) patient reported. Radiographs provide abundant information about the periodontium and until now, this information has not been obtained by any other non - invasive methods. But radiographs give a 2D representation of 3D structures and their limitations are well known. Complex anatomic structures, such as cortical plates of teeth may be superimposed on the region of interest. In addition, the exact form of periodontal defects including hemiseptal defects, intrabony defects and furcation involvements cannot be determined from radiographs. To overcome the inherent difficulties of conventional radiography, 3D image analysis by CBCT has been introduced and used in this study.

Considering the paramount literature available and for the ease of understanding, the review of literature has been segregated into four parts

1. Review of studies on Guided Tissue Regeneration (GTR)
2. Review of studies on Bone grafts
3. Review of studies on the combination therapy (GTR + Bone grafts)
4. Review of studies on the methods of analysis of regeneration

1. Review of Studies on Guided Tissue Regeneration (GTR)

Nyman S et al. (1982)[12] did a study to examine whether new cementum and new attachment would form during healing of wound, if preference is given to periodontal ligament (PDL) cells to repopulate the wound area adjacent to root surgically deprived of its PDL and cementum layer. The study experiment was conducted on three adult monkeys wherein a Millipore filter was placed on maxillary lateral incisors and mandibular canines. After the reflection of mucoperiosteal flap and removal of approximal levels of alveolar bone, notches were prepared along root surface for demarcation and Millipore membrane was placed. After repositioning the flap, the jaws were removed after 6 months for histological sections. In all the specimens, new cementum with inserting collagen fibers was observed on the curetted root surfaces and the newly formed cementum was thicker in the apical part than at coronal level. At the coronal part healing was frequently characterized by connective tissue adhesion to the root surface without signs of cementum formation and fibrous attachment. The finding of extensive cementum formation including fibrous attachment and absence of signs of ankylosis in the present material can therefore be explained by the limited possibility for bone cells to invade the wound adjacent to the roots. The findings suggest that the design of the surgically treated wound and the ability of the PDL cells helped to repopulate the curetted root surfaces.

Pontoriero R et al. (1987)[13] evaluated the regenerative potential of the periodontal tissues in human degree II and III furcation defects using the principles of GTR. The study comprised of 37 subjects with bilateral furcation defects wherein the Control group were treated with open flap debridement (OFD) alone and the Test group

received OFD and a Teflon membrane was placed to cover the entrance of the furcation and surrounding bone apical to the alveolar crest. 6 months evaluation demonstrated that in the treatment of degree II furcation defects at mandibular molars using the principles of GTR 19 sites out of 21 resulted in the resolution of the furcation defect. Conventional therapy showed resolution of furcation defect in less than 20% of the cases. The degree III furcation defects also responded well to GTR therapy where complete resolution occurred in 4 out of 16 sites, partial resolution occurred in 9 out of 16 sites while at 3 sites, a degree III involvement persisted. The Control group in treatment of degree III furcation involvements failed to result in the resolution of any of the furcation defects. Thus, there are reasons to suggest that GTR therapy is a superior alternative to conventional treatment of degree II furcation involvements in mandibular molars. Also, the findings suggest that the healing of degree II mandibular furcation defects might occur after single GTR procedure and healing of degree III furcation defect may require 2 or more surgical interventions. The first procedure would convert a degree III to degree II involvement and the second surgical procedure might result in closure of furcation defect.

Andersson B et al. (1994)[14] compared the healing of buccal Class II furcation defects in mandibular molar after treatment by GTR technique or coronally positioned flap (CPF) technique. The study comprised of total of 18 bilateral furcation defects in 8 subjects, where 9 defects were treated with GTR technique and 9 defects with CPF technique. Clinical assessment for plaque index (PI), gingival index (GI), PPD, probing attachment level (PAL) and bleeding on probing (BOP) were made at baseline, 3 months and 6 months post-operatively. The radiographic examination was performed using conventional radiographs at baseline and 12 months postoperatively and digital

subtraction imaging was performed to evaluate the difference at baseline and at 12 months. The results demonstrated a statistically significant reduction in PPD at 6 and 12 months post-operatively and a tendency for gain of PAL at 6 months postoperatively in the GTR treated teeth. The teeth treated with CPF technique group showed a statistically significant reduction of PPD at 6 months and a tendency to reduction after 12 months. On radiological assessment after 12 months, 2 furcation defects of GTR group demonstrated gain of interradicular bone tissue whereas the CPF group did not demonstrate any gain of bone tissue. Even though no statistically significant differences were observed in any parameters between the 2 treatment modalities, the findings suggest effective healing in treatment of Class II molar furcation defects using GTR technique.

Wang HL et al. (1994)[15] designed a study to evaluate the effect of Type I bovine collagen for GTR in treatment of Class II furcation defects. The study comprised of 12 patients, 6 males and 6 females diagnosed with advanced periodontitis exhibiting bilateral Class II mandibular furcation defects with attachment loss ≥ 6mm. After the completion of initial therapy, the defects were randomly assigned into Test group who received flap debridement and collagen membrane and Control group who received flap debridement alone. All clinical data including probing depth (PD), CAL, gingival recession (GR), PI and GI and measurements from stent to base of defect, crestal bone to base of defect, width of defect, and mobility were recorded at baseline and 2, 4 and 6 months post-operatively and at 12 month re-entry surgery. Statistical analysis was performed utilizing the paired t test. On comparison of 12 month re-entry to baseline, both Control and Test groups demonstrated significant improvement in all the clinical parameters. However, there was no significant difference in PD, CAL, GR, width of

defect, and mobility between Control and Test groups. The Test group treated with collagen membrane showed statistically greater improvement in defect fill and vertical bone fill than Control group at re-entry. A significant improvement of furcation horizontal bone repair and defect improvement was noted in the collagen membranes-treated sites as compared to the presurgery status. No adverse tissue reaction, infection or delayed healing was observed in either group during this study. This study suggests that positive role of collagen membrane in the treatment of Class II furcation defects.

Hugoson A et al. (1995)[16] conducted a multi-center study with 38 patients demonstrating atleast two contralateral molar Class II furcation defects to evaluate the GTR therapy using a bioresorbable matrix barrier (Test) and a non-resorbable expanded polytetrafluoroethylene (ePTFE) barrier (Control). After initial therapy, mucoperiosteal flap were reflected followed by placement of barriers membranes to cover the furcation entrance and flaps repositioned to completely cover the barriers. A second surgical procedure was performed 4 to 6 weeks after implantation to remove the non-resorbable barrier at the Control sites. At baseline and 12 months post-surgically PI, GI, PD, CAL, and position of gingival margin were recorded for all the patients. The change in clinical attachment level in the horizontal direction (CAL- change) was used as a primary variable to address the efficacy of the barriers. Statistical analysis revealed reduction in PD at both the sites and more pronounced GR at Control sites. The gain of attachment in both horizontal and vertical direction was more pronounced at the Test sites as compared to the Control sites and was found to be statistically significant ($p \leq 0.05$). At Control sites, only the gain of attachment in horizontal direction was statistically significant. Also, the postsurgical complications were more frequent following the treatment at Control site ($p \leq 0.05$). Thus, the findings of the study suggest that use of

bioresorbable barrier results in significant gain in CAL in horizontal direction, lesser GR and also less post-operative complications.

Eickholz P et al. (2000)[17] clinically and radiographically compared the effects of GTR using 2 different bioabsorbable barriers comprising of experimental (Test), polydioxanon membrane to standard (Control) polylactideacetyltributyl citrate membrane. The study consisted of 21 patients, exhibiting 22 pairs of similar contra lateral defects (30 intrabony and 14 Class II furcation lesions) and each defect was randomly assigned for treatment with either Control or Test barrier membrane devices. PI, GI, PD, and vertical and horizontal clinical attachment loss (CAL-V; CAL-H) and standardized radiographs were obtained at baseline and 12 months post-surgery. The differences in the changes from baseline to 12 months in both the groups were compared using paired-*t* test Wilcoxon signed rank test. At 4 weeks post-surgery 61% of all barriers were exposed to some extent. At 12 months, all the defects in both the groups observed a significant reduction in GI, PD and CAL-V gain. However, no statistically significant difference for PI, GI, PD and CAL-V was seen between both the groups at baseline and at 12 month. The analysis revealed significant bony fill within intrabony defects and a significant but small CAL-H gain within furcation defects. The findings suggest that the use of both bioabsorbable barriers may be considered for GTR therapy.

Cury PR et al. (2003)[18] evaluated the results and long term stability of GTR using a bioabsorbable membrane in Class II furcation defects, compared to OFD. A total of 18 furcation defects in 9 patients diagnosed with chronic periodontitis and demonstrating 2 comparable Class II furcation defects were selected and randomly assigned to either

Test (GTR) or Control (OFD) group. The clinical measurements and standardized vertical bitewing radiographs were obtained for all the patients at baseline, and at 6, 12, 18, and 24 months. The radiographs were taken with long cone paralleling technique and analyzed by subtraction radiography. There were statistical significant reduction in the PD for both groups ($p<0.007$, $p<0.0005$, respectively); however the differences between groups were not significant at any examination. The intra-group and inter-group differences in the gain of CAL-V were not significant ($p>0.05$). The gain in CAL-H was significant in the Test group over 24 months but was not significant for compared to Control ($p<0.03$). In the sites treated with GTR, 2 sites showed complete closure, one was converted to Class I, and one tooth was lost due to root resorption. In the sites treated with OFD alone, 2 defects progressed to Class III over 24 months. The Test group showed gain in bone height at 12 and 18 months, however the gain was significant only at 24 months ($p=0.015$) and the bone height was stable for Control group at 24 months. The findings of the study suggest that GTR may provide promising results over OFD, in terms of greater horizontal CAL gain and greater possibility for complete closure of some defects and stability of the outcome over time.

Kothiwale SV (2013)[19] conducted a foremost innovative study to evaluate the effect of GTR using CM in periodontal pocket therapy clinically and radiographically. The study was a single blind randomized controlled clinical trial comprising of 10 patients (6 males and 4 females) with moderate to severe periodontitis with mean age of 38.2 years. The quadrants were divided into Study and Control sites wherein the Study sites received conventional periodontal pocket therapy and placement of CM whereas the Control sites received conventional periodontal pocket therapy alone. All the sites were evaluated clinically for GI, PI, PPD and relative attachment level (RAL) at baseline and

12 months and radiographically using radiovisuography (RVG) by paralleling technique for bone gain (BG) and density at baseline, 6 months and 12 months. After stastical analysis using student *t* test, stastically significant differences were found for all clinical parameters in both the sites at 12 months. The study site showed highly significant reduction in GI (0.40 ± 0.08) with p = 0.0001, PI (0.41 ± 0.18), PPD (2.50 ± 0.53) with p = 0.0431 and an increased BG of 0.86 ± 0.18. Also, the radiographic findings of the sites treated with CM demonstrated significant BG and density than the Control sites. The author thus concluded that CM when used along with conventional periodontal pocket therapy can have influence on clinical parameters.

Chakraborthy S et al. (2015)[20] carried out a study to clinically evaluate and compare the efficacy of amnion membrane (AM) and CM in combination with coronally advanced flap in the treatment of gingival recession. A total of 12 systemically healthy patients exhibiting at least 2 bilateral Miller's Class I or Class II GR were selected and randomly allocated to Group I where recession defects were treated with amnion allograft along with coronally advanced flap and Group II where recession defects were treated with chorion allograft along with coronally advanced flap. All the clinical parameters such as GI, PI, GR, RAL, width of the recession defect, width of keratinized gingiva and percentage of root coverage were evaluated at baseline, 3 and 6 months post-surgery. In Group I the mean decrease in length of recession was 1.58 ± 1.14 mm, gain in attachment level was 2.17 ± 1.53 mm and total mean percentage of root coverage was 22%. In Group II the mean decrease in length of recession was 2.00 ± 1.54 mm, gain in attachment level was1.58 ± 1.22 mm and total mean percentage of root coverage was 34%. Considering the results, both AM and CM prove to be versatile allografts and novel tissue engineered biomaterials in the treatment of root coverage.

Holtzclaw D (2015)[21] conducted case series to present a simplified method of Schneiderian membrane perforation repair with amnion-chorion membranes (ACM) in 9 cases with Schneiderian membrane perforation via lateral window technique. A retrospective record was obtained in which 77 cases were identified with a total of 104 sinus augmentations, of which nine perforations were noted. Of these 9 cases, all occurred due to manual hand instrumentation and none of them were aborted mid-procedure and all perforations were repaired with ACMs. Following repair of the membrane, the sinus augmentation was done using combination of allograft and xenograft particulate bone. A total of 23 dental implants were placed in the augmented sites after an average of 4.9 months. A total of 158 dental implants were placed in non-perforated augmented sinuses. Of these only 1 failure was noted in the repaired site while only 3 failures were noted in non-perforated site which occurred during pre-loading phase of healing. The findings of the case series suggest that ACM is a potential and viable treatment option for repair of Schneiderian membrane perforation.

Hassan M et al. (2017)[22] used a split-mouth, single-blind, randomized trial design to compare the effectiveness of amnion-chorion membranes (ACM) and dense polytetrafluoroethylene membranes (dPTFE) membranes in ridge preservation, particularly when they are intentionally left exposed. 22 non molar sites on the same arch were used to compare treatments with the two membranes. Evaluation for ridge dimensions was done clinically and radiographically with CBCT prior to and 3 months after ridge preservation. The study also evaluated postoperative discomfort which was recorded with Visual Analog Scale (VAS). A mixed ANOVA model was used to compare the differences between the treatments. There was no statistical difference in the clinical and radiographic ridge dimensions between the two treatments. Also,

significantly more osteoid and higher bone volume density but less graft particles and bone surface density were seen at ACM sites compared with dPTFE sites. However, there was no statistical difference in mineralized bone area and soft tissue area between the two treatments. Importantly, sites treated with ACM had significantly lower postoperative VAS scores than those treated with dPTFE. Hence, it can be concluded that ACM membrane when intentionally exposed is equally effective in ridge preservation and also aids in reducing postoperative VAS scores due to its healing properties, and potentially result in better bone quality for implant placement.

2. Review of Studies on Bone grafts

Quintero G et al. (1982)[23] clinically evaluated the osteogenic potential of DFDBA at 6 months in the treatment of human periodontal osseous defects. A total of 27 osseous defects in 11 patients with one-, two- or three- wall morphology were treated with cortical bone which was obtained from human donor within 24 hours after death under sterile conditions, decalcified, freeze-dried, ground and sieved to particle size of 250 to 500μm. After completion of pre-surgery, clinical record measurements were made using a stent and graduated periodontal probe before surgery, at the time of surgery, and at re-entry, which were supplemented with radiographs and photographs. The mean bone fill of defect of one-, two- and three- wall was 61.5%, 62% and 73% respectively. The combined mean regeneration for all osseous defects was 2.4 mm, or 655 of defect fill. The results of the study demonstrate that DFDBA has some potential as an osseous grafting material in treatment of periodontal osseous defects.

Fucini SE et al. (1993)[24] suggested that efficacy of periodontal grafting procedures might improve with different ranges of DFDBA particle sizes and compared the bony defect resolution obtained using two different particle size ranges of DFDBA in 11 patients with intrabony defects. Paired interproximal intrabony defects in 11 patients received the DFDBA graft material obtained from cortical bone of single donor which was ground to particle sizes of 250µ to 500µ or 850µ to 1000µ. Initial soft and hard tissue measurements were made immediately before the procedure and at re-entry after 6 months using an electronic constant-force probe. The bony defect fill for the large particle group was 1.66 mm (38.6%) and 1.32 mm (34.9%) for the small particle group, however this difference was not statistically significant. Hence, the choice of the particle size is a matter of personal preference and depends on the clinician's choice based on handling characteristics.

Reynolds MA et al. (1996)[25] histologically examined the fate of DFDBA used for regeneration in intrabony defects and secondarily compared the amount of new attachment apparatus formation, including component tissues, in relation to the presence or absence of residual graft material. Histologic data from prior studies was obtained to examine role of DFDBA in the formation of new attachment apparatus. In present study intrabony defects associated with 32 teeth in 12 patients were treated with and without DFDBA and were late recommended for extraction. The teeth were removed at 6 months en bloc and submitted for histologic examination. Histologic sections from 12 patients with 32 grafted defects revealed that 72% of the grafted defects exhibited residual DFDBA particles. The amalgamation of DFDBA particles within the new viable bone was clearly evident. Foe a within subject comparison, data was extracted from 14 sites of 5 patients treated with DFDBA graft material to compare the amount of

regeneration in relation to the presence or absence of residual graft material. The within subject comparison revealed that defects harbouring residual graft particles exhibited significantly greater amounts of new attachment apparatus formation (1.72 mm vs. 0.20 mm), including new bone (2.33 mm vs. 0.23 mm), cementum (1.74 mm vs. 0.23 mm), and associated periodontal ligament than sites without evidence of graft matrix ($p < 0.05$). There was no apparent difference in the nature of the new attachment apparatus or component tissues but only in amount of formation. The study thus suggests that inflammation and graft containment may be important factors that influence the fate of DFDBA and the regenerative response.

Zhang M et al. (1997)[26] approved of the fact that osteoinductive potential of demineralised bone matrix (DBM) is an important factor to consider before clinical use and hence conducted an invivo and invitro study to establish a quantitative means for assessment of the osteoinductive potential of DBM. Intramuscular and subcutaneous sites in 8-10 week old athymic mice were implanted with DBM, obtained from 14 cadaveric donors for invivo and invitro assay. A total of 82 (2 implants per mouse) athymic mice for in vivo assay were used which were then euthanized at suggested time interval. The calcium content of explants, as an indicator of new bone formation, were assayed and expressed as a change in the weight percent calcium in the explant as compared to the weight percent of calcium in the implanted material. The calcium content of DBM before implantation was 0.5 ± 0.048 weight percentage. The yield in calcium content at muscular implantation was found to be more osteoinductive (increase of 10.0 ± 0.4 calcium weight percent of explant, which was almost 20 times the calcium content of dry DBM) than subcutaneous implantation (increase of 1.62 ± 0.27 calcium weight percent of explant).There was no statistical difference in the weight of explanted

material and implanted material between weeks 1 to 4, however, the increase in explant weight began by week 5. Week 4 was chosen as the optimum time for explantation in the in vivo assay in that sufficient calcium levels had been achieved and increase in linear fashion was observed after 5 weeks. Dose response curves with DBM demonstrated maximal activity at the 20 mg dose. Human periosteal (HPO) cells were chosen for in vitro bioassay for DBM. Alkaline Phosphatase (ALP) activity was considered as an indicator of osteoblast induction which reached its highest level on day 5 of DBM treatment. The dose response studies for invitro assay with revealed that quantities approximating 5 to 10 mg DBM provided for maximal levels of alkaline phosphatase in cell extracts. Between in vivo calcium remineralization assay and the in vitro alkaline phosphatase assay of osteoinductivity of DBM a liner correlation was observed (R^2 = 0.7397). Thus it can be suggested that in vitro assay may be a good substitute for the in vivo assay in assessing the osteoinductive potential of DBM.

Zhang M et al. (1997)[27] investigated the relationship between residual calcium levels and particle size of ground DBM and its osteoinductive potential using in vitro and in vivo assays. The osteoinductive potential of the bone-derived biomaterial was assessed by measuring the degree of new bone formation (change in percent calcium content after 4 weeks of implantation) in the in vivo assay and levels of ALP activity associated with cultures of HPO cells in the in vitro assay, respectively. Both the assays demonstrated certain degree of osteoinductive potential in slightly DBM and overly DBM and showed maximum osteoinductive potential when bone was demineralized to levels of approximately 2% residual calcium. The DBM particles ranging from 500 to 710 microns provided for the highest level of calcium deposition (increase of 8.1 weight percent calcium) after 4 weeks of implantation in muscle pouches, whereas particles

less than 250 microns showed the lowest level of calcium deposition (increase of only 2.8 weight percent calcium). Also, with respect to age and gender, the study revealed that DBM from female donors in the 31 to 40 years old age group and male donors in the 41 to 50 year age group possess the highest osteoinductive potential. The DBM derived from donor bone from both female and male donors in the 51 to 60 year age group presented the lowest osteoinductive potential. However, there was no significant difference in osteoinductive potential for DBM derived from male and female donors.

Kimble KM et al. (2004)[28] conducted a clinical trial to determine whether the addition of DFDBA significantly influences the outcome of collagen membrane GTR-based root coverage procedures. A total of 20 patients exhibiting Miller's Class I or II recession defect participated in the study, of which, one defect per patient was treated with a collagen membrane covered by a CPF with or without placement of DFDBA. At baseline and 3 and 6 months post-surgery clinical parameters recorded for Test and Control sites included: recession depth, recession width, width of keratinized tissue, clinical CAL, and PD. All the surgical procedures were performed by single surgeon. Pre-surgery and post-surgery (6-month) data were obtained and stastical analysis for parametric data was carried by using Student's paired t test and for non-parametric data was carried by using the Wilcoxon matched pairs test. The percentage of root coverage for the sites treated with collagen membrane and with collagen membrane and DFDBA was 68.4% ± 15.2 % and 74.3% ± 11.7% respectively but the difference between the groups was not statistically significant. Also, both the groups demonstrated statistical significant improvement in recession depth, recession width, gain in CAL and width of keratinized gingiva at 6 months compared to baseline ($p < 0.05$); however, difference between the groups was not statistically significant. Thus the results of the study

suggest that application of both the GTR techniques with or without DFDBA result in better clinical outcomes with respect to decrease in recession depth and width, gain in CAL and width of keratinized tissue. Hence both GTR techniques are clinically effective in attaining root coverage.

