

# Clinical and Radiographic Evaluation of Autogenous Dentin Graft and Demineralized Freeze-Dried Bone Allograft with Chorion Membrane in the Treatment of Grade II and III Furcation Defects:- A Randomized Controlled Trial

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## Abstract

**Background:** Periodontal Regeneration of any tissue type is a complex biological process in itself, requiring a triad of cells, locally acting growth factors, systemic hormones, and the extracellular matrix components in which these interact. **Aims:** The aim of this study was to compare the effectiveness of autogenous dentin graft (ADG) and demineralized freeze-dried bone allograft (DFDBA) with chorion membrane in the treatment of Grade II and III Furcation defects in patients with moderate-to-severe chronic periodontitis. **Subjects and Methods:** A total of 20 Grade II and III furcation defects in patients with moderate-to-severe chronic periodontitis were randomly assigned to either Group I (ADG + chorion membrane) or Group II (DFDBA + chorion membrane) and evaluated clinically for Gingival Index (GI), probing pocket depth (PPD), clinical attachment level (CAL), vertical bone depth (VBD), and horizontal bone depth (HBD) and radiographically for furcation bony defect (FBD). **Results:** Intragroup comparisons of clinical parameters GI, PPD, and CAL have shown a statistically significant reduction at the end of 3 months and 6 months, but intergroup comparison was not statistically significant. At the end of 6 months, there was a significant reduction in VBD in Group I ( $2.65 \pm 0.71$  mm) compared with Group II ( $4.00 \pm 1.26$  mm) and HBD ( $1.84 \pm 0.59$  mm) compared with Group II ( $3.95 \pm 1.74$  mm), respectively. At the end of 3 months and 6 months, FBD depth was significantly reduced in Group I ( $1.21 \pm 1.10$  and  $0.43 \pm 0.22$  mm<sup>2</sup>, respectively) compared with the Group II ( $3.04 \pm 2.45$  and  $2.68 \pm 2.50$  mm<sup>2</sup>, respectively). **Conclusions:** The results of the present study indicate that the use of ADG and chorion membrane improved all the clinical parameters. Individuals treated with ADG and chorion membrane showed significant reduction for VBD, HBD, and FBD in the treatment of Grade II and III furcation defects than in the individuals treated with DFDBA and chorion membrane.

**Keywords:** Autogenous dentin graft, chorion membrane, demineralized freeze-dried bone allograft, furcation bone depth, furcation defects, horizontal bone depth, smart dentin grinder, vertical bone depth

## INTRODUCTION

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or a group of specific microorganisms, resulting in progressive destruction of the periodontal ligament (PDL) and alveolar bone with pocket formation, recession, or both.<sup>[1]</sup> Maintenance of the natural dentition in health and comfortable function is the primary goal of periodontal therapy.<sup>[2]</sup> Periodontal regeneration of any tissue type is a complex biological process in itself, requiring a triad of cells, locally acting growth factors, systemic hormones, and the extracellular matrix components, in which, these interact. In periodontium, such regeneration involves the creation of new alveolar bone, cementum, and

PDL.<sup>[3]</sup> The progression of periodontitis into the bifurcation and trifurcation areas of multirrooted teeth leads to furcation involvement. Furcation is that part of a root complex that is located between separated root cones or roots.<sup>[4]</sup> A variety of bone grafts in combination with GTR membrane resulted in superior bone fill, probing depth reduction, and clinical

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attachment gain when compared to bone grafts used alone in human Grade II and Class III furcation defects.<sup>[5]</sup>

Extracted tooth is considered as clinical waste. It is a known concept that alveolar bone and teeth develop from neural crest cells, and these contain proteins which are similar to the dentin, bone, and cementum. The tooth consists of 85% of dentin. It contains growth factors, which can be used in humans for defect fill, and it has been proven in animal studies that bovine dentin can be processed into graft, which is slowly and gradually replaced by the bone.<sup>[6]</sup>

One of the oldest biomaterials used for scaffolds is the fetal membrane. The fetal membrane was first used for the transplantation of skin in 1910.<sup>[7]</sup> It has gained importance because of its ability to reduce scarring and inflammation, enhance wound healing, and serve as a scaffold for cell proliferation and differentiation as a result of its antimicrobial properties. In addition, the chorionic membrane (CM), a fetal membrane, is a biomaterial that can be easily obtained, processed, and transported.<sup>[8]</sup> The present study was conducted to compare the efficacy between autogenous dentin graft (ADG) and demineralized freeze-dried bone allograft (DFDBA) with chorion membrane clinically and radiographically in the treatment of Grade II and III furcation defects in patients with moderate-to-severe chronic periodontitis.

