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ORIGINAL ARTICLE

Evaluation of the Effect of Diode Laser on Healing after Gingivectomy

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ABSTRACT
Objective: To evaluate the effect of photobiomodulation of diode laser on the healing of gingiva following gingivectomy. **Material and Methods:** 15 patients with inflammatory gingival enlargement in the age group 18-65 years were enrolled for the study according to the inclusion and exclusion criteria and were divided into two groups, i.e., control and test, with a total of 30 sites. Gingivectomy was performed with a scalpel, and the test site was irradiated with a diode laser, which was repeated after 7 days, finally, the patients were recalled after 21 days for re-evaluation. Soft tissue parameters were recorded at baseline and 21 days, and visual analog scale was recorded at 7 days. Also, tissue was excised and sent for histological analysis both at baseline and 21 days, and the data was subjected to statistical analysis. **Results:** A clinically significant improvement was observed in patient response to pain in the test group compared to the control group (p=0.001). There was an observable improvement in tissue color in the soft tissue analysis (p=0.001) and a significant reduction in the inflammatory component in the histological analysis in the test group as compared to the control group (p=0.001). **Conclusion:** Diode laser has a photobiomodulation effect on wound healing after gingivectomy, thus enhancing the rate of healing and decreasing post-operative pain.

Keywords: Gingivectomy; Lasers; Wound Healing.

Introduction

Gingival enlargement is the overgrowth of the gingiva characterized by an expansion and accumulation of the connective tissue with the occasional presence of an increased number of cells [1]. It may be related to various etiologic factors such as dental plaque, mouth breathing, hormonal imbalance, and medications [2]. Gingival enlargement may hamper patient esthetics if present in the anterior areas and may also lead to further plaque accumulation due to altered gingival contours, thus causing further destruction. It may hamper routine oral hygiene maintenance, resulting in poor plaque control, which may result in difficulty in speech, mastication, and eruption, causing functional problems as well. Thus, gingival enlargement frequently needs to be treated with surgical intervention. Gingivectomy is the most commonly performed procedure for the treatment of gingival enlargements [3].

After the gingivectomy procedure, the tissues undergo healing by secondary intention, which takes around five weeks [4]. After the procedure, surface epithelialization is complete within 5-14 days, but keratinization is still not complete. Complete repair of connective tissue takes around 7 weeks, thereby delaying the wound healing process [5].

Due to delayed wound healing, patients may experience prolonged discomfort during this phase. In an attempt to enhance wound healing, several studies have been conducted using topical medicaments, use of anti-bacterial or amino acids, and other compounds that result in improved secondary intention healing [6,7]. However, the application of lasers for the stimulation of wound healing has been recently studied and is a developing field with great promise.

A low-level laser is a red or infrared light having a wavelength ranging from 600 to 1000 nm and power < 0.5 W. It has a low absorption power in water and due to its wavelength, is capable of penetrating into soft and hard tissues. Low-level laser therapy (LLLT), also called 'Soft Laser Therapy', has shown excellent therapeutic benefits [8]. LLLT as a treatment option came into significance due to the work of Mester, who, after the application of a low-energy (1 J/cm^2) ruby laser, noticed improved wound healing [9].

The impact of low-level laser is through its non-heating effects, which results in a wide range of effects at cellular and molecular levels [10,11]. The sum of these events stimulated by LLLT results in the restoration of tissue homeostasis and speeds up the repair process with minimal side effects. LLLT, therefore, is being used in treatments of various biological tissues and immune modulation.

Difficulties associated with delayed wound healing after gingival surgical procedures have generated a need to boost the wound healing process. Amongst the variable options available, LLLT is the latest treatment modality suggested for enhancing wound healing through the process of photobiomodulation (PBM). Thus, the present study was undertaken to assess the effect of PBM of diode laser on gingival wound healing after scalpel gingivectomy. Based on this concept, a null hypothesis was proposed that differences in these outcomes shall be statistically significant.

Material and Methods

Study Design and Ethical Clearance

A randomized split-mouth clinical study was conducted to assess the effect of photobiomodulation of diode laser on gingival wound healing after scalpel gingivectomy. The study was conducted in accordance with the Helsinki Declaration [12], approved by the institutional ethical committee (TMU/IEC/2019/05). Informed consent was obtained from the subjects included before the treatment.

