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Comparison of Chorion Allograft and Subepithelial Connective Tissue Autograft in the Treatment of Gingival Recession- A Randomized Controlled Clinical Trial

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Abstract

Background: To compare the clinical outcomes of subepithelial connective tissue graft and chorion membrane along with coronally advanced flap in the treatment of gingival recession

Methods: A total of 12 patients with 24 sites showing isolated bilateral Miller's class I and II gingival recessions were randomly allocated into two treatment sites. One site, connective tissue graft, (n=12 sites) while on the contra-lateral site, chorion membrane (n=12 sites) was used with coronally advanced flap. Clinical parameters: probing depth, recession depth, recession width, width of keratinized gingiva, relative attachment level, thickness of keratinized gingiva were recorded at the baseline, 3 months, and 6 months. Amount of root coverage was evaluated after 6 months.

Results: Statistically significant differences were observed between test and control sites in terms of recession depth, recession width, width of keratinized gingiva and thickness of keratinized gingiva at 6 months. The test sites presented 66.17±18.85% and the control site showed 87.17±18.33% of root coverage at 6 months.

Conclusion: Very limited amount of recession coverage with chorion membrane and did not serve as an alternative to connective tissue graft**.**

Trial registration: CTRI/2017/12/010964

Keywords: Chorion Membrane, Gingival Recession, Root Coverage, Subepithelial Connective Tissue Graft, Thickness of Keratinized Gingiva

Keymessage: Chorion membrane showed improvement in clinical parameters in the treatment of gingival recession but not comparable to connective tissue graft.

Introduction

Apical migration of the gingival margin leads to the exposure of root surface to the oral cavity, results in higher susceptibility to root caries, dentinal hypersensitivity and unfavourable aesthetics. [1,2]

Among various root-coverage procedures, the subepithelial connective tissue graft technique is considered to be the standard approach. It has an excellent prognosis with good esthetic results. [3] However, it is time-consuming, traumatic and difficulty in obtaining graft of uniform thickness. [4]

A unique allograft, chorion membrane, was introduced as a root coverage material to overcome all these obstacles. Chorion contains different types of collagen, proteoglycan, laminin, and bioactive factors which help in binding gingival epithelial cells to the root surface. Being a potent stem cell reservoir it promotes cell differentiation, stimulate healing and help in revascularization. Thus it has widespread application in the periodontology. [5-7]

The aim of this study was to clinically evaluate the efficacy of chorion membrane (CM) and subepithelial connective tissue graft (SCTG) with coronally advanced flap (CAF) in the treatment of gingival recession. The study had clinically evaluated and compared the amount of root coverage and improvement in gingival thickness obtained in both CM and SCTG sites.

Materials & Methods

Study design and participants: This was an interventional split mouth study included 12 systemically healthy subjects with 24 sites. It included male and female patients in the age range of 18-50 years, presenting with Miller's class I and II bilateral recession having adequate width of keratinized gingiva. The exclusion criteria were followed: 1) history of any systemic disease or medication 2) Miller's class III and IV gingival recession 3) root caries or crowns at CEJ 4) pregnancy/lactation 5) poor oral hygiene (PI<2) 6) smokers and alcoholics. The study was approved by the Ethical Committee of the JSS Academy of Higher Education & Research, Mysuru. Written consent was obtained from all the subjects before the examination. Only qualifying patients who were willing to participate in the study for 6 months were selected from outpatients at the Department of Periodontology, JSS Dental College & Hospital, Mysuru.

Sample size calculation: 12 patients were selected and 24 sites were estimated based on an earlier conducted clinical study (amnion membrane and connective tissue graft). Significance level and standard deviation = 0.60 (α=0.05, β=0.2). [8]

Pre-surgical therapy: Before non-surgical therapy (zero day), Plaque Index (PI) and Gingival Index (GI) were recorded. Proper oral hygiene instruction was given. Phase I therapy was performed. Impressions taken and casts poured to prepare the stent. Intraoral periapical radiograph of both the sites were taken to confirm the bone level. 2 months' post therapy, a periodontal assessment was carried out and two sites were randomly allocated into test (CM+CAF) and control (SCTG+CAF) sites using a coin-toss method.

