

Surgical Wound Healing Made with Diode Lasers: A Pilot Study

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ABSTRACT

Background: The aim of this study is to evaluate the comparative effects of the diode lasers in animal models. Many surgical instruments like scalpel, electrosurgery and different laser types can be used for surgical incisions. Each of these has their own advantages and disadvantages.

Methods: Dorsal skin incisions, 10 mm in length and 1 mm in depth, were created on male Wistar albino rats via 3 different laser wavelengths. The animals were sacrificed at post-operative 24 h, 3 days or 1 week. All incision sites were removed by excisional biopsies and the specimens underwent histological examination.

Results: There were no statistically significant differences in the thermal changes, inflammation levels and fibrosis in all wavelength groups ($p>0.05$).

Conclusion: Pulsed mode of lasers accelerates the epithelisation regardless of the wavelengths.

Keywords: Diode lasers, Wound healing, Continuous wave lasers, Pulsed lasers

INTRODUCTION

Wound healing is divided into four topics as coagulation, inflammation, proliferation and maturation. The main events occurred during the healing are angiogenesis, epithelisation, granulation tissue formation and collagen deposition in the proliferation phase [1-3].

Numerous surgical instruments have been introduced in dental practice for surgical purpose. These include scalpel, electrosurgery, CO₂, Nd:YAG, Er:YAG and diode lasers. Each of these devices has their own advantages and disadvantages. Steel scalpel also known as cold knife has been commonly preferred for many years because of its ease of use accuracy and minimal damage to the surrounding tissues [4,5]. The use of devices that provide both incision and coagulation during the procedure has increased in both medicine and dentistry [6-8].

Electrosurgery is a surgical process that uses high frequency electrical currents [7]. Electrosurgery provides adequate hemostasis by sealing the vessels before cutting. On the other hand, wound healing is delayed by extensive thermal injury and also electrosurgery units have the disadvantage of causing muscle fasciculation [5-8].

The use of soft tissue lasers for the surgical procedures in dentistry aims to provide benefits to both the dentist and the patient [7,9]. Medical and dental researchers began to study different type of laser for intraoral surgical procedures. Diode lasers are the most common laser type owing to its compact size affordability and possibility to be used in many applications such as endodontics, soft tissue surgery, periodontics, orthodontics, bleaching and low level laser therapy (LLLT). It was introduced in mid-90 by Harris and Pick in 1995. Latest reports have suggested that diode laser with wavelengths between 810 to 970 nm in continuous and pulsed mode is a possible equipment for soft tissue surgery in the oral region [5,9].

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Furthermore, diode lasers have a fiber optic tip that provides opportunity to work with or without contact depending on the clinical requirements. Great absorption in hemoglobin with result of giving a good bleeding control is another reason of this choice. This feature provides bloodless area and clear view of the operative field and also there is often no need for a suture after the incision [4,10,11]. Heat produced during laser incision causes protein denaturation, drying, vaporization and carbonization of the application area. As a result of that pain, receptors at the incision area are inhibited. This eliminates the necessity of anesthetics injection and disinfection of the field. A better and faster healing process provides a good comfort to the patient after the operation so no medication is needed [7,12].

There are different wavelengths of diode lasers that were produced by various companies in the market and each diode laser has continuous and pulsative application mode. It has a crucial importance to determine the soft tissue damage resulting from standardized incision using different wavelengths and application modes [8,13]. However, no studies have assessed the effectiveness of each diode laser and the efficiency of the mode of application as pulsative or continuous. Therefore, this study aims to evaluate the comparative effects of the diode lasers of 3 different wavelengths in animal models. The first null hypothesis of the study is that there is no effect of the wavelength and the second one is that there is no effect of mode of application on the wound healing in animal models.