Sample Size Calculation

The sample size of 20 was selected by using simple random sampling without replacement (SRSWOR) technique using tippet's random no. table. The inclusion criteria were 80% (approximately), and the exclusion criteria were 20% (approximately) of the total sample size. However, the sample size was calculated by using $N=4*\alpha^2/L^2$ by putting standard deviation ($\alpha=0.4950$) and least permissible error ($L=0.1105$) (i.e., power of study 89% or 0.89), respectively. After putting all these values in the formulae, a sample of size 15 was obtained.

A total of 15 participants with inflammatory gingival enlargement, both male (12 subjects) and female (3 subjects), with an average age ranging from 18-65 years, participated in this study. The following inclusion criteria were adopted: patients with gingival enlargement without bone defects involving either the maxilla or mandible or both; patients willing to comply with the surgical protocol and report for the required number of recall visits.

The following exclusion criteria were adopted: patients with systemic disorders; patients taking any medication known to affect the outcome of periodontal therapy; pregnant/lactating females; smokers; and patients taking any medication known to cause gingival overgrowth as a side effect.

Site Selection

Cach patients had two sites, which were divided into control and test, and therefore, had a total of 30 sites. The selected subjects having gingival enlargement on the mandibular or maxillary arch were divided randomly into two groups, with one site per subject serving as a control group and the other site of the same subject as a test group.

- Control group – the site undergoing conventional gingivectomy only.
- Test group – the site undergoing conventional gingivectomy followed by PBM by diode laser (Diode LASER, Denlase, IDS Denmed Pvt. Ltd., India).

Clinical Protocol

After selection, the subjects were taken up for phase I therapy. Oral hygiene instructions were reinforced, and recording of the Plaque Index (PI) [13] was carried out, followed by blood investigations. Patients were recalled after three weeks of phase I therapy, and then, with the help of a toss of a coin, the sites were divided into two groups, i.e., control and test.

Conventional gingivectomy was performed after the administration of local anesthesia using a blade and scalpel. The excision was carried out to the desired depth after marking the pockets, followed by gingivoplasty to contour the gingiva.

The test site was subjected to LLLT by irradiating with a Denlase Diode laser of 980nm for PBM at 0.3W using the continuous wave mode immediately after surgery. The irradiation was made in a non-contact mode with a distance of approximately 1-2 mm from the tissues. During the delivery of light, the delivery tip was kept perpendicular to the tissue surface and moved with a scanning velocity of approximately 1mm/sec for a period of 80 seconds. The excised tissue was sent for histological evaluation.

Patients were recalled after 7 days for another application of LLLT therapy at the test site. Monitoring of post-surgical soft tissue wound healing was done clinically on the 21st day. A full-mouth PI was again taken during this visit, following the same protocol as the initial visit. A 1 mm tissue was taken from both the control and the test site, making sure that the underlying bone was not exposed and that the physiological sulcus was maintained. The tissue procured was subjected to histological analysis. The evaluation of wound healing at surgical sites was done clinically using the healing index of Landry et al. [14].

Visual Analog Scale

Patient perception of pain was also evaluated on the 7th day post-operative between the control and test sites based on the visual analog scale (VAS) as proposed by Freyd [15].

Statistical Evaluation

The data collected were analyzed using SPSS version 22.0 (IBM Corp., Chicago, IL, USA), and p-values ≤0.05 were considered statistically significant. The descriptive statistics included mean and standard deviation, whereas the intragroup comparison for the different time intervals was done using paired t-test and repeated measures ANOVA, and intergroup comparisons for the different mean scores between two groups were done using independent t-test.

Results

The histopathological sections at baseline (day 0) and on 21 days of control and test groups were evaluated at 40 x magnification under a light microscope. Figure 1A demonstrates the para-keratinized stratified squamous epithelium at baseline with a fibrovascular connective tissue stroma along with proliferating endothelial lined blood capillaries of varying sizes. In Figure 1B, it is possible to observe that the para-keratinized stratified squamous epithelium on 21 days of the control group; however, Figure 2 demonstrates a fibrous connective tissue stroma on 21 days of the test group.