Clinical parameters: A single periodontist (R.M) recorded all clinical periodontal measurements at baseline, 3 and 6 months without information of the sites treated. Clinical parameters included: PI, GI, probing depth (PD) measured from the gingival margin to the sulcus depth, recession depth (RD) measured from CEJ to gingival margin, recession width (RW) measured at the widest point from mesial gingival margin to distal gingival margin, width of keratinized gingiva (WKG) recorded by roll method. Personalized acrylic stents were used for relative attachment level (RAL) recordings

in order to standardize the measurements. A horizontal and vertical groove was made on the stent to place the probe in position. RAL were measured by adding gingival margin level (GML) with PD. Thickness of keratinized gingiva (TKG) was measured 2 mm apical to the gingival margin with 25k endodontic file with rubber stopper. All the recordings were measured using UNC-15 probe (Hu-Freidy, Chicago, IL).

After 6 months, the percentage of root coverage (RC) was calculated: [pre-operative gingival recession depth - post-operative recession depth]/[preoperative recession depth] * 100%

Surgical Procedure: All the surgical procedures were carried out by a single periodontist (S.M). 0.12% chlorhexidine digluconate rinse was used before surgery, and povidone iodine solution for extra-oral antisepsis followed by application of local anesthesia (2% Lignocaine HCl containing 1: 80,000 adrenaline). The CAF procedure proceeded with the placement of two horizontal incisions mesially and distally at the level of CEJ of the involved tooth (Fig.1,5). An intra-crevicular incision given using no. 12 blade. Two vertical releasing incisions were extended beyond the mucogingival junction. Using 15c blade, a split thickness flap was reflected by sharp dissection. Muscle tension was released apical to the bone dehiscence in order to mobilize the flap. The facial portion of the interdental papillae was de-epithelialized to form connective tissue bed for final placement of the flap margin. Thorough root planing was done and convexities were reduced. Test site was treated with freeze dried CM (commercially available from Tata Memorial Hospital, Mumbai) which was contoured according to the size of the defect while covering at least 2 mm of bone all around the defect. For proper adherence to the recipient site, the CM was soaked in normal saline for 1 minute (Fig.2). The contralateral control site was treated with connective tissue graft harvested from the palatal region by trap-door method (Fig.6). The graft was then immediately transferred to the recipient site and secured with 5-0 vicryl suture. The flaps were coronally advanced and sutured with 5-0 vicryl. 3-0 silk suture placed on donor site. Light finger pressure was applied for 5 minutes to remove dead spaces. Periodontal pack (Coe-Pak, GC America, Chicago, IL) along with tin foil was placed as a dressing material.

Post-surgical care: Antibiotics and analgesics were prescribed. Patients were advised to avoid tooth brushing in the treated area for the first 2 weeks and to rinse with 0.12% Chlorhexidine solution three times a day for 4 weeks. The silk sutures were removed after 2 weeks. Patients were instructed to use soft toothbrush and brushing technique was modified. Recall appointments were scheduled weekly during the first month and then at 3rd (Fig.3,7) and 6th month (Fig.4,8) to assess the clinical parameters.

Statistical analyses: For every parameter and for every assessment time point, mean values and standard deviations (SD) were calculated. The repeated-measures ANOVA was used to assess the effect of time and treatment on continuous variables. The Bonferroni post hoc test was applied for multiple intra group comparisons. The intergroup differences were statistically explored using paired sample t-test. The level of significance was set at $p<0.05$ and statistical analyses were conducted using commercially available software SPSS (Statistical Package for Social Science version 22).

