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Laser as an adjunct to Tetracycline root conditioning gel and bioactive bone graft material in the treatment of infra-bony defect: A split mouth clinical trial

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## Abstract

**Background:** Periodontal regeneration in intrabony defects has associated challenges despite the advancements in the field of regeneration. Several treatment modalities have been applied over the years to reconstruct these defects surgically. All these techniques are mainly aim at regenerating the periodontium and establishment of health.

**Objectives:** To evaluate the effectiveness of tetracycline gel and bioactive bone graft along with the application of diode laser in intra bony defects in chronic periodontitis patients.

**Methods:** A total of 40 sites (20 in each group) were randomly divided into Group I (Open flap debridement with tetracycline gel and bioactive bone graft) and Group II (Open flap debridement with tetracycline gel and bioactive bone graft with application of diode laser). The clinical parameter evaluated were Plaque index (PI), Gingival index (GI), Probing pocket depth (PPD), and Relative attachment level (RAL) at baseline, 3-month, 6 month & 9 month postoperatively. Radiographic parameters recorded osseous defect depth, amount of defect fill and percentage of defect fill at baseline, 6 month & 9 month.

**Results:** Results showed that gingival and plaque index were reduced in a time-dependentmanner from baseline to 9 months. Both the group showed reduction in mean PPD and RALwhich was statistically significant when comparing baseline (Group I:  $5.15 \pm 0.72$ , Group II:  $5.1 \pm 0.83$  at 3 month, Group I:  $4.01 \pm 0.73$ , Group II:  $3.45 \pm$  0.58 at 6 month to 9 month (Group I:  $3.52 \pm 0.79$ , Group II:  $2.5 \pm 0.5$  respectively). Group II showed greater reduction in

PPD (p= 0.005). Conclusion: The diode laser can be safely and effectively used in combination with tetracycline gel and bone graft in treatment of intrabony defects and helps to achieve better treatment outcomes and aids in tissue healing and enhanced periodontal regeneration.

**Keywords:** Tetracycline Gel, Bioactive Bone Graft, Diode Laser.

## Introduction

Periodontal diseases are caused by microbes that colonize on the periodontium, resulting in loss of supporting connective tissue and alveolar bone around the tooth. Advanced periodontitis are characterized by clinical attachment loss, pocket formation and osseous lesion. Osseous lesion represents the amniotic sequelae of the apical spread of the periodontitis.

Periodontal therapy aims to seize the disease progression and regenerate the lost attachmentapparatus. Deep intraosseous defects represent high risk sites for disease progression. Suchdefects are treated with various regenerative treatment modalities including bone grafts, bonegraft substitute Guided Tissue Regeneration (GTR), Root conditioning agent and biologicmediators.

Studies have suggested that PDL cells can be triggered to reproduce and move to thepreconditioned dentin surface, especially when tetracycline (TTC-HCl) has been used. It hasbeen seen that this preconditioning disrupts the smear layer and to some extent demineralizes the dentin surface to reveal the underlying collagen fibers.<sup>2,3,4</sup>.Tetracycline's anti proteolytic properties, together with their specific anticollagenase, activity has resulted in inhibitingbone resorption.<sup>5</sup> Bone resorption induced by parathyroid hormone, prostaglandins of the Eseries and bacterial endotoxin.6

Schalhorn RG interpreted the considerations that rule the selection of a material as follows:

biological acceptability, predictability, clinical feasibility, minimum operative hazards, minimal post-operative sequelae and patient acceptance.<sup>7</sup>

Alloplasts are synthetic bone grafts which can be mineralized and are associated withosteoconduction. It is a physical effect by which the matrix of the graft forms a scaffold thatfavors outside cells to penetrate graft and form new bone. They are biocompatible, inorganicbone grafting material. Alloplasts fall into two classes: Ceramics and polymers. Eg.Polymethylmethacrylate (PMMA), Polyhydroxyethylmethacrylate (PHEMA), Calcium