## SUBJECTS AND METHODS

Patients with untreated periodontitis satisfying the inclusion and exclusion criteria were enrolled in the study selected from the outpatient section, Department of Periodontics and Implantology, Kamineni Institute of Dental Sciences, Narketpally, Nalgonda (Dist), Telangana. A detailed case history was recorded, the nature and purpose of the study was explained to the patients in their native language, and written informed consent was obtained. The Institutional Ethical Committee approved the study (KIDS/IEC/2016/31).

### Inclusion criteria

- Systemically healthy patients
- Age group between 25–55 years
- Grade II and III furcation defects
- Horizontal bone loss  $\geq 2$  mm in multirrooted tooth in the furcation area using Naber's probe
- Extracted teeth due to advanced periodontal bone loss or other indications such as wisdom teeth or orthodontic indications or fractured teeth (which cannot be restored) for ADG
- Clinical attachment level  $\geq 3$  mm
- Evidence of radiolucency in the furcation area on an intraoral periapical.

### Exclusion criteria

- Systemically compromised patients and those on medications (corticosteroids/bisphosphonate) that may interfere with wound healing
- Pregnant women and lactating mother

- Active periodontal treatment within the last 6 months
- Smokers
- Root canal-treated teeth which cannot be used for preparing ADG.

### Study design

Convenience sampling was done, and ten patients were recruited in each group. The power of the study was 76% with 30.99% confidence interval, and the level of significance was 5% or 0.05.

The study consists of 20 Grade II and III furcation defects in patients with moderate-to-chronic periodontitis, which were randomly assigned by coin test into two groups:

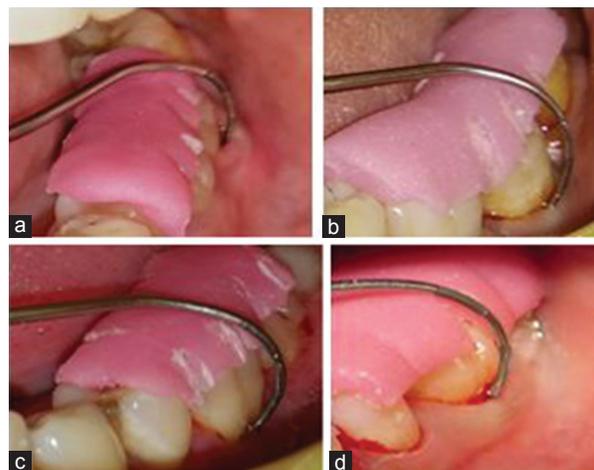
- Group I (Test): Ten Grade II and III furcation defects were treated by the placement of ADG with chorion membrane
- Group II (Control): Ten Grade II and III furcation defects were treated by the placement of DFDBA bone graft with chorion membrane.

### Clinical diagnosis

Gingival Index (GI), probing pocket depth (PPD), clinical attachment level (CAL), vertical bone depth (VBD), and horizontal bone depth (HBD) of each tooth were recorded using University of North Carolina Probe-15 and Naber's Probe. Custom-made occlusal acrylic stents were used to standardize the probe angulation and position. Clinical parameters were recorded at baseline, 3 months, and 6 months after treatment [Figure 1a-d].

### Radiographic diagnosis

Bone fill was recorded using digital radiovisiography. All the radiographs were analyzed using a metal ball of known diameter (3.95 mm). Areas of the furcation defect were obtained by calibrating spatial measurements [Figure 2] in the radiographic software (UTHSCA). Radiographically, furcation bony defect (FBD) was recorded at baseline, 3 months, and 6 months after treatment [Figure 3a-d].



