

Comparative Evaluation of the Efficacy of Laser Therapy and Fibroblastic Growth Factor Injection on Mucosal Wound Healing in Rat Experimental Model



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Abstract

Introduction: The aim of the present study was to compare the effects of laser and basic fibroblastic growth factor (bFGF) treatment on operative wound healing in a rat model.

Methods: Sixty-six male Wistar rats were employed in this study. A 10-mm surgical wound was created on the buccal mucosa of each rat, under anesthesia, and then the rats were divided into 3 groups of 22: (1) GF group (received subcutaneous injection of bFGF), (2) laser group (treated with low-level laser irradiation), and (3) control group (received no treatment). On day 5, half of the rats in each group and on day 10 the other half, were sacrificed. Afterward, samples were taken from rats' buccal mucosa for histological assay and scoring. The data were analyzed using Mann-Whitney test ($\alpha = 5\%$).

Results: On day 5 there was not any significant difference between GF and control groups; however, the laser group showed clinically delayed wound coverage, compared to other groups ($P < 0.05$). On day 10, histological examination demonstrated marked vascular granulation tissue (GT) in GF group. Collagen production was significantly prominent in laser group compared to GF treated samples ($P = 0.004$). Inflammation of GT in GF and laser groups was significantly less than that in control samples ($P = 0.005$ and $P = 0.001$, respectively).

Conclusion: The components of wound matrix induced by GF and laser treatment were significantly different. Although bFGF or laser treatment of oral wounds, under the conditions of the present study, did not accelerate wound healing, they showed some other notable effects on the quality of healing.

Keywords: bFGF; Granulation tissue; Laser; Oral; Wound healing.

Introduction

Mouth sores are common ailments that appear on any site of the oral cavity, including the lips, cheeks, gums, tongue, floor, and roof of the mouth. Most ulcers are benign and resolve spontaneously.¹ These lesions can arise as a result of a vast number of factors, including local causes (e.g. chemical, mechanical and thermal injuries; infection; neoplasia; ischemia; radiation), systemic conditions (autoimmune and inflammatory disorders, side effects of various drugs, systemic infections, hematologic status or inherited conditions)² or they may be of an unknown cause like idiopathic aphthous stomatitis.³

Mouth ulcers can be painful when eating, drinking or brushing teeth.⁴ On the other hand, the mouth is the first barrier against foreign bodies and microorganisms,⁵ which can be compromised by wounds.

The wound healing process is a dynamic one which can be divided into three phases: inflammatory, proliferation, and maturation. During the first stage, clot is formed and eventually, vessels dilate to allow essential cells and agents to reach the wounded area. The proliferation phase is characterized by the formation of granulation tissue (GT) and also new vessels. The surface of the wound is then covered with epithelium. In the last stage (maturation), remodeling of collagen would occur.⁶ The structure and composition of GT in the proliferation stage is often the indicator of how the wound healing process would go on. Better perfusion of tissue with new vessels would result in promotion of healing; on the other hand, fibroblasts and collagen formation have also a key role in the healing process.^{7,8}

In spite of the fact that our understanding of wound

healing has vastly increased over the last decade, unfortunately, there is not a universally approved treatment for oral and cutaneous wounds.⁹ The best treatment is the modality which would both accelerates the healing process and relieves pain. For pain relief of oral ulcers, practitioners usually prescribe a manually made mouth wash that includes dexamethasone or betamethasone and diphenhydramine or lidocaine in addition to nystatin drop.¹⁰ This cocktail would only alleviate patient symptoms, with no effect on the healing process.^{7,11}

In previous studies, several modalities have been introduced for acceleration of wound healing, such as traditional medicine^{12,13} and chemical drugs like phenytoin.¹⁴

Laser therapy, introduced in 1976 for wound healing,¹⁵ is proposed not only as a modality to relieve pain¹⁶ but also for its healing promotion effects.^{14,17,18}

The exact mechanism underlying laser efficacy in accelerated wound healing has not been demonstrated yet.¹⁹ In the past, the terms 'photobioactivation' and 'biostimulation' were frequently used based on the stimulatory effects, later replaced by 'biomodulation', because inhibitory effects were also noted.²⁰ There are contradictory statements on the efficacy of laser on wound healing; this is largely due to different devices and laser parameters applied in these studies as well as the variety of study models, including animal and human, which make the comparison difficult. Lucas et al²⁰ found that animal experiments and clinical studies that address the biological effects of LLLT on wound healing have been conducted simultaneously, rather than in sequence.

Yasukawa et al²¹ investigated the effect of a He-Ne laser on operative wound healing in rats. They histologically examined various laser parameters on its biologic impacts and showed that with higher laser power and considering intervals between laser sessions, a more desirable healing process can be achieved.

