Comparative evaluation of effectiveness of concentrated growth factors (CGF) enriched bone graft matrix (sticky bone) with hydroxyapatite reinforced beta tricalcium phosphate and bioabsorbable membrane in treatment of class ii furcation defects - A clinical study

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Abstract

Aim - To compare the effectiveness of concentrated growth factors enriched bone graft matrix (Sticky bone) with hydroxyapatite reinforced Beta TCP and bioabsorbable membrane in treatment of class II furcation defects.

Method - Total 24 patients with class II furcation defects were included in the study. The parameters recorded were plaque index (PI), papillary bleeding score (PBI), horizontal probing depth (HPD), vertical probing pocket depth (V-PPD), relative clinical attachment level (R-CAL), relative gingival marginal level (R-GML) on the day of surgery and after 6 months.

Results- Comparison between mean PPD reduction between groups at 6 months indicated remarkable reduction in β-TCP + GTR group by 0.83 ± 0.93mm. Similarly, mean R-CAL gain at 6 months indicated notable gain in β-TCP + GTR group by 1 ± 1.27 mm. The mean gain of R-GML (0.25 ± 1.13 mm) and HPD reduction (0.16 ± 1.02 mm) between groups at 6 months showed no statistical significance.

Conclusion – Sticky bone group showed remarkable improvement from baseline in measured clinical parameters. Percentage of defects converted to class I from class II were higher in sticky bone group (83.3%) compared to HA/β-TCP + GTR group (66.6%) showed regenerative potential which can be compared to combination therapy.

Introduction

The ideal goal of the therapy of furcation involved tooth is to preserve it by achieving complete closure of the defect through periodontal regeneration thus improving the prognosis of the involved tooth. Amongst the various classes of furcation, Class II furcation defects with their unique anatomy continue to pose challenge to the regenerative capabilities of the oral cavity. To obtain a desired result, numerous treatment modalities have been utilized with combination therapy being widely used. It employs use of GTR membrane along with bone replacement graft thus enhancing graft only therapy by inhibiting epithelial ingrowth and bone graft containment. It offer an advantage over monotherapeutic approaches and is considered as a definitive treatment modality for numerous furcation type defects, among which exceptional results can be seen in class II involvement.

Most widely used synthetic scaffold made up of ceramic granules used in periodontal regeneration is beta-tricalcium phosphate. It acts as a highly selective carrier and scaffold for in growth of cells which later get absorbed and eventually replaced by newly formed bone. Various human and animal studies have proved its osteoconductive property and biodegradable nature. But when used in combination with hydroxyapatite, effectivity of β-TCP is enhanced thus provide bone volume gain. Particulate form of β-TCP with > 99% crystalline structure consists of 60% HA and 40% β-TCP. Preclinical evidence suggested that the use of this ratio allow bioabsorbable ability of the particulate graft thus accelerating new bone formation.
To enhance the regenerative potential of bone replacement graft alone, GTR membranes are used in combination. It prevents containment of graft, exclude epithelial ingrowth, contamination by microbes and variable inductivity of the graft. When effectivity of bioabsorbable and non-resorbable membrane is compared in human class II furcation defects, it is shown to possess remarkable significance.\textsuperscript{10} The GTR technique using polylactic acid (PLA), polyglycolic acid (PGA) bioabsorbable membrane prevent need for second surgical site preparation for its removal. Previous studies have demonstrated that PLA/PGA is biocompatible, nonallergic and does not produce any inflammatory response. However, it has been claimed that the healing of periodontal defects using GTR therapy occurs by new attachment.\textsuperscript{11}