**Figure 1:** (a) Horizontal bone depth at baseline in Group I, (b) horizontal bone depth at the end of 6 months in Group I, (c) horizontal bone depth at baseline in Group II, and (d) horizontal bone depth at the end of 6 months in Group II

### Presurgical therapy

Once the diagnosis was made presurgical therapy consisted of scaling and root planing under local anesthesia and occlusal adjustment, if necessary, 6 weeks following completion of presurgical therapy patient's response to the therapy and to determine the need for periodontal surgery.

### Autogenous dentin graft preparation

Individuals who were included in Group I should have at least one tooth to be extracted. These extracted teeth [Figure 4a] were used to obtain ADG by using smart dentin grinder (SDG) as per the manufacturer's instructions. The procedure included the removal of restorations such as crowns and fillings, carious lesions, discolored dentin, PDL, and calculus were cutoff using tungsten carbide bur. Teeth were grinded in the grinding sterile chamber of a newly designed, Smart Dentin Grinder™ [Figure 4b]. The SDG was capable of grinding the tooth completely in 3 s and then by vibrating movement (sorting) of the grinding chamber for 20 s. The particles <300 μm fell into a Lower chamber. Particulate (<300 μm) was considered as a nonefficient particulate size for bone grafting. The collecting drawer chamber consisted of dentin particles between 300 and 1200 μm efficient for grafting [Figure 4c]. The particulate dentin from the drawer was immersed in basic alcohol for 10 min, in a small sterile glass container which was provided with SDG. The basic alcohol cleanser consisted of 0.5M of NaOH and 30% alcohol (v/v) for defatting, dissolving all organic debris, bacteria, and toxins of the dentin particulate. After decanting with the basic alcohol cleanser, the particulate was washed twice in sterile phosphate-buffered saline (PBS) [Figure 4d]. The PBS decanted [Figure 4e] leaving wet particulate dentin ready to graft into alveolar bone defects. Alternatively, the wet particulate was kept on a hot plate (140°C) for 5 min [Figure 4f], and the dry bacteria-free particulate autologous dentin was obtained that was served for immediate or future grafting procedures. The process from tooth extraction until grafting took approximately 15–20 min. The efficiency of selecting the dentin particulate of specific size for grafting was more than 95%. The volume of the particulate dentin obtained was more than twice of the original root volume (≥2cc).

### Surgical procedure

After anesthetizing the area with 2% lignocaine with adrenaline (1:80,000) solution, a sulcular incision was given using BP blade no. 15, and a full-thickness mucoperiosteal flap was elevated (Kirkland flap). Thorough debridement was performed using area-specific curettes and Quentin furcation curettes (Hu-Friedy, USA), and the anatomy of the furcation bony defect was clinically confirmed and the defect was filled either with ADG [Figure 5a] and chorion membrane (Group I) [Figure 5b] (or DFDBA [Figure 5c] and chorion membrane (Group II) [Figure 5d]). Flaps were approximated using a 3–0 nonabsorbable silk suture, and periodontal dressing was given.