Up to now, many studies have examined different growth factors on wound healing, such as epidermal growth factor (EGF), transforming growth factor (TGF β ²²), recombinant platelet-derived growth factor (rPDGF)^{23,24} and basic fibroblastic growth factor (bFGF).²⁵

bFGF is a recognized potent stimulator of endothelial cells and neo-vessel formation.¹⁸ This agent has shown promising effects in healing promotion^{24,25}; however, there is no evidence on its efficacy on mucosal wounds. Therefore, the present study was conducted to compare healing promotion effects of low-level laser therapy and fibroblastic growth factor injection on mucosal wounds, clinically as well as microscopically, in rat experimental models.

Methods

Animals

Sixty-six male Wistar rats (each group consisting of 22

rats) aged 8 weeks (average body weight of 230 g) were used in this study.

Rat Buccal Mucosal Wound Model

A wound with a length of 10 mm, width of 5 mm and depth of 5 mm was created with scalpel in the left buccal mucosa of all experimental rats under anesthesia with isoflurane (by means of inhalation). The wounds were not sutured so they were supposed to heal by secondary intention. To control the bleeding, intensive pressure with sterile gauze was used. Moreover, enrofloxacin (Sigma Aldrich, USA) intra-muscular injection was used at 5 mg/kg, once a day for 3 days to prevent infection.

Laser Therapy Protocol

A diode laser (Fionoe, Jiangsu, China), with parameters summarized in Table 1, was used for laser-assisted treatment of experimental wounds in the laser group. This procedure was carried out after superficial inhalation sedation for each rat and opening of its mouth with a molt mouth gag (Figure 1). The sequence of irradiation was every other day (first, third and fifth day after the operation).

Treatment by Growth Factor

One milliliter of bFGF (Sigma Aldrich, USA) with a concentration of 0.1 μ g/mL was injected submucosally in the bed of the wounds every other day (first, third and fifth day after the operation).

Table 1. Laser Parameters and Specifications

Parameter	Explanation
Type	Diode: GaAlAs
Wavelength	810 nm
Cross section of laser tip	1 cm ²
Power	0.1 W
Intensity	0.1 W/cm ²
Dose	4 J/cm ²
Irradiation time	40 s
Irradiation mode	CW
Distance from area	In Contact

Abbreviation: CW, continuous wave.



Figure 1. Laser Application in Rat Experimental Buccal Wound.

Clinical Assessment of Wound Closure

The edge of each wound was traced on to a glass slide and the wound area was determined by quantifying the surface area of an open wound. The trace taken immediately after the operation was used as the reference or original area and all further areas were recorded as a percentage of the original area. Wound closure percentage was calculated as follows:

$$\%wound\ closure = \frac{(area\ on\ day0 - open\ area\ on\ final\ day)}{area\ on\ day0} \times 100$$

Preparation of Mucosal Specimens

On the fifth day after the operation, half of the animals in each group, and on day 10, the other half were sacrificed with an intraperitoneal overdose of chloroform. Afterwards, rectangular specimens (each with a width of 10 mm and length of 30 mm) were removed from the operation site of each rat and were fixed in 10% formalin solution. The specimens were paraffin embedded and the samples were sectioned in thin slices (5µm) for slide preparation. Finally, hematoxylin and eosin (H&E) staining was carried out for histopathological assessment under a light microscope.

Histological Examination

The scoring of the samples was done by a blinded pathologist, similar to the histological scoring system used in the study by Taheri et al¹⁴ with some minor modifications (Table 2).

Statistical analyses

Analysis of the histological and clinical rankings was performed using the Kruskal-Wallis test with individual comparisons performed by Mann-Whitney test (p<0.05). The analysis was performed using the SPSS 23 package.

Results

Day 5

In the control group, 63.3% of the cases showed less than 50% epithelial migration, and PMN infiltration was moderate to marked in more than 80% of them (Figure 2). Mean reduction in wound size in GF and control groups was significantly larger than that in the laser group. This result was also demonstrated in microscopic view where wound coverage with epithelium in the group treated with GF was significantly larger than that in the laser group. Although GF group received higher mean scores of parameters indicative of wound healing promotion

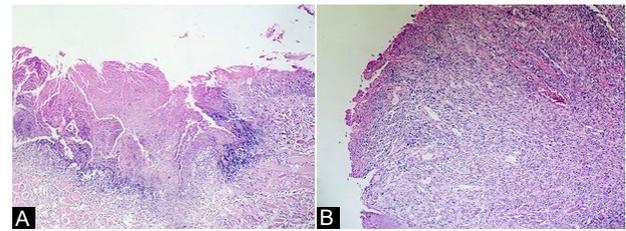


Figure 2. A, B. Histological Findings 5 Days After Wounding in Control Group. There is not any epithelial coverage on the wound (thickness of cut edges) and the GT is filled with PMNs (H&E; ×40).

than the control group, this difference was not statistically significant (Figure 3). *P* values are given in Table 3. Similarly, the highest scores of collagen production belonged to samples of the laser treated group, although the difference was not significant (Figure 4). On the other hand, wound closure percentage of laser group was significantly smaller than the control group (*P*=0.034).