Recently, a biocompatible product known as sticky bone which utilizes patients own blood with altered centrifugation speed (2700–3000 rpm for 1–2 min) have been introduced\textsuperscript{12}. In this, graft material is entrapped in fibrin mesh which prevents it from scattering. The autologous fibrin glue helps in faster healing of tissues and prevents bone loss at the time of healing. It contains vital elements necessary for bone formation as well as growth factors for stimulation and migration of cells.\textsuperscript{13} It has been used in ridge preservation procedure\textsuperscript{14} and in dehiscence defects\textsuperscript{15} and has shown to possess bone regenerative capacity. Along with new bone formation it also helps to enhance the density of the newly-formed bone.\textsuperscript{15} Since its regenerative potential in infrabony defects like furcations have not been evaluated so far, the current clinical study was carried out to assess its effectivity for treatment of Class II furcation defects in mandibular molars for the first time and its potential is associated with bio absorbable PLA-PGA membrane along with HA reinforced Beta-TCP.

**Materials And Methods**

A total of 24 patients, age range between 23 to 65 years (mean age- 45.75 years) were included in the period from December 2017 to September 2018. Patient selection and recruitment was done from the department of Periodontics and Implantology. The study was approved with approval no. DMIMS (DU)/IEC/2017-18/6730 by the Institutional ethics committee of Datta Meghe Institute of Medical Sciences. Mandibular molars with buccal class II furcation defects, as confirmed by thorough clinical examination were included. Defects with a horizontal and vertical defect depth of \( \geq 3 \text{mm} \) were considered. Teeth with sufficient keratinized gingival width, and with the gingival margin coronal to the furcation fornix were included. Only those teeth with proximal bone height coronal to inter-radicular bone level, intact and vital tooth surfaces adjacent to the furcation area were selected. Non-compliant patients, smokers or use of tobacco products in any other form, patients with loosening of involved tooth or allergic to graft material, patient with known history of regenerative therapy done with the involved tooth or at the selected site were excluded from the study.

**Pre-surgical therapy**

At the beginning, patients were explained about purpose of the study and design. Informed consents were signed by every patient. Following examination and diagnosis, initial therapy consisting of scaling and root planing was performed. A re-evaluation examination was performed after 6 weeks to determine
patient's response to the therapy and to confirm the need for periodontal surgery. After preparatory phase and before initiation of the surgery, all the subjects were categorized into 2 groups depending upon the treatment modality provided. In group I (n=12) furcation defects, sticky bone was used and in group II (n=12) bone graft material (HA/β-TCP) along with a PLA-PGA bio-absorbable membrane was utilized. All patients were recalled for re-evaluation after 3- and 6-months after surgery.

Measurements of all the clinical parameters including indices (Plaque index, papillary bleeding index) to record plaque and bleeding score were carried out on the day of surgery and over the span of 3 and 6 months after that. Pocket depth, level of clinical attachment and gingival margin were documented on the day of surgery and after 6 months. To measure the clinical parameters, occlusal acrylic stents with a groove was fabricated. At the surface of furcation defect, mesial, distal and midbuccal/midlingual line angle were used to record horizontal probing depth (HPD) (Fig. 4a, 5a), vertical probing pocket depth (V-PPD), relative clinical attachment level (R-CAL), relative gingival marginal level (R-GML).

**Surgical procedure**

Prior to initiation of surgery, 0.2% chlorhexidine gluconate solution (Hexedine, ICPA Health products Ltd., India) was given to the patients and advised to rinse their mouth for 1 minute. 2% Xylocaine containing 1:80,000 concentration of epinephrine (Ligno-Ad local anesthetic, Proxim Remedies, India) anesthetic solution was used for nerve block and infiltration anesthesia. Extraoral antisepsis was performed with povidine iodine solution. To expose the defect and to get proper visualization for debridement of the defect area, reflection of full thickness mucoperiosteal flap was done with the help of a periosteal elevator (24 G Hu-Friedy, USA). Careful curettage should be done on the subsurface of flap to remove diseased pocket epithelium. Furcation area was thoroughly cleaned by removing diseased granulation tissue. Later, hand (Gracey curettes, HU-Friedy, USA), furcation curettes and machined driven (EMS- mini PIEZON) instruments were used to clean the bare root surface along with roof of the furcation. Scaling and planing of involved root surfaces were performed thoroughly to obtain smooth hard consistency. The defect was prepared by removing excess fluid with moist gauze to achieve hemostasis (Fig 4b, 5b).