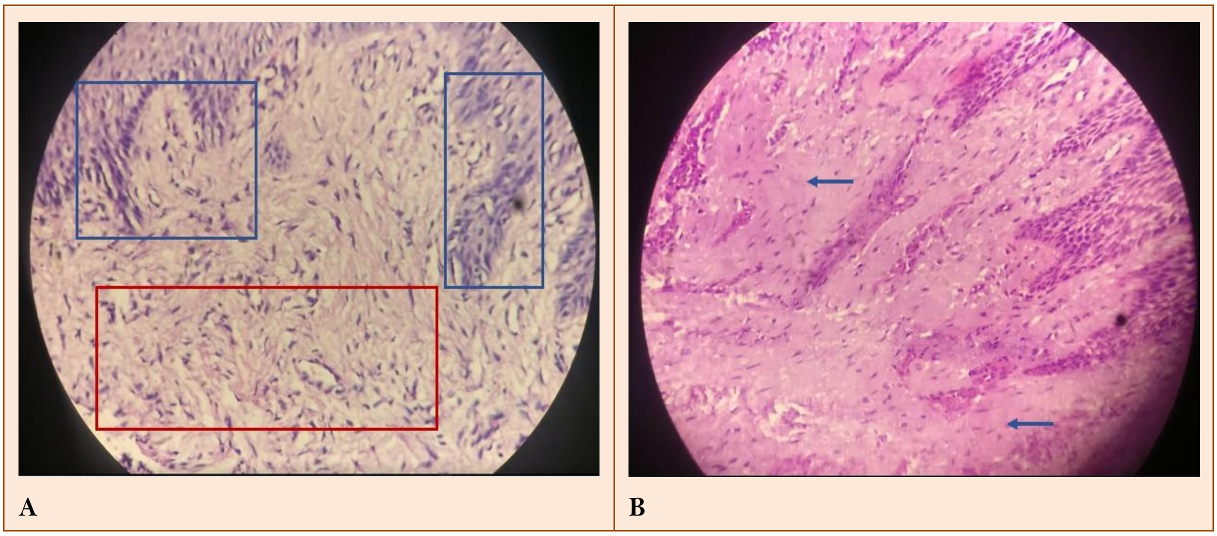

Figure 1. (A) The histopathological section at baseline (day 0) demonstrates para-keratinized stratified squamous epithelium (Blue) with a fibro-vascular connective tissue (Red) stroma and proliferating endothelial lined blood capillaries of varying sizes. Stroma also shows numerous bundles of collagen fiber along with fibroblasts, and few inflammatory cells infiltrate, chiefly consisting of lymphocytes. (B) The histological section of the Control group at 21 days demonstrates para-keratinized stratified squamous epithelium. Connective tissue stroma shows dense fibrous bundles (Blue arrow), and moderate inflammatory cells infiltrate chiefly consisting of lymphocytes.

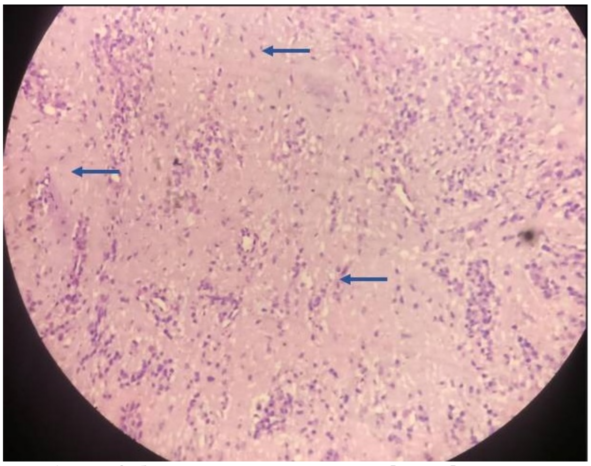

Figure 2. The histological section of the Test group at 21 days demonstrates fibrous connective tissue stroma. Connective tissue stroma comprises of endothelial lined blood capillaries of varying sizes. Stroma also shows dense collagen fiber bundles along with fibroblasts (Blue arrow), and few inflammatory cells infiltrate, chiefly consisting of lymphocytes.