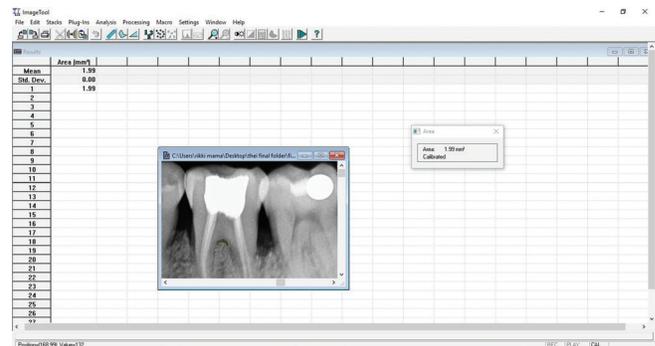


Figure 2: Calibration of metal ball and area of radiographic defect

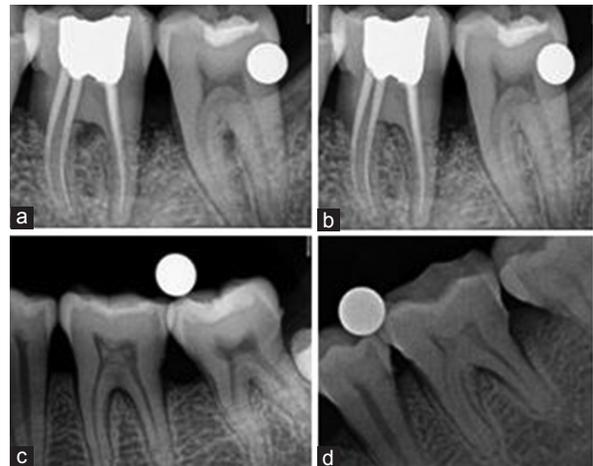


Figure 3: (a) Radiovisiography at baseline in Group I, (b) radiovisiography at 6 months in Group I, (c) RVG at baseline in Group II, and (d) radiovisiography at 6 months in Group II

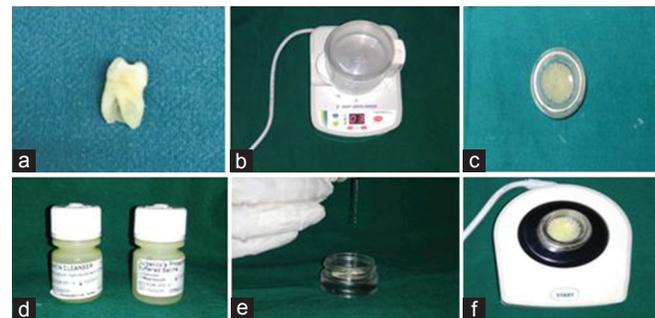


Figure 4: (a) Extracted tooth for graft preparation, (b) smart dentin grinder, (c) autogenous dentin graft, (d) cleanser and buffer solutions, (e) disinfection of the graft, and (f) hot plate

### Postsurgical care

All patients received systemic antibiotic therapy (capsule amoxicillin 500 mg thrice daily and capsule metrogyl 400 mg thrice daily) for 5 days and analgesics (tablet voveran 50 mg twice daily) for 3 days to prevent postoperative pain and edema. Postoperative instructions were given to the patient. 0.2% chlorhexidine mouth rinse was advised twice daily. Healing of soft tissues was visualized, and the patient was asked for any symptoms regarding discomfort, pain, and swelling. The patient was recalled after 1 week for suture removal.

### Statistical analysis

Data were analyzed using SPSS software version 24.0 program (SPSS Inc., Chicago, IL, USA) and statistically analyzed. Intragroup comparison for GI, PPD, CAL, VBD, HBD, and FBD scores was done by repeated-measures ANOVA test. Intragroup pairwise comparison for GI, PPD, CAL, VBD, HBD, FBD scores was done by Bonferroni *post hoc* test. Intergroup comparison for GI, PPD, CAL, VBD, HBD, and FBD scores was done by Independent sample *t*-test.  $P < 0.05^*$  was considered to be statistically significant.

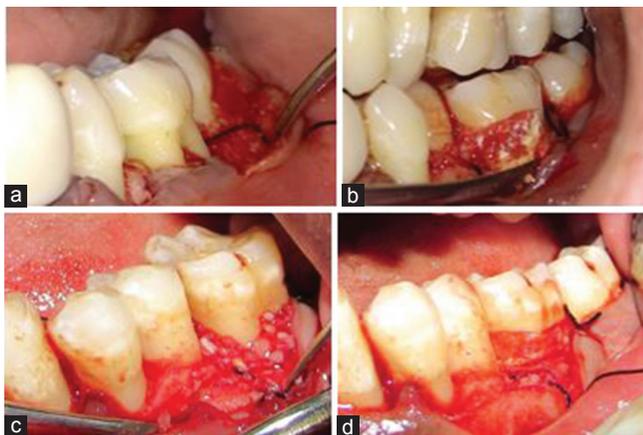
### RESULTS

All the patients were compliant, and healing was uneventful for both groups. Table 1 shows reduction in GI, PPD, CAL, VBD, HBD, and FBD scores in all the patients at the end of 3 months and 6 months when compared to baseline and were statistically significant ( $P < 0.05^*$ ). Table 2 shows intragroup pairwise comparison of mean GI, PPD, CAL, VBD, and HBD scores was statistically significant from baseline to 3 months, baseline to 6 months, and 3 months to 6 months, FBD was not significant at 3 months to 6 months in Group I, whereas in Group II of mean GI, PPD, CAL, and VBD scores were