Hydroxide, Hydroxyapatite, Tricalcium Phosphate, Calcium Phosphor-Silicate. They have anadvantage of easier availability, eliminating the need of a donor site and carry no risk of disease transmission.<sup>8</sup>PerioGlass is a absorbable osteoconductive bone graft synthetic substitute composed of acalcium phosphosilicate bioactive glass; Bioglass composed of silicon dioxide (46 mole %), calcium oxide (24.4 mole%), phosphorous pentoxide (2.6 mole %).<sup>9</sup> The particulate form is ofsize range 90-170µm. At the time of use, the device is mixed with saline or patient's ownblood to form a wet sandy paste that is applied to the defect. The use of laser therapy has been investigated as an adjunctive tool to conventional, mechanical procedures commonly employed in the treatment of periodontal and periimplantdisease. Mechanical instrumentation of root surface for the reduction of bacteria and removalof soft and hard tissue deposits results in partial removal of pocket epithelium and healing, by formation of a long junctional epithelium. This has shown to retard epithelial

downgrowthand help in formation of new connective tissue attachment.<sup>9</sup>

Hence the present study is carried out to compare Laser as an adjunct to tetracycline rootconditioning gel and bioactive bone graft material in the treatment of infrabony defects. Toour knowledge this is the first study where diode laser has been incorporated with root biomodification and alloplastic bone graft for periodontal regeneration.

## **Material & Method**

Healthy patients with moderate to severe chronic periodontitis were selected for this studyfrom the Outpatient Department of Periodontology, Rungta College of Dental Sciences andResearch, Bhilai, Chhattisgarh.

**Study Design:** The study was double blinded, randomized controlled clinical trial. Patientsdiagnosed with chronic periodontitis based on clinical and radiographical criteria proposed bythe 1999 International World Workshop were selected for the study.<sup>1</sup> A total of 40 sites wereselected for this clinical study, and Phase I therapy was performed in all the patients.

Four weeks following Phase I therapy a periodontal reevaluation was performed to confirm the suitability of the sites for the study. The selected sites were randomly divided into Group Iand Group II according to the type of treatment rendered to them. Experimental Site A and Experimental Site B according to the type of treatment render to them by using split mouthdesign.

**Experimental Site** A - 20 surgical sites treated with tetracycline gel and bioactive bone graft.

**Experimental Site B** -20 surgical sites treated with tetracycline gel and bioactive bone graftwith application of diode laser.

## **Inclusion Criteria**

1. Age group of 20 -55 years.

3. Patient having at least two contra-lateral periodontal intra-bony defects

4. Radiographic evidence of interproximal alveolar bone loss.

5. Systemically healthy patients.

6. Patients who had not received any type of periodontal therapy for the past 6 months.

#### **Exclusion Criteria**

1. Subjects taking medications, such as corticosteroids, calcium channel blockers orimmunosuppressive drugs, which are known to interfere with periodontal woundhealing.

2. Subjects allergic to medications

3. Subjects who are pregnant or lactating.

4. Smokers /Tobacco users

5. Subjects showing poor oral hygiene maintenance after initial phase I therapy.

The proposed study was carried out on patients with chronic periodontal disease as assessedby clinical and radiographical findings. Patient's verbal and written informed consent wasobtained from all the patients before the commencement of the study. A brief history of eachpatient was recorded on a case history Proforma. Four weeks following phase I therapy aperiodontalreevaluation was performed to confirm the suitability of the sites for the study.

### **Clinical Parameter:**

1. Plaque Index (PI) (Silness and Loe, 1964)<sup>10,11</sup>

2. Gingival Index (GI) (Loe and Silness, 1964)

3. Probing pocket depth (PPD)<sup>12</sup> i.e. measured from the gingival margin to the base of the periodontal pocket recorded with the help of UNC-15 probe

4. Relative attachment level (RAL)<sup>13</sup>-distance from the wire on the stent to thegingival margin recorded with help of UNC-15 probe.

**Radiographic Evaluation** 

1. Orthopantomogram (OPG)

2. Digital Radiovisiography (RVG) with millimetre grid mount. OPG, RVG and routinehaematological investigations were done preoperatively at baseline and post-operatively at 6and 9 months.

### **Treatment Protocol**

**1.Presurgical Procedure:** Initial therapy consisting of full mouth supragingival and subgingival scaling and root planning was carried out. Detailed instruction regarding self-performed plaque control measures were given to the patients. After four weeks, studysubjects were recalled and only those maintaining optimum oral hygiene were furthersubjected to the surgical procedure. The PPD & RAL were recorded for each selected site inall patients.