MATERIALS AND METHODS

Male Wistar albino rats with a weight of 250-300 g body mass and 5 months old were used in the study. Ethical clearance was taken from Committee on Animal Research of Bezmialem Vakif University (Protocol number: 2015/243). The rats were anesthetized by intramuscular injection of ketamine 35 mg/kg and xylazine HCL 5 mg/kg. Following anesthesia, the dorsum (operative field) of each rat was shaved with an electric shaver. After

marking the incision line on every rat, 3 different laser wavelengths; 810 nm (Gigaa, Cheese, China), 940 nm (Epic 10, Biolase, USA) and 980 nm (SIROLaser Advance, Sirona, Bensheim, Germany) were used to create an incision (10 mm in length and 1 mm in depth) on animals' dorsal side to the subcutaneous level using the lasers in continuous (3 W/cm²) or chopped modes (3 W/cm² 50% Duty cycle). Totally each rat had 6 incisions that were cut at least 1 cm apart.

The animals were sacrificed after 24 h, 3 days or 1 week. Incision areas of the dorsal side of each rat were removed by excisional biopsy and the specimens underwent histological examination. The specimens were fixed in formalin (10%) processed in the usual manner and embedded in paraffin blocks. They were sectioned and stained with standard hematoxylin and eosin solution. Light microscope (CX 31; Olympus, Tokyo, Japan.) was used for histological examination. Sections were evaluated by a pathologist and thermal changes, closure of epithelium granulation tissue, inflammation and fibrosis were recorded (grading range 0 to 3+).

Statistical analysis was executed with Statistical Package for Social Science (SPSS v.22. Chicago,IL, USA) for Windows 10.0 and where appropriate Kruskal Wallis and Mann Whitney U tests were for pairwise comparisons among groups ($\alpha=0.05$).

RESULTS

There were no statistically significant difference in the thermal changes, epithelial closure grade, levels of granulation tissue, inflammation levels and fibrosis among first day, third day and seventh days of wavelengths when pulsed and continuous mode were used (**Table 1**). For all wavelengths; there were no statistically significant differences between the pulsed and continuous modes in terms of thermal change levels, epithelial closure grades, granulation tissue levels, inflammation levels and fibrosis levels on the first day, third day and seventh days (**Table 2**).

Table 1. Evaluation of individual wavelengths in day and modes in terms of thermal change, epithelial closure grade, granulation tissue, inflammation and fibrosis.