**Surgical Procedure for sticky bone group**

From the antecubital vein, 10 ml blood was withdrawn by venipuncture. Unlike PRF where blood was collected in plane glass test tubes, for preparation of sticky bone sterile plastic tubes (10 ml) without any anti-coagulant were used. Test tubes were immediately placed in centrifugal machine at 3000 rpm for 1-2 min. The time require for preparation of AFG varies from 2-12 mins. Higher concentration of growth factors can be obtained by using centrifugation time of 1 min. After 1 min, AFG tube was taken out of centrifuge showing 2 different layers (Fig.1). Uppermost layer was an AFG which was obtained with a syringe (Fig.2) while the bottom red blood cells layer was disposed of. Particulate bone graft then immediately mixed with AFG (Fig.2) and kept for polymerization. The optimum time require for polymerization is up to 5-10 mins. Sticky bone thus obtained (Fig 3) was adapted in such a way that defect should be covered completely (Fig. 4c).
**Surgical procedure for HA/β-TCP + GTR group**

Bone graft (β-Ostin TCP, Basic Healthcare, New Delhi) along with trimmed PLA/PGA membrane (BioMesh, Biodegradable membrane, Samyang pharmaceuticals, Korea) was placed over the defect so as to cover it completely followed by suturing (Fig 5c).

The flap was coronally repositioned and sutured in such a way that the flap margin was located 1 to 2 mm coronal to CEJ. A simple interrupted interproximal suture (4-0 non-resorbable surgical black silk sutures, USP braided, Ethicon, Johnson Ltd.) were given to hold flap in desired position in both the groups. The surgical site was dressed with a periodontal surgical dressing (Coe-Pak, TM, GC, America Inc, ALSIP, IL, USA).

**Post-operative Care**

After surgery, patients were instructed to avoid brushing at operated area. 0.2% chlorhexidine gluconate (Hexidine – ICPA) solution were prescribed to the patients and advised to rinse two times a day for 1 minute, for 4-6 weeks. A Zerodol-SP containing Aceclofenac (non-steroidal anti-inflammatory) 100 mg, Paracetamol 325 mg and Serratiopeptidase 15 mg two times a day along with Amoxicillin 500 mg, an antibiotic for five days; three times a day after the surgery were prescribed. The patients were instructed to report after 1, 3- and 6-months following procedure where all clinical measurements were documented cautiously.

**Statistical Analysis:**

“For all parameters, the mean and standard deviation (mean ± SD) values were measured. The mean data were analyzed using traditional statistical methods for statistical significance. Student's paired t-test was used to compare the data from baseline to 6 months for each group while comparison between the groups was performed using Student's unpaired t-test. The observed difference was considered significant if the probability value ($p$) was < 0.05 and if it was > 0.05 it was deemed to be non-significant.”

**Results**

During study period of 6 months, wound healing was uneventful and none of the sites were eliminated from the study. All the patients were satisfied with treatment provided to them.

Plaque and papillary bleeding index were calculated at baseline, 3 and 6 months and follow-up showed marked reduction in both groups (Table 1).

Clinical parameters V-PPD, V-RAL, RGML, HPD were compared at baseline and 6 months in each group (Table 1) and between both groups which showed remarkable changes (Table 2). Mean PPD reduction (0.83 ± 0.93 mm) and mean CAL gain (1 ± 1.27 mm) in β-TCP + GTR group was remarkably high compared to sticky bone group while no statistically significant reduction of HPD and changes in RGML
were noted between two groups (Table 2). Frequency of furcation closure in both the groups showed remarkable regenerative capability.

**Discussion**

In view of the evidences found in the literature\(^{16,17,18,19}\) which imply the advantages of combination therapy in the improvement of furcation defects, present study utilized bone graft along with a bioabsorbable membrane and was compared with a newly introduced concentrated growth factor (CGF) enriched bone graft matrix (sticky bone) for the management of class-II furcation involvements.