**2.Surgical Procedure:** Prior to the surgical procedure, the patients were instructed torinse with 0.2% chlorhexidine gluconate for 1 minute. The surgical protocol emphasized complete asepsis and infection control. Incision and Flap Reflection: The operative site wasanaesthetized with 2% lignocaine HCL with adrenaline (1:80,000). After achieving adequate

anesthesia, crevicular incisions were given on the facial and lingual sides reaching the tip of the interdental papilla using B.P knife with blade no. 12 and an interdental incision with bladeno. 15. A full thickness mucoperiosteal flap was reflected using the periosteal elevator, takingcare that the interdental papillary tissue was preserved or retained as much as possible.

Meticulous defect debridement and root planning was performed using hand instruments and area-specific curettes. Suture was passed through the elevated flaps as per the relevanttechnique prior to performing the regenerative procedure. In group A: Root conditioning was done using tetracycline gel for 3 minutes. The wound was rinsed 2-3 times with sterile saline solution. Then mixed Bioactive Glass(perioglas®, Nova Bone Products, USA) with sterile normal saline solution were placed overdefect site.

In group B: Wavelength, 980 nm, average output-0.5 W, with an energy density of 4-5J/cm2 in continuous wave output aiming beam will be used to remove all visible epithelium in the inner side of the flap from the free gingival margin. Root Conditionings were appliedusing tetracycline gel for 3 minutes. The wound was rinsed 2-3 times with sterile saline solution, and non-contact mode was used with the tip pointed into the sulcus for 20 seconds to he gingiva of teeth over each surface covering the entire oral cavity. This was followed byplacement of bioactive Glass (perioglas<sup>®</sup>, Nova Bone Products, USA) with sterile normal saline solution over defect site. Finally, the mucoperiosteal flaps was repositioned and securedin place using 3-0 nonabsorbable black braided silk surgical suture and the surgical area wascovered with non-eugenol periodontal dressing's.



Fig:1 Pre-operative clinical view (a) & bioactive bone graft placed (b) in group A

Fig:2 De-epithilization was done with fibro-optic tip (a) Bioactive bone graft is placed(b) followed by bio stimulation (c) in group B.

**Post-operative care:** After the surgical procedure, patients in both groups were prescribed medications. Analgesics and anti-inflammatory drugs and antibiotics instructed for 5 dayspost-operatively. Then postoperative instructions were given to the patients.

**Post-operative recall visits:** Patients were recalled after one-week post operatively forremoval of periodontal dressing and the sutures. Recall appointments were given at 3 month,6 month & 9 month post-surgery and the relevant clinical and radiographic parameters wererecorded during these visits. At each visit, oral hygiene instructions were reinforced.

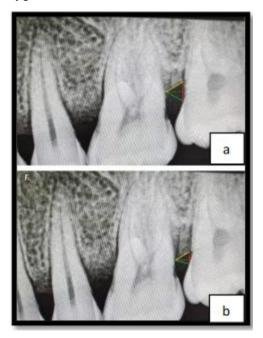


Fig. 3: (a) baseline & (b) 9month of group A

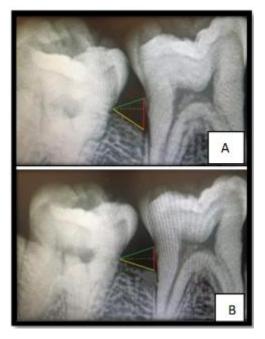


Fig. 4: (a) baseline & (b) 9month of group B Statistical software: The Statistical software namely SPSS v.16.0, was used for the analysis of the data and Microsoft office and Excel programs used to generate graphs, tables etc.

**Statistical Methods:** Descriptive statistical analysis was carried out in the present study.

**Independent t-test:** Independent student t test was used for the comparison of mean Plaqueindex, Gingival index, PPD, RAL, Radiographic Defect Depth and Amount of Defect Fill. Itis applied to a data of observation for same variables between two samples. Analysis ofvariance (ANOVA) is a collection of statistical models, and their associated procedures, inwhich the observed variance in a particular variable is partitioned into componentsattributable to different sources of variation.Mann-whitney u test: A one-sided research hypothesis is used if interest lies in detecting a positive or negative shift in one population as compared to the other.

#### Result

Results showed that the gingival and plaque index were non-significantly at compared on differenttime line.

However, gingival and plaque index were reduced in a time-dependentmanner from baseline to 9 months. The gingival index range at 9 month was 0.3-1 and plaque index was 0.4-1.2 at 9 months.