Grunisky BS et al. (2004)[29] conducted a randomized controlled clinical trial to evaluate the use of DFDBA in combination with enamel matrix derivative (EMD) [EMD + DFDBA] compared to enamel matrix derivative [EMD] alone in the treatment of human intrabony periodontal defects. A total of 40 patients with 67 intrabony defects ≥ 3 mm were selected. 20 patents with 34 sites received treatment with EMD alone and 20 patients with 33 sites received combination treatment of EMD + DFDBA. All clinical soft and hard tissue measurements were made by UNC 15 probe at baseline and at 6 months re-entry surgery. The soft tissue measurements include PD, CAL, and recession (REC); whereas the hard tissue measurements include defect depth, alveolar crestal resorption, and defect wall morphology. Statistical analyses were performed using unpaired and paired Student *t* tests to compare hard and soft tissue measurements. Both the groups showed excellent soft tissue healing and no adverse complications. The sites treated with EMD alone exhibited reduction in PD of 4.0 ± 0.3 mm and CAL gain of 3.2 ± 0.3 mm. The sites receiving combination therapy showed reduction in PD of 3.6 ± 0.2 mm and gain in CAL of 3.0 ± 0.3 mm. There was significant improvement in soft tissue measurements at 6 months for both treatment groups ($P < 0.001$); however difference between the groups was not statistically significant. The mean bone fill in the EMD + DFDBA group was 3.7 ± 0.2 mm (74.9%), while the EMD alone group demonstrated a mean bone fill of 2.6 ± 0.4 mm (55.3%). The combination therapy yielded greater than

50% and 90% of gain in bone fill when compared to EMD alone. Thus application of EMD in addition to DFDBA may result in enhancement of hard tissue parameters.

Gajiwala AL et al. (2006)[30] clinically and radiographically assessed the osteogenic potential of DFDBA in the repair of human periapical osseous defects associated with devitalized teeth. The DFDBA was indigenously prepared for the first time in India by Tata Memorial Hospital Tissue Bank. After removal of periapical lesions associated with devitalized teeth, DFDBA was placed in periapical osseous defects in 10 healthy patients. Clinical and radiographic measurements were made for all the patients. Pre- and post-operative IOPA were made to assess osseous fill of the radiolucent periapical defects. All the patients responded well to therapy with no evidence of post-operative infection or any complication. At the end of 6-month, all the patients showed remarkable decrease in the grades of mobility, and 9 out of the 10 patients showed more than 50 % of mineralization of periapical osseous defects which was radiographically confirmed by evidence of normal bony trabeculae. The findings indicate that DFDBA is a cost effective, biocompatible material with osteogenic potential and is a viable treatment option for treatment of osseous defects.

Wood RA et al. (2012)[31] conducted a histological study to histologically evaluate and compare the percentage of new bone formation in the healing of non-molar extraction sockets grafted with freeze dried bone allograft (FDBA) versus DFDBA for ridge preservation. The secondary aim of this study was to compare dimensional changes in ridge height and width after grafting with these two materials. A total of 40 patients were randomly divided into two groups, comprising of 20 patients each where extraction sockets were filled with either FDBA or DFDBA. All the graft material was

procured from single donor and ground to particle size of 250 – 750 μm and DFDBA demineralised to 3.3 % residual calcium. Thus the only difference in the two materials was the percentage mineralization of the final bone graft. Approximately 19 weeks after grafting, a trephine drill was used to obtain a core biopsy of 2-mm diameter and ≈ 8 mm in length from each grafted site. A total of 32 biopsies (16 in each group) were analyzed histologically to determine percentage of vital bone, residual graft particles, and connective tissue / other non-bone components. There were no significant differences when comparing changes in alveolar ridge dimensions and percentage connective tissue / other non-bone components between groups. The sites treated with DFDBA showed significantly greater percentage of vital bone at 38.42% versus FDBA at 24.63% and lower percentage of residual graft particles at 8.88% compared to FDBA at 25.42%. This study provided the first human histologic and clinical evidence directly comparing the healing in ridge preservation with DFDBA versus FDBA in and demonstrated significantly greater new bone formation and lesser residual graft particles with DFDBA.

Ogihara S et al. (2014)[32] conducted a controlled randomized clinical trial to determine the relative efficacy of EMD/FDBA versus EMD/DFDBA in treatment of infrabony defects. A total of 69 patients exhibiting not more than 1 intrabony defect were randomly assigned to EMD/FDBA (EF) group (n=23), EMD/DFDBA (ED) group (n=23) and EMD alone without graft material (E) as a negative Control group (n=23). Minocycline (10mg/ml) was added to the composite graft material containing either FDBA or DFDBA. The clinical measurements were made at baseline and at 1 year and 3 year follow up. All groups demonstrated significant reduction in PD reduction and gain in CAL gain from baseline. The EF and ED groups showed better treatment

outcomes than the control group at 1 year and 3 years. There was no statistically significant difference between the EF and ED groups at 1 year and 3 years. Also all the groups demonstrated significant radiographic BG from baseline; but differences between the groups was not statically significant between 1 and 3 years for any group. Both EF and ED groups resulted in greater soft tissue improvement at 1 and 3 years follow-up compared with E group alone. Thus it is evident that both graft materials result in better soft issue and hard tissue improvements when combined with EMD and are better alternative in management of deep intrabony defects.

3. Review of Studies on the Combination therapy (GTR + Bone grafts)

Anderregg CR et al. (1991)[33]conducted a study to evaluate clinically the bone repair potential of DFDBA combined with a barrier material in the treatment of human molar furcation defects (Experimental) as compared to the barrier technique alone (Control). The study group comprised of fifteen pairs of Class II or III furcation invasion defects which were randomly treated with an e-PTFE and DFDBA or the e-PTFE membrane alone. Clinical measurements were made using calibrated periodontal probes to determine soft tissue REC, PD, and attachment levels. Intra-surgical measurements were made to determine crestal resorption, vertical and horizontal open probing attachment. Each site was surgically re-entered and measurements were repeated at six months post- treatment. Both the treatment modalities demonstrated minimal soft tissue REC. Sites treated with combined technique showed statistically significant improvement in PD reduction and CAL gain. Hard tissue changes were comparable for alveolar crestal resorption. Statistically significant difference was found for both horizontal and vertical bone repair favouring the use of combined technique.

Wallace SC et al. (1994)[34] compared the periodontal soft and hard tissue repair using ePTFE membrane with and without DFDBA. A total of 6 patients with 17 mandibular Class II buccal molar furcal invasions were recruited in the study, of which 10 teeth were randomly selected as Test sites (ePTFE + DFDBA) and 7 as Controls (ePTFE alone). Soft tissue measurements were made to assess PD, REC, and PAL at baseline. After flap reflection, open surgical measurements were made to determine alveolar crestal height (CEJ-AC) and vertical (CEJ-BDF) and horizontal (HPDF) defect depth. The ePTFE membranes were removed at 6 weeks. The sites were surgically re-entered after 6 months to obtain both soft tissue and open surgical measurements. Both the treatment resulted in decreased PD, increased REC, gain in PAL, decreased CEJ-BDF, increased CEJ-AC and decreased HPDF. However, none of the changes were statistically significant. The addition of DFDBA to the GTR procedure did not significantly improve any of the mean soft tissue and open surgical measurements between Control (ePTFE alone) and Test (ePTFE + DFDBA) groups in mandibular Class II buccal furcation. The study thus suggests no added benefit of combined graft and GTR over GTR alone.

Luepek PG et al. (1997)[35] evaluated a bioresorbable barrier with and without DFDBA in the treatment of human molar furcation. 14 subjects exhibiting paired Class II mandibular molar furcation defects were randomly treated with either the resorbable barrier alone or resorbable barrier in combination with DFDBA. Clinical measurements including GR, PD, clinical attachment, and bone fill were measured at baseline and 6 months post-surgery. All sites were surgically re-entered. There was significant reduction in PD reduction ($p < 0.01$) and vertical BG ($p < 0.02$) at defects treated with combination therapy as compared to resorbable barrier alone. The non-smokers for both

treatment groups revealed greater reduction in PD, and greater vertical and horizontal BG. Also, within the non-smoking group, PD reduction was significantly higher for combination therapy than the resorbable group ($p < 0.02$) alone. The study suggests that improvements in soft tissue measurements are better in non-smoker patients treated with combination therapy of resorbable barrier and DFDBA.

Lamb JW et al. (2001)[36] conducted a 9 month re-entry study to compare the regenerative healing using porous (P) and nonporous (NP) Teflon barrier membranes plus DFDBA in Class II buccal/lingual furcation defects. Twenty-four patients diagnosed with adult periodontitis and one Class II furcation defect were randomly selected to receive the NP treatment and 12 received the P membrane. All the linear and volumetric measurements were performed by a single masked examiner using graduated periodontal probe at baseline and at 9 months during surgical re-entry. There was no statistically significant differences ($p>0.05$) between NP and P groups at any time with respect to clinical indices and open and closed open or closed probing measurements. There was significant gain in CAL and improvement in mean open horizontal probing depth for both the NP and P groups at 9 months. Bone fill of $\geq$ 50% was obtained in 100% of intrabony defects. Thus use of porous and non-porous barrier membranes with a DFDBA graft would be effective in treatment of Class II furcation defects in humans, with no distinct difference between the two therapies.

Kothiwale SV. et al (2009)[37] evaluated and compared the efficacy of DFDBA and bovine derived xenogenic bone graft (BDX) [Bio-Oss] with amniotic membrane as GTR in the treatment of human periodontal Grade II buccal furcation defects, clinically and radiographically. 10 patients diagnosed with chronic periodontitis and exhibiting

bilateral Grade II buccal furcation defect, were randomly treated using DFDBA with amniotic membrane (Experimental site A) or using BDX with amniotic membrane (Experimental site B). All the clinical and radiographic parameters were recorded at baseline, 6 and 9 months. At 9 months after therapy, both Experimental site A and Experimental site B showed reduction in PD and RAL gain. Percentage BG in Experimental site A and Experimental site B was 76.3% and 79.6% respectively. But there was no statistical difference between the two materials in all measurements. Hence, it can be concluded that use of GTR with either of the material results in improvement of soft and hard tissue measurements.

Kher VK et al. (2013)[38] compared the effectiveness of GTR by using a collagen membrane barrier with or without DFDBA in the treatment of periodontal infrabony defects characterized by unfavourable architecture. Sixteen systemically healthy patients with 20 periodontal infrabony defects with depth of intrabony component ≥3 mm as assessed by clinical and radiographic measurements were selected for the study. Clinical measurements including PI, papillary bleeding index, PPD, GR, CAL and radiographic defect depth (DD) were measured at baseline. The defects were randomly assigned to either the Test group (collagen membrane plus DFDBA) or the Control group (collagen membrane only). On statistical analysis, there was significantly greater reduction in PPD, gain of CAL and radiographic DD in the GTR + DFDBA group compared with the GTR group at 6 months compared to baseline. The results of the present study indicate that the use of a GTR membrane with bone graft leads to greater improvement in clinical parameters as compared with the use of bioresorbable membrane alone in the treatment of infrabony defects characterized by unfavourable architecture.

Shah R et al (2014)[39] presented a case report to demonstrate the use and potential of DFDBA in conjunction with human CM in management of ridge preservation. A 22-year-old male patient with history of root canal treatment and exhibiting Grade II mobility clinically underwent tooth extraction due to a failed endodontic treatment. A missing buccal wall of socket was observed on extraction and was managed by GTR using DFDBA in conjunction with the human CM. On 6 month evaluation, the soft tissue appeared healthy but with slight loss of tissue height (0.36 mm) and tissue width (2.04 mm). There was increase in thickness of gingiva post-operative (0.93 mm) as compared to pre-operative (0.76 mm). Also, there was no loss of bone height on post-operative radiographic evaluation. The case demonstrates that use of DFDBA in conjunction with human CM proved to be effective in minimizing soft/hard tissue loss and also helped in some amount of tissue gain.

Rosen PS et al (2015)[40] conducted a case series to show a combined regenerative approach layering root modification with recombinant platelet derived growth factor (PDGF)BB, a composite allograft with mesenchymal stem cells (MSCs) along with a barrier derived from human amnion-chorion to treat Class III/ IV mandibular furcation defects. A total of 5 mandibular Class III and one borderline Class IV furcation were treated with combination approach. On 6 month evaluation 3 of the mandibular Class III/ IV furcation were completely closed, 2 of furcation were reduced to Class I furcation on their buccal aspect, whereas the lingual aspect were completely closed. However, there was no improvement with 1 furcation defect. The results of the case series suggest that the regenerative approach proves to be a promising therapy in dramatically changing the prognosis of poor to hopeless teeth and prolonging the life of Class II/ IV mandibular molars.

Pajnigara NG et al. (2017)[41] evaluated volumetric changes to assess the regenerative efficacy of DFDBA with and without AM in Grade II furcation defects both clinically and radiographically using CBCT. A total of 20 patients in mean age range of 43.75 ± 6.22 years exhibiting at least 1 pair of bilateral Grade II furcation defects were randomly assigned to Group I (DFDBA) and Group II (DFDBA + AM). All the clinical parameters such as PPD, CAL, GR, and horizontal PD were recorded at baseline, 3 months, and 6 months. Radiographic assessment included measurements of the defect height, width, depth, and volume at baseline and 6 months postoperatively. On statistical analysis, defects treated with DFDBA + AM resulted in significant improvement in clinical and radiographic parameters when compared with DFDBA alone. The mean reduction in radiographic defect volume for Group I was 11.15 ± 6.39 mL and 17.02 ± 10.86 mL for Group II. The study suggests combination therapy of bone graft (DFDBA) in conjunction with membrane (AM) results in greater the reduction in defect volume indicating greater amount of healed regenerated tissue.

Mehta DP (2018)[41] designed a split mouth study to evaluate the efficacy of autologous platelet rich fibrin (PRF) as a membrane as compared to collagen membrane along with DFDBA in treatment of Grade II furcation defects in molars. The study comprised of 18 patients exhibiting 2 sites of Grade II furcation defects each, that were randomly allocated to the Test group (autologous PRF membrane + DFDBA) and Control group (collagen membrane + DFDBA). The clinical measurements including PI, PD, relative vertical clinical attachment level (RVCAL), gingival marginal level (GML), and radiographic bone levels were recorded at baseline, 3 months, and 6 months postoperatively. On statistical analysis, intragroup comparison revealed statistically significant difference ($p < 0.001$) in clinical outcome (PI, PD, CAL) from baseline to 3

and 6 months for both the groups. However, there was no statistical difference between the groups on intergroup comparison. There was statistical difference in GML at baseline to 3 and 6 months for both the groups. The results indicate a significant role of autologous PRF membrane as regenerative material in treating furcation defects and use of graft and barrier membranes for treatment of furcation defects.

4. Review of Studies on Analysis of Regeneration

Yukna RA (1994)[43] clinically evaluated treatment response of HTR polymer, a biocompatible microporous composite of PMMA (poly-methyl-methacrylate), PHEMA (poly-hydroxyl-ethyl-methacrylate), and calcium hydroxide as a bone replacement graft in human periodontal furcation defects in 9 patients with 15 pairs of mandibular Class II furcation defects. Of these 10 first molars and 5 second molars received HTR whereas 8 first molars and 7 second molars received autogenous osseous coagulum (AOC). The defects were randomly assigned to one of the treatment and were surgically re-entered after about 6-12 months to obtain documentation. All the clinical soft measurements, direct clinical measurements and radiographic parameters were recorded at baseline and at re-entry. Both the treatments responded well and improved clinical status of furcation defects. Also there was significant reduction in original defect depth and original PD; however, there was no difference between the two difference replacement graft treatments. Differences were found and in favour of HTR polymer with $P \leq 0.05$ for horizontal residual furcation depth (2.4 mm vs 3.9 mm), horizontal furcation fill (1.9 mm vs 0.8 mm) and percent horizontal furcation fill (44.4 % vs 17.1%). The results of the study suggest that HTR polymer appears to be clinically beneficial biocompatible

alloplastic material and also indicates that use of bone replacement graft materials as regenerative therapy are of some beneficence in treatment of Grade II furcation defects.

Harris RJ (1999)[44] conducted a human histologic study to evaluate the regeneration using combination of DFDBA with a bioabsorbable polylactic acid membrane softened with citric acid esters (PLACA) in furcation defects. After obtaining the consent, 3 maxillary molar teeth with buccal furcation involvement in a 47 year old woman who was to receive an immediate maxillary denture were included in the study. After reflection of full-thickness flap, a groove was placed into the roots with ½ round bur which were then planed with hand and ultrasonic instruments. The furcation defects were filled with DFDBA, covered with PLACA membrane and the flap were sutured back to its original position. At 6 months post-operatively, the teeth were being extracted with a small piece of tissue from the furcation. On clinical examination 2 out of 3 teeth demonstrated gain in CAL. Histologic evaluation demonstrated formation of new cementum, connective tissue fibers embedded into cementum and bone and no intervening epithelium. Also, cells were apparent in the lacunae indicating that bone was vital. Hence, it can be concluded that irrespective of the quantity, some amount of regeneration could be obtained in furcation defects using DFDBA and PLACA membrane.

Misch KA et al. (2006)[45] compared linear measurements of periodontal defects obtained through CBCT images to traditional methods. Artificial osseous defects of varying depth and width were created on premolar and molar of mandibles of dry skulls. For each investigation, CBCT scan, periapical radiograph, and direct measurements using a periodontal probe were compared to defect measurements made by impression

material for standard reference by an electronic caliper. For linear measurements there were no statistical differences between bone sounding, radiography, and CBCT. A significant difference was observed on comparing isolated interproximal measurements using a probe versus the caliper ($p < 0.001$) but no significant difference for CBCT or radiography. All bony defects were identifiable and measurable directly or with CBCT. In comparison, buccal and lingual defects could not be measured with radiographs. It was concluded that all three modalities are useful for identifying interproximal periodontal defects. Compared to radiographs, the 3D capability of CBCT offers a significant advantage because all defects can be detected and quantified.

Vandenberghe B et al. (2008)[46] explored the diagnostic values of digital intraoral radiography and CBCT in the determination of periodontal bone loss, infrabony craters and furcation involvements. Assessment of the imaging modalities was conducted through bone level measurements, infrabony crater and furcation involvement classifications. 71 human cadaver and dry skull bony defects were measured and evaluated by 3 observers. The CBCT images were obtained at 120 kV and 23.87 mAs, and observations were made on a 5.2 mm panoramic reconstruction view and on 0.4 mm thick cross-sectional slices. Intraoral radiographs were obtained with charge-coupled device (CCD) sensor using the paralleling technique, at 60 kV and 0.28 mAs exposure. Comparison was made with the gold standard. The mean error (gold standard deviation) of bone level measurements was 0.56 mm for intraoral radiography and 0.47 mm for the CBCT images. There were no significant differences ($P = 0.165$) between the two methods. However, on 0.4 mm thick cross-sections, the mean error was 0.29 mm and the Wilcoxon signed-rank test indicated a significant difference when compared with the CCD ($P = 0.006$). The detection of crater and furcation involvements

failed in 29% and 44% for the CCD, respectively, in contrast to 100% detectability for both defects with CBCT. CBCT on the panoramic 5.2 mm reconstruction view allowed comparable measurements of periodontal bone levels and defects as with intraoral radiography. Thus CBCT with 0.4 mm thick cross-sections demonstrated values closer to the gold standard, indicating more accurate assessment of periodontal bone loss.

Walter C et al. (2010)[47] assessed the accuracy of CBCT in detecting furcation involvement (FI) in maxillary molars and aimed to compare the intra-surgical assessment of FI to data generated by CBCT. A total of 14 patients diagnosed with generalized advanced chronic periodontitis were recruited and treated non-surgically. For maxillary molars with increased PPD ≥ 6mm / increased FI were subjected to periodontal surgery and further re-evaluated by CBCT images for the degree of FI. 25 maxillary molars were subjected to furcation surgery and intra-surgical FI assessments were compared with data derived from CBCT images. On comparison of FI, 84% of the CBCT data were confirmed by the intra-surgical findings (95% CI). On comparison with intra-surgical analysis, 14.7% (11 sites) were underestimated (CBCT less than intra-surgical value), and only 1.3% (one site) revealed overestimation (CBCT more than intra-surgical value). Also, there was greatest agreement between both assessments in distal furcation entrances, followed by buccal and mesial entrances. Hence, it was concluded that there is substantial agreement between CBCT and intra-surgical assessment for maxillary FI and CBCT enables accurate assessment of bone loss and classification of the degree of FI in maxillary molars.

Cimbaljevic MM et al. (2015)[48] compared the use of periodontal probing and CBCT images in the diagnosis of FI in patients with chronic generalized severe periodontitis. A

total of 174 furcation sites (38 maxillary and 30 mandibular molar teeth) in 15 patients were assessed for FI at three sites (buccal, mesiopalatal, and distopalatal) of maxillary molars, and at two sites (buccal and oral) of mandibular molars. FI was assessed both clinically (using Naber's periodontal probe) and on CBCT images, using a dichotomous scale (present/absent). Overall, FI were more often detected by CBCT evaluation than by clinical examination. The agreement between the evaluation methods was present in 46.9% of cases, with a stronger agreement in maxillary sites than in mandible (63.3% and 45.0% respectively). FI detected clinically was confirmed by means of CBCT in 24% of the evaluated sites. The strongest agreement between two methods of 73.7% was found in FI detection in the distopalatal maxillary sites, whereas the smallest agreement (36.6%) was found in the buccal sites of the mandibular molars, in which 63.3% of FI were detected using CBCT only, but not clinically. FI were more often detected by CBCT than clinically. The results of the study indicate greater accuracy of CBCT in assessing FI than clinical probing and that CBCT can be used as an adjunct tool in diagnosing FI in cases of surgical intervention.