Results

Total 15 subjects (13 males 2 females), meeting the inclusion criteria with bilateral recession were randomly divided into test and control sites. The test sites (n=15)

were treated CM+CAF and the control sites (n=15) were treated with SCTG+CAF. 3 subjects lost the 3 months' follow-up in the study. As a result, 12 subjects mean age of 36.83±8.69 years were analysed over a period of 6 months.

Table 1 presents the patient and defect-related characteristics. There were no significant differences in the characteristics of teeth between the sites. Except, in one subject, where canine was considered as the test tooth, bilateral 1st premolar was taken as the control.

Table 2 shows the comparison of mean PI and GI scores at zero day, baseline, 3 and 6 months. Both showed statistically significant reduction at each time interval as compared to zero day indicating a good standard of oral hygiene.

Intragroup comparison of clinical parameters (mean±SD) over the 6-month experimental period are presented in table 3. Both the site showed reduction in mean PD at 3 and 6 months. The mean RD value at the test site decreased from 3.00±0.7 at baseline to 1.08±0.9 and 1.00±0.9 at 3 and 6 months, respectively (p=0.00). Similarly, in the control site, the mean RD value decreased from 3.33±0.9 at baseline to 0.58±0.8 and 0.42±0.8 at 3 and 6 months, respectively (p=0.00). Mean RW and WKG at the control sites showed statistical significant result as compared to test site at 3 and 6 months. Both the sites showed significant result in RAL gain and increase in TKG.

Table 4 shows the intergroup comparison. Statistically significant differences between test and control sites in terms of RD, RW, WKG, TKG and % of RC ($p<0.05$)

The mean percentage of RC was calculated at 6 months and compared with baseline. There was 66.17±18.85 and 87.17±18.33 mean percentage of RC at the test and control sites after 6 months' follow-up respectively (p=0.002) (Table 4).

After being treated with chorion, no subjects complained of pain whereas, 7 out of 12 patients complained of severe pain at the control site along with discomfort while taking food. Delayed healing of the palatal wound was noted in 1 subject.

Discussion

Out of the numerous surgical techniques for gingival recession, SCTG+CAF is considered the benchmark for root coverage therapy. [9,10] A bilayer vascular supply nourishes the graft yielding better esthetic outcome. However, the downside of this technique has led us in search of other regenerative materials. [11] Recent evidence from several in-vivo studies indicated that placental membrane may be a powerful tool for periodontal regeneration. [8,12-14] With this concept, the study was designed to test a versatile substitute, CM, for root coverage and compared with SCTG.

The experimental therapy resulted in significant PD reduction gain demonstrating the bioactivity of chorion and its intrinsic healing potential. The mean PD reduction noted was 0.75 mm at 6 months from baseline. The results were similar to the case series reported by Esteves et al, (0.81 ± 0.75). [15] This is probably because the matrix of the chorion contains abundant growth factors that promote periodontal regeneration and provide an environment for accelerated healing. [16] The study showed the reduction in PD at the control site as well but was not significant after 6 months when compared with baseline. The result is in contrast to the study conducted by Gharoudi et al. in which PD increased by 0.19 mm in the SCTG treated site. However, when the two sites were compared, our study revealed no significant changes ($p>0.05$).

The achieved mean RAL gain at the test sites after 6 months was 2.67 mm. The result was in accordance with studies done by Brain (amnion membrane), [17]

Chakraborthy et al. [13] (chorion membrane) and Esteves et al. [15] where 1.2±1.51 mm, 2 mm and 3.48±1.21 respectively was achieved. The previous studies set down the RAL gain to laminins that may have promoted regeneration, accelerated tissue adhesion, which are key factors in improved healing of gingival lesions. Furthermore, the secretory leukocyte proteinase inhibitor I, lactoferrin, defensin, and elafin which are the antimicrobial agents might improve wound healing. At 3 months the mean RD decreased by 1.92 mm and 2.75 mm in the test and control sites, respectively. On intergroup comparison significant values were obtained at 3 and 6 months. As limited literature is available on CM, this study was compared with the studies done on amnion, since amnion and chorion are known to share similar properties like immunomodulative, antimicrobial, anti-inflammatory and regenerative.[18,19] The achieved reduction of RD in the test sites is similar with the studies done by Ghahroudi et al., Chakraborthy et al.[8,13] The decrease of RD can be explained with the fact that chorion is rich in collagen, proteoglycans, laminin, and fibronectin which promotes cell attachment, growth, and differentiation.[20,21]