Table 1: Gingival index and plaque index betweencontrol and test group

Tests		Baseline	3 months	6 months	9 months	p val0ue
Gingival	Range	1.6-2.8	0.64-2.1	0.3-1.7	0.3-1	
index						
	Mean ±SD	2.01 ±	1.22 ±	1.14 ±	0.94 ±	0.074"
		0.36 0.34		0.26	0.15	0.071
Plaque	Range	1.6-2.8	1-2.2	1-1.6	0.4-1.2	
index						
	Mean ±SD	$2.1\pm0.44$	1.35 ±	1.16 ±	0.84 ±	0.0214 <sup>#</sup>
			0.30	0.17	0.27	

Data are represented as mean  $\pm$  SD (n=20), Significantly different at \*p<0.05, \*\*p<0.01and \*\*\*p<0.001 in comparison to controlgroup. #p<0.05, Not significant Table 2: Comparison of mean probing pocket depth between control and test groups.

Groups	Mean	SD	Minimum to	Median	95% Cl of difference	p-value	Significant
Control	4.90	1.51	maximum 1.52-5.92	4.58	2.49 to 7.30		Not
Track	4.59	2.10	2.50.7.20	4.27	1 24 4- 7.02	o#	
Test	4.58	2.10	2.50-7.30	4.27	1.24 to 7.93	0.434 *	Significant

In comparison of mean probing pocket depth between control and test groups showspatient-level analysis with means, standard deviations (SD), medians, range from minimumto maximum for both test and control and lower and upper 95% Confidence interval ofmean. Pvalues for the patient-level analysis were calculated with the nonparametric Mann-Withney U-test and nonparametric paired Wilcoxon test to assess the influence of time.

Significantly different at \*p<0.05, \*\*p<0.01 and \*\*\*p<0.001 in comparison to controlgroup. #p<0.05, Not significant.

The result of relative attachment level, shows the comparison of mean relative attachment levelbetween

control and test groups. Patient- level analysis with means, standard deviations (SD),medians, range from minimum to maximum for both test and control and lower and upper 95% Confidence interval of mean.

 Table 3: Comparison of mean relative attachment level

 between control and test groups.

Groups	Mean	SD	Minimumto	Median	95% Cl	р	Significant
			maximum		of	value	
					difference		
Control	8.71	1.97	4.37-8.30	8.42	5.57 to		
					11.85	0.242 *	Not Significant
Test	8.40	2.71	5.45-11.80	8.15	4.08 to	1	Significant
					12.72		

P-values for the patient-level analysis were calculated with the nonparametric Mann-Withney U- test and nonparametric paired Wilcoxon test to assess the influence of time.

Significantly different at \*p<0.05, \*\*p<0.01 and \*\*\*p<0.001 in comparison to controlgroup. #p<0.05,(Not significant).

Radiographic data of infra bony defects at baseline, at 9 months and 9 month of defectfill.Results showed that bony defects were significantly reduced in both control (p<0.05) and testgroup (p<0.01) in a time-dependent manner. The defect depth in control group at 9 month was1.5-3 and in test group in was reduced to 1.2-3 at 9 month. The percentage of defect fill was40.4-66 and 40-78.1 in control and test groups, respectively. Data are represented as mean  $\pm$  SD (n=20), Significantly different at \*p<0.05, \*\*p<0.01 and\*\*\*p<0.001 in comparison to control group. #p<0.05, Not significant, p-values for the patient-level analysis were calculated with the nonparametric Mann-Withney U- test and nonparametric paired Wilcoxon test to assess the influence of time. Significantly different at \*p<0.05,\*\*p<0.01 and \*\*\*p<0.001 in comparison to control group. #p<0.05, (Not significant).

Table 4: Radiographic data of infra bony defects atbaseline, at 9 months and 9 month ofdefect fill.

		Defect depth		Amountof	Percentage of		
				defect	defect	р-	
				Fill	Fill	value	Significant
Groups		Baseline	9 months	9 months	9months		
	Range	4.2-8.3	1.5-3	1.7-5.3	40.4-66		Significant
Control						0.05*	
	Mean	5.23 ±	2.32 ±	3.01 ±	55.32 ±		
	± SD	1.02	0.48	0.89	9.67		
	Range	4-5.5	1.2-3	1.7-4.8	40-78.1		Significant
Test						0.01**	
	Mean	5.03 ±	1.85 ±	3.15 ±	62.8 ±		
	± SD	0.50	0.53	0.71	10.87		

Significantly different at \*p<0.05, \*\*p<0.01 and \*\*\*p<0.001 in comparison to control group.#p<0.05, (Not significant).