Mode	Day	Wave lengths	Thermal change	Epithelial closure grade	Granulation tissue	Inflammation	Fibrosis
			Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)
Pulsative	1 st day	810 nm	2.83 ± 0.41 (3)	0 ± 0 (0)	1 ± 1.1 (1)	2.33 ± 0.52 (2)	0 ± 0 (0)
		940 nm	2.83 ± 0.41 (3)	0 ± 0 (0)	0.67 ± 0.82 (0.5)	2 ± 0.89 (2)	0 ± 0 (0)
		980 nm	2.83 ± 0.41 (3)	0 ± 0 (0)	1 ± 1.1 (1)	1.5 ± 0.84 (1)	0 ± 0 (0)
		p	1.000	1.000	0.836	0.181	1.000
	3 rd day	810 nm	2.33 ± 0.82 (2.5)	0.17 ± 0.41 (0)	1.67 ± 0.82 (1.5)	2.33 ± 0.82 (2.5)	0.17 ± 0.41 (0)
		940 nm	2.67 ± 0.52 (3)	0.33 ± 0.52 (0)	2 ± 0.89 (2)	2 ± 0.63 (2)	0.17 ± 0.41 (0)
		980 nm	2.67 ± 0.52 (3)	0 ± 0 (0)	2 ± 0.89 (2)	1.5 ± 0.55 (1.5)	0.33 ± 0.52 (0)
		p	0.683	0.322	0.728	0.139	0.738
	7 th day	810 nm	1.67 ± 1.03 (2)	1 ± 1.1 (1)	3 ± 0 (3)	2 ± 0.89 (2)	0.5 ± 0.55 (0.5)
		940 nm	2.17 ± 0.98 (2.5)	0.83 ± 0.41 (1)	2.67 ± 0.82 (3)	1.67 ± 0.82 (1.5)	0.67 ± 0.82 (0.5)
		980 nm	2.33 ± 0.82 (2.5)	0.67 ± 0.52 (1)	2.5 ± 0.55 (2.5)	2 ± 0.63 (2)	0.67 ± 0.82 (0.5)
		p	0.474	0.845	0.161	0.663	0.953
Continuous	1 st day	810 nm	2.33 ± 0.82 (2.5)	0 ± 0 (0)	0.67 ± 0.82 (0.5)	1.83 ± 0.98 (1.5)	0.17 ± 0.41 (0)
		940 nm	2.83 ± 0.41 (3)	0 ± 0 (0)	0.83 ± 0.98 (0.5)	1.67 ± 0.82 (1.5)	0.33 ± 0.82 (0)
		980 nm	2.83 ± 0.41 (3)	0 ± 0 (0)	0.83 ± 0.98 (0.5)	1.83 ± 0.98 (1.5)	0 ± 0 (0)
		p	0.299	1.000	0.954	0.954	0.586
	3 rd day	810 nm	2.67 ± 0.52 (3)	0.17 ± 0.41 (0)	2.17 ± 0.75 (2)	2.67 ± 0.52 (3)	0.33 ± 0.52 (0)
		940 nm	2.17 ± 0.75 (2)	0.5 ± 0.55 (0.5)	2.33 ± 0.52 (2)	2.17 ± 0.41 (2)	0.33 ± 0.52 (0)
		980 nm	2.67 ± 0.52 (3)	0.17 ± 0.41 (0)	2.17 ± 0.98 (2.5)	2.33 ± 1.03 (3)	0.5 ± 0.55 (0.5)
		p	0.328	0.351	0.948	0.349	0.802
	7 th day	810 nm	1.83 ± 0.98 (1.5)	0.5 ± 0.55 (0.5)	3 ± 0 (3)	2.33 ± 0.82 (2.5)	0.5 ± 0.55 (0.5)
		940 nm	2.33 ± 1.03 (3)	0.67 ± 0.52 (1)	2.5 ± 0.84 (3)	2.33 ± 0.82 (2.5)	0.67 ± 0.52 (1)
		980 nm	2.67 ± 0.82 (3)	0.5 ± 0.55 (0.5)	2.67 ± 0.52 (3)	2.17 ± 0.98 (2.5)	0.33 ± 0.52 (0)
		p	0.283	0.809	0.298	0.954	0.533

Kruskal Wallis Test

Table 2. Evaluation of individual modes in day and wavelength in terms of thermal change, epithelial closure grade, granulation tissue, inflammation and fibrosis.