statistically significant from baseline to 3 months and baseline to 6 months, whereas CAL, VBD, HBD, and FBD were not significant when observed from baseline to 6 months and 3 months to 6 months' time interval ( $P < 0.05^*$ ). Mean GI, PPD, and CAL scores were not statistically significant from baseline to 3 months and baseline to 6 months [Graphs 1-3]. Table 3 shows mean reduction in VBD and HBD at 6 months, which was greater for Group I (ADG + chorion membrane) than Group II (DFDBA + chorion membrane), the difference was statistically significant ( $P < 0.05^*$ ) [Graphs 4 and 5]. Reduction in furcation defect depth was greater for Group I (ADG + chorion membrane) than Group II (DFDBA + chorion membrane) at 3 months and 6 months, and the difference was statistically significant ( $P < 0.05^*$ ) [Graph 6].

### DISCUSSION

Regeneration of the periodontium within the furcation defect is considered one of the most challenging aspects of periodontal therapy.<sup>[9]</sup> It has been reported that molars with periodontitis involving furcation are having a higher rate of periodontal breakdown, and furcation-involved molars have responded less favorably to nonsurgical therapy than in molars without furcation involvement or single-rooted teeth.<sup>[10]</sup> Clinically, successful regeneration at furcation sites is determined as the elimination or reduction of the horizontal and vertical components of the lesion (that is, gain of clinical attachment level and bone fill), but conclusive evidence of true regeneration can only be achieved by histological means.<sup>[11]</sup> Machtei<sup>[12]</sup> stated that from clinical point of view, complete elimination of the interradicular defect appears to be the most important outcome. Thus, the main outcome variables for studies evaluating the efficacy of regenerative techniques in furcations are change of furcation status (conversion into Class I or complete closure) and horizontal hard tissue fill. Changes in direct bone measurements (horizontal probing bone level at surgery and during reentry) serve as primary outcome variables for evaluating clinical success, while clinical attachment level gain (horizontal/vertical probing attachment level), probing depth reduction (horizontal/vertical), and radiographic assessments may serve as secondary outcomes. Bone fill during a reentry procedure is the only component of a regenerated periodontium that can be accurately assessed



**Figure 5:** (a) Autogenous dentin graft placed in relation to 36, (b) autogenous dentin graft and chorion membrane placed in relation to 36, (c) demineralized freeze-dried bone allograft placed in relation to 36, (d) demineralized freeze-dried bone allograft and chorion membrane placed in relation to 36

**Table 1: Intragroup comparison of mean scores in Group I and Group II at different study intervals by repeated-measures ANOVA test**

	Group I				Group II			
	Baseline	3 months	6 months	P	Baseline	3 months	6 months	P
GI	1.89±0.61	1.22±0.63	0.65±0.30	<0.001*	1.96±0.40	1.23±0.42	0.68±0.16	<0.001*
PPD	5.63±1.41	3.39±0.82	2.41±0.67	<0.001*	5.62±1.79	3.65±1.30	3.18±0.99	<0.001*
CAL	6.70±1.40	4.49±0.95	3.43±1.02	<0.001*	6.68±1.64	4.13±1.27	3.97±1.65	<0.001*
VBD	6.55±1.89	3.73±1.31	2.65±0.71	<0.001*	5.95±1.59	4.30±1.48	4.00±1.26	<0.001*
HBD	5.25±1.27	2.75±0.59	1.84±0.59	<0.001*	5.80±2.67	3.55±1.54	3.95±1.74	<0.001*
FBD	3.77±2.17	1.21±1.10	0.43±0.22	<0.001*	3.94±3.78	3.04±2.45	2.68±2.50	<0.001*

\*Significant. GI: Gingival Index, PPD: Probing pocket depth, CAL: Clinical attachment level, VBD: Vertical bone depth, HBD: Horizontal bone depth, FBD: Furcation bony defect