Pajnigara N et al. (2017)[49] evaluated the dimensions of 200 Grade II furcation defects clinically (pre-and post-surgery), intra-surgically, and by CBCT (pre and post-surgery) in forty patients with a mean age of 38.05 ± 4.77 years, diagnosed with chronic periodontitis. After the pre surgical clinical and CBCT measurements, DFDBA was placed in the furcation defect and the flaps were sutured back. Six months later, these defects were evaluated by recording measurements clinically, i.e., post-surgery clinical measurements and also post-surgery CBCT measurements (40 defects each). Pre-surgery clinical measurements (vertical 6.15 ± 1.71 mm and horizontal 3.05 ± 0.84 mm) and CBCT measurements (vertical 7.69 ± 1.67 mm and horizontal 4.62 ± 0.77 mm)

underestimated intra-surgery measurements (vertical 8.025 ± 1.67 mm and horizontal 4.82 ± 0.67 mm) in both vertical and horizontal aspects, and the difference was statistically not significant (vertical p = 1.000, 95% CI; horizontal p = 0.867, 95% CI). Further, post-surgery clinical measurements (vertical 2.9 ± 0.74 mm and horizontal 1.52 ± 0.59 mm) underestimated CBCT measurements (vertical 3.67 ± 1.17 mm and horizontal 2.45 ± 0.48 mm). The difference between presurgery clinical – presurgery CBCT (p< 0.0001, 95% CI) versus postsurgery clinical – postsurgery CBCT (p< 0.0001, 95% CI) values in both vertical and horizontal aspects was statistically significant. It was concluded that the use of CBCT appears to be prudent for accurate diagnosis of furcation defects in advanced periodontal diseases.

MATERIALS AND METHODS

Furcation involvement in multi-rooted teeth greatly brings down the prognosis of the tooth. Reliable detection, assessment and measurement of furcation and the degree of its involvement is a unique and challenging task. An optimal treatment outcome for a furcation defect relies on early and accurate diagnosis and appropriate treatment modality. Of the various surgical and non-surgical treatment modalities to treat furcation involvement, various barrier membranes for GTR have been evaluated over the years.

The present study was undertaken to evaluate DFDBA alone and in conjunction with CM in the treatment of Grade II furcation defects. The evaluation was done clinically and by CBCT.

One of the most commonly used classifications for furcation involvement was the one given by Irvin Glickman in 1953.[5] Accordingly, the furcation involvements are classified into four different grades and the present study utilizes Grade II furcation defects as mentioned in this classification.

Grade I- It is the incipient or early stage of furcation involvement. The pocket is suprabony and primarily affects the soft tissues. Early bone loss may have occurred with an increase in probing depth, but radiographic changes are not usually found.

Grade II- It can affect one or more furcation of the same tooth. The furcation lesion is initially a cul-de-sac with a definite horizontal component. If multiple defects are present, they do not communicate with each other because a portion of alveolar bone remains attached to the tooth. The extent of horizontal probing determines whether the defect is early or advanced. Vertical bone loss may be present. Radiographs may or may not detect the furcation involvement, particularly with maxillary molars because of the overlap of roots.

Grade III- The bone is not attached to the dome of furcation. In early Grade III furcation involvement, the opening may be filled with soft tissue and may not be visible. It may not be possible to pass a periodontal probe entirely and completely through the furcation because of interference with bifurcational ridges or facial/lingual bony margins. However, if the buccal and lingual probing dimensions are added and what obtained is a cumulative probing measurement that is equal to or greater than the buccal/lingual dimension of the tooth at the furcation orifice, it must be concluded that a

Grade III furcation exists. Properly exposed and angled radiographs of early Grade III furcation display the defect as radiolucent area in the crotch of the tooth.

Grade IV- The interdental bone is destroyed and soft tissues have receded apically so that furcation opening is clinically visible. A tunnel therefore exists between the roots of such an affected tooth. Thus, the periodontal probe passes readily from one aspect of the tooth to another.

Clinical research reported remarkable gains in clinical attachment levels using DFDBA in human intraosseous lesions, proving DFDBA of high osteogenic potential. The demineralized particles of DFDBA undergo biochemical change that induces remineralization of the particles that attracts the osteoblasts from the neighbouring adjacent bone leading to successive layering of bone.

The CM used has numerous advantages owing to its structure and composition. Structurally, it consists of the following layers: reticular, basement membrane and trophoblasts. The extracellular matrix comprises of collagen type I, III, IV, V, and VI, proteoglycans, fibronectin, and laminin. Collagen is well-tolerated and bio-absorbable, has hemostatic properties and encourages migration of adjacent autogenous connective tissue. Fibronectin is involved in many cellular processes, including tissue repair, blood clotting, cell migration and adhesion. Laminin has a high affinity for binding epithelial cells and thus this membrane, in contrast to traditionally available membranes, allows for rapid epithelial cell growth rather than epithelial exclusion. Additionally, the matrix of the chorion contains abundant growth factors which promote periodontal regeneration and provide a natural environment for accelerated healing. Furthermore, the ability of this allograft to self–adhere eliminates the need of suturing, thus making it easier to use in posterior defects.

So, it was felt necessary to conduct clinical trial and evaluate its efficacy in the treatment of Grade II furcation defects. Both DFDBA particle size 500 -1040 μm and CM 2 × 2.59 cm were obtained from TATA Memorial Cancer Research Hospital, Mumbai.

20 patients (13 females and 7 males) affected with moderate to severe chronic periodontitis and comprising of both the sexes in age range of 30-60 years (mean age of 40.85 ± 7.32 years) were selected from those visiting the Department of Periodontology of our Institute. Each patient demonstrated bilateral Grade II furcation defects in molars. The study was initiated after the clearance from the Institutional Ethics Committee of our institute. A special proforma was designed so as to have systematic and methodological recording of observation and information. This included a detailed case history, clinical examination, radiographic evaluation, periodontal indices and written consent of the patient.

INCLUSION CRITERIA

1. Patients with moderate to advanced chronic periodontitis as assessed by PPD ≥ 5mm and CAL ≥ 5mm.
2. Patients with at least one pair of bilateral Grade II furcation defects in either maxillary or mandibular arches.

EXCLUSION CRITERIA

1. Patients with history of known systemic diseases, allergies or drug usage.
2. Patients who have undergone periodontal therapy in last 6 months.
3. Patients or lactating women.

CLINICAL PROCEDURE

Twenty (13 females and 7 males) systemically healthy patients with moderate to severe chronic periodontitis in age range of 30-60 years, exhibiting bilateral Grade II furcation defects, were included in the present study. The selected sites were randomly assigned to Group I that is Control group (DFDBA) and Group II that is Test group (DFDBA + CM). Each patient was explained about the treatment procedure to be performed and an informed consent was obtained prior to beginning of the study.

PRESURGICAL HYGIENE THERAPY

All the selected patients were subjected to presurgical hygiene therapy and received thorough scaling and root planing session, oral hygiene instructions, and any occlusal adjustment were corrected prior to surgery. Three weeks after the initial therapy the patients were re-evaluated to assess the plaque control and overall oral hygiene. On the day of the surgical procedure, prior to surgery, recording of clinical data was carried out by the same examiner in all the patients. For evaluation of oral hygiene and gingival health, PI and GI were obtained at baseline, 3 months and 6 months.

ALLOTMENT

The selected sites were randomly assigned by a computer-generated random table number by the operator, who is the second examiner to Group I that is Control group (DFDBA) and Group II that is Test group (DFDBA + CM). Each patient was explained about the treatment procedure to be performed and its outcome and a written informed consent was obtained prior to beginning of the study.

A. PLAQUE INDEX (PI) (TURESKY–GILMORE–GLICKMAN MODIFICATION OF QUIGLEY-HEIN 1970)[50]

Plaque was assessed on labial/ buccal and lingual surfaces of all teeth after using a disclosing agent.

Score	Criteria
0	No plaque.
1	Separate flecks of plaque at the cervical margin of the tooth.
2	A thin, continuous band of plaque (upto 1mm) at the cervical margin.
3	A band of plaque wider than 1mm but covering less than one third of the crown.
4	Plaque covering atleast one third but less than two thirds of the crown.
5	Plaque covering two thirds or more of the crown.

Calculations

The total plaque score for the entire mouth per person was obtained by totalling all of the plaque scores and dividing by the number of surfaces examined.

$$\text{PI} = \frac{\text{Total plaque score}}{\text{No of surfaces examined}}$$

Plaque scores	Condition
0	Excellent
0.1 – 0.9	Good
1.0 – 1.9	Fair
2.0 – 3.0	Poor

B. GINGIVAL INDEX (GI) (LOE AND SILNESS, 1963)[51]

The severity of gingivitis was scored on mesial, distal, buccal and palatal/lingual surfaces of selected index teeth. The teeth selected as the index teeth were-

16 - Maxillary Right First Molar

12 - Maxillary Right Lateral Incisor

24 - Maxillary Left First Premolar

36 - Mandibular Left First Molar

32 - Mandibular Left Lateral Incisor

44 - Mandibular Right First Premolar

A UNC-15 periodontal probe was used to assess the bleeding potential of gingival margin according to following criteria.

Score	Criteria
0	Absence of gingival inflammation/normal gingiva.
1	Mild inflammation, slight change in colour, slight oedema, no bleeding on probing.

2	Moderate inflammation, moderate glazing, redness, oedema, hypertrophy, bleeding on probing.
3	Severe inflammation, marked redness and hypertrophy, ulceration, tendency to spontaneous bleeding.

Calculations

If the scores around each tooth are totalled and divided by four, the gingival index score for the tooth is obtained. To obtain the GI score for the individual the total index score for each of the teeth are totalled and divided by total number of teeth examined.

$$\text{GI} = \frac{\text{Total GI scores of all teeth}}{\text{Number of teeth examined}}$$

The numerical scores of the GI may be associated with varying degrees of clinical gingivitis as follows:

Gingival scores	Condition
0.1-1.0	Mild gingivitis
1.1-2.0	Moderate gingivitis
2.1-3.0	Severe gingivitis

After the hygiene phase of therapy, soft tissue measurements were determined to the nearest millimeter mark by using UNC-15 graduated periodontal probe from the

cementoenamel junction (CEJ) to free gingival margin (FGM) and from the CEJ to the base of periodontal pocket (BP). Soft tissue REC and PPD were also recorded. Custom made occlusal acrylic stents were used to standardize the probe angulation and position. Occlusal stents were fabricated with cold cured acrylic resin on a cast model obtained from an alginate impression. The occlusal stents covered the occlusal surface of the tooth being treated and occlusal surfaces of at least one tooth in mesial and distal directions. Stents also extended apically on the buccal and lingual surfaces so as to cover the coronal third of the teeth. A groove (guide plane) was made on the stent in relation to each involved tooth to guide the periodontal probe while taking measurements. This technique provided a fixed reference point and fixed angulations for measurements at each site.

CBCT ANALYSIS

CBCT measurements were taken for each Group i.e. the Control and the Test group at baseline and at 6 months. The CBCT analysis included the measurement of bone defect height, bone defect depth and the bone defect width. These three parameters generate the total volume of the bone defect.

SURGICAL ARMAMENTARIUM

Instruments were arranged in a definite order on a sterilized drape placed on a surgical trolley. All the equipments were autoclaved. The surgical armamentarium consisted of -

- Mouth mirrors.
- UNC-15 periodontal probe (Hu-Friedy, USA).
- Straight probe.
- Naber's probe

- Explorer number 23 and number 17.
- Tweezer.
- Disposable gloves.
- Disposable face masks.
- Disposable syringe – 5ml and 2ml.
- Local anesthetic (2% Xylocaine HCl with adrenaline 1:200000).
- Bard parker handles.
- No. 11, 12, and 15 blades.
- Periosteal elevator (24G Hu-Friedy, USA).
- Gracey curettes.
- Scissors – straight and curved.
- Tissue forceps.
- Needle holder.
- Mersilk suture material.
- Cotton swabs.
- Kidney tray with saline and irrigation syringe.
- Dappen dish.
- Coe – pak.
- Normal saline.
- Denatured spirit.
- 0.2 % Chlorhexidine gluconate.

Materials Used in Periodontal Regeneration

Demineralized freeze dried bone allograft

DFDBA was procured from the Tissue Bank of TATA Memorial Hospital and Research Centre, Mumbai. It comprises of cortical bone which is harvested in a sterile manner within 12 hours of death of the donor.

Cortical bone is less antigenic than cancellous bone and has a higher concentration of bone inductive proteins. Sterilization of the DFDBA was done either by procuring the tissue under sterile condition or by using a specific sterilization process. Tissues were stored in deep freezer for 4 weeks which reduces the immunogenicity. Then the tissues are cut and washed with bio-filtered water followed by pasteurization and alcohol therapy, terminal sterilization is done by gamma irradiation. Pasteurization removes most vegetative bacteria, fungi and viruses including HIV (Human Immunodeficiency Virus). Alcohol therapy is done to remove any remaining HIV, bacteria and spores. These processed allografts are irradiated by gamma rays at a dose of 25 Kilo Gray units from Cobalt 60 source at Microtrol Sterilization centre Pvt. Limited in Bangalore. The preservation of tissue is done by either Freeze drying or deep freezing. Freeze drying removed approximately 95% of water from bone by a process of sublimation in vaccum. This enables the biologic material to be stored for long periods of time at room temperature, without fear of detrimental enzymatic activity. Biologically useful properties of the graft are also preserved.

DFDBA primarily works through both the principles of osteoconduction and osteoinduction. On demineralization, the crude particles of the DFDBA graft may expose/ activate the bone inductive proteins, such as BMPs located within the matrix that aids in mesenchymal cell migration and osteogenesis when implanted into the bony

defects. Occasionally, the part of graft does not activate bone growth thereby serving as a scaffold for the natural bone to grow in and then eventually gets resorbed and replaced by new bone. The particle size used in this study was 500-1040 μm.

Chorion membrane

The CM was procured from the Tissue Bank of TATA Memorial Hospital and Research Centre, Mumbai. The membrane is cleaned of blood, pasteurized at 60°C in saline, treated with 70% alcohol, washed, lyophilised using a dose of 25 kGy. The CM being of placental origin is immunoprivilege, and possesses anti-inflammatory and antibacterial properties. The CM reduces inflammation and provides a protein-rich matrix that accelerates tissue adhesion, facilitates migration of cell and promotes regeneration. All of these properties play a key role in improved healing of periodontal lesions and might result in reduction in PPD and decrease in clinical attachment loss.

SURGICAL PROCEDURE

After the completion of initial therapy and baseline examinations, the patients entered into the surgical phase of the regenerative therapy. The respective sites to be treated were anesthetized with local anaesthesia containing 2% Xylocaine HCl with adrenaline (1:200000). After adequate anaesthesia the surgical procedure was initiated.

Incision

Intrasulcular incision extending atleast one tooth mesial and distal to the treatment site were made to release full thickness mucoperiosteal flaps. An attempt to preserve the interdental papilla was made wherever possible.

Reflection of Flap

A full thickness mucoperiosteal flap was reflected to gain access for the defect debridement. All granulation tissue from the bony wall of the defects was removed with the help of curettes. The root surfaces were scaled and planed using hand instruments. Baseline hard tissue measurements were recorded at the time of surgery from the CEJ to the base of furcation defect. Horizontal probing defect depth was also measured.

Placement of Graft Material and Membrane

For Test group, DFDBA bone graft was added with saline and taken in small increments and placed in defect site. The defect was measured for selection of proper membrane size and the membrane was trimmed according to the size of the defect. The trimming of the CM was done in such a way that the membrane covered 2-3mm of the adjacent alveolar bone laterally as well as apically. Upon placement, the processed dehydrated CM became hydrated and self - adhered to the area thus eliminating the need for suturing the membrane. Immediately after placing the membrane, the reflected flap was repositioned over the CM and secured with interrupted sutures (3-0 mersilk suture). Periodontal dressing was placed. For Control group, the sites received the same treatment without placement of the membrane.

POST-SURGICAL CARE

All the patients were given appropriate oral hygiene and post-surgery instructions. Patients were placed on antibiotics (Amoxicillin + Clavulanic acid: 625 mg) two times a day for five days. Analgesics (Ketorolac tromethamine 10mg) were prescribed to control postsurgical discomfort. Sutures and Coe-pak were removed after seven days. Patients were instructed to use a Chlorhexidine mouth rinse (10 ml twice daily) for 15

days and refrain from chewing hard or sticky foods, forcefully brushing the treated sites, or using any interdental aids until the next appointment at 3 months. Adverse effects were recorded at recall visits, and supragingival deposits were removed.

POST SURGICAL EVALUATION

The patients were evaluated clinically at 3 and 6 months and by CBCT at 6 months intervals. Using UNC 15 graduated periodontal probe the measurements of CAL, PPD and gingival recession were taken similar to the pre procedures.

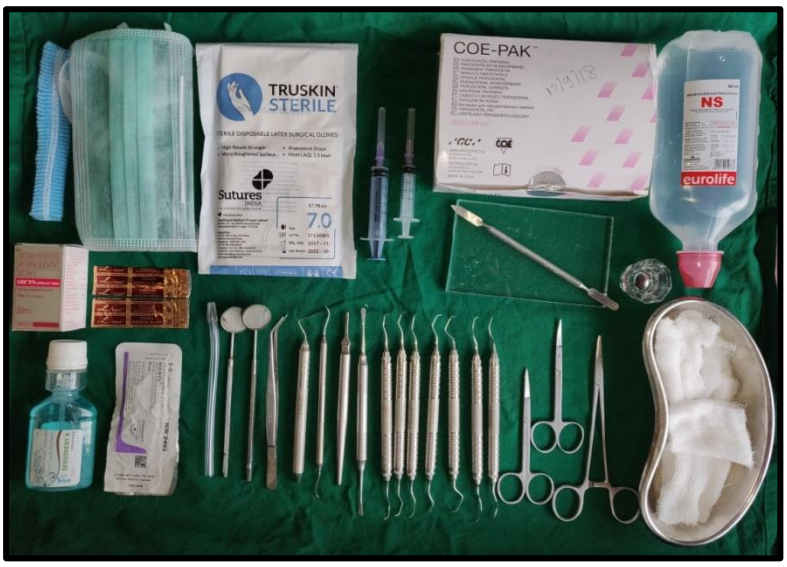
CBCT MEASUREMENTS

All the sites in both Test and Control groups were subjected to CBCT assessment. The Orthophos® XG 3D manufactured by Sirona Dental Systems GmbH, Germany using 3Diagnosys 4.2 Imaging software was used for the CBCT assessment. Patient was asked to remove all metal objects and wear a lead apron. The patient was asked to bite gently and naturally on the bite block without joining the incisors. The upper incisors centered with the bite block. The patient was adjusted using two positional laser beams-

- The mid-sagittal positioning laser beam
- The 3D FoV positioning laser beam

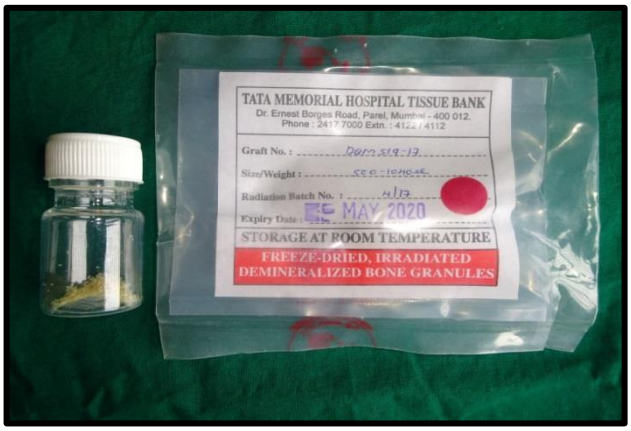
The digital readout was seen on the computer screen.