Day 10

Clinical assay of wound closure percentage on day 10 showed more than 70% reduction in size of the open area in the group treated with subcutaneous injection of bFGF. Clinical wound closure and microscopic epithelial wound coverage were significantly accelerated in GF group compared to laser-treated cases. Furthermore, GF group showed markedly augmented vascular formations compared to laser and control groups (Figure 5). It was observed that laser induced significantly greater collagen synthesis than bFGF (Figure 6).

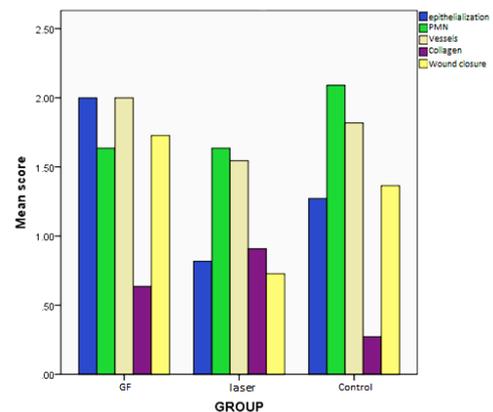


Figure 3. Day 5 Mean Scores of Each Measured Parameter in Separate Groups.

Table 2. Scores of Each Clinical and Histological Parameter

Score	Epithelialization	PMNs	Collagen	Vessels	Clinical Wound Closure Percent
0	The thickness of cut edges	Absent	Absent	Absent	No reduction
1	Migration of cells (<50%)	Mild	Mild	Mild	Less than 50%
2	Migration of cells (>50%)	Moderate	Moderate	Moderate	More than 50%
3	Bridging the excision	Marked	Marked	Marked	Almost 100%

Table 3. P Values of Differences in Pathological Scores of Groups Compared

Groups	Examined Pathological Indices					
	Wound Size	Epithelial Coverage	Inflammation	Collagen	Vascular Formation	
GF day 5	LLLT day 5	0.001*	0.005*	0.847	0.478	0.217
	Control day5	0.151	0.056	0.171	0.243	0.056
	GF day 10	0.04*	0.001*	0.013*	0.133	0.076
LLLT day 5	C day 5	0.034*	0.243	0.365	0.088	0.478
	GF day10	0.001*	0.000*	0.652	0.004*	0.000*
LLLT day 10	C day 10	0.002*	0.028*	0.001*	0.133	0.365
	LLLT day 5	0.217	0.019*	0.016*	0.005*	0.133
GF day 10	C day10	0.193	0.088	0.005*	0.3	0.002*

C, control.

*Significant difference.

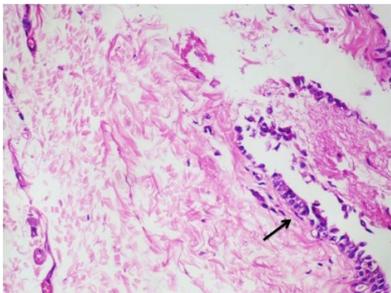


Figure 4. Collagen Deposition in GT in a Laser-Treated Wound on Day 10 of the Experiment. Note the inconsistent epithelium (arrow) (H&E; ×40)

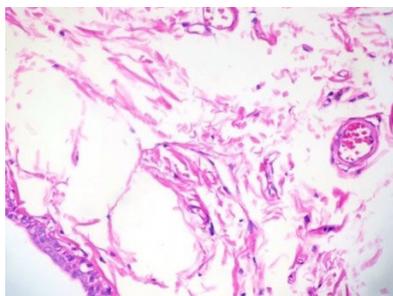


Figure 5. Histologic View of GT 10 Days After Wounding in a Sample Treated With bFGF. High vascular GT and a complete coverage of epithelium are evident (H&E; ×100)

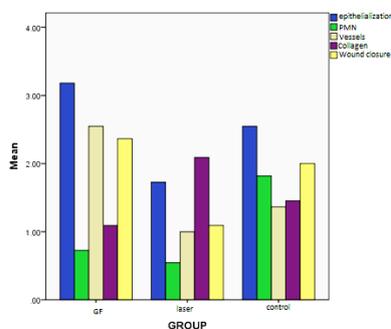


Figure 6. Day10 Mean Scores of Each Measured Parameter in Separate Groups.