Table 1 shows an improved tissue color and reduction in an overall inflammatory component in the test group (p=0.001) as compared to the control site, with a mean difference of 0.67 and 0.93, respectively. Histological analysis showed an increase in the amount of collagen bundles in the test sites with a mean difference of 0.9 between the control sites, which was statistically significant (Table 1).

Table 1. Intergroup comparison of soft tissue and histological parameters in both groups followed by low-level laser therapy.

	Parameter	Group				Mean Difference	p-value*
		Control		Test			
		Mean	SD	Mean	SD		
Soft Tissue	Tissue color	4.33	0.617	5.0	0.01	0.67	0.001#
	Bleeding on palpation	2.86	0.351	3.00	0.001	0.14	0.05
	Granulation tissue	2.80	0.414	3.00	0.001	0.2	0.072
	Incision margin	3.00	0.001	3.00	0.001	0.0	1.0
	Suppuration	2.00	0.001	2.00	0.001	0.0	1.0
		3	0.2	3.2	0.01	0.2	0.001#
Histological	Epithelial thickness	2.8	0.414	3.0	0.001	0.2	0.072
	Fibroblast	2.8	0.414	3.0	0.001	0.2	0.072
	Collagen fibers	1.8	0.56	2.73	0.45	0.9	0.001#
	Blood vessels	2.73	0.457	2.80	0.414	0.07	0.49
	Inflammatory component	1.6	0.507	2.53	0.516	0.93	0.001#
		2.1	0.237	2.81	0.159	0.71	0.001#

SD: Standard Deviation; *Independent t-test; #Statistically Significant.

PI scores were comparable in both control and test sites. However, a significant difference was observed in the pain perception measured by the VAS, with patients reporting reduced pain in the test sites with a mean difference of 1.06, which was statistically significant (p=0.001) as shown in Table 2.

Table 2. Intergroup comparison of plaque index (PI) and visual analog scale (VAS).

Variables	Site	Mean	S.D.	Mean Difference	p-value*
Plaque Index (PI)	Control	1.09	0.201	0.02	0.79
	Test	1.11	0.206		
Visual Analoge Scale (VAS)	Control	1.46	0.74	1.06	0.001#
	Test	0.40	0.51		

*Independent t-test; #Statistically Significant.

Discussion

A randomized split-mouth clinical and histological study including 30 sites was conducted to assess the effect of photobiomodulation of diode laser on gingival wound healing after scalpel gingivectomy. The red and near infrared laser radiation is absorbed by the respiratory chain present in the cell mitochondria which results in a series of biochemical reactions intracellularly and involve a number of cellular components, of which cytochromes are the primary photo acceptors. They are able to absorb red and near infrared light irradiation at appropriate wavelengths. This leads to activation of the respiratory chain, resulting in a reduction in the oxidation state and an increase in ATP synthesis. Concurrently, the membrane ions (e.g., sodium (Na^+), potassium (K^+), and calcium ($Ca2^+$)) are also activated, which can control cell metabolism and proliferation [16].

These physical and chemical alterations in the photoreceptor molecules cause an intracellular biochemical chain of reactions, which produce a local and systemic response. This cellular action is important in order to achieve a controlled anti-edematous, anti-inflammatory response and to promote angiogenesis, thus leading to improved healing and an appreciable analgesic response. Thus, the application of LLT, i.e., PBM, is useful in wound healing [16].

Following gingivectomy, an open wound is formed, and it may take more than five weeks to heal completely. During this period, the patient may experience pain due to the open wound and secondary repair in order to reduce the patient's discomfort after gingivectomy. Clinical trials have been done on the effects of LLLT on gingival healing through its PBM effect [17].

In a study by Amorim et al. [18], a 685nm wavelength was used with an output power of 50 mW, with an energy achieved of 4 J/cm2 to treat gingivectomy sites for PBM using LLLT in a split-mouth design taking control and test group. Immediately post-surgery and on subsequent days weekly after surgery, the clinical and biometrical evaluation was done. The results showed significant improvement in healing at test sites at 21 and 28 days [18].