INSTRUMENTATION



Surgical Armamentarium

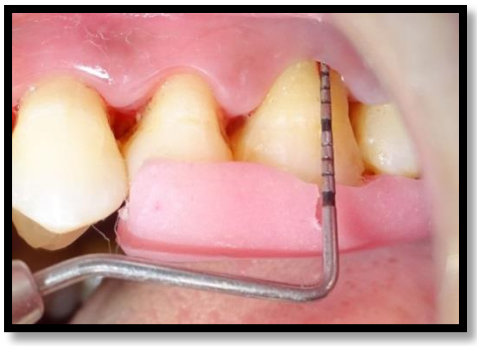
MATERIALS USED

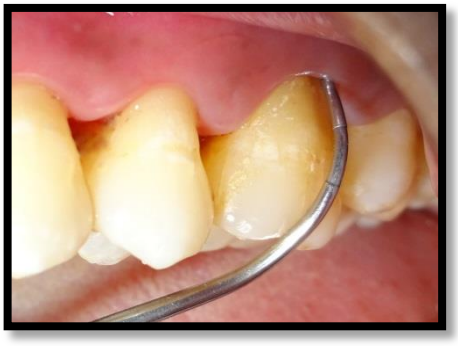


Demineralized Freeze Dried Bone Allograft (DFDBA)

Chorion Membrane (CM)

SURGICAL PROTOCOL FOR DEMINERALIZED FREEZE DRIED BONE ALLOGRAFT

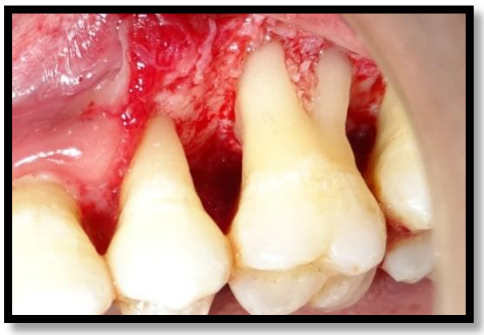




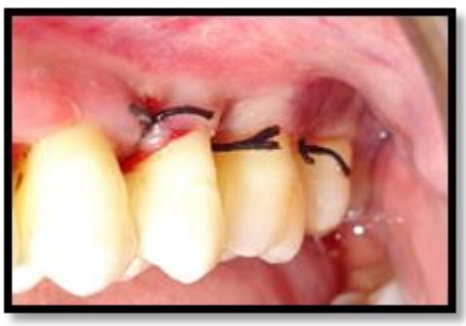
Pre-operative Photograph

Vertical Probing Depth

Horizontal Probing



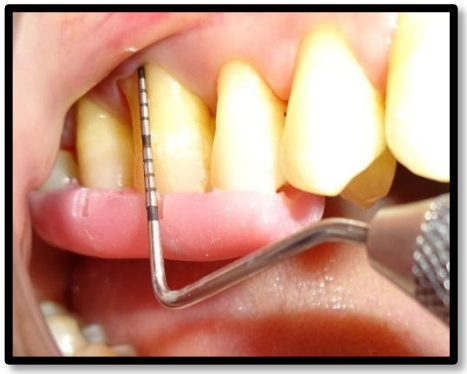
Placement of DFDBA

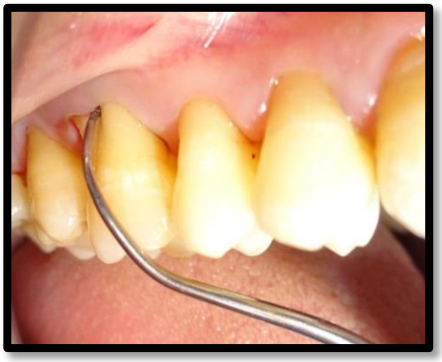


Sutured flap

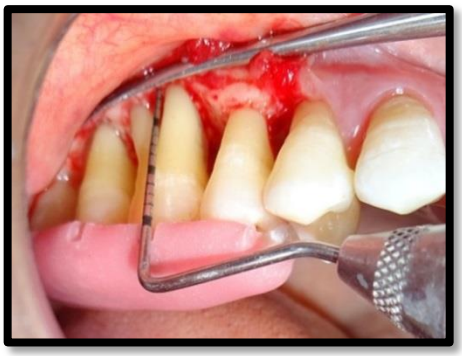
Periodontal Dressing

SURGICAL PROTOCOL FOR DEMINERALIZED FREEZE DRIED BONE ALLOGRAFT PLUS CHORION MEMBRANE

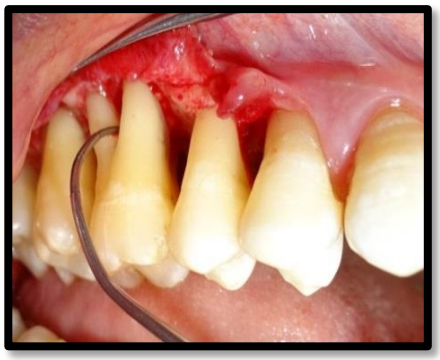




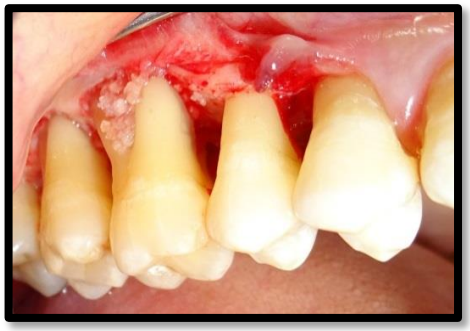
Pre-operative Photograph

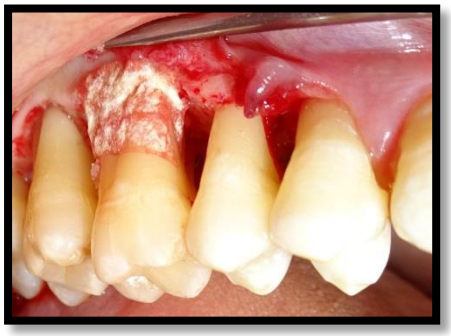


Vertical Probing Depth

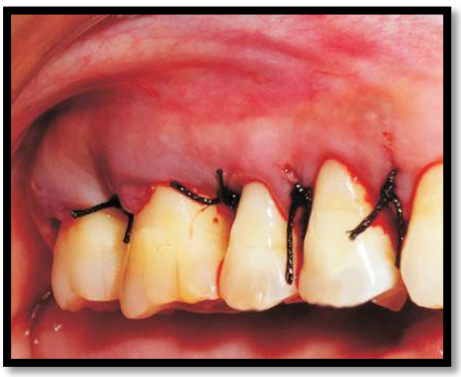


Horizontal Probing Depth

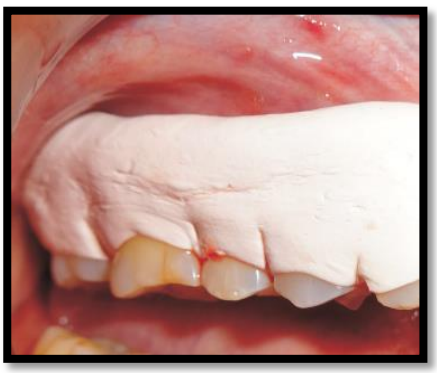




Placement of DFDBA + CM

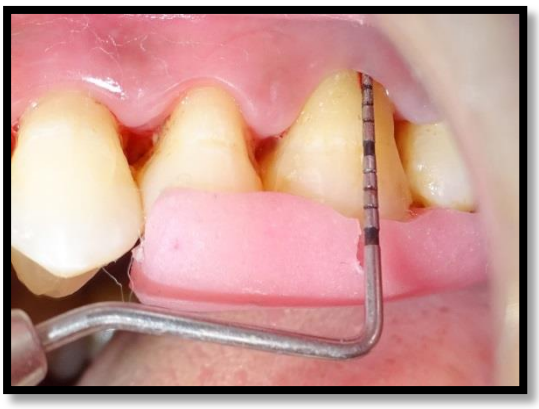


Sutured flap

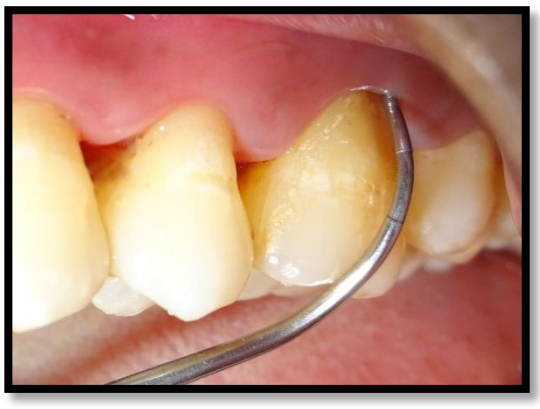


Periodontal Dressing

RECALL: CLINICAL PARAMETERS FOR DFDBA

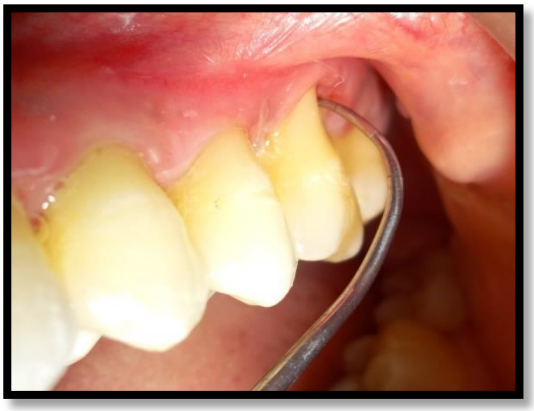


Baseline

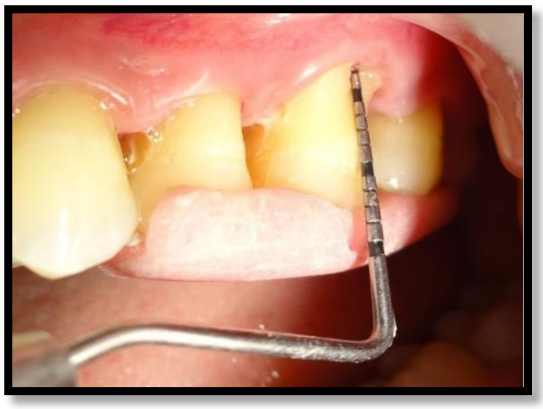


Baseline

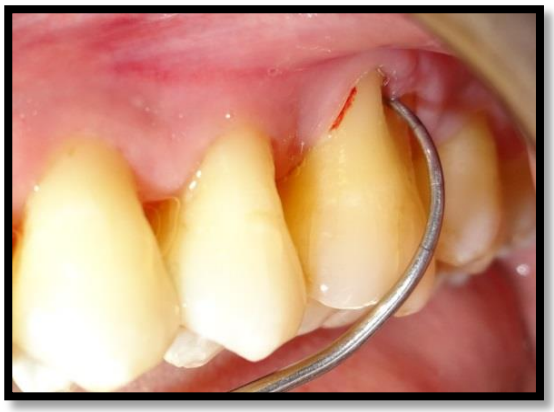
3 months



3 months

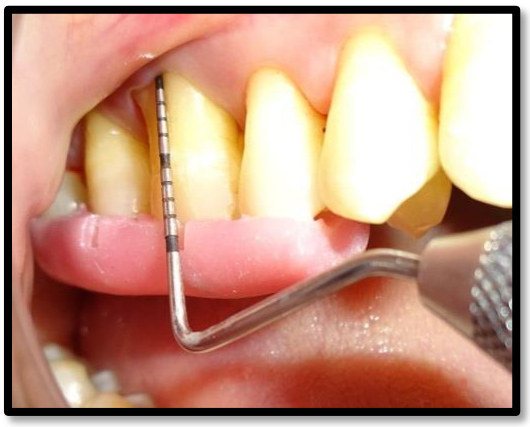


6 months

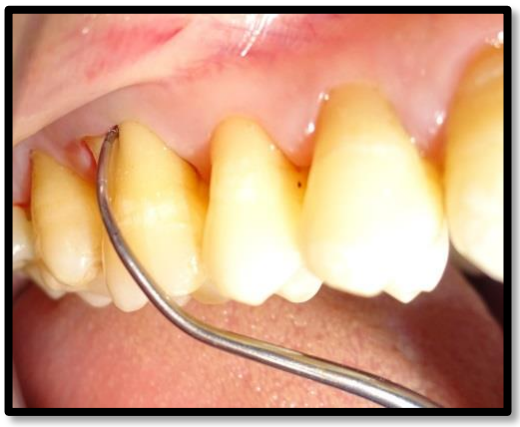


6 months

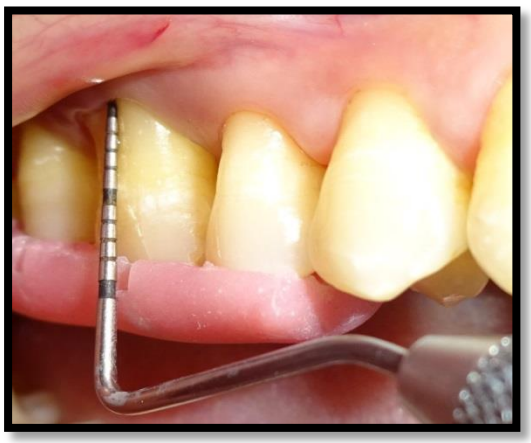
RECALL: CLINICAL PARAMETERS FOR DFDBA + CM



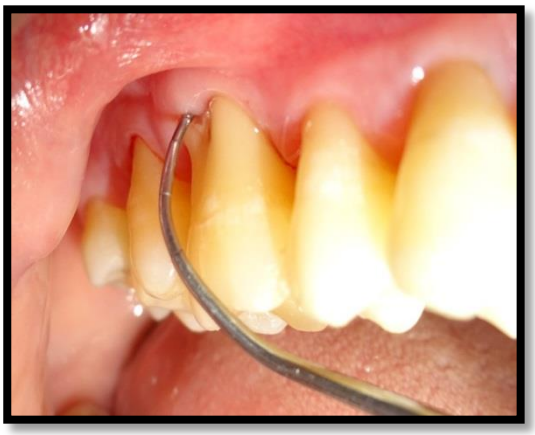
Baseline



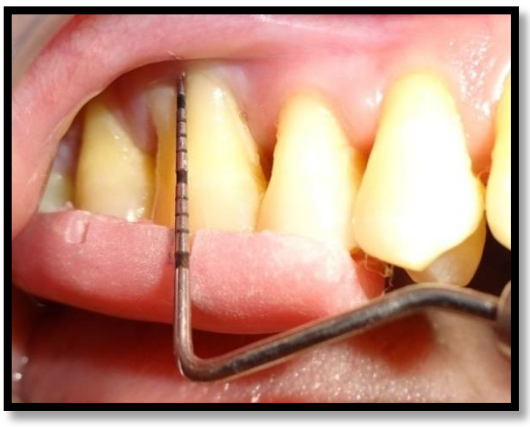
Baseline



3 months



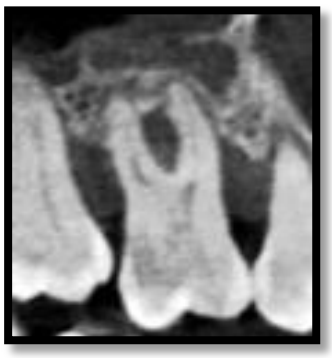
3 months



6 months

6 months

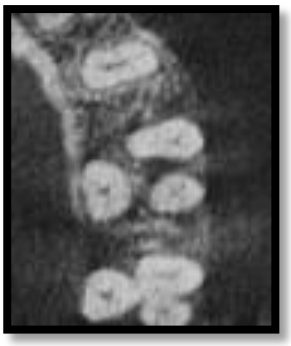
CBCT PARAMETERS FOR DFDBA



Baseline

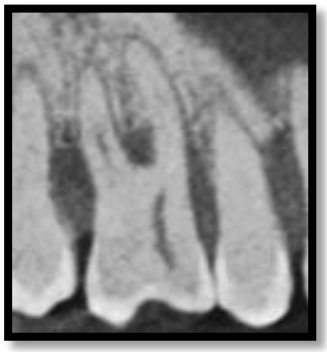
Baseline

6 months



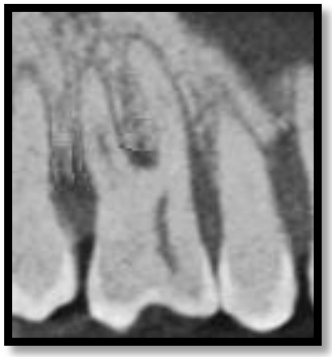
6 months

CBCT PARAMETERS FOR DFDBA + CM

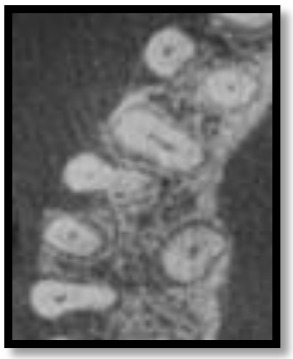


Baseline

Baseline



6 months



6 months

RESULTS

At baseline, the parameters PI, GI, PPD, CAL, GR and horizontal PD were assessed clinically and height, width and depth of the defect were assessed by CBCT. PI, GI, PPD, CAL, GR, horizontal PD was measured at all recall visits (3 months and 6 months). The CBCT analysis for defect height, width and depth was done at recall interval of 6 months.

STATISTICAL ANALYSIS

The data was analyzed using the STATA Version 20.0. The p value was taken as significant when less than 0.05.

1. The continuous variables (age, PI, GI, PPD, CAL, REC, Horizontal PD, Defect height, Defect width, Defect depth, Volume of defect) were presented as Mean ± SD.

2. PI, GI, PPD, CAL and Horizontal PD were compared at different time point in each group by performing repeated measures of Analysis of Variance (ANOVA). Pairwise comparisons were made by Tukey's multiple comparison tests.

3. Mean changes in these study parameters were compared between Group I and Group II by independent t-test.

4. Defect height, defect width, defect depth were compared before and after 6 months by performing paired t- test for normalized data. Mean change in defect height, width and depth between 2 groups were compared by independent t-test.

5. Volume of defect was compared before and after 6 month by Wilcoxon Signed Rank test for non-normalized data. Changes in volume of defect between 2 groups were compared by performing Wilcoxon Rank Sum Test (Mann-Whitney test).

20 (13 females and 7 males) systemically healthy patients affected with chronic periodontitis with a mean age of 40.85 ± 7.32 years (range 30- 60 years) presenting with 20 bilateral Grade II furcation defects were included in the present study. The selected sites were randomly included into Group I (DFDBA) and Group II (DFDBA + CM).

All the patients returned for follow-up evaluation at 3 and 6 months postoperatively. During the course of study, wound healing was uneventful in both groups and post grafting healing revealed excellent soft tissue response to both materials without any signs of infections or adverse complications. There was no untoward local and systemic reaction, indicating the biocompatibility of the CM as well as DFDBA.

Clinical indices findings at baseline, 3 months and 6 months:

In general, patients showed good oral hygiene through-out the complete duration of the study. Baseline full mouth plaque score was 2.98 ± 0.27, while at 3 months, it decreased to 2.23 ± 0.46, while at 6 months, the mean PI was 1.52 ± 0.35. The difference in PI scores when compared with baseline measurements versus 3 months, showed statistically significant decrease in plaque score ($p<0.0001$). At 6 months post-surgical PI measurements showed statistically significant decrease ($p<0.0001$) when compared to baseline, also, the reduction of plaque score from 3 months to 6 months was statistically significant ($p<0.0001$). **(Table 1) (Graph 1)**

The mean GI dropped from 1.64 ± 0.29 at baseline to 1.32 ± 0.22 at 3 months and to 1.05 ± 0.09 at 6 months. GI scores when compared with baseline to 3 months showed statistically significant decrease ($p<0.0001$) and also when compared at 6 months, the difference was statistically significant ($p<0.0001$). The mean decrease in GI from 3 months to 6 months was also statistically significant ($p<0.0001$) **(Table 1) (Graph 1).**

Soft tissue clinical parameters at baseline, 3 months and 6 months:

Probing pocket depth (PPD)

In Group I, the mean PPD at baseline was 5.45 ± 0.68 mm and that at 3 months was 4.15 ± 0.74 mm and in Group II, the mean PPD at baseline was 5.80 ± 0.83 mm and that at 3 months was 3.80 ± 0.69 mm **(Table 2) (Graph 2, 3).** At baseline there was no significant difference between Group I and Group II ($p=0.156$). At 3months, the mean PPD reduction was 1.3 ± 0.36 mm for Groups I and 2.0 ± 0.46 mm for Group II. There was a statistically significant reduction in PPD for Group I and Group II at 3 months ($p<0.0001$) and also the difference at 3 months was statistically significant between the two groups ($p<0.0001$) **(Table 2, 5) (Graph 4).**

In the Group I, the mean PPD at baseline was 5.45 ± 0.68 mm and that at 6months was 3.35 ± 0.87 mm and in Group II the mean PPD at baseline was 5.80 ± 0.83 mm and that at 6 months was 2.85 ± 0.67 mm **(Table 3) (Graph 2, 3)**. There was statistically significant reduction at 6 months compared to 3 months in both the groups. However, the reduction in PPD at 3-6 months between both the groups was not statistically significant. **(Table 4, 5) (Graph 6)** At 6 months, the mean PPD reduction was 2.10 ± 0.47 mm for Group I and 2.85 ± 0.74 mm for Group II. There was statistically significant reduction in PPD for Group I and Group II at 6 months when compared to baseline ($p<0.0001$) and in Group II when compared to Group I ($p=0.009$) **(Table 3, 5) (Graph 5).**

Clinical attachment level (CAL)

In Group I, the mean CAL at baseline was 5.80 ± 0.76 mm and that at 3 months was 4.85 ± 0.74 mm. The mean CAL at baseline in Group II was 6.10 ± 1.16 mm and that at 3 months was 4.4 ± 1.18 mm **(Table 2) (Graph 2, 3).** At baseline there was no significant difference between Group I and Group II ($p=0.342$). A mean CAL gain of 0.95 ± 0.39 mm was observed in Group I and Group II exhibited a mean CAL gain of 1.7 ± 0.57 mm. Both groups exhibited a statistically significant increase in CAL at the end of 3 months ($p<0.0001$) **(Table 5) (Graph 4).**

In Group I, the mean CAL at baseline was 5.80 ± 0.76 mm and that at 6 months was 4.17 ± 0.87 mm. Group II showed a mean baseline CAL of 6.1 ± 1.16 mm and at 6 months, it was 3.55 ± 0.99 mm **(Table 3) (Graph 2, 3).** There was statistically significant gain in CAL at 6 months compared to 3 months in both the groups. However, the gain in CAL at 3-6 months between both the groups was not statistically

significant. **(Table 4, 5) (Graph 6)** The mean CAL gain at 6 months in Group I was 1.63 ± 0.65mm and in Group II was 2.55 ± 0.67 mm. There was statistically significant CAL gain for Group I and Group II at 6 months when compared to baseline ($p<0.0001$). There was a statistically significant more CAL gain at 6 months in Group II when compared to Group I ($p=0.004$) **(Table 3, 5) (Graph 5).**

Gingival recession (GR)

In Group I, the mean GR at baseline was 0.35 ± 0.46 mm and that at 3 months was 0.70 ± 0.47 mm. The mean GR at baseline in Group II was 0.30 ± 0.57 mm and that at 3 months was 0.60 ± 0.75 mm **(Table 2) (Graph 2, 3)**. A mean increase in GR of 0.35 ± 0.41 mm was observed in Group I and Group II exhibited a mean increase in GR of 0.30 ± 0.57 mm. Group I exhibited a statistically significant increase in GR ($p=0.042$) and Group II also exhibited a statistically significant increase in GR ($p=0.030$) at the end of 3 months. However, the difference between the groups was no statistically significant ($p=0.758$) **(Table 5) (Graph 4)**.