**Table 2: Intragroup pairwise comparison between Group I and Group II at different study intervals by Bonferroni *post hoc* test**

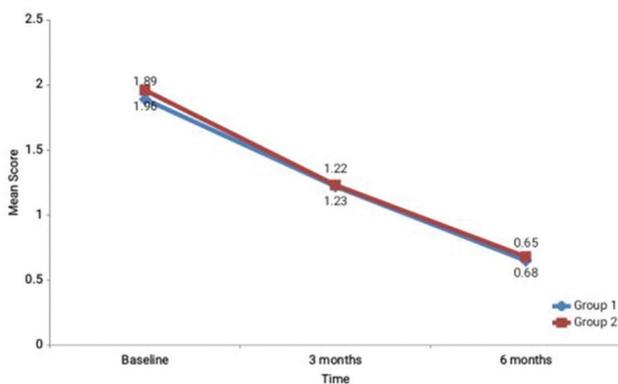
Group	Time	P					
		GI	PPD	CAL	VBD	HBD	FBD
Group I	Baseline						
	3 months	0.001*	<0.001*	0.001*	0.002*	<0.001*	0.003*
	6 months	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.003*
Group II	Baseline						
	3 months	0.003*	0.006*	0.005*	0.01*	0.006*	0.11 (NS)
	6 months	<0.001*	0.001*	<0.001*	0.006*	0.003*	0.25 (NS)
Group I	Baseline						
	3 months	<0.001*	0.001*	<0.001*	0.006*	0.003*	0.25 (NS)
	6 months	<0.001*	0.001*	0.003*	0.004*	0.11 (NS)	0.11 (NS)
Group II	Baseline						
	3 months	0.001*	0.007*	1.00 (NS)	0.15 (NS)	1.00 (NS)	0.07 (NS)
	6 months	0.001*	0.007*	1.00 (NS)	0.15 (NS)	1.00 (NS)	0.07 (NS)

\*Significant. GI: Gingival Index, PPD: Probing pocket depth, CAL: Clinical attachment level, VBD: Vertical bone depth, HBD: Horizontal bone depth, FBD: Furcation bony defect, NS: Not significant

**Table 3: Intergroup comparison between Group I and Group II at different time intervals by independent sample *t*-test**

	Group	GI	PPD	CAL	VBD	HBD	FBD
Baseline, mean±SD	Group I	1.89±0.61	5.63±1.41	6.70±1.40	6.55±1.89	5.25±1.27	3.77±2.17
	Group II	1.96±0.40	5.62±1.79	6.68±1.64	5.95±1.59	5.80±2.67	3.94±3.78
P		0.76 (NS)	0.99 (NS)	0.98 (NS)	0.45 (NS)	0.56 (NS)	0.90 (NS)
3 months, mean±SD	Group I	1.22±0.63	3.39±0.82	4.49±0.95	3.73±1.31	2.75±0.59	1.21±1.10
	Group II	1.23±0.42	3.65±1.30	4.13±1.27	4.30±1.48	3.55±1.54	3.04±2.45
P		0.97 (NS)	0.60 (NS)	0.48 (NS)	0.38 (NS)	0.14 (NS)	0.04*
6 months, mean±SD	Group I	0.65±0.30	2.41±0.67	3.43±1.02	2.65±0.71	1.84±0.59	0.43±0.22
	Group II	0.68±0.16	3.18±0.99	3.97±1.65	4.00±1.26	3.95±1.74	2.68±2.50
P		0.78 (NS)	0.06 (NS)	0.39 (NS)	0.009*	0.002*	0.01*

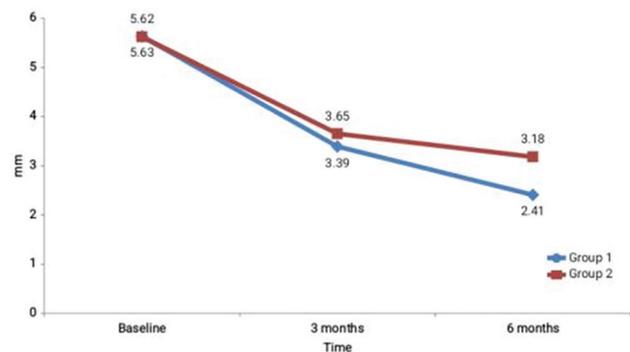
\*Significant. GI: Gingival Index, PPD: Probing pocket depth, CAL: Clinical attachment level, VBD: Vertical bone depth, HBD: Horizontal bone depth, FBD: Furcation bony defect, NS: Not significant