In the present study, there was statistically significant decrease in RW when compared between the groups at 3 and 6 months ($p<0.05$). The result is inconsistent with the study conducted by Chakraborthy et al. [13] where the sites treated with CM showed a significant improvement in RW. However, our study showed a significant decrease of RW at the CAF+SCTG treated sites but the result is contradicting with the study by Gharoudi et al [8] where the control site treated with CTG showed no significant result.

We obtained statistically significant increase in mean WKG between test and control sites at 3 and 6 months when compared with baseline. Mean WKG at the test and control sites were 2.50±0.7 and 2.83±0.7 respectively after 6 months. CAF and the keratinocyte growth factor released by the CM, might help in keratinization which maintained the mucogingival junction in its position. Many studies have emphasized the positive role of SCTG in increasing keratinized gingival width [9,11,22-25] because of its ability to induce epithelial cell differentiation at the recipient site. [26]

Thin biotype can be a cause of recession. There was significant increase in mean TKG by 0.42 mm in CM+CAF treated site and 0.92 mm in SCTG+CAF after 6 months. It was in consensus with the study done by Kothiwale. [14] However, the result we obtained is in contrast with the study done by Rehan et al. [27] with amnion membrane which was followed up over a period of 18 months with insignificant results. The increase may be due to the presence of large number of pro-angiogenic growth factors which promote endothelial recruitment and better vascularisation. [28] Immunohistochemical staining analysis done on CM showed increased concentration of laminin. [29] These helped in binding the epithelium on the root surface. [30] The presence of tissue inhibitor of metalloproteinases (TIMPs) suppresses matrix metalloproteinases (MMPs) in turn reduces inflammation and collagenous degradation. [28]

The mean percentage of RC at the CM+CAF treated site was 66.17±18.85%, while SCTG+CAF treated site showed 87.17±18.33%. Only 1 out of 12 test site came out with 100% RC while, 7 out of 12 control sites showed complete RC. The achieved RC in the test site was better as compared to the study by Chakraborthy et al. [13] Also, the present study differed from the case series which obtained 89.92%±15.59% RC. [15] The success of CM+CAF is credited to its anti-inflammatory and antimicrobial properties and large number of growth factors. Also, this allograft contains some cytokines that

affect progenitor cells, which may play vital role in activating cells at the site to participate in regeneration and tissue maturation. [28]

The present study came across various advantages of CM over SCTG. Elimination of a second surgical site was found to be the most important advantage. CM has a self-adherent nature that reduces surgical time, makes procedure easier for the clinician and comfortable to the patient. Graft rejection is also minimal due to its non-immunogenic property. Since commercially available, an ample amount is available to use in multiple recession cases. Though there are some limitations of this study. Being technique sensitive procedure, improper handling of flap and harvesting of CTG can be a reason for failure in getting complete root coverage. Only one tooth treated with SCTG in mandibular jaw showed complete coverage. This is probably due to thin mucosa apical to the recession which tends to pull the CAF in downward direction.

Conclusion

The result of the study showed very limited amount of recession coverage with chorion membrane and did not serve as an alternative to connective tissue graft. Hence, CTG can still be considered the gold standard. Further long-term clinical trials and histopathologic studies are necessary to know the predictability of the membrane.