### Discussion

Successful periodontal therapy depends on anti-infective procedures aimed at suppressingpathogenic organisms found in dental plaque associated with the tooth surface in the oral cavity.Nonsurgical mechanical therapy alone may not effectively eliminate the periodontal diseasecompletely, particularly in deep pockets. Hence, surgical therapy is performed, which provides improved visualization of the root surface and defects.<sup>14</sup>The ideal goal is the restoration of the periodontium by predictable regeneration of bone and connective tissue attachment, which has been destroyed by periodontal disease. Healingfollowing conventional therapy is most likely to occur mainly through the formation of a longjunctional epithelium.<sup>15</sup>

No study till date has evaluated the efficacy of laser with bioactive glass as bone graft andtetracycline gel as root conditioning in infrabony defects. This study compares the combination of bioactive bone graft and tetracycline gel with or without use of laser therapy.

Both the group showed reduction in mean PPD and RAL which was statistically significant whencomparing baseline (Group I:  $5.15 \pm 0.72$ , Group II:  $5.1 \pm 0.83$  at 3month, Group I:  $4.01 \pm 0.73$ , Group II:  $3.45 \pm 0.58$  at 6

month and 9 month (Group I:  $3.52 \pm 0.79$ , Group II: 2.5  $\pm$  0.5respectively). Group II showed greater reduction in PPD (p= 0.005). There was significant reduction in defect depth (Group I:  $3.01 \pm 0.89$  and Group II:  $3.15 \pm 0.71$ ) and increase inpercentage of defect fill (0.05 & 0.01) in both groups.

In the present study tetracycline were uses as root conditioning gel for root bio modification. Itcomprises a group of broad-spectrum antimicrobial agents that were introduced into clinicalpractice in the late 1940s (Duggar 1948). They are used extensively in the management ofperiodontal diseases and the agents used for this indication include tetracycline hydrochloride, doxycycline and minocycline.

Terranova V, Wang HL and Bharadwaj A suggested that PDL cells can be triggered toreproduce and move on to the preconditioned dentin surface, especially when tetracycline (TTC-HCl) has been used. It was observed that this preconditioning by TTCHCl disrupts the smearlayer and to some extent demineralizes the dentin surface to reveal the underlying collagenfibres.<sup>15,16,17</sup>

The use of tetracycline showed demineralization during regenerative periodontal surgery whichmay demineralize cementum which facilitated a cell and fiber attachment to the cementumsurface and was an important initial step in the natural healing process and new attachmentformation. A matrix is thereby provided supporting migration and proliferation of cells related to periodontal wound healing.<sup>18</sup>

Listgarten et al.19 observed a decrease in the percentage ofsubgingival spirochetes and motile rods in patients given a single course of scaling and rootplanning. The reduction of probing depth and gain of clinical attachment were significant in alltreatment groups. These improvements might simply reflect a change in

tissue composition ofperiodontal tissues, rather than a true gain of new attachment.

Bioactive glasses (BGs) bone grafts are being used for bone tissue engineering applications and they form a very good material to produce platform for bone regeneration. This is because they have reasonable mechanical strength and hence can withstand stress, they do notundergo corrosion, and they are biocompatible and biodegradable. These properties can bealtered on the basis of the application by varying their composition.<sup>20,21</sup> Certain BGs are observed to form a mechanically strong and firm bond with the bone, and some compositions have alsobeen observed to bond well with soft tissues as well as bone. BGs can be used for efficient bonetissue engineering applications as they can enhance revascularization, osteoblast adhesion, enzyme activity, and differentiation of mesenchymal stem cells.<sup>22,23</sup>

In this study, some defects were three-walled defects, while others were one-walled or acombination of one and two walls.