Wave length	Day	Mode	Thermal change	Epithelial closure grade	Granulation tissue	Inflammation	Fibrosis
			Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)
810 nm	1 st day	Pulsative	2.83 ± 0.41 (3)	0 ± 0 (0)	1 ± 1.1 (1)	2.33 ± 0.52 (2)	0 ± 0 (0)
		Continuous	2.33 ± 0.82 (2.5)	0 ± 0 (0)	0.67 ± 0.82 (0.5)	1.83 ± 0.98 (1.5)	0.17 ± 0.41 (0)
		p	0.211	1.000	0.600	0.306	0.317
	3 rd day	Pulsative	2.33 ± 0.82 (2.5)	0.17 ± 0.41 (0)	1.67 ± 0.82 (1.5)	2.33 ± 0.82 (2.5)	0.17 ± 0.41 (0)
		Continuous	2.67 ± 0.52 (3)	0.17 ± 0.41 (0)	2.17 ± 0.75 (2)	2.67 ± 0.52 (3)	0.33 ± 0.52 (0)
		p	0.465	1.000	0.268	0.465	0.523
	7 th day	Pulsative	1.67 ± 1.03 (2)	1 ± 1.1 (1)	3 ± 0 (3)	2 ± 0.89 (2)	0.5 ± 0.55 (0.5)
		Continuous	1.83 ± 0.98 (1.5)	0.5 ± 0.55 (0.5)	3 ± 0 (3)	2.33 ± 0.82 (2.5)	0.5 ± 0.55 (0.5)
		p	0.867	0.423	1.000	0.495	1.000
940 nm	1 st day	Pulsative	2.83 ± 0.41 (3)	0 ± 0 (0)	0.67 ± 0.82 (0.5)	2 ± 0.89 (2)	0 ± 0 (0)
		Continuous	2.83 ± 0.41 (3)	0 ± 0 (0)	0.83 ± 0.98 (0.5)	1.67 ± 0.82 (1.5)	0.33 ± 0.82 (0)
		p	1.000	1.000	0.794	0.495	0.317
	3 rd day	Pulsative	2.67 ± 0.52 (3)	0.33 ± 0.52 (0)	2 ± 0.89 (2)	2 ± 0.63 (2)	0.17 ± 0.41 (0)
		Continuous	2.17 ± 0.75 (2)	0.5 ± 0.55 (0.5)	2.33 ± 0.52 (2)	2.17 ± 0.41 (2)	0.33 ± 0.52 (0)
		p	0.212	0.575	0.484	0.598	0.523
	7 th day	Pulsative	2.17 ± 0.98 (2.5)	0.83 ± 0.41 (1)	2.67 ± 0.82 (3)	1.67 ± 0.82 (1.5)	0.67 ± 0.82 (0.5)
		Continuous	2.33 ± 1.03 (3)	0.67 ± 0.52 (1)	2.5 ± 0.84 (3)	2.33 ± 0.82 (2.5)	0.67 ± 0.52 (1)
		p	0.715	0.523	0.598	0.176	0.859
980 nm	1 st day	Pulsative	2.83 ± 0.41 (3)	0 ± 0 (0)	1 ± 1.1 (1)	1.5 ± 0.84 (1)	0 ± 0 (0)
		Continuous	2.83 ± 0.41 (3)	0 ± 0 (0)	0.83 ± 0.98 (0.5)	1.83 ± 0.98 (1.5)	0 ± 0 (0)
		p	1.000	1.000	0.799	0.527	1.000
	3 rd day	Pulsative	2.67 ± 0.52 (3)	0 ± 0 (0)	2 ± 0.89 (2)	1.5 ± 0.55 (1.5)	0.33 ± 0.52 (0)
		Continuous	2.67 ± 0.52 (3)	0.17 ± 0.41 (0)	2.17 ± 0.98 (2.5)	2.33 ± 1.03 (3)	0.5 ± 0.55 (0.5)
		p	1.000	0.317	0.733	0.125	0.575
	7 th day	Pulsative	2.33 ± 0.82 (2.5)	0.67 ± 0.52 (1)	2.5 ± 0.55 (2.5)	2 ± 0.63 (2)	0.67 ± 0.82 (0.5)
		Continuous	2.67 ± 0.82 (3)	0.5 ± 0.55 (0.5)	2.67 ± 0.52 (3)	2.17 ± 0.98 (2.5)	0.33 ± 0.52 (0)
		p	0.338	0.575	0.575	0.670	0.465

Mann Whitney U Test

Figure 1 shows the thermal changes on the tissues after laser irradiations groups. There were gradually decrease day by day in all wavelengths and application modes;

however, there were no statistically differences among the tested days ($p > 0.05$).