In Group I, the mean GR at baseline was 0.35 ± 0.46 mm and that at 6 months was 0.82 ± 0.88 mm. Group II showed a mean baseline GR of 0.30 ± 0.57 mm and at 6 months, it was 0.70 ± 0.73 mm (**Table 3) (Graph 2, 3)**. There was increase in GR at 3-6 months in both the groups, however, the increases was not statistically significant. Also, the increase in GR at 3-6 months between both the groups was not statistically significant. **(Table 4, 5) (Graph 6)**The mean increase of GR at 6 months in Group I was 0.47 ± 0.59 mm and in Group II was 0.40 ± 068 mm. There was statistically significant increase in GR for Group I ($p=0.001$) and Group II ($p=0.004$) at 6 months when compared to baseline. However, the difference between the groups was no statistically

significant (p=0.758). There was increase in GR at 6 months compared to 3 month in Group I (p=0.225) and Group II (p=0.155) but that was not statistically significant. **(Table 3, 5) (Graph 5).**

Horizontal probing depth (PD)

In Group I, the mean horizontal PD at baseline was 4.5 ± 0.60 mm and that at 3 months was 3.55 ± 0.60 mm and in Group II, the mean horizontal PD at baseline was 4.95 ± 0.99 mm and that at 3 months was 3.75 ± 0.91mm **(Table 2) (Graph 2, 3).** At baseline, there was no statistical significant difference between the two groups (p= 0.093). At 3 months, the mean horizontal PD reduction was 0.95 ± 0.22 mm for Group I and 1.2 ± 0.41mm for Group II. There was a statistically significant reduction in horizontal PD for Group I as well as Group II at 3 months compared to baseline (p<0.0001). Also, the reduction in horizontal PD was significantly greater in Group II as compared to Group I (p=0.022) **(Table 5) (Graph 4).**

In the Group I, the mean horizontal PD at baseline was 4.5 ± 0.60 mm and that at 6 months was 2.85 ± 0.48 mm and in Group II the mean horizontal PD at baseline was 4.95 ± 0.99 mm and that at 6 months was 2.5 ± 0.51 mm **(Table 3) (Graph 2, 3).** There was statistically reduction in horizontal PD at 6 months compared to 3 months in both the groups. Also, the reduction in horizontal PD was statistically greater in Group II as compared to Group I (p=0.016). **(Table 4, 5) (Graph 6)** At 6 months, the mean horizontal PD reduction was 1.65 ± 0.58 mm for Group I and 2.45 ± 0.82 mm for Group II**.** There was statistically significant reduction in horizontal PD for Group I and Group II at 6 months when compared to baseline (p<0.0001). There was a statistically

significant reduction in horizontal PD at 6 months in Group II when compared to Group I (p=0.001) **(Table 3, 5) (Graph 5).**

Radiographic parameters at baseline and 6 months

CBCT analysis of Bone defect height

The mean bone defect height at baseline for Group I was 2.97 ± 0.66 mm and for Group II it was 2.91 ± 0.49 mm. At 6 months, the mean bone defect height for Group I was 2.01 ± 042 mm, showing a mean reduction of 0.96 ± 0.53 mm of bone defect height. The mean bone defect height at 6 months for Group II was 1.91 ± 0.34 mm thus exhibiting a reduction of 1.0 ± 0.37 mm of bone defect height. There was a significant reduction of bone defect height in both the groups (p<0.0001) at 6 months. The reduction of bone defect height was not statistically significant between Group II and Group I (p=0.759) **(Table 6, 7) (Graph 7, 8, 9).**

CBCT analysis of Bone defect width

The mean bone defect width at baseline for Group I was 2.22 ± 036 mm and for Group II it was 2.31 ± 0.37 mm. At 6 months, the mean bone defect width for Group I was 1.69 ± 0.35 mm, showing a mean reduction of 0.52 ± 0.18 mm of bone defect width. The mean bone defect width at 6 months for Group II was 1.42 ± 0.36 mm thus exhibiting a reduction of 0.88 ± 0.32 mm of bone defect width. There was a significant reduction of bone defect width in both the groups (p<0.0001) at 6 months. The higher reduction of bone defect width was statistically significant for Group II when compared to Group I (p<0.001) **(Table 6, 7) (Graph 7, 8, 9)**

CBCT analysis of Bone defect depth

The mean bone defect depth at baseline for Group I was 2.85 ± 0.49 mm and for Group II it was 3.12 ± 0.53 mm. At 6 months, the mean bone defect depth for Group I was 1.98 ± 0.35 mm, showing a mean reduction of 0.87 ± 0.38 mm of bone defect depth. The mean bone defect depth at 6 months for Group II was 1.81 ± 0.47 mm thus exhibiting a reduction of 1.3 ± 0.32 mm of bone defect depth. There was a significant reduction of bone defect depth in both the groups ($p<0.0001$). The higher reduction of bone defect depth was statistically significant for Group II when compared to Group I ($p<0.001$) **(Table 6, 7) (Graph 7, 8, 9)**

CBCT analysis of Bone defect volume

The mean bone defect volume at baseline for Group I was 18.87 ± 6.69 mm^3 and for Group II it was 21.62 ± 7.70 mm^3. At 6 months, the mean bone defect volume for Group I was 6.69 ± 2.56 mm^3, showing a mean reduction of 12.08 ± 4.69 mm^3 of bone defect volume. The mean bone defect volume at 6 months for Group II was 4.99 ± 2.14 mm^3 thus exhibiting a reduction of 16.62 ± 6.35 mm^3 of bone defect volume. There was a significant reduction of bone defect volume in both the groups ($p<0.0001$). The increased reduction of bone defect volume was statistically significant for Group II when compared to Group I ($p=0.015$) **(Table 6, 7) (Graph 7, 8, 9)**

DISCUSSION

The main goal of periodontal treatment is elimination of inflammatory processes in order to arrest the progression of the disease and maintenance of the natural dentition in health and comfortable function. When periodontal disease has caused loss of attachment apparatus, optimal care seeks to regenerate the periodontium to its pre-disease state. Regeneration refers to the reproduction or reconstitution of a lost or injured part, in contrast to repair, which describes healing of a wound by tissue that does not fully restore the architecture or the function of the part.[52] Regenerative periodontal therapy attempts to restore lost periodontal structures and functional attachment through the regeneration of cementum, periodontal ligament, and alveolar bone.

Inflammatory involvement of the periodontium in the furcation area of the bifurcation and trifurcation of the teeth presents a great challenge to clinicians to have access for adequate instrumentation. Various treatment modalities such as surgical debridement / resective or regenerative procedures based on the degree of furcation involvement have been established as effective means to correct the unfavourable anatomic structure, arrest its progression and promote regeneration in these areas.

The concept of guided tissue regeneration was introduced more than 30 years ago.[12, 53, 54] Melcher in 1976 presented the concept of "compartmentalization," in which the periodontal connective tissues of the periodontium were briefly divided into gingival corium, the periodontal ligament, the cementum, and the alveolar bone.[55] Under normal healing conditions, epithelial cells rapidly migrate in an apical direction to reach the most apical portion of the instrumentation area, forming a long junctional epithelium and preventing the formation of a new attachment. The aim of regenerative procedures is to limit the epithelial attachment at a more coronal position than before treatment, allowing cells from PDL and bone to repopulate the root surface and to form a new periodontal attachment. The principle of GTR is based on the principle of exclusion of gingival connective tissue cells from the wound area and to prevent the epithelial downgrowth and allow cells with regenerative potential to enter into the wound site first. Thus, placement of a barrier membrane would ensure that the detached root becomes repopulated with cells from PDL capable of forming bone, PDL and cementum. An alternative explanation is that the membrane provides sufficient space for optimal wound stability, an essential requisite for periodontal regeneration to occur with. The introduction of resorbable barrier membranes, the second generation barrier membranes, need for second surgical procedure to remove the barrier membrane was

completely eliminated. In this study a resorbable chorion membrane (CM) has been used.

GTR combined with the osseous grafting technique was invented for better clinical and histological outcomes manifested by elimination of vertical and/or horizontal defect components by bone fill. Urist and co-workers showed through numerous animal experiments that demineralization of a cortical bone graft induces new bone formation and greatly enhances its osteogenic potential.[10] Demineralization with hydrochloric acid exposes the bone inductive proteins located in the bone matrix. These proteins are collectively called bone morphogenetic protein (BMP). They are composed of a group of acidic polypeptides that have been cloned and sequenced. In addition, there appears to be homology among bone inductive proteins between mammalian species. BMP stimulates the formation of new bone by osteoinduction. That is, the demineralized graft induces host cells to differentiate into osteoblasts, whereas an undemineralized allograft is thought to function by osteoconduction as it acts as a scaffold for new bone formation. **Libin et al. (1975)**[56] were the first to report the use of cortical and cancellous DFDBA in humans.

Amniotic membranes, comprising of amnion and chorion, are the membranes building the amniotic sac that surrounds and protects the embryo and have been in the field of medicine for various purposes. The CM has numerous advantages owing to its structure and composition. The structure of CM histologically consists of reticular layer, basement membrane, and trophoblasts. The reticular layer of CM contains collagen Types I, III, IV, V, VI, and VII along with proteoglycans. The basement membrane is composed of collagen Type IV, fibronectins, and laminins. CM possesses various antimicrobial and antibacterial properties as it is immuno-privileged in nature and hence

is a well-tolerated, biodegradable membrane for use in periodontology. According to studies, fresh and dehydrated chorion has greater growth factor and cytokine load compared to fresh and dehydrated amnion.[57] The thickness of CM is about 4 times the AM accounting for its higher content of growth factors and cytokine than AM. [58] Thus, CM may aid in better reduction of inflammation. Moreover, the membrane is dry and hence quickly hydrates with blood at surgical site, making it very pliable for handling and allowing close adaptation to the contours of the underlying alveolar bone. Also, its self-adherent in nature circumvents the need to be fixed into place using sutures. It is resorbable, avoiding secondary surgical intervention for its removal. Thus CM is an allograft with many unique properties making it a promising new substitute in the field of Periodontology. In this study the Test sites were treated with combination of DFDBA and CM.

Studies in the literature have suggested the effectiveness of amnion/amniotic membrane in combination with DFDBA for periodontal regeneration;[37, 41] however, there is paucity in literature evaluating the effectiveness of the use of CM as a barrier in the treatment of Grade II furcation defects. Hence, through this study we would like to evaluate and demonstrate the superior qualities of CM as a physical barrier in combination with DFDBA in bringing about regeneration of the bone.

Thus, the present study was aimed to evaluate the efficacy of combined therapy of DFDBA + CM and DFDBA alone in the treatment of human Grade II furcation defects, clinically and by CBCT.

A total of 40 sites were selected in 20 patients with chronic periodontitis in the age group of 30 to 60 years (mean age of 40.85 ± 7.32 years) of either sex who met the

inclusion criteria were recruited and randomly assigned to each treatment sequence. All the selected patients had PPD of ≥ 5mm, CAL ≥ 3mm and radiographic evidence of identical furcation defects in either of the molar region of the mouth bilaterally. A split-mouth design was employed because it allows the comparison of outcome between the treatments. It has the ability to greatly facilitate the interpretation of trials by minimizing the effects of inter-patient variability. At baseline, no significant differences in any of the investigated parameters were observed between Group I and Group II indicating that the randomization process was effective. One defect was treated with DFDBA alone and other with DFDBA + CM. The clinical and radiographic parameters in the study were evaluated until 6 months as the dimensional alteration of the periodontal tissues after periodontal therapy occurs within the first 6 months.

During the course of study, wound healing was uneventful in Group I and Group II without any signs of infection and complications. There was no clinical evidence of untoward local and systemic immune response was detected in Test group as well as Control group, indicating the biocompatibility of CM as well as DFDBA. These findings were in accordance with the findings of **Rummelhart et al. (1989)**[59]**, Holtzclaw and Toscano (2012)**[60] and **Kothiwale et al. (2014)**[19]. **Rummelhart et al.** in the treatment of periodontal osseous defects observed continuity of bone within the defect and surrounding alveolar bone and no evidence of presence of original particulate graft material. **Holtzclaw and Toscano** and **Kothiwale et al.** reported CM's unique biologic and physical attributes that reduces the complexity of trimming, suturing and placement of barriers, minimizing the chances of post-operative complications.

Each patient participating in the study showed good oral hygiene level with healthy clinical gingival condition throughout the duration of study and demonstrated lower PI

score at the end of six months. This indicates maintenance of good oral hygiene which was the result of repeated oral hygiene instructions given to the patients throughout the study period. Plaque control is essential for the long term stability of clinical outcomes. Bacterial plaque is a major and important factor in the etiology of periodontal destruction and successful therapy depends upon its removal subsequent to treatment. The decrease in PI was statistically significant at the end of 3 months and 6 months compared to baseline. The statistical decrease in plaque index scores in our study are in accordance with the finding of **Cortellini et al. (1993)**[61] **Pontoriero et al. (1988)**[62] and **Mehta DP et al. (2018)**[42]. **Cortellini et al.** evaluated the clinical measures after periodontal regeneration in intrabony defects and observed a significant decrease in full mouth plaque score at 1 year follow up. Similarly **Pontoriero et al.** and **Mehta DP et al.** on evaluating the regenerative potential of GTR technique in Grade II furcation defects reported decrease in PI at the end of study period. The result of the above mentioned studies in accordance with our study accentuates the significance of repetitive reinforcement of oral hygiene instructions for maintenance of improved oral hygiene.

The GI reduction was found to be statistically significant, similar to the study by **Pontoriero et al. (1988)**[62] who had evaluated the regenerative potential of guided tissue regeneration Grade II mandibular molar furcation defects and found reduction in both GI and PI scores.

In the present study, the vertical clinical periodontal probing were performed by using a UNC-15 probe and customized acrylic stents with guiding grooves for reproducible probing sites. In Group I, the mean PPD at baseline was 5.45 ± 0.68 mm and that at 3 months was 4.15 ± 0.74 mm. In Group II, the mean PPD at baseline was 5.80 ± 0.83

mm and that at 3 months was 3.80 ± 0.69 mm. At 3 months, the mean PPD reduction for Group I was 1.3 ± 0.36 mm and for Group II was 2.00 ± 0.45 mm and this difference between the groups was statistically significant. Also, there was statistically significant difference in PPD in both the groups at 3 months compared to baseline. In Group I, the mean PPD at baseline was 5.45 ± 0.68 mm and that at 6 months was 3.35 ± 0.87 mm. In Group II, the mean PPD at baseline was 5.80 ± 0.83 mm and that at 6 months was 2.85 ± 0.67 mm. There was statistically significant difference in PPD in both the groups at 6 months compared to baseline. At 6 months, the mean PPD reduction for Group I was 2.1 ± 0.47 mm and for Group II was 2.85 ± 0.74 mm and this difference between the groups was statistically significant. These results were similar to those reported by **Anderegg et al. (1991)**[33] who evaluated the potential of DFDBA combined with a barrier material in the treatment of human molar furcation defects as compared to the barrier technique alone and found a significant improvement in probing depth reduction at 6 month and greater reduction of probing depth in sites treated with barrier membrane along with DFDBA. Similar reduction in the mean probing depths in both Experimental and Control group with significantly greater reduction in experimental group at end of 6 months were reported by **Luepke et al. (1997)**[35] who treated Grade II furcation defects with resorbable barrier alone (Control group) and resorbable barrier in combination with DFDBA (Experimental group). The resemblance in the findings of both the previous studies suggest that the addition of DFDBA in combination with a barrier is advantageous to barrier alone GTR technique. Similar reduction in PPD has been reported by **Kothiwale et al. (2009)** in Grade II furcation defects and **Kiany et al. (2015)** in intrabony defects. **Kothiwale et al. (2009)**[37] compared the efficacy of DFDBA and BDX as separate regenerative materials along with amniotic membrane as GTR in Grade II buccal furcation defects and reported significant reduction in PPD at 6

months and 9 months for both the treatment modalities. **Kiany et al. (2015)**[63] evaluated and compared the efficacy of AM with BBM and a CM with BBM in GTR for the treatment of intrabony periodontal defects in 6 month clinical trial and reported significant PPD reduction.

However, contradictory results were shown by **Kruger et al. (2000)**[64] who found no statistically significant reductions in PPD in 2 groups, one receiving a bioresorbable membrane and another treated only with OFD over a period of one year. The authors have attributed these deviating results to the fact that the pre-conditions of every individual varied considerably, defects were diverse in nature and treatment was performed with or without antibiotics. Contradictory results were also shown by **Gantes et al. (1988)**[65] where they compared DFDBA to OFD and found no statistically significant differences in PPD reduction when observed over a period of 12 months.

The most commonly used soft tissue clinical outcome variable in regenerative studies is the clinical attachment level (CAL). In Group I the mean CAL at baseline was 5.8 ± 0.76 mm and that at 3 months was 4.85 ± 0.74 mm. In Group II, the mean CAL at baseline was 6.1 ± 1.16 mm and that at 3 months was 4.4 ± 1.18 mm. At 3 months, the mean CAL gain for Group I was 0.95 ± 0.39 mm and for Group II was 1.7 ± 0.57 mm and this difference between the groups was statistically significant. Also, there was statistically significant difference in CAL in both the groups at 3 months compared to baseline.

In Group I, the mean CAL at baseline was 5.8 ± 0.76 mm and that at 6 months was 4.17 ± 0.87 mm. In Group II, the mean CAL at baseline was 6.1 ± 1.16 mm and that at 6 months was 3.55 ± 0.99 mm. At 6 months, the mean CAL gain for Group I was 1.63 ± 0.65 mm and for Group II was 2.55 ± 0.67 mm and this difference between the groups

was statistically significant. Also, there was statistically significant difference in CAL in both the groups at 6 months compared to baseline.

These finding of our study correlate to the findings of **Wang et al. (1994)**[15] who observed a CAL gain of 1.67 mm in class II furcation defects treated with Collagen membrane compared to 0.67 mm in OFD at the end of 12 month. The lesser amount of CAL gain in the above mentioned study as compared to our study could be due to variations in the defects sizes, the material used, etc. As barrier membranes without bone graft was used in the study there might be a possibility that the membrane had collapsed into the defect which could have resulted in less amount of CAL gain. Also, **Luepke et al. (1997)**[35] observed a CAL gain of 1.37 mm in sites treated with bioresorbable barrier membranes alone compared to CAL gain of 1.83 mm in sites treated with combination of barrier membrane and DFDBA indicating a significant CAL gain in both the sites when compared to presurgery status. The findings of our study correlate with the findings of the study by **Mehta DB et al. (2018)**[42] who observed a CAL gain of 2.8 mm and 3.0 mm at the end of 6 months in Grade II furcation defects treated with PRF membrane in combination with DFDBA and collagen membrane in combination with DFDBA respectively. Both the groups showed statistical significant CAL gain at 3 months and 6 months which could be due to either the presence of partially resorbed particles of the graft that may hinder the probe or true regeneration of connective attachment due to the presence of the graft alone or in combination with membrane overlying the graft that may have created and maintained a space underneath the membrane or true bone formation.

A statistical significant CAL gain at 3 months and 6 months in our study and above mentioned two studies could be due to either the presence of partially resorbed particles

of the graft that may hinder the probe or true regeneration of connective attachment due to the presence of the graft alone or in combination with membrane overlying the graft that may have created and maintained a space underneath the membrane or true bone formation.

It is a known fact that in majority of the clinical trials for treatment of Grade II furcation defects wherein regenerative therapies have been utilized, there is reduction in PPD as well as gain in CAL postoperatively. In our clinical trial, we substantiated the above findings. Also, the PPD reduction and CAL gain observed in our clinical trial with combination therapy (DFDBA + AM) was better as compared to the monotherapy (DFDBA). This could be due to the fact that, the membrane prevents epithelial migration and the bone graft material resorbs and provides space for progenitor cells to occupy these voids thereby leading to regeneration. Moreover, the immunohistochemical staining analysis of chorion membranes showed intense concentrations of laminin and laminin‑5 throughout the barrier which is of particular importance due to its high affinity for binding gingival epithelial cells for better adaptation to the root surface.[66, 67] These characteristics may have contributed in better PPD reduction and CAL gain in the Group II.

The degree of gingival recession after surgery is one of the several factors influencing the process of regeneration, as the more the gingiva recedes, the shorter the root surface portion that is available for PDL cell repopulation and hence we have observed the change in the level of marginal gingival level pre- and post- surgical therapy.

In Group I, the mean GR at baseline was 0.35 ± 0.48 mm and that at 3 months was 0.70 ± 0.47 mm. In Group II, the mean GR at baseline was 0.30 ± 0.57 mm and that at 3 months was 0.60 ± 0.75 mm. At 3 months, the mean increase in GR for Group I was

0.35 ± 0.41 mm and for Group II it was 0.30 ± 0.57 mm and this difference was statistically significant compared to baseline for both the groups. However, there was no statistically significant difference in GR between both the groups at 3 months.