Inflammatory cell infiltration was a predominant phenomenon in most wounds in control group. However, this feature was significantly less observed in GF and laser groups (Figure 2).

Discussion

The GT color and condition can foreshow the wound healing process.²³ Thick GT with dominance of vessels was observed in most samples of GF group. This result was consistent with previous investigations on bFGF application, which showed accumulation of only provisional matrix (filled with neovessels) after bFGF injection into wounds.²³ Moreover, in the present investigation, the bFGF treated group showed a higher percentage of wound closure compared to the laser and control groups. This can be related to the effect of bFGF on endothelial cells and proliferation of vessels which cause a bloody bed in the wound. The drainage of epithelium would make wound coverage faster. It should be investigated in future studies if the strength of scar tissue after complete healing is adequate enough with such treatment modality. Nevertheless, wound coverage in GF group (clinically and microscopically) did not statistically supersede the control group, which may be attributed to the low number of samples in each group. However, in Pierce et al's examination,²³ bFGF injection was followed by accelerated wound healing. This confliction may be justified by the difference in the site of operation, which was skin in the aforementioned study; Possibly, because the skin contains a lower number of vessels than mucosal tissues,²⁶ bFGF prescription could play a valuable role in augmentation of vascular formations and cause a significant difference between bFGF and control groups. Oda et al²⁷ investigated bFGF biological mechanism in rat palatal wound healing; they found higher levels of fibroblast growth factor receptor 1 (FGFR1)-positive cells in the group treated by single dose topical bFGF. So stimulated cell proliferation was reported as the mechanism of bFGF action in wound healing. However, stimulation of collagen maturation was also reported in palatal wounds treated with bFGF; which is in confliction

with the results of the present investigation. This may be due to the methodological differences between these two studies, as Oda et al²⁷ investigated cellular and subcellular effects of this modality, whereas in the present study only microscopic view of tissues was examined and it turns out that many cellular interactions would be overlooked.

There are a large number of studies which demonstrated healing promotion effects for laser.²⁸⁻³⁰ The mechanism of low-level laser therapy in healing promotion is not completely understood yet; however, it is supposed that laser efficacy is not only due to its heating effects but rather to its photochemical, photophysical and/or photobiological effects; many name these as photobiomodulation. It seems that laser illumination can improve local circulation, cell proliferation, and collagen synthesis by increasing cell access to ATP.³¹ However, similar to *in vitro* and *in vivo* surveys of Solmaz et al,⁸ the present study showed that laser induced a delayed healing process of oral wounds in rats. This conflation might possibly be attributed to different laser parameters, the difference between the employed methods (such as the site of the wound, animal or human study, scales to measure, healing stage) and laser parameters. For example, in the study of Tabakoglu et al,²⁸ the wound size was half the size of the ulcer examined in the present study; therefore, it is predicted that wound closure in that study was more due to the contraction mechanism rather than healing by secondary intention; and as the wounds in laser group have higher amounts of collagen, the susceptibility to contract is higher in this group than control group.³²

Collagen fibers in laser group showed considerably higher levels than GF group. This characteristic of laser therapy was previously reported by Taheri et al,¹⁴ which resulted in a more firm tissue despite increasing the risk of keloid scar tissue creation. Yet, laser-induced collagen production did not differ significantly with the amount of collagen accumulated normally in the same stage of wound healing in control group; this result might become significant if a larger number of cases are employed. Inhibition of collagen aggregation caused by bFGF has already been reported in the literature; this effect is in line with making penetration of neovessels to tissues easy and would last until the latest phase of healing.²³ According to the results of the present study, it is advisable to use bFGF injections in earlier phases of wound healing to achieve vascular GT which facilitates wound closure, and employ laser therapy in later stages to induce collagen production and induce GT maturation.

Both bFGF and laser treatments reduced inflammation considerably in the final phase of healing. This may be attributed to the reported pain reduction capabilities of laser therapy on wounds.³³

Conclusion

The results showed that bFGF injection of oral wounds did not lead to a faster wound closure, and laser therapy

caused a delay in this process. bFGF induced GT was more vascular; in contrast, laser caused matured GT filled with collagen bundles.

Ethical Considerations

The experiment was conducted in compliance with the protocol approved by the Institutional Animal Care and Use Committee of Esfahan University of Medical Sciences.

Conflict of Interests

The authors declare that they have no competing interests.

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