In present study, all the cases showed complete healing of operated area without any complications. Also, strict adherence to plaque control and oral hygiene maintenance throughout the study period showed reduced PI and PBI score at 6 months follow-up in both the groups which plays important role in maintaining clinical attachment gain obtained after periodontal therapy.\(^{20}\)Reduction of probing pocket depth is a primary objective of periodontal therapy\(^{21}\) which was prominently reduced in favour of HA/\(\beta\)-TCP + GTR group (0.83 ± 0.93 mm; \(p < 0.05\)) compared to sticky bone group at 6 months follow-up. This finding has evidences from the literature showing, although limited, a mean PPD reduction by using HA/\(\beta\)-TCP + GTR compared to monotherapy in management of furcation defects.\(^{22,23}\)Similarly, statistically significant clinical attachment gain of 3 ± 1.12 mm was seen in HA/\(\beta\)-TCP + GTR group compared to sticky bone group 2 ± 0.85 mm at 6 months (mean CAL gain- 1 ± 1.27; \(p < 0.05\)). These results are comparable with findings stated in a literature where HA/\(\beta\)-TCP along with PLA-PGA membrane\(^{23}\), collagen membrane\(^{24}\) in class II furcation type defects reported a significant CAL gain after 6 months. Placement of a PLA-PGA membrane over DFDBA as a therapeutic modality in furcation defects had also indicated regenerative potential.\(^{25}\) The percentage of class II furcation defects that showed complete closure was higher in HA/\(\beta\)-TCP + GTR group (16.6%; \(n = 2\)) while 66.6% percent of defects converted from class II to class I showing remarkable regenerative potential. Evidences available in literature have demonstrated that GTR therapy helps in conversion of Class II to Class I furcation thus increases the long-standing prognosis of tooth.\(^{26}\) The combination therapy has advantages over grafting only therapy. It contains the graft in defect area, support the membrane to ideal position and enhance epithelial exclusion.\(^3\) Long term results demonstrated gain in attachment up to 4.5 mm in GTR therapy in furcation management.\(^{27,28}\) All the remaining defects in HA/\(\beta\)-TCP + GTR group (\(n = 2\)) also showed significant reduction of defect depth compared to baseline values.

To the best of our knowledge, no studies have been carried out evaluating the efficacy of sticky bone in furcation management. Therefore, comparison of the use of sticky bone in furcation defects cannot be made. Thus, it stands for exogenous comparison of sticky bone with per se established treatment modality for regeneration in furcation defects. Since, clinical bone fill is considered as a primary outcome in regenerative therapy of furcation defects, significant horizontal defect depth reduction at 6 months in sticky bone group (Fig. 4d) compared to HA/\(\beta\)-TCP + GTR group (Fig. 5d) substantiated the bone filling potential of sticky bone. Also, percentage of defects converted from class II to class I in sticky bone group
(n = 10; 83.3%) showed remarkable regenerative potential which can be compared to combination therapy. The change in furcation grade of the teeth i.e. conversion to lower class I or complete elimination of defect and/or hard-tissue fill horizontally are regarded as main variables to evaluate effectivity of regenerative therapy like GTR.29

Considering all these characteristics of sticky bone, to eliminate the extra cost required for the barrier membrane and to prevent the bacterial contamination due to membrane exposure, sticky bone was used for the first time as a treatment modality in furcation type defects. Although, studies are available showing regenerative potential of sticky bone in ridge defects, improvement in clinical parameters like PD reduction and CAL gain in management of furcation defects can be achieved by application of barrier membrane like CGF membranes along with sticky bone. Also, the probability of success of treatment modality is regulated by numerous factors like patient compliance, defect anatomy, treatment method etc.20 These factors have great influence on treatment outcome thus comments can be given only after better understanding of these factors. As a result, analysis was performed in relation to treatment modality in the present study. Long term follow up and histologic studies are needed to support the outcomes obtained in the current study. To govern the stability of the results and to quantify the regeneration, randomized control trials with larger sample size and radiological analysis need to be planned.