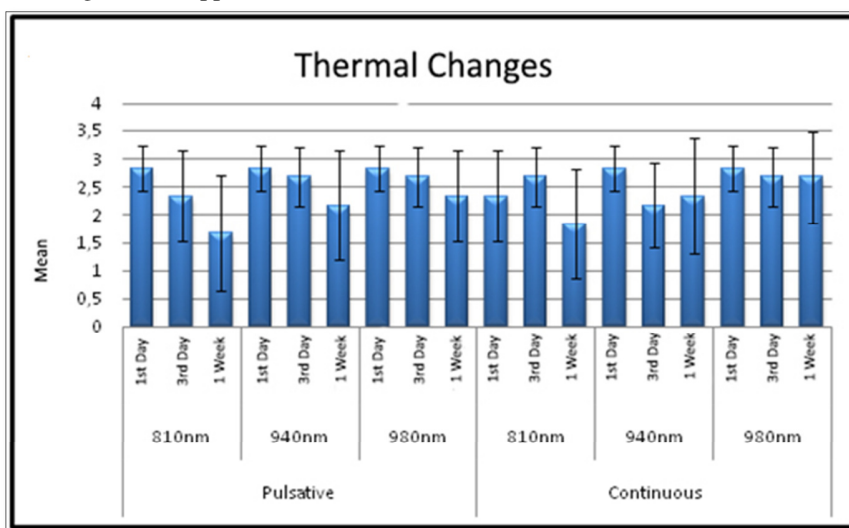


Figure 1. The mean degree of thermal changes.

After bilateral comparisons to determine the days of significance; the level of epithelial closure in the seventh day was found significantly higher on the first day in all wavelength groups ($p < 0.05$). There was no significant

difference between epithelial closure grades on the first day and on the third day in all wavelength groups ($p > 0.05$) (Figure 2).

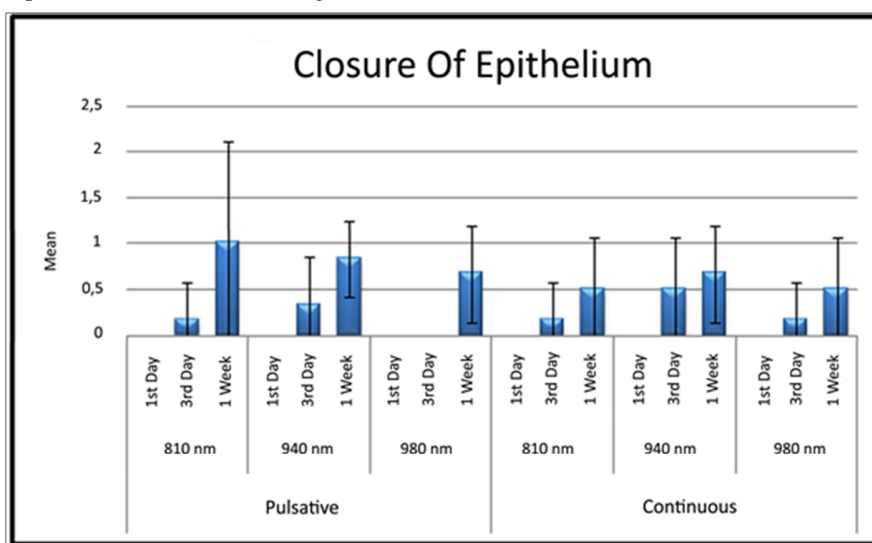


Figure 2. The mean degree of closure of epithelium.

DISCUSSION

In dentistry, the used wavelengths of the diode lasers are 655, 810, 940, 1064 nm, respectively [14]. They are used for different clinical applications such as cutting/destroying tissues and coagulating bleeding. 810, 940 and 980 nm wavelengths are ideal for soft tissue applications due to the well absorption characteristics by melanin and hemoglobin with a penetration depth

between 0.5 to 3 mm. Not only the wavelength but the power, pulse duration, fiber diameter and frequency may also affect the cutting efficiency of lasers on the soft tissues. Thermal damage of the tissue may be influenced by the following factors: output power, wavelength, affinity with the target tissue, terms transmission and type of the optical fiber [14]. Yammie et al. [14] investigated diode lasers with three different wavelengths 810, 940,

980 nm similar to our study. They have created 10 incisions measuring 10 mm in length and 2 mm in depth on fourteen male rabbits' ventral surface of their tongues. The laser parameters were 2 W with continuous mode delivered using a 400 nm optical fiber. They have detected similar thermal denaturation such as carbonization in different wavelength groups. They have reported that there was no statistically significant difference for the cutting depth and the incision's width. They have observed that 940 nm groups showed a wider marked carbonization zone but there was no statistically significant difference between the groups. The highest necrotic zone reported at 980 nm group but there was no statistically significant difference, either. They also reported no significant difference for inflammatory zones among the groups [14]. Similar to Yammie et al.'s [14] study, our results showed no statistically significant difference of the different wavelengths for the inflammatory zones; we also found no statistically significant difference between chopped and continuous modes.