In Group I, the mean GR at baseline was 0.35 ± 0.48 mm and that at 6 months was 0.82 ± 0.88 mm. In Group II, the mean GR at baseline was 0.30 ± 0.57 mm and that at 6 months was 0.70 ± 0.73 mm. At 6 months, the mean increase in GR for Group I was 0.47 ± 0.59 mm which was statistically significant and for Group II was 0.40 ± 0.68 mm which was statistically significant compared to baseline. But, there was no statistically significant difference in GR between both the groups at 6 months. It was found that there was no significant difference between two groups regarding increase in GR between 3-6 months.

Similar findings were reported by **Wang et al. (1994)** and **Bouchard et al. (1997)**. **Wang et al. (1994)**[15] observed an increase in GR of 0.08 mm at sites treated with collagen barrier membrane in combination with OFD whereas an increase of 1.08 mm at sites treated with OFD alone at the end of 12 months, however, there was no statistical difference between both the treatment groups. **Bouchard et al. (1997)**[68] reported a change in depth of recession of 0.6 mm and 0.8 mm at 12 months at sites treated with e-PTFE membrane and PLA/ PGA membrane respectively in mandibular buccal Class II furcation defects with no significant difference between both the groups.

The primary response variable in the treatment of furcation defects is the gain in horizontal probing depth. In Group I, the mean horizontal PD was 4.50 ± 0.60 mm, 3.55 ± 0.60 mm and 2.85 ± 0.48 mm at baseline, 3 month and 6 months respectively. In Group II, the mean horizontal PD was 4.95 ± 0.99 mm, 3.75 ± 0.91 mm and 2.50 ± 0.51 mm at baseline, 3 month and 6 month respectively. There was statistically significant

difference in horizontal PD in both the groups at 3 and 6 months compared to baseline. At 3 months, the mean horizontal PD reduction for Group I was 0.95 ± 0.22 mm and for Group II was 1.20 ± 0.41 mm and this difference between the groups was statistically significant. At 6 months, the mean horizontal PD reduction for Group I was 1.65 ± 0.58 mm and for Group II was 2.45 ± 0.82 mm and this difference between the groups was statistically significant. These results were similar to those reported by **Leonardis et al. (1999)**[69] who evaluated and compared GTR + DFDBA (Test group) and GTR (Control group). The clinical efficacy of the 2 treatment modalities was evaluated at 6 and 12 months postoperatively. The placement of DFDBA in the furcation defect under the bioabsorbable membrane resulted in a greater mean reduction of horizontal PD when compared to the regenerative therapy alone. An analogous study conducted by **Pajnigara N et al. (2017)**[41] on evaluation of the regenerative efficacy of DFDBA with or without amnion membrane in Grade II furcation defects reported higher mean horizontal PD reduction in group treated with DFDBA in combination with amnion membrane than in group treated with DFDBA graft alone.

However, contradictory results have been reported by **Tsao YP et al. (2006)**[70] who evaluated the association between factors and clinical parameters that may influence the outcomes of three different treatment modalities (open flap debridement alone [OFD], bone graft [BG], and bone graft plus a bioabsorbable collagen membrane [BG + C]), in mandibular Class II furcation defects and reported no significant correlation between reduction in horizontal PD and higher probability of achieving improvement of clinical outcomes.

The teeth with Grade I FI can be treated predictably with non-regenerative therapies / surgical debridement alone, whereas teeth with Grade II/ Grade III FI may require

invasive regenerative surgical procedures. Hence, accurate pre-surgical diagnosis of degree of FI is essential to elect the indicated mode of periodontal therapy. However, current diagnostic approaches including clinical probing and intraoral radiography have shown several limitations in their reliability.

Clinical probing is dependent on the probing force, while periapical radiographs or bitewing radiographs may over or underestimate the amount of bone loss due to projection errors. One of the main drawbacks of intraoral radiography is the overlap of anatomical structures and lack of 3D information. This often hinders a true distinction between the buccal and lingual cortical plate and complicates the evaluation of periodontal bone defects, especially the infrabony lesions, also denoted as craters, and furcation involvements. Several efforts for optimizing these diagnostic tools have been made over the past few years. Conventional CT solves this problem by providing axial slices throughout the object of interest but has major drawbacks, including high radiation dose, high cost and low resolution. CBCT is a recently developed imaging modality, reinforcing this 3D assessment of bone defects. When compared with conventional CT, CBCT considerably reduces radiation exposure to patients. Minute changes within the alveolar bone in terms of gauging the height, width and depth of a defect such as Grade II furcation defect can be recorded with greater accuracy in CBCT, which no other tool will be in a position to record. Also, digital images can be stored easily in computer memories and can be easily reconstructed for viewing from any direction at any time.

In the current clinical trial, the mean bone defect volume at baseline for Group I was 18.87 ± 6.31 mm^3 and that at 6 months was 6.69 ± 2.56 mm^3 showing a mean reduction of 12.08 ± 4.69 mm^3. Group II showed a mean bone defect volume of 21.62 ± 7.70 mm^3

at baseline which decreased to 4.99 ± 2.14 mm^3at 6 months, exhibiting a mean reduction of 16.62 ± 6.35 mm^3. Both groups showed statistically significant reduction in bone defect volume at 6 months when compared to baseline. However, Group II showed the greater reduction of bone defect volume when compared to Group I and this difference was statistically significant. Group II showed statistically significant bone defect volume reduction compared to Group I indicating better results for combination therapy over monotherapy.

These findings are in accordance to the similar results with regards to bone volume shown by **Rajnay et al. (1996)**[71] who evaluated the volume of defect fill at histological level, treated with GTR membrane in baboons. Groups consisted of ePTFE membrane, polylactic acid membrane and OFD in treated molar furcations. From the histologic data, a computer calculated the volume of new bone, connective tissue, epithelium and cementum as a percentage of original defect size. The results indicated that digital imaging technology is a useful research tool for determining the volume of defect fill. It is however worthy to note that the findings of our study indicated radiographic bone fill.

Though it is one of the very few clinical trials which calculated volumetric bone fill radiographically, it is pertinent to note that periodontal regeneration can only be assessed by histologic evaluation. Since the healing pattern in our clinical trial is similar to other trials which have assessed results histologically, it can be well be said that the radiographic bone fill which was evident in CBCT was due to the regenerative process which had taken place within the surgical wound. **Ito et al. (2001)**[72] compared the efficacy of 3DCT and conventional IOPA to check the regenerative capacity of GTR membrane in furcation defect and found that no significant differences were seen in IOPA after 1 year, however, the CT images showed a significant regrowth of bone

coronally and the entrance of furcation area was much narrower when the results were observed at the end of 1 year.

Mishra PR et al. (2018) [73] clinically and radiographically evaluated the concerted effect of synthetic anorganic bone matrix/cell-binding peptide (ABM/P-15) along with OFD in treatment of infrabony defects and reported a greater mean reduction of radiographic defect depth at the end of 6 months and observed greater defect resolution which could be attributed to the obvious defect fill by the bone. This results of our study are found to be in accordance with **Khanna D et al. (2012)**[74] who had treated buccal mandibular Grade II furcation defects with hydroxyapatite bone grafting (HA) along with GTR and compared the results with OFD alone and reported that on comparisons between the initial and six-month postoperative radiographs revealed better radiographic bone level improvement in the GTR group, in which an increased radio-opacity around the furcation area appeared as a pattern, at the six-month postoperative evaluation. The synergistic effect of DFDBA combined with GTR has also been evaluated by **Kher et al. (2013)**[38]in intrabony defects where the authors found significantly greater radiographic DD reduction in DFDBA plus GTR group post-operatively at 6 months as compared to GTR alone. **Rosen P (2013)**[75] in a case report treated a Class III furcation on the first molar which was having 1+ degree mobility. A composite allograft containing mesenchymal cells was used to fill the defect, and it was layered by a barrier derived from human amnion–chorion tissue. Six months after the completion of surgery, the furcation was closed to a Nabers probe from both the buccal and lingual aspects and radiographically by CBCT, the area was radiopaque suggestive of bone fill/containment. **Grimard et al. (2009)**[76]compared intra-oral radiographs (IRs) and cone-beam volumetric tomography (CBVT) to direct surgical evaluations to assess bone level

changes following regenerative therapy and concluded that compared to direct surgical measurements CBVT images reflect more precise and accurate bony defect dimensions than IR. Thus CBVT is an equivalent substitution to direct surgical measurements and can obviate the need for surgical re-entry to assess regenerative therapy outcomes. Also, **Braun et al. (2014)**[77] concluded CBCT images offer an advantageous alternative to the conventional single-tooth radiograph while taking the higher exposure of radiation into account and also a considerably more precise analysis of periodontal defects is possible due to the third dimension. The accuracy in assessing the volumetric changes in periodontal infrabony defect volume using CBCT have been evaluated by **Wanikar I et al. (2018)**[78] in grade II furcation defects and by **Bodhare G et al. (2018)**[79] in intrabony defects. **Wanikar et al. (2018)**[78] evaluated the synergistic efficacy of 1% alendronate (ALN) gel in combination with platelet rich fibrin (PRF) and PRF alone in treating Grade II furcation teeth and confirmed radiographically by CBCT images that there was greater mean reduction in bone defect volume of 11.98 ± 4.13 mm^3 for PRF + ALN than PRF alone. Similarly, **Bodhare G et al. (2018)**[79] compared the clinical changes and radiographic bone fill acquired by pre-operative and post-operative CBCT images for Bioactive Glass (BG) with and without autologous PRF in intrabony defects and reported an achievement of greater bone fill with BG+PRF than with PRF alone.

The literature suggests that CM and DFDBA when used alone and in combination can result in periodontal regeneration on a previously diseased contaminated root surface, but due to constraints we could not do histological analysis and thus cannot confirm that CM and DFDBA results in true periodontal regeneration. However, if we correlate our study with the previous studies, then it will not be unreasonable to consider that the Test group yielded healing which was quite similar to that of true periodontal regeneration as

was assessed by clinical and radiographic parameters. Like the studies mentioned above CBCT analysis also enabled us to assess the volumetric healing characteristics to a certain extent.

Like the studies mentioned above, CBCT analysis also enabled us to assess the volumetric healing characteristics to a certain extent and helped in the analysis of regeneration radiographically with relatively less invasion.

CONCLUSION

The present randomized controlled clinical and CBCT study was undertaken to compare the effectiveness of DFDBA plus CM and DFDBA alone in the treatment of Grade II furcation defects. 20 systemically healthy subjects with 20 bilateral Grade II furcation defects were selected for the study. Baseline measurements included PI, GI, PPD, CAL, GR and bone defect height, depth and width by CBCT. At the time of surgery, defects were randomly assigned to either Group I i.e. Control group (DFDBA) or Group II i.e. Test group (DFDBA + CM). PI, GI, PPD, CAL and GR were assessed at 3 and 6 months while the CBCT analysis was done at 6 months.

During the course of study, healing was uneventful in both Group I and Group II, without any signs of infection or complications. There was no untoward local and systemic reaction indicating the biocompatibility of the materials used. No clinical evidence of undesirable, immune response was detected and no evidence of tissue reaction was seen.

The reductions in PI and GI indicated satisfactory maintenance of oral hygiene by patients throughout the study period. A statistically significant greater reduction in PPD was observed in Group II than in Group I at 3 months and 6 months. There was a mean CAL gain of 1.7 ± 0.65 mm and 2.35 ± 0.67 mm in Group I and Group II respectively at 6 months was. The horizontal PD reduction in Group II was significantly greater than in Group I at 3 months and 6 months.

The mean reduction in bone defect height, width and depth in Group II was statistically significantly greater than in Group I at 6 month evaluation. Also, there was statistically significant reduction in volume of defect in Group II as compared to Group I at 6 month evaluation.

From the analysis of results, following conclusions were drawn:

1. DFDBA + CM resulted in statistically significant reductions of PPD at 3 months and 6 months, compared to DFDBA alone.
2. DFDBA + CM showed statistically significant CAL gain at 3 months and 6 months, compared to DFDBA alone.
3. DFDBA + CM showed significantly better results in terms of reduction of bone defect volume at 6 months, compared to DFDBA alone.

In conclusion, within the limits of the present study, both the treatment groups resulted in significant periodontal regeneration as assessed by clinical and radiographic parameters, but use of DFDBA + CM could be more beneficial in achieving better results in terms of periodontal regeneration.

Attempting to identify the most accurate method for evaluating hard tissue changes after periodontal therapy is an important task. To date, re-entry procedure appears to be the gold standard and, while no single method can produce similar information consistently. The images obtained by CBCT, combined with clinical measurements, will definitely increase our ability to determine the treatment outcome without the use of re-entry procedure.

It should be noted that the differences in healing patterns, microbial pathogens, study designs, patient population, measurement techniques and human defect variations make it difficult to compare clinical results. Also, different methods like clinical, histological and radiographic evaluations have been used in various studies for assessing the outcomes of treatments. This could be some of the reasons for variations observed amongst clinical trials. Further studies that exploit properties of CM in the field of periodontal regeneration and tissue engineering are encouraged.

LIMITATIONS

The following limitations were observed in the present study:

1. The sample size in the present study was limited to 20 bilateral furcation defects. The primary results of the study should be substantiated by similar investigations on larger number of sample defects.

2. Long term analysis is needed to determine the stability of the results and to improve the radiographic assessment of the results.

3. The nature of attachment between the newly regenerated tissue and root surface could not be assessed since the ethical considerations as well as associated patient non-acceptance restricted the re-entry surgery to assess bone fill and extraction of the treated teeth for histological examination.

4. The design of the study did not allow us to evaluate as to what extent of clinical improvement could be attributed to chorion membrane alone, as use of chorion membrane alone was not included in the study.

5. The operator was the assessor in the present study and there were no blinded examinations. Therefore possibility of operator bias to some extent cannot be ruled out.

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Table 1: Comparison of clinical indices among the study population

	Plaque Index	**Gingival Index**
Baseline	2.98 ± 0.27	1.64 ± 0.29
3 months	2.23 ± 0.46	1.32 ± 0.22
Mean difference at 3 months	0.75 (p<0.0001, HS)	0.32 (p<0.0001, HS)
6 months	1.52 ± 0.35	1.05 ± 0.09
Mean difference at 6 months	1.46 (p<0.0001, HS)	0.59 (p<0.0001, HS)
Mean difference between 3 months – 6 months	0.71 (p<0.0001, HS)	0.27 (p<0.0001, HS)

(HS: highly significant)

Table 2: Comparison of clinical parameters (in mm) in both the groups at 3 months

	Group I			**Group II**		
	Baseline	**3 months**	**p-value**	**Baseline**	**3 months**	**p-value**
PPD	5.45 ± 0.68	4.15 ± 0.74	<0.0001, HS	5.80 ± 0.83	3.80 ± 0.69	<0.0001, HS
CAL	5.80 ± 0.76	4.85 ± 0.74	<0.0001, HS	6.1 ± 1.16	4.4 ± 1.18	<0.0001, HS
GR	0.35 ± 0.48	0.70 ± 0.47	0.042, S	0.30 ± 0.57	0.60 ± 0.75	0.030, S
HPD	4.50 ± 0.60	3.55 ± 0.60	<0.0001, HS	4.95 ± 0.99	3.75 ± 0.91	<0.0001, HS

(HS: highly significant; S-significant)

Table 3: Comparison of clinical parameters (in mm) in both the groups at 6 months

	Group I			Group II		
	Baseline	**6 months**	**p-value**	**Baseline**	**6 months**	**p-value**
PPD	5.45 ± 0.68	3.35 ± 0.87	<0.0001, HS	5.80 ± 0.83	2.85 ± 0.67	<0.0001, HS
CAL	5.80 ± 0.76	4.17 ± 0.87	<0.0001, HS	6.1 ± 1.16	3.55 ± 0.99	<0.0001, HS
GR	0.35 ± 0.48	0.82 ± 0.88	0.0001, S	0.30 ± 0.57	0.70 ± 0.73	0.004, S
HPD	4.50 ± 0.60	2.85 ± 0.48	<0.0001, HS	4.95 ± 0.99	2.50 ± 0.51	<0.0001, HS

(HS: highly significant; S: significant)

Table 4: Comparison of clinical parameters (in mm) in both the groups at 3-6 months

	Group I			Group II		
	3 months	**6 months**	**p-value**	**3 months**	**6 months**	**p-value**
PPD	4.15 ± 0.74	3.35 ± 0.87	<0.0001, HS	3.80 ± 0.69	2.85 ± 0.67	<0.0001, HS
CAL	4.85 ± 0.74	4.17 ± 0.87	<0.0001, HS	4.4 ± 1.18	3.55 ± 0.99	<0.0001, HS
GR	0.70 ± 0.47	0.82 ± 0.88	0.225, NS	0.60 ± 0.75	0.70 ± 0.73	0.155, NS
HPD	3.55 ± 0.60	2.85 ± 0.48	<0.0001, HS	3.75 ± 0.91	2.50 ± 0.51	<0.0001, HS

(HS: highly significant; NS: non-significant)

Table 5: Comparison of clinical parameters (in mm) between both the groups at different time intervals

	Baseline v/s 3 months			**Baseline v/s 6 months**			**3 months v/s 6 months**		
	Group I	**Group II**	**p-value**	**Group I**	**Group II**	**p-value**	**Group I**	**Group II**	**p-value**
PPD reduction	1.30 ± 0.36	2.0 ± 0.45	<0.0001, HS	2.10 ± 0.47	2.85 ± 0.74	0.009, HS	0.80 ± 0.48	0.95 ± 0.67	0.114, NS
CAL gain	0.95 ± 0.39	1.70 ± 0.57	<0.0001, HS	1.63 ± 0.65	2.55 ± 0.67	0.004, HS	0.68 ± 0.63	0.85 ± 0.74	0.651, NS
GR increase	0.35 ± 0.41	0.30 ± 0.57	0.758, NS	0.47 ± 0.59	0.4 ± 0.68	0.547, NS	0.12 ± 0.59	0.10 ± 0.41	0.225, NS
HPD reduction	0.95 ± 0.22	1.2 ± 0.41	0.022, S	1.65 ± 0.58	2.45 ± 0.82	0.001, S	0.70 ± 0.57	1.25 ± 0.78	0.016, S

(HS: highly significant, S: significant, NS: non-significant)

Table 6: Comparison of radiographic parameters in both the groups at different time interval

	Group I			Group II		
	Baseline	**6 months**	**p-value**	**Baseline**	**6 months**	**p-value**
Bone defect height (in mm)	2.97±0.66	2.01±0.42	<0.0001, HS	2.91 ± 0.49	1.91 ± 0.34	<0.0001, HS
Bone defect width (in mm)	2.22 ± 0.36	1.69 ± 0.35	<0.0001, HS	2.31 ± 0.37	1.42 ± 0.36	<0.0001, HS
Bone defect depth (in mm)	2.85 ± 0.49	1.98 ± 0.35	<0.0001, HS	3.12 ± 0.53	1.81 ± 0.47	<0.0001, HS
Bone defect volume (in mm^3)	18.87 ± 6.31	6.69 ± 2.56	<0.0001, HS	21.62 ± 7.70	4.99 ± 2.14	<0.0001, HS

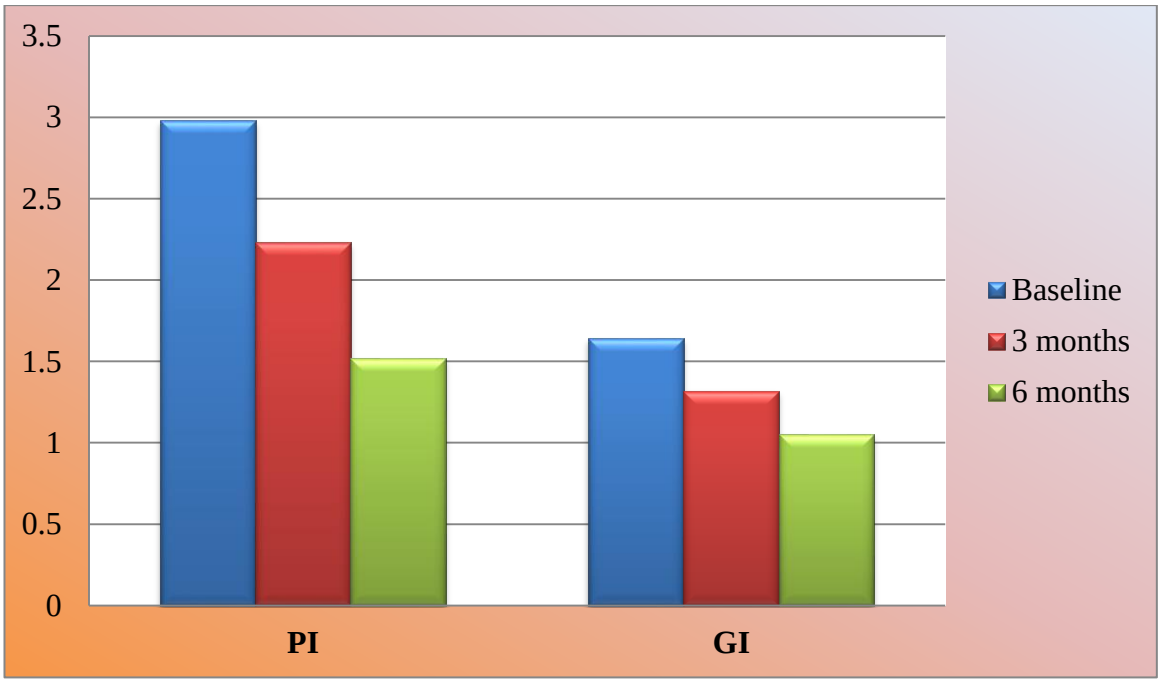
(HS: highly significant)