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Legend Tables and Figures

Table 1: Patient and defect-related characteristics

Variable	Test Site (CM + CAF) (n=12 sites)	Control Site (CTG + CAF) (n=12 sites)
Age (years: mean)	36.83 ± 8.69 years	36.83 ± 8.69 years
Male/Female	Male: 12 Female: 0	Male: 12 Female: 0
Maxilla	07 Canine: 05 Molar: 02	07 Canine: 05 Molar: 02
Mandible	05 Canine: 01 1st Premolar: 04	05 1st Premolar: 05
Recession defect	Miller's class I: 09 Miller's class II: 03	Miller's class I: 09 Miller's class II: 03

CM: Chorion membrane; CAF: Coronally advanced flap; CTG: Connective tissue graft

Table 2: Comparison of mean plaque and gingival scores at different time intervals among study participants

Clinical parameter	Zero day (1) Mean±SD	Baseline (2) Mean±SD	3 months (3) Mean±SD	6 months (4) Mean±SD	Statistical inference†	Post hoc comparison‡
PI	1.75±0.4	1.00±0.00	1.00±0.00	1.00±0.00	F value: 33.00 df: 3 p value: 0.00*	1 Vs 2: 0.001* 1 Vs 3: 0.001* 1 Vs 4: 0.001* 2 vs 3: 1.00 2 Vs 4: 1.00 3 Vs 4: 1.00
GI	1.28±0.3	0.17±0.03	0.17±0.04	0.17±0.04	F value: 152.98 df: 3 p value: 0.00*	1 Vs 2: 0.00* 1 Vs 3: 0.00* 1 Vs 4: 0.00* 2 vs 3: 1.00 2 Vs 4: 1.00 3 Vs 4:1.00

† Repeated Measures Analysis of Variance, ‡ Bonferroni post hoc test. 1: zero day, 2: baseline, 3: 3 months, 4: 6 months, p <0.05 is statistically significant*

PI: Plaque index; GI: Gingival index

Table 3: Changes in clinical parameters (mean ± SD) over the 6-month experimental period

Parameters	Sites	1 (Mean± SD)	2 (Mean± SD)	3 (Mean± SD)	1vs2 (p value) †	1vs3 (p value) †	2vs3 (p value) †	Statistical inference‡
PD	Test	1.50±0.7	0.92±0.5	0.83±0.6	0.08	0.04*	1.00	F: 7.21 df: 2 p: 0.004*
	Control	1.50±0.9	0.83±0.4	0.83±0.4	0.07	0.07	1.00	F: 6.77 df: 2 p: 0.005*
RD	Test	3.00±0.7	1.08±0.9	1.00±0.9	0.00*	0.00*	1.00	F: 51.12 df: 2 p: 0.00*
	Control	3.33±0.9	0.58±0.8	0.42±0.8	0.00*	0.00*	0.49	F: 126.23 df: 2 p: 0.00*
RW	Test	3.50±0.5	2.75±1.1	2.67±1.1	0.17	0.13	1.00	F: 4.79 df: 2 p: 0.01*
	Control	3.58±0.7	1.25±1.4	1.08±1.4	0.001*	0.002*	1.00	F: 22.98 df: 2 p: 0.00*
WKG	Test	2.33±0.8	2.42±0.7	2.50±0.7	1.00	0.49	1.00	F: 1.57 df: 2 p: 0.23
	Control	2.08±0.3	2.83±0.7	2.83±0.7	0.005*	0.005	1.00	F: 17.47 df: 2

								p: 0.00*
RAL	Test	12.92±2.1	10.42±2.0	10.25±2.1	0.00*	0.00*	0.5	F: 132.55 df: 2 p: 0.00*
	Control	13.42±2.4	10.08±1.7	9.92±1.6	0.00*	0.00*	0.5	F: 112.95 df: 2 p: 0.00*
TKG	Test	1.25±0.6	1.67±0.5	1.67±0.5	0.05*	0.05*	1.00	F: 7.86 df: 2 p: 0.003*
	Control	1.25±0.4	2.17±0.4	2.17±0.4	0.00*	0.00*	1.00	F: 121.00 df: 2 p: 0.00*