On the contrary, Ozcelik et al. [19] reported that wound healing on the LLLT irradiated sites showed complete healing clinically at around 21 days, as compared to the healing observed on the 24 days on the control site, suggesting there was no appreciable effect on enhancing wound healing. Furthermore, in a clinical study conducted by Damante et al. [20], the LLLT effect on wound healing after gingivoplasty was not appreciable.

So, our study was undertaken to evaluate and compare the efficacy of LLLT on wound healing after gingivectomy. In our study, we used both clinical and histological parameters to assess the results. Histological evaluation of the excised tissue taken during gingivectomy was analyzed. The results showed no significant changes in the epithelial thickness, amount of angiogenesis and amount of fibroblasts between the control and test sites. However, the overall histological observations in the present study show a difference in the mean values of test and control sites.

The decrease in the inflammatory component can be attributed to the increase in the cell metabolism due to activation of the respiratory chain cytochrome c oxidase and local reduction of inflammatory neutrophil cell infiltration. Studies have also indicated inhibition of prostaglandin (PG) E2, tumor necrosis factor (TNF) α, COX2 and interleukin (IL) 1β. The subsequent down-regulation of the inflammatory markers and increased cellular ATP uptake could be the factor responsible for the decrease in inflammation after LLLT [20].

Increased cellular respiration achieved after LLLT irradiation and changes in redox signaling have a profound effect on fibroblast proliferation and increased activity. This increase in cell number and activity could attribute to the increased collagen distribution observed in the samples [20].

Studies have also suggested the increase in human keratinocytes motility promoting early epithelialisation, increased proliferation of fibroblast and collagen matrix synthesis due to upregulation of fibroblast growth factors and enhanced revascularization which is integral to successful wound healing rate [20,21].

In the present study, the parameters of BOP, presence of granulation tissue, suppuration and incision margin showed no significant differences between the control and test site, but the tissue color showed better healing after 21 days in the test site. Since patient comfort level is important in determining the degree of treatment outcome, the present study even evaluated the patient response to pain at both Control and test sites during the healing phase using the VAS Scale. Our results showed a statistically significant result with a difference of the mean value of 1.06, which corresponded to a clinically appreciable lesser pain response on the 7th day post-operative in the test site as compared to the control site similar to the studies by Kaarthikeyan et al. [17] and Sanz-Moliner et al. [22], which suggests a positive effect on the patient's pain response after LLLT.

LLLT may reduce pain by subsequent reduction of inflammation due to lowering the levels of PGE2, IL2, and TNF-α. A neural hypothesis has also been proposed by Chow et al. [23], who showed a positive relationship between pain relief and neural blockade achieved by Nd-YAG laser irradiation. Studies by Kasai et al. [24] and Wakabayashi et al. [25], showed blocking of action potential in nociceptors after LLLT.

Other proposed mechanisms include alteration in the release of neurotransmitters upregulation of serotonins and endorphins with downregulation of acetylcholine and substance p and improved lymphatic flow which separately or in tandem may be contributory to decreased pain response after LLLT [26].

The present study also considered the oral hygiene of the patients so that plaque accumulation does not impair healing on the results, a complete plaque index was recorded at start of treatment and on 21st day after procedure. The results showed equal improvement, with no difference between the mean values at test and control sites, therefore suggesting that plaque has not been a hindrance to the healing process in either of the cases. However, within the limit of the existing literature, gingival wound healing may be improved and patient discomfort is also reduced using lasers, which is also in concurrence with our study [29-33].

The small sample size is one of the limitations of this study. Furthermore, the study did not include medically compromised patients who are otherwise fit to undergo surgical procedures, which could have given us a better understanding of the efficacy of PBM in healing wounds for individuals who are in more need of such treatment advancements.

Conclusion

Low-level laser therapy using diode laser can be used after gingivectomy for faster wound healing and reduction of post-operative discomfort to patients. The findings manifested in reduced post-operative pain and accelerated wound repair, which was also evident histologically. However, the present study did not include any medically compromised individuals, so further studies can be done including patients who are medically compromised to provide comfort to those who are in real need.

Authors' Contributions

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ML	https://orcid.org/0009-0005-1127-1281	Conceptualization, Methodology, Formal Analysis, Investigation, Writing - Original Draft and Writing - Review and Editing.
All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.		

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None.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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