Table 7: Comparison of radiographic parameters between both the groups

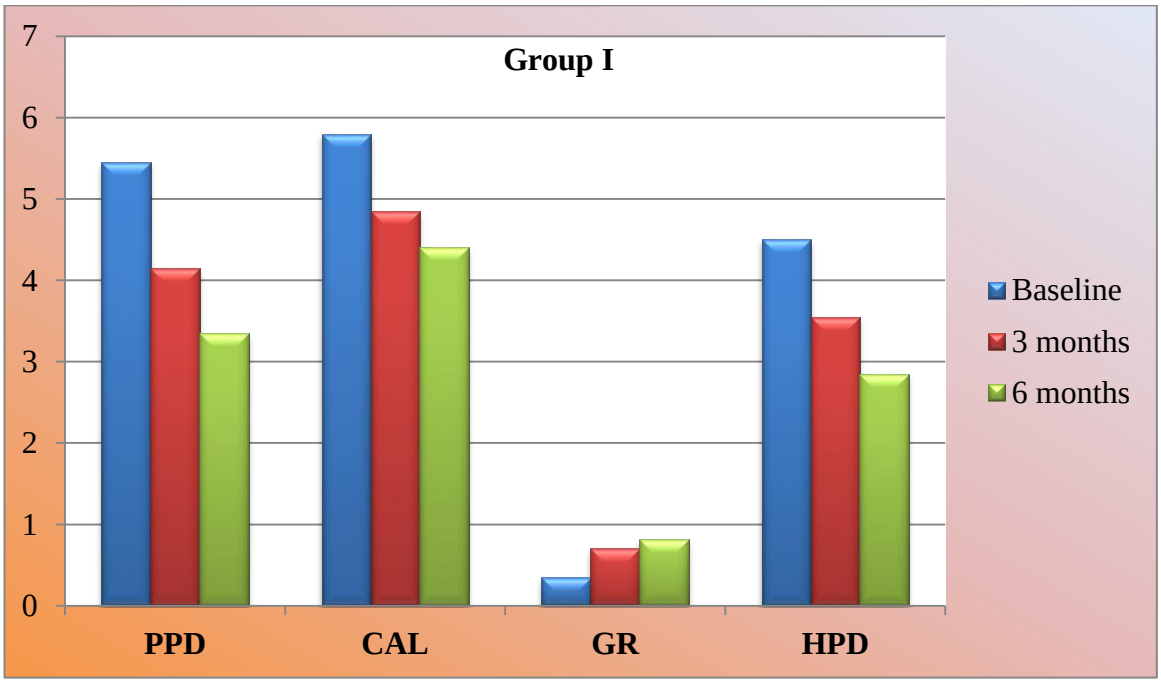
	Group I	**Group II**	**p-value**
Gain inBone defect height (in mm)	0.96 ± 0.53	1.0 ± 0.37	0.759, NS
Gain in Bone defect width (in mm)	0.52 ± 0.18	0.88 ± 0.32	<0.001, S
Gain in Bone defect depth (in mm)	0.87 ± 0.38	1.3 ± 0.32	<0.001, S
Gain in Bone defect volume (in mm^3)	12.08 ± 4.69	16.62 ± 6.35	0.015, S

(HS: highly significant)

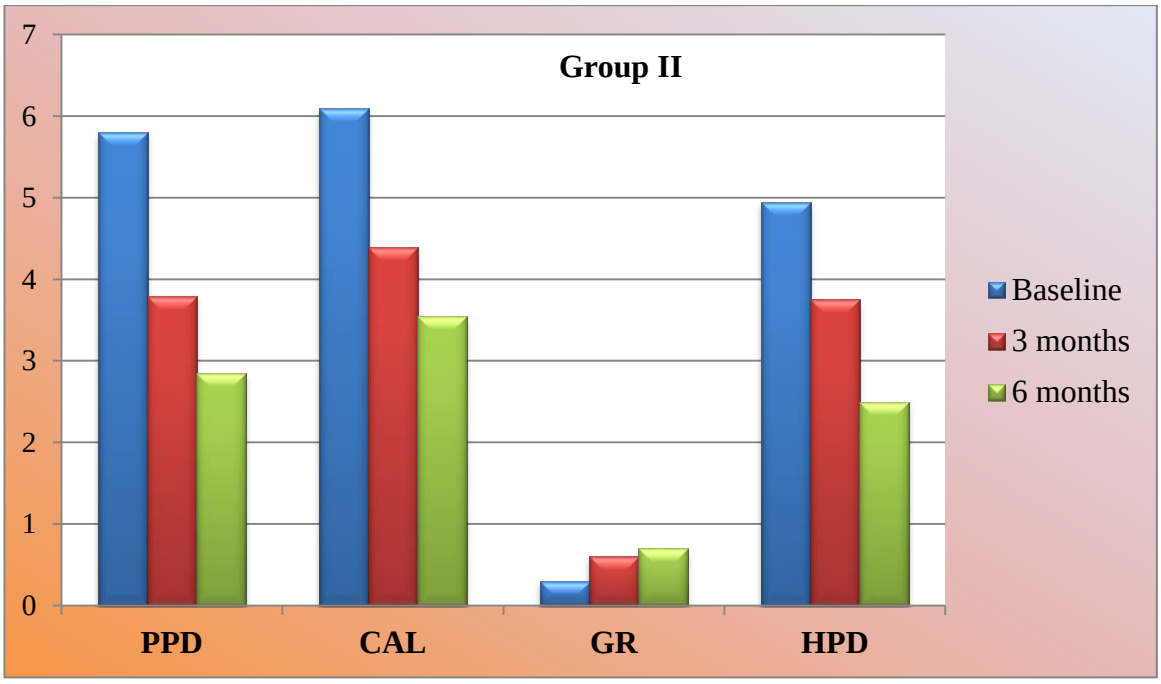
Graph 1: Comparison of clinical indices among the study population



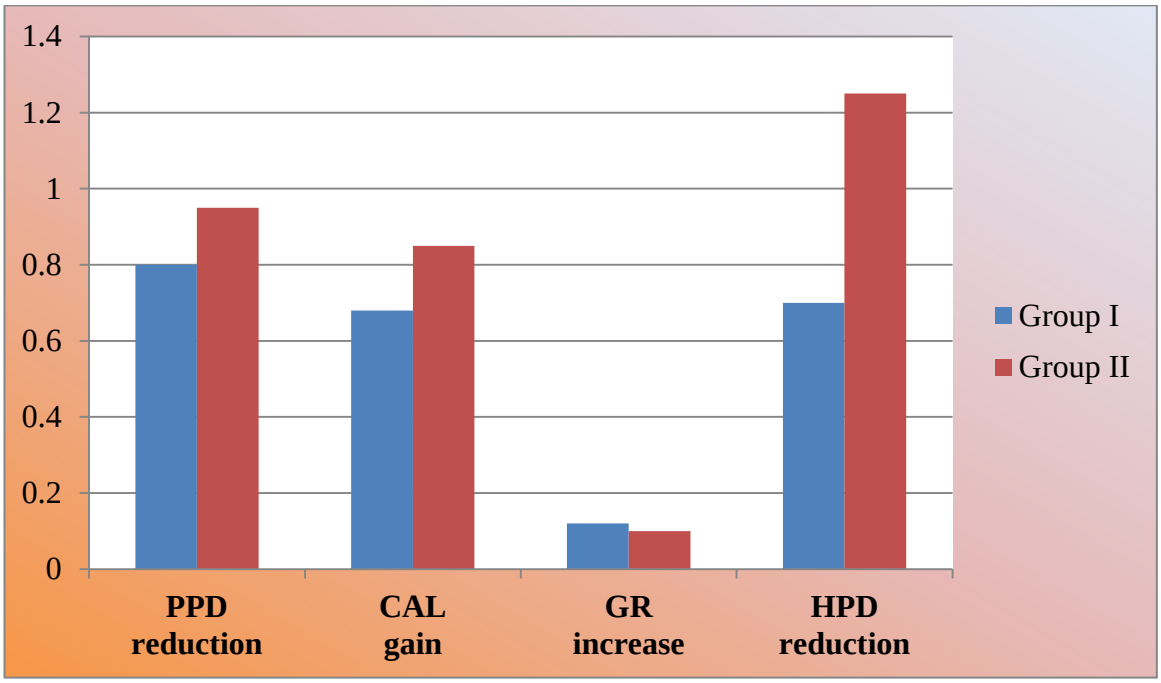
Graph 2: Comparison of clinical parameters at different time intervals in Group I



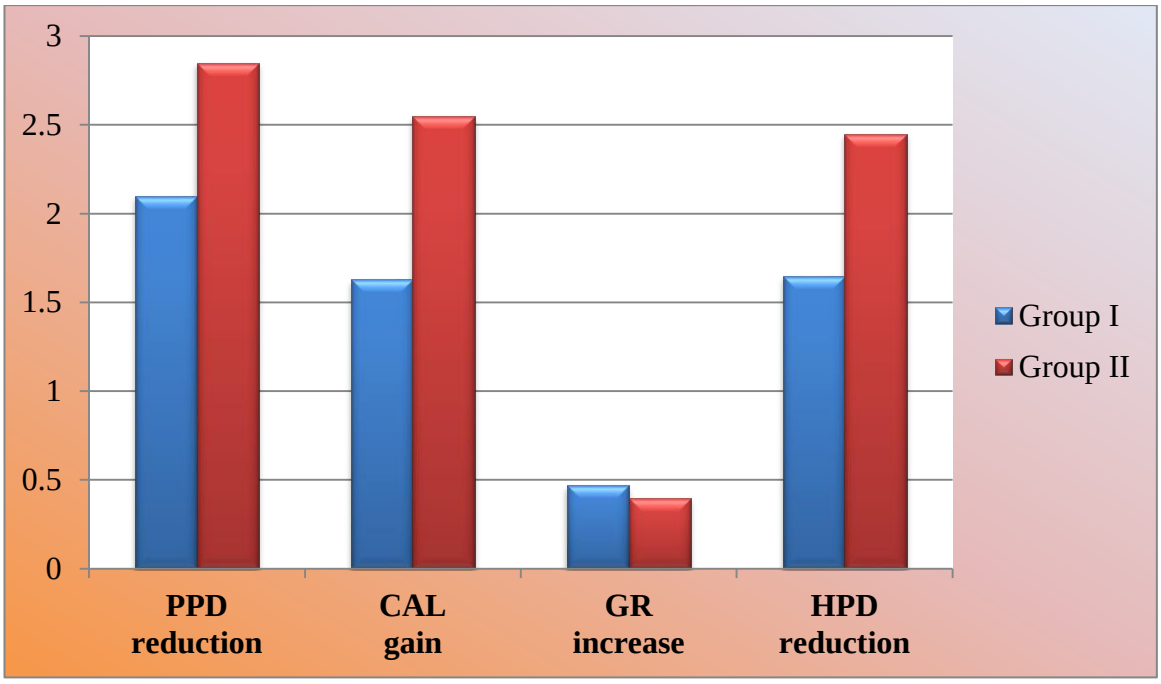
Graph 3: Comparison of clinical parameters at different time intervals in Group II



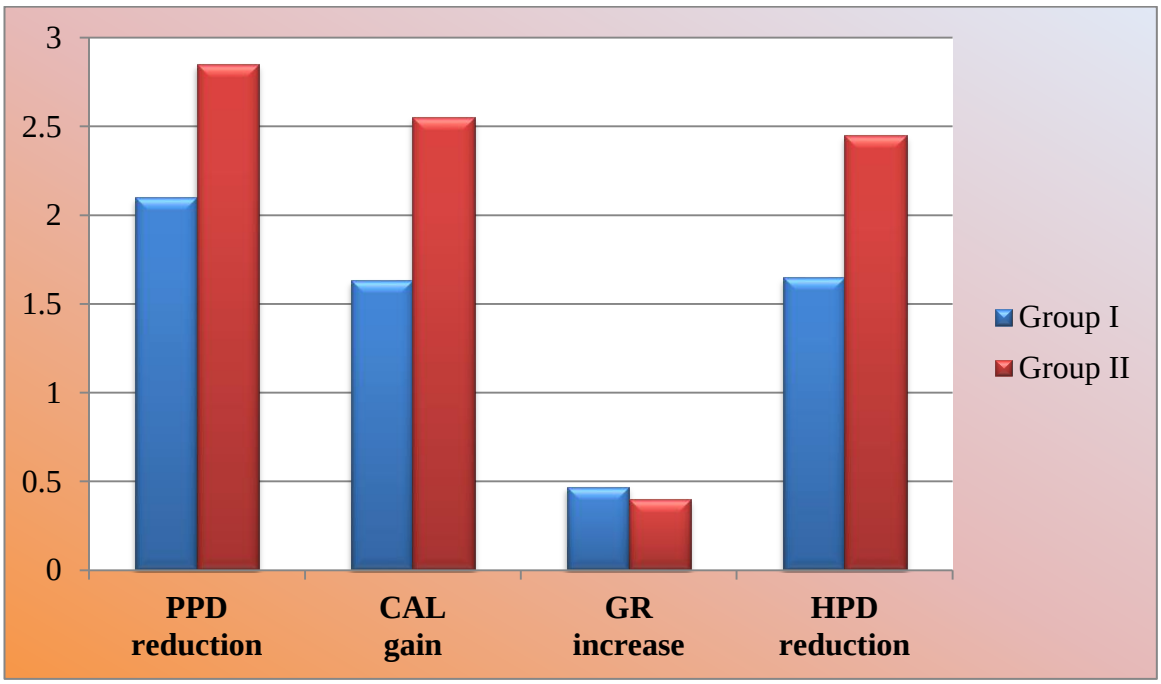
Graph 4: Comparison of clinical parameters between both the groups at 3 months



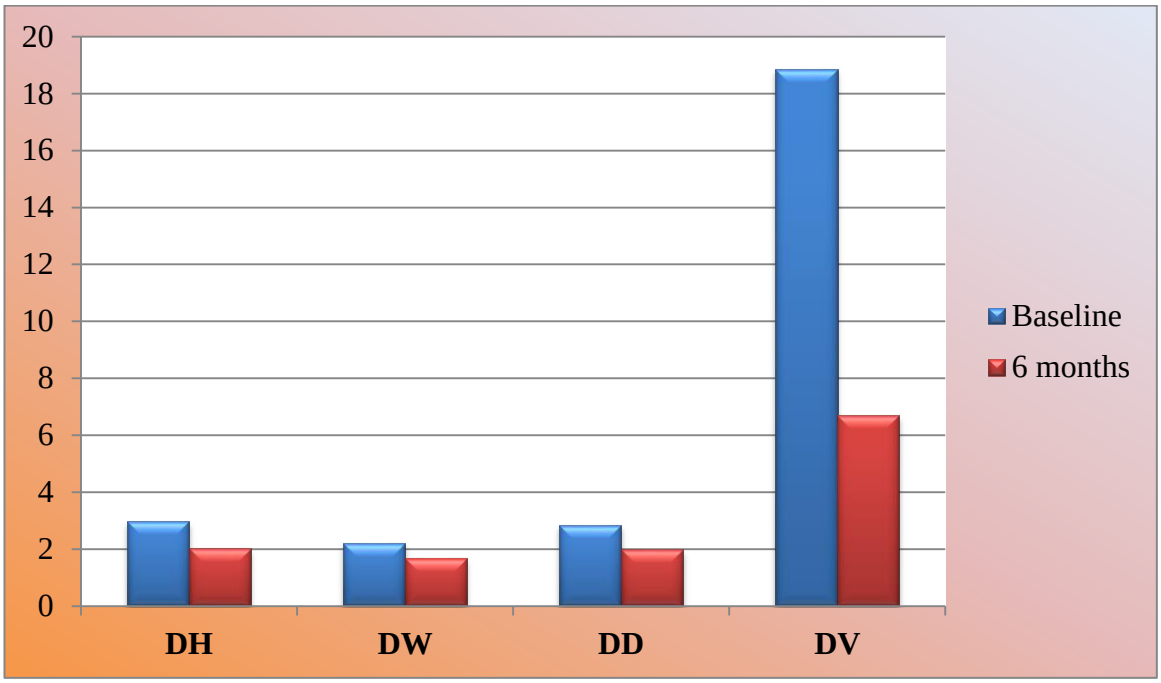
Graph 5: Comparison of clinical parameters between both the groups at 6 months



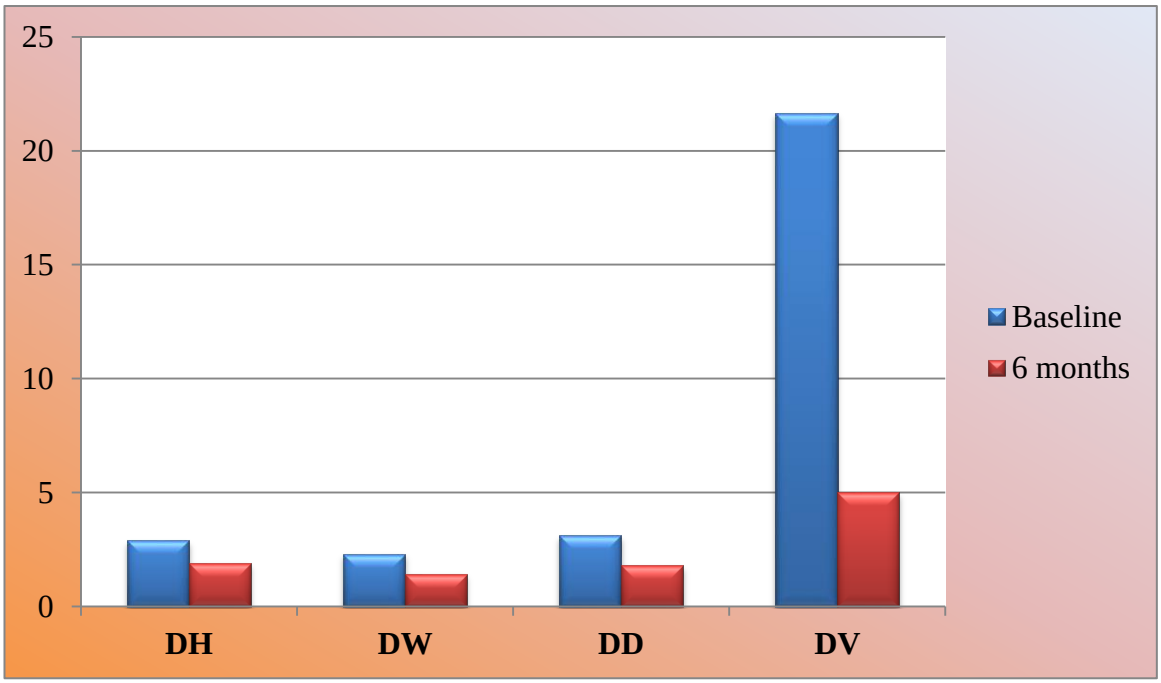
Graph 6: Comparison of clinical parameters between both the groups at 3-6 months



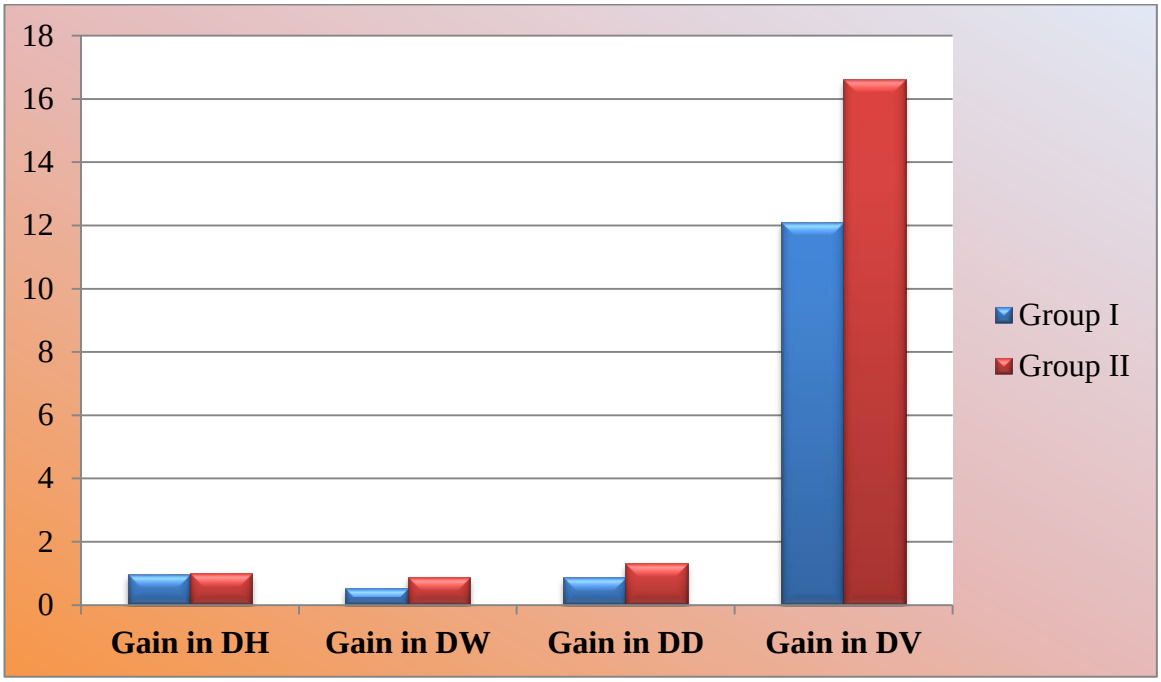
Graph 7: Comparison of radiographic parameters in Group I at different time interval



Graph 8: Comparison of radiographic parameters in Group II at different time interval



Graph 9: Comparison of radiographic parameters between both the groups at 6 months