**Abbreviations**

HPD- Horizontal probing depth
V-PPD- Vertical probing pocket depth
R-CAL- Relative clinical attachment level
R-GML- Relative gingival marginal level
GTR- Guided tissue regeneration
HA- Hydroxyapatite
β-TCP- Beta- Tricalcium phosphate
PD- Pocket depth
DFDBA- Demineralized freeze dried bone graft
PLA- Polylactic
PGA- Polyglycolic acid

**Declarations**
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### Table 1

**Comparison of clinical parameters between baseline, 3 Months and 6 months Follow-Up (Mv±Sd)**

| Parameter               | Sticky bone group | P       | HA/β-TCP + GTR group | P       |
|-------------------------|-------------------|---------|----------------------|---------|
| Plaque index            |                   |         |                      |         |
| Baseline                | 1.35 ± 0.15       | <0.001  | 1.29 ± 0.16          | <0.001  |
| 3 months                | 0.94±0.30         |         | 0.86±0.19            |         |
| 6 months                | 0.72±0.29         |         | 0.64±0.20            |         |
| Papillary bleeding index|                   |         |                      |         |
| Baseline                | 1.44±0.24         | <0.001  | 1.34±0.10            | <0.001  |
| 3 months                | 0.98±0.30         |         | 0.925±0.11           |         |
| 6 months                | 0.67±0.31         |         | 0.72±0.18            |         |
| V- PPD                  |                   |         |                      |         |
| Baseline                | 4.16 ± 0.71       | <0.001  | 4.5 ± 0.67           | <0.001  |
| 6 months                | 2.75 ± 0.75       |         | 2.25 ± 0.45          |         |
| V-RAL                   |                   |         |                      |         |
| Baseline                | 10.5±0.79         | <0.001  | 11.5 ± 0.90          | <0.001  |
| 6 months                | 8.5 ± 1.24        |         | 8.5 ± 1              |         |
| HPD                     |                   |         |                      |         |
| Baseline                | 4.5 ± 0.52        | <0.001  | 4.41± 0.51           | <0.001  |
| 6 months                | 2.08 ± 0.51       |         | 1.83± 0.93           |         |
| R-GML                   |                   |         |                      |         |
| Baseline                | 6.33 ±0.65        | 0.02    | 7 ± 0.42             | 0.002   |
| 6 months                | 5.75 ± 0.86       |         | 6.16 ± 0.83          |         |

### Table 2

**Comparison of clinical parameters between sticky bone group and β-TCP + GTR group at 6 months follow-up.**

(MV ± SD; in mm)
| Parameters     | Sticky bone group | β-TCP + GTR group | Difference | P value |
|---------------|-------------------|-------------------|------------|---------|
| PPD Reduction | 1.41±0.51         | 2.25±0.75         | 0.83±0.93  | 0.01    |
| CAL Gain      | 2±0.85            | 3±1.12            | 1±1.27     | 0.02    |
| GR            | 0.58±0.79         | 0.83±0.71         | 0.25±1.13  | 0.46    |
| HPD reduction | 2.41±0.51         | 2.58±0.79         | 0.16±1.02  | 0.58    |

**Table 3**

**Frequency of Clinical Furcation changes at 6 months in sticky bone group and β-TCP + GTR group**

| Parameters                              | Sticky bone group (n=12) | β-TCP + GTR group (n=12) |
|-----------------------------------------|--------------------------|--------------------------|
|                                         | No. of sites (%)         | No. of sites (%)         |
| Complete closure (HPD = 0)              | 0 (0%)                   | 2 (16.6%)                |
| Changes from Class II to Class I (HPD < 3) | 10 (83.3%)               | 8 (66.6%)                |
| No change                               | 0 (0%)                   | 0 (0%)                   |

*S-* Significant, p<0.05; **NS-* Non significant, p>0.05 compared to baseline