**Graph 1:** Comparison of the mean Gingival Index between different time intervals in Groups I and II

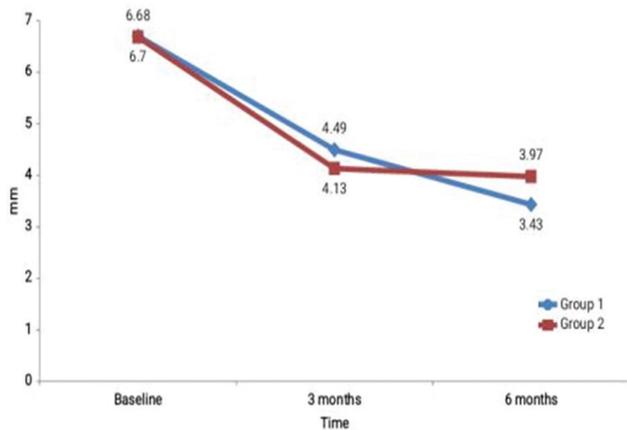
clinically. The placental allografts possess antibacterial and antimicrobial properties. They reduce inflammation and provide a matrix highly rich in protein and thereby facilitate migration of cells at the area of the defect.<sup>[13]</sup>

In our present study, in terms of PD and CAL, there is a statistically significant difference noted in both the groups, which is in accordance with the study conducted by Kothiwale *et al.*<sup>[14]</sup> To evaluate, anti-inflammatory effect of chorion as a

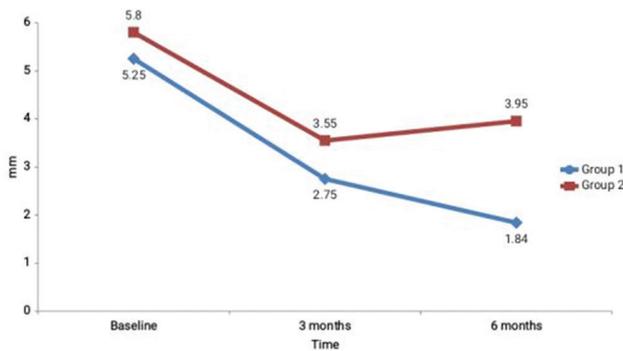


**Graph 2:** Comparison of the mean probing pocket depth between different time intervals in Groups I and II

barrier membrane in periodontal pocket therapy by assessing interleukin 11 (IL-11) level in gingival crevicular fluid (GCF) was conducted, where they have used OFD + CM in one group and OFD in another group. PPD, CAL, and IL-11 in GCF were assessed. They have concluded that adjunctive use of chorion membrane in flap surgery provides an additive anti-inflammatory effect along with improvement in clinical outcomes enhancing the long-term prognosis. Autogenous bone grafts are considered to be the gold standard since there is



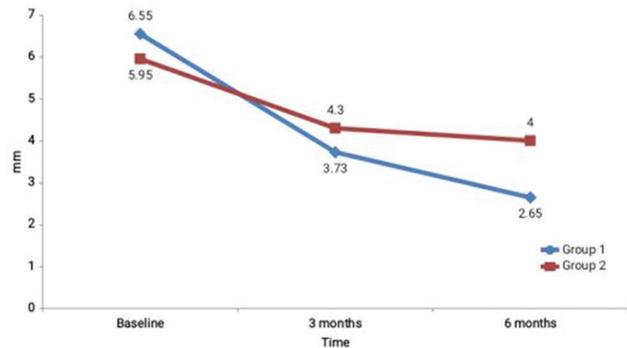
**Graph 3:** Comparison of the mean clinical attachment level between different time intervals in Groups I and II