MASTER CHART

PLAQUE INDEX

Sr.No	Baseline	3 months	6 months
1	3.04	2.72	1.58
2	2.79	1.98	1.28
3	2.96	2.32	1.76
4	3.29	2.84	1.74
5	2.67	1.97	1.37
6	3.25	2.31	1.76
7	2.54	1.76	1.21
8	2.69	1.95	1.42
9	3.43	2.87	1.69
10	2.87	1.96	1.36
11	3.29	2.54	1.82
12	2.88	1.97	1.51
13	3.13	2.74	1.95
14	2.95	2.17	1.43
15	2.56	1.13	0.73
16	2.74	1.82	1.42
17	3.28	2.78	1.97
18	2.88	1.72	1.18
19	3.43	2.8	2.26
20	2.93	2.35	0.96

GINGIVAL INDEX

Sr.No	Baseline	3 months	6 months
1	1.54	1.01	0.88
2	1.93	1.78	1.01
3	1.63	1.16	1.08
4	1.34	1.21	1.06
5	2.21	1.74	1.32
6	1.78	1.25	1.05
7	1.33	1.2	1
8	1.89	1.32	1.17
9	2.12	1.56	1.04
10	1.75	1.42	1.14
11	1.66	1.31	1.12
12	1.43	1.17	0.97
13	1.53	1.16	1.08
14	1.37	1.25	1
15	1.87	1.34	1.14
16	2.07	1.79	1.12
17	1.15	1	0.92
18	1.56	1.28	1.01
19	1.25	1.16	1.04
20	1.58	1.32	0.98

CLINICAL PARAMETERS

BASELINE

	Group I – Control DFDBA				Group II – Test DFDBA + CM			
	PPD	**CAL**	**GR**	**HPD**	**PPD**	**CAL**	**GR**	**HPD**
	(mm)	**(mm)**	**(mm)**	**(mm)**	**(mm)**	**(mm)**	**(mm)**	**(mm)**
1	5	5	0	5	5	5	0	4
2	6	6	0	4	5	5	0	5
3	5	5	0	4	6	7	1	4
4	5	6	1	5	7	7	0	6
5	5	5	0	4	6	6	0	5
6	5	6	1	5	5	5	0	4
7	7	7	0	6	6	6	0	6
8	5	5	0	5	5	5	0	5
9	5	5	0	4	5	5	0	5
10	5	5	0	4	5	5	0	6
11	6	7	1	5	7	7	0	5
12	5	6	1	4	5	6	1	6
13	5	5	0	5	7	9	2	3
14	6	6	0	4	6	6	0	5
15	7	7	0	4	7	8	1	6
16	5	6	1	5	6	6	0	5
17	5	5	0	4	5	5	0	6
18	6	7	1	4	6	7	1	6
19	5	6	1	5	5	5	0	4
20	6	6	0	4	7	7	0	3

CLINICAL PARAMETERS

3 MONTH RECALL

	Group I – Control DFDBA				Group II – Test DFDBA + CM			
	PPD	**CAL**	**GR**	**HPD**	**PPD**	**CAL**	**GR**	**HPD**
	(mm)	**(mm)**	**(mm)**	**(mm)**	**(mm)**	**(mm)**	**(mm)**	**(mm)**
1	3	4	1	4	3	3	0	3
2	4	5	1	3	3	3	0	4
3	4	5	1	3	4	5	1	3
4	4	5	1	4	5	5	0	5
5	4	4	0	3	4	4	0	4
6	4	5	1	4	3	3	0	3
7	6	6	0	5	4	4	0	5
8	4	4	0	4	3	3	0	4
9	4	4	0	4	3	3	0	4
10	4	4	0	3	3	3	0	4
11	5	6	1	4	5	6	1	4
12	4	5	1	3	3	4	1	4
13	3	4	1	4	4	6	2	2
14	4	5	1	3	4	5	1	3
15	5	6	1	3	5	6	1	4
16	4	5	1	4	4	5	1	4
17	4	5	1	3	4	4	0	5
18	5	6	1	3	4	6	2	5
19	3	4	1	4	4	4	0	3
20	5	5	0	3	4	6	2	2

CLINICAL PARAMETERS

6 MONTHS RECALL

	Group I – Control DFDBA				**Group II – Test DFDBA + CM**			
	PPD	**CAL**	**GR**	**HPD**	**PPD**	**CAL**	**GR**	**HPD**
	(mm)	**(mm)**	**(mm)**	**(mm)**	**(mm)**	**(mm)**	**(mm)**	**(mm)**
1	3	3	0	3	3	3	0	2
2	4	4	0	3	2	2	0	3
3	4	4	0	3	3	3	0	2
4	3	5	2	3	4	5	1	3
5	3	3	0	3	2	2	0	3
6	3	5	2	3	3	3	0	2
7	6	6	0	3	3	3	0	3
8	3	4	1	3	3	3	0	3
9	3	3	0	3	2	3	1	2
10	3	4	1	2	3	4	1	3
11	4	6	2	3	4	5	1	2
12	3	4	1	3	3	4	1	3
13	2	3	1	4	3	4	1	2
14	3	4	1	2	3	4	1	2
15	4	4	0	2	4	5	1	3
16	4	4	0	3	2	4	2	2
17	3	4	1	3	3	3	0	2
18	3	5	2	2	2	4	2	3
19	2	4	2	3	2	2	0	3
20	4	4	0	3	3	5	2	2

CBCT MEASUREMENTS OF DEFECT HEIGHT, DEFECT WIDTH, DEFECT DEPTH OF GROUP I

	CONTROL - GROUP I					
SR. No.	**Baseline**			**6 Months**		
	DH (mm)	**DW (mm)**	**DD (mm)**	**DH (mm)**	**DW (mm)**	**DD (mm)**
1	4.3	2.1	2.7	1.9	1.8	2.3
2	2.4	1.4	2.8	1.8	1.1	1.9
3	2.6	2.3	3.1	2.2	1.7	1.5
4	3.1	2.3	4.2	2.2	1.6	2.3
5	3.8	2.1	2.3	1.9	1.5	1.8
6	2.2	2.6	2.6	1.4	1.7	1.6
7	4.6	1.8	3.2	3.2	1.1	2.5
8	2.8	2.4	3.4	2.1	1.8	2.6
9	2.1	2	2.9	1.8	1.6	1.9
10	2.8	2	1.8	1.7	1.8	1.5
11	3.1	1.9	2.8	2.4	1.2	2.1
12	3.4	3.1	3.1	2.2	2.6	1.9
13	2.8	2.6	3.2	1.9	2.1	2.3
14	2.7	2.4	2.5	2.2	1.9	1.9
15	2.1	2.6	2.3	1.5	1.8	1.4
16	3	2.3	3.1	2.7	2.1	2.3
17	2.6	1.9	2.8	1.9	1.5	1.7
18	2.8	2.1	3	1.6	1.5	2.3
19	3.3	2.3	2.6	2.1	1.8	1.6
20	2.9	2.2	2.6	1.5	1.7	2.2

CBCT MEASUREMENTS OF DEFECT HEIGHT, DEFECT WIDTH, DEFECT DEPTH OF GROUP II

	TEST - GROUP II					
Sr. No.	**Baseline**			**6 Months**		
	DH (mm)	**DW (mm)**	**DD (mm)**	**DH (mm)**	**DW (mm)**	**DD (mm)**
1	3.1	1.8	2.9	2.2	0.9	1.7
2	3.4	2.1	3.2	2.1	1.5	2.3
3	2.3	2	2.6	1.8	1.1	1.5
4	3.8	2.4	4.2	2.2	1.4	2.5
5	2.3	2	3.2	1.4	1.3	1.6
6	2.4	2.9	2.7	1.3	2.1	1.2
7	3.4	2.2	3.6	1.8	1.2	1.5
8	2.6	2.4	3.2	1.9	1.6	2.1
9	3.2	2.6	2.9	2.4	1.2	1.4
10	3.2	2.5	3.5	2.1	1.9	2
11	2.4	3.1	2.9	1.9	2.1	1.4
12	2.8	2.4	3.4	1.7	1.3	2.3
13	2.2	2.1	2	1.6	1.8	1.1
14	3.5	2.2	3.3	2.4	1.6	2.5
15	2.9	2.4	3.7	1.6	1.8	2.3
16	3	2.6	3.2	2.4	1.1	2.1
17	3.2	2.4	3.9	1.8	1.4	2.4
18	3.6	2.7	3.1	2.2	1.2	1.9
19	2.4	1.8	2.6	2	0.9	1.1
20	2.6	1.6	2.3	1.4	1.1	1.4

CBCT MEASUREMENTS OF BONE DEFECT VOLUME

	CONTROL – GROUP I		TEST – GROUP II	
Sr. No.	**Baseline**	**6 Months**	**Baseline**	**6 Months**
	(mm^3)	**(mm^3)**	**(mm^3)**	**(mm^3)**
1	24.381	7.866	16.182	3.366
2	9.408	3.762	22.848	7.245
3	18.538	5.61	11.96	2.97
4	29.946	8.096	38.304	7.7
5	18.354	5.13	14.72	2.912
6	14.872	3.808	18.792	3.276
7	26.496	8.8	26.928	3.24
8	22.848	9.828	19.968	6.384
9	12.18	5.472	24.128	4.032
10	10.08	4.59	28	7.98
11	16.492	6.048	21.576	5.586
12	32.674	10.868	22.848	5.083
13	23.296	9.177	9.24	3.168
14	16.2	7.942	25.41	9.6
15	12.558	3.78	25.752	6.624
16	21.39	13.041	24.96	5.544
17	13.832	4.845	29.952	6.048
18	17.64	5.52	30.132	5.016
19	19.734	6.048	11.232	1.98
20	16.588	5.61	9.568	2.156

COMPARATIVE EVALUATION OF CHORION MEMBRANE IN COMBINATION WITH DEMINERALIZED FREEZE DRIED BONE ALLOGRAFT AND DEMINERALIZED FREEZE DRIED BONE ALLOGRAFT ALONE IN TREATMENT OF GRADE II FURCATION DEFECTS: A CLINICORADIOGRAPHIC STUDY

CASE HISTORY PROFORMA

NAME: **OPD NO.**

AGE/SEX: **DATE:**

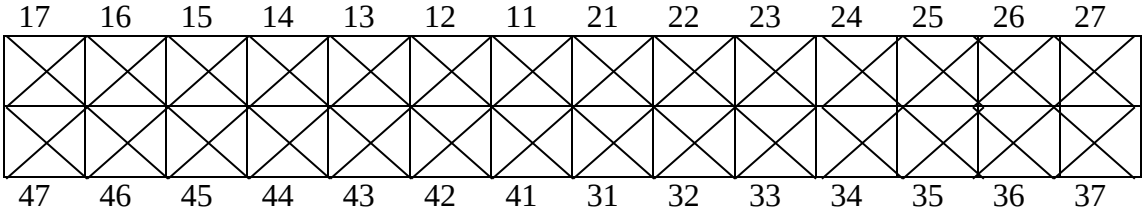
OCCUPATION: **ADDRESS:**

CHIEF PERIODONTAL COMPLAINT:

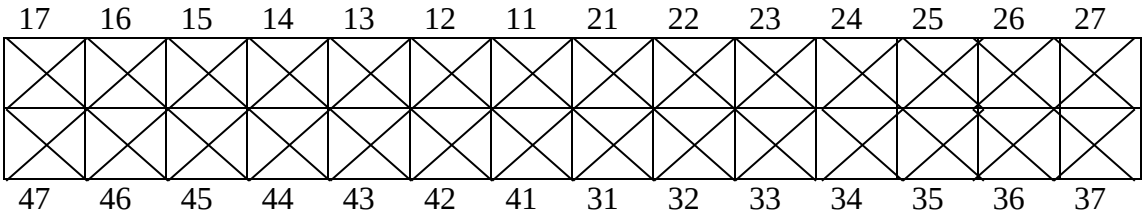
PAST DENTAL HISTORY:

PAST MEDICAL HISTORY:

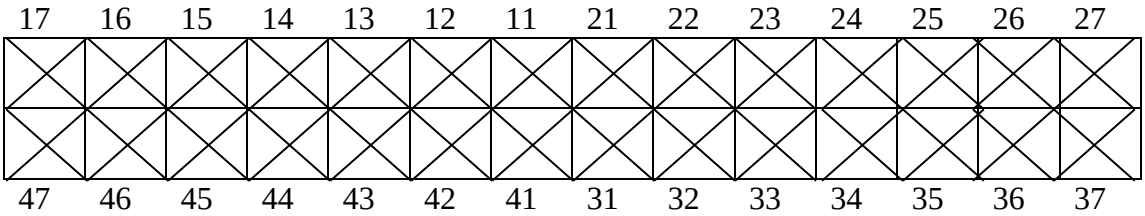
PROBING POCKET DEPTH (mm) (BASELINE):



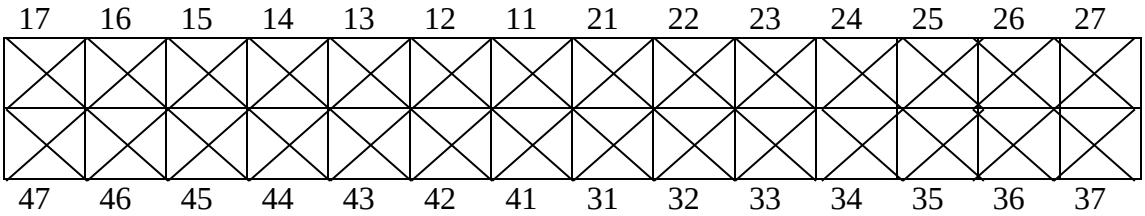
PROBING POCKET DEPTH (mm) (3 MONTHS):



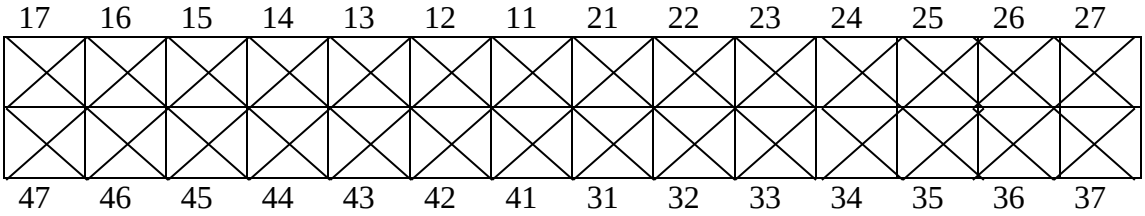
PROBING POCKET DEPTH (mm) (6 MONTHS):



CLINICAL ATTACHMENT LEVEL (mm) (BASELINE)



CLINICAL ATTACHMENT LEVEL (mm) (3 MONTHS)



CLINICAL ATTACHMENT LEVEL (mm) (6 MONTHS)

17	16	15	14	13	12	11	21	22	23	24	25	26	27
47	46	45	44	43	42	41	31	32	33	34	35	36	37

RECESSION:

Baseline-

3 Months-

6 Months

INDICES

1. PLAQUE INDEX (PI) ***(Turesky-Gilmore-Glickman Modification of Quigley-Hein 1970)*** **(BASELINE)**

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

$$PI = \frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}} =$$

PLAQUE INDEX (PI) ***(Turesky-Gilmore-Glickman Modification of Quigley-Hein 1970)*** **(3 MONTHS)**

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

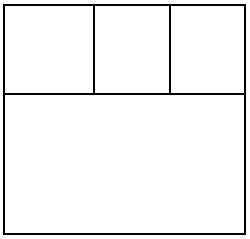
$$PI = \frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}} =$$

PLAQUE INDEX (PI) ***(Turesky-Gilmore-Glickman Modification of Quigley-Hein 1970)*** **(6 MONTHS)**

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

$$PI = \frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}} =$$

2. GINGIVAL INDEX (GI) (BASELINE)



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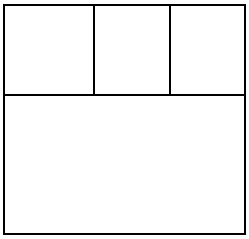

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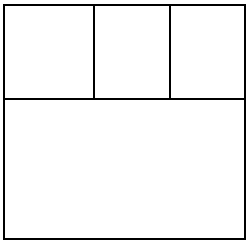
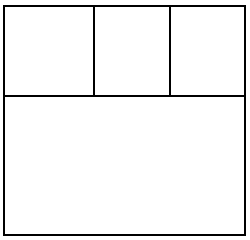


24



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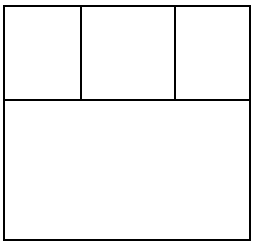

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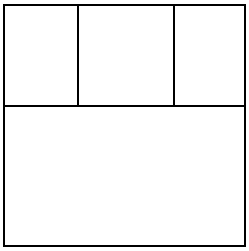
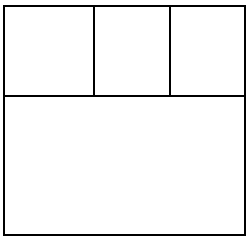


36

GI = Total GI scores of all teeth = ☐

Number of teeth examined

GINGIVAL INDEX (GI) (3 MONTHS)



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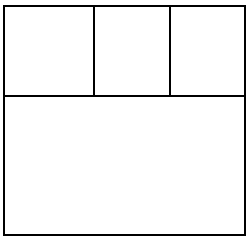
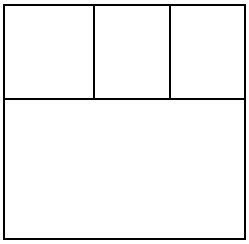


12



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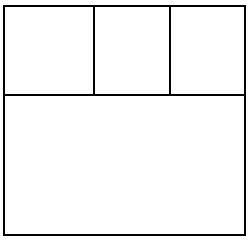

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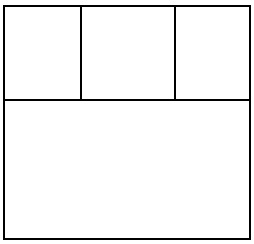

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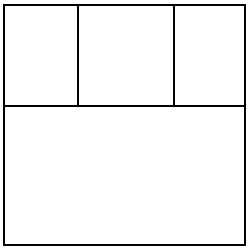
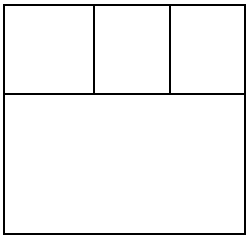


36

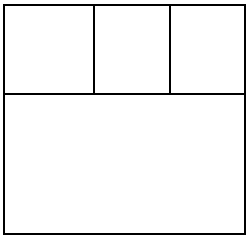
GI = Total GI scores of all teeth =

Number of teeth examined

GINGIVAL INDEX (GI) (6 MONTHS)



16



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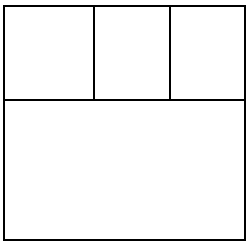

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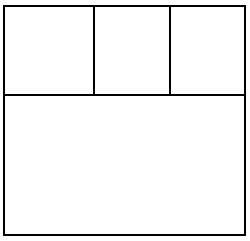

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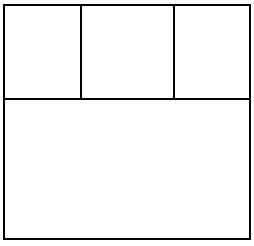

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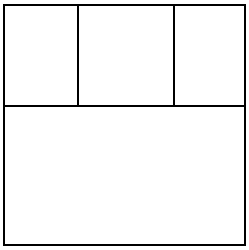

36

GI =Total GI scores of all teeth =

Number of teeth examined

FURCATION INVOLVEMENT CLINICALLY:

Tooth No.	Baseline horizontal probing depth (with Nabers)	3 months horizontal probing depth(with Nabers)	6 months horizontal probing depth (with Nabers)

FURCATION INVOLVEMENT CLINICALLY AFTER FLAP REFLECTION:

Tooth No.	Material used	Clinical Baseline horizontal furcation depth (UNC 15)	Clinical Baseline vertical furcation height (UNC 15)

FURCATION INVOLVEMENT (CBCT):

Tooth No.	Material Used	Baseline Bone Defect Height	Bone Defect Height (6 Month)	Baseline Bone Defect Width	Bone Defect Width (6 Month)	Baseline Bone Defect Depth	Bone Defect Depth (6 Month)

Tooth No.	Baseline Bone Defect Volume	Bone Defect Volume (6 Months)

(Confidential)

Informed Consent Form

"Comparative Evaluation of Chorion Membrane in Combination with Demineralized Freeze Dried Bone Allograft and Demineralized Freeze Dried Bone Allograft Alone in Treatment of Grade II Furcation Defects: A Clinicoradiographic Study"

Mr./Master/Mrs./Miss._____
Resident of: _____
_____aged _____ years,
excercising my free will/choice, without any pressure/lure of incentive in any form, hereby give my consent for the project to be conducted.

I acknowledge the receipt of "patient's information sheet", and also the doctor has informed me about this research project suitably and sufficiently to my satisfaction. I agree to let my X-rays, photographs, blood investigations, other investigations to be taken as required. I agree to take part in this project and will not mix any other projects during the period of this trial. I shall report to the dental hospital or other place where called on given appointment dates and time. I shall inform the doctor on any adverse effects or unusual symptoms noticed by me. I shall co-operate with the doctors and paramedical staff, in all respects. I permit to publishing the results of my participation in this study. I shall not be given any reimbursement or compensation. I have been informed of my right to opt out of this research project at any time without giving any reason for doing so. I hereby record my consent for participation in the said trial.

_____	_____	_____	_____
Patient's name	Signature/thumbprint	Date	Time

_____	_____	_____	_____
Investigator's Name	Signature	Date	Time