†Repeated Measures Analysis of Variance, ‡Bonferroni post hoc test. 1: baseline, 2: 3 months, 3: 6 months, p <0.05 is statistically significant*

PD: Probing depth; RD: Recession depth; RW: Recession width; WKG: Width of keratinized gingiva; RAL: Relative attachment level; TKG: Thickness of keratinized gingiva

Table 4: Intergroup comparison of clinical parameters (mean ± SD) over the 6 months experimental period

Parameters	Sites	1 (Mean ± SD)	2 (Mean ± SD)	3 (Mean ± SD)
PD	Test	1.50±0.7	0.92±0.5	0.83±0.6
	Control	1.50±0.9	0.83±0.4	0.83±0.4
Statistical inference†		t: 0.00 df: 11 p: 1.00	t: 0.56 df: 11 p value: 0.59	t: 0.00 df: 11 p: 1.00
RD	Test	3.00 ± 0.7	1.08±0.9	1.00±0.9
	Control	3.33 ± 0.9	0.58±0.8	0.42±0.8
Statistical inference†		t: 1.77 df: 11 p: 0.10	t: 2.17 df: 11 p: 0.05*	t: 2.24 df:11 p: 0.05*
RW	Test	3.50±0.5	2.75±1.1	2.67±1.1
	Control	3.58±0.7	1.25±1.4	1.08±1.4
Statistical inference†		t: 0.43 df: 11	t: 3.59 df: 11	t: 3.80 df:11

		p: 0.67	p: 0.004*	p:0.003*
WKG	Test	2.33±0.8	2.42±0.7	2.50±0.7
	Control	2.08±0.3	2.83±0.7	2.83±0.7
Statistical inference†		t: 1.39 df: 11 p: 0.19	t: 2.80 df: 11 p: 0.01*	t: 2.34 df: 11 p: 0.04*
RAL	Test	12.92±2.1	10.42±2.0	10.25±2.1
	Control	13.42±2.4	10.08±1.7	9.92±1.6
Statistical inference†		t: 1.15 df:11 p: 0.27	t: 0.71 df: 11 p: 0.49	t: 0.65 df: 11 p: 0.53
TKG	Test	1.25±0.6	1.67±0.5	1.67±0.5
	Control	1.25±0.4	2.17±0.4	2.17±0.4
Statistical inference†		t: 0.00 df: 11 p: 1.00	t: 3.32 df: 11 p: 0.007*	t: 3.32 df:11 p: 0.007*
% of RC	Test			66.17±18.85
	Control			87.17±18.33
Statistical inference†				t– value: 3.93 df: 11 p value: 0.002*

†Paired sample t-test. 1: baseline, 2: 3 months, 3: 6 months, $p<0.05$ is statistically significant*

PD: Probing depth; RD: Recession depth; RW: Recession width; WKG: Width of keratinized gingiva; RAL: Relative attachment level; TKG: Thickness of keratinized gingiva