Cortellini et al.<sup>24</sup> reported that two-walled and threewalled defects had the highest potential forregeneration when grafting procedures were used. In addition, the defects varied according todepth and width, while there is well-documented evidence reporting better predictability withdeep, narrow defects than with shallow, wide ones.<sup>25</sup>

Ong et al.<sup>26</sup> and Dybvik et al.<sup>27</sup> found only a slight improvement in periodontal parameters ininfra-bony defects treated with bioactive glass in comparison with those of controls treated withopen debridement only, and the difference was not statistically significant. However, notably,they selected teeth with poor prognosis because of deep osseous defects, severe bone loss andhypermobility. Our results are in good conformity with those of other authors who treated infrabony defects with bioactive glass. For example, attachment gains of 2.7 mm, 3.0 mm and 2.8 mm, respectively, were reported, and comparable reductions in pocket probing depth of 3.7 mm, 4.4mm and 3.9 mm, respectively, were recorded.<sup>28,29,30</sup>

In the present study we have used a 980nm Gallium Aluminum-Arsenide (GaAlAs) diodelaser. This laser does not interact with dental hard tissues therefore it is an excellent soft tissuesurgical laser.

Kreisler et al.31 studied the adhesion of periodontal ligament cells to the rootsurface after treatment with an 810 nm diode laser, concluding that the diode laser has nodeleterious repercussions on the root surface.

Moritz et al.32 has reported pocket irradiation witha diode laser (805nm) with subsequent scaling showed considerable bacterial elimination fromperiodontal pockets especially in terms of Aggregatibacter actinomycetemcomitans. Further fromthese findings it is signified that the diode laser can be used safely in the periodontal pocket inclose proximity to hard tissues. It has been observed that a diode laser also assistsbacterial elimination from periodontal pockets, ensuing in better healing.

Nevertheless, there are very less reports of the use of GaAlAs diode laser as an adjunct tomechanical debridement in access flap surgery, although it is the most commonly used laser. Theadjunctive effects of diode laser in open flap debridement have been evaluated in this studybased on clinical and radiological parameters.

Our results are in agreement with those of other investigations carried out both in vivo and invitro and demonstrating that low-power laser accelerates fibroblast proliferation and differentiation of osteoblasts. Along

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with a studies that carried out a histological assessment of the effect of laser therapy on the healing of bone defects associated with autologous bone graft.

Laser therapy was carried out every other day for 15 days (830 nm, 50 mW and fluence 10J/cm2). In the laser groups, bone remodelling was more evident, both quantitatively and qualitatively, than in the subjects in the other groups, indicating that the use of laser had resultedin a positive bio modulatory effect on the healing of bone defects associated with autologousbone grafts.<sup>33</sup> Radiographic bone measurement is a non-invasive, painless, alternative to direct bonemeasurement and we used it in our study to monitor alveolar bone changes and to measure theoutcome. It has been reported that both radiographic interpretation and changes in measurements of clinical attachment level over time are reliable in assessing the outcome of treatment of infrabony defects. In other words, the use of attachment levels and radiological evaluation areeffective indicators of the outcome of periodontal regenerative therapies.34 When interpreting the findings of the present study, it has to be pointed out that the changes in clinical attachment levelconcur with the gain in radiological alveolar bone level. In addition, post-surgical healing

indicated good soft tissue responses both to the bioactive glass alone and when combined withsoft laser, resulting in no adverse complications. Moreover, the patients'good standards of oralhygiene and inflammation-free periodontal tissue in the postoperative phase improved thetreatment outcome.

Our results have confirmed a positive effect of the soft laser in accelerating de novo boneformation and mineralization, which are essential for endosseous bone healing, resulting in asignificant improvement in the quality of recovery and a decrease in recovery time; this isparticularly important in the presence of local and systemic conditions, which could retard thehealing process, for example in patients withuncontrolled diabetes. In summary, our studyattempted to evaluate the biological effect of GaAlAs laser irradiation in improving the inductive cellular activities leading to accelerated bone healing.

Use of tetracycline gel &bioactive bone graft along with laser therapy seems to be promising for the treatment of infrabony defects.

### Conclusion

The diode laser can be safely and effectively used as an adjunct to the treatment of chronicperiodontitis with the advantage of decreased gingival inflammation as there was significant reduction in PI, GI, PPD and gain in RAL at 3 months, 6 months & 9 months post-operatively.

Tetracycline gel and bone graft in combination with laser has shown significant clinicalimprovement therefore it can be safely and effectively used to achieve the better treatmentoutcome and can aid in tissue healing. However, no significant difference was observed inintergroup comparison.

Use of tetracycline gel &bioactive bone graft along with laser therapy seems to bepromising for the treatment of infrabony defects. Future studies with more critically designedprotocols, larger sample size and inclusion of histological examination as a criterion forperiodontal regeneration are warranted to further explore this potential of the laser therapy.

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