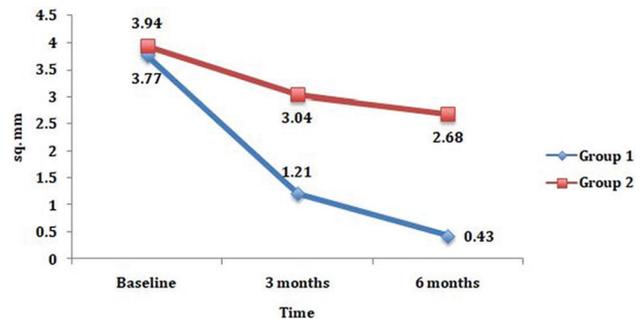
**Graph 5:** Comparison of the mean horizontal bone depth between different time intervals in Groups I and II

a possibility to retain cell viability and graft revascularization, and there is no possibility of disease transmission, but the added operating time and morbidity associated with their harvest and the limited available volume of autogenous intraoral bone at times associated with periodontal disease.<sup>[15]</sup>

Teeth and bones share many similarities. The teeth, cartilages, nerves, and maxillofacial bones all embryologically originated in the neural crest, sharing identical origin.<sup>[16]</sup> Clinicians support the intramembranous bone formation pathway, when intraoral bone grafting is achieved.<sup>[17,18]</sup> Tooth is a composite structure consisting of inorganic components, including the calcium phosphate lineage and organic components such as collagen. Tooth minerals consist of five biological calcium phosphates: hydroxyapatite, tricalcium phosphate, octacalcium phosphate (OCP), amorphous calcium phosphate (ACP), and dicalcium phosphate dihydrate.<sup>[19]</sup> The organic parts of dentin and cementum include type I collagens and various growth factors such as bone morphogenic proteins. Type I collagen occupies about 90% of the organic parts of tissues, with the rest noncollagenous proteins (NCPs), biopolymers, lipid, citrate lactate, etc., NCPs include phosphoryn, sialoprotein, glycoprotein, proteoglycans, osteopontin, osteocalcin, dentin matrix protein-1, osterix, and cbfa1 (runx 2). These proteins



**Graph 4:** Comparison of the mean vertical bone depth between different time intervals in Groups I and II



**Graph 6:** Comparison of the mean area of radiographic defect between different time intervals in Groups I and II

are known to trigger the bone resorption and generation processes.<sup>[20]</sup>

Autogenous tooth bone graft material (auto BT) was first developed in 2008 and has been used mainly for guided bone regeneration to supplement dental implants.<sup>[21]</sup> Tooth graft has first been introduced by Korea Tooth Bank R and D Center and has satisfied many clinicians and patients for its osteoconduction as well as osteoinduction capacity. Kim artificially processed tooth as a graft material.<sup>[22]</sup> Dentin tooth can be classified into three groups according to the degree of demineralization: undemineralized dentin (UDD), partially demineralized dentin matrix (DDM) (70% decalcified), and DDM. It has been shown that UDD is less effective in bone formation, whereas other studies have shown that DDM is biocompatible and also osteoinductive, similar to demineralized bone matrix.<sup>[23]</sup>

The osteogenic capacity of a demineralized tooth was verified as early as 1967, and it has been generally accepted that autogenous and allogenic demineralized teeth are osteoinductive or osteoconductive graft materials.<sup>[24]</sup> In tooth-based graft materials there is higher mineralization and crystallinity when compared with bone.<sup>[25]</sup> However, tooth demineralization is time-consuming (usually 2–6 days), thus limiting the use of fresh demineralized tooth (FDT) as a graft material. Nevertheless, FDT has shown great potential in alveolar bone regeneration.<sup>[26]</sup>

Another drawback of demineralization is that prolonged acid exposure may negatively affect NCPs involved in new bone formation.<sup>[27]</sup> Thus, in our study, ADG obtained from SDG was undemineralized, was not subjected to any acid treatment, and was immediately used in defects without delaying the time and interfering with the action of NCPs.

## CONCLUSIONS

The results of the present study show superior and promising results by the use of ADG and chorion membrane and improvement in all the clinical and radiographic parameters. VBD, HBD, and FBD significantly reduced using ADG and chorion membrane in the treatment of Grade II and III furcation defects when compared to DFDBA and chorion membrane.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

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