Figures

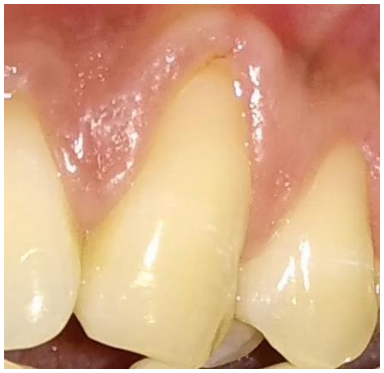

Fig.1: Pre-operative view of test site

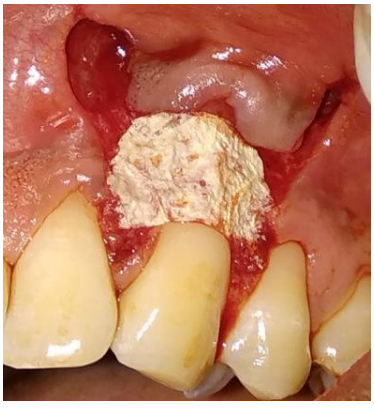

Fig.2: Chorion membrane placed on the test site

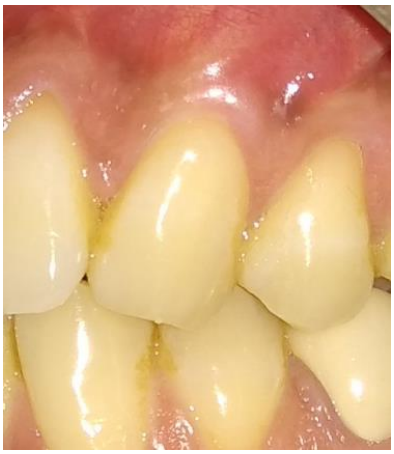

Fig.3: 3 months postoperative view of test site

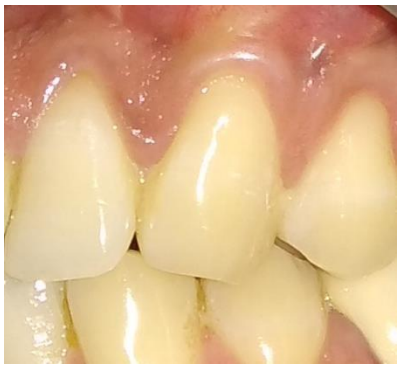

Fig.4: 6 months postoperative view of test site

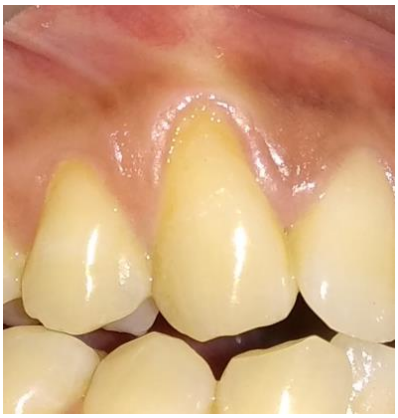

Fig.5: Pre-operative view of control site

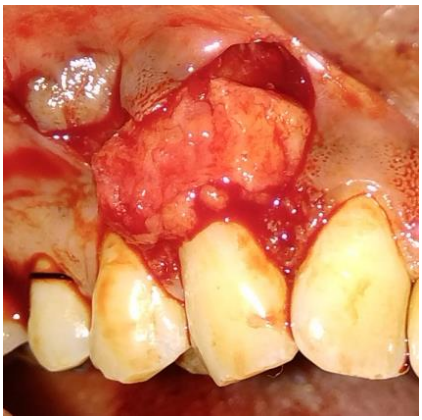

Fig.6: CTG secured on the control site

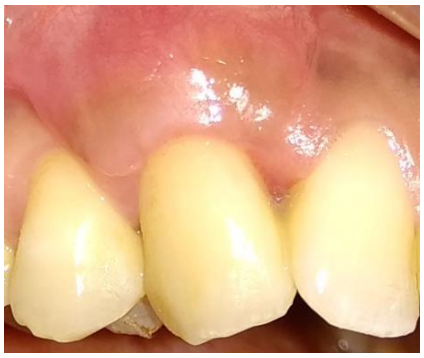

Fig.7: 3 months postoperative view of control site

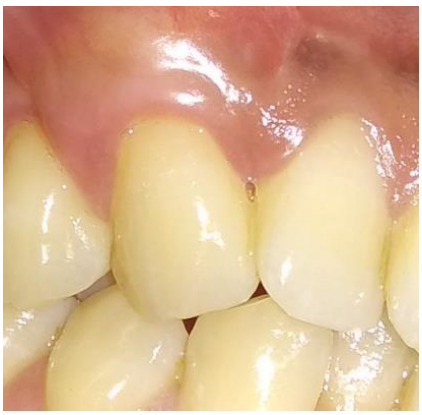

Fig.8: 6 months postoperative view of control sit