Jin et al. [15] investigated guinea pig mucosa wound healing on the 15 mm length incisions created by a scalpel, diode laser and Er, Cr:YSGG laser. The laser used in this study is 810 nm diode lasers with 2 W of power, pulse length of 0.5 ms continuous wave and aiming beam of 635 nm. They used continuous mode and with non-contact technique. The inflammatory response at diode laser group had the lowest values at post surgery of the first day but at postsurgical 3 and 5 day groups the inflammation at the diode laser group was the highest [15]. They also investigated TNF- α and TNF- β 1 expression in the groups and concluded that diode laser was a good device for oral mucosal incisions but the tissue damage created by diode laser was higher than the scalpel or Er, Cr:YSGG laser. Kaur et al. [16] investigated two different techniques as scalpel and diode laser (810 nm) for uncovering the implants, found no statistical significant difference between healing index scores.

Saperia et al.'s [17] research which was the first study to evaluate the effect of laser on wound healing using the technique of molecular biology, they made whole-layer wounds in the back of pig and created three different groups as irradiation with Helium-Neon laser, tungsten light and no light each other. In laser and tungsten groups, Types I and III procollagen mRNAs were expressed more than in non-irradiation group. They proposed that laser irradiation increased collagen synthesis [17].

Suzuki et al. [18] investigated the wound healing efficacy of a 660 nm diode laser with different energy densities in a rat incisional wound model. Samples were divided into groups to receive 660 nm diode laser irradiation 24 h after surgery at an energy density of 0 (control), 1, 5 or 10 J/cm². Tissue sections were stained with hematoxylin-

eosin and an antibody for ED1 to determine the number of macrophages around the wound. They found that 660 nm diode laser with energy density of 1 and 5 J/cm² enhanced wound healing in a rat incisional wound whereas increased laser irradiation failed to yield such enhancement.

Mun et al. [19] have performed an animal study to clarify the effect of diode laser on wound healing based on microscopic findings. Laser diode of 655, 785 and 850 nm wavelengths were irradiated to the rat skin wound for 9 days, 20 min a day. They concluded that group of 850 nm resulted in the most abundant collagen formation so diode laser had beneficial effects on the formation of fibroblast and collagen and resulted in better wound healing. In our study, there was no statistically significant difference in the fibrosis between first, third and seventh days for three wavelength when pulsed and continuous mode were used. This result may come from that the method difference. Saperia et al. [17] and Mun et al. [19] and Suzuki et al. [18] did not create the wounds with laser, they just apply laser after the wound created to assess the healing properties. There are many studies assessing the healing effects of different kind of lasers on wounds/scars, but the studies assessing healing of wounds created by lasers are limited.

Paysse et al. [20] compared structural and functional outcomes and efficiency of diode laser photocoagulation for retinopathy of prematurity (ROP) when delivered in a pulsed mode versus a near-continuous mode. They concluded that there were no differences at functional outcome, complications, structural outcome found between using pulsed and near-continuous mode diode laser delivery for high-risk ROP.

Bryant et al. [21] compared the incisional wound healing created by scalped and CO₂ laser on canine oral mucosa and monitored at 3, 7, 14 days after the incision, they found a significant delay in the laser group and they linked the situation to excess thermal damage of the continuous wave laser beam. They also reported that short pulsed-free electron laser caused by the delay from the scalpel group on wound healing shortens. They concluded that the importance of laser pulse duration was much more important on the wound healing than the wavelength. Our study's results which are compatible with Bryant et al.'s [21] findings, chopped mode laser applications and showed faster epithelization than the continuous mode independent from the wavelength.

Havel et al. [22] compared 940 nm and 1470 nm wavelength laser diode system in vitro and in vivo. In vitro setup was on porcine liver and turkey muscle tissue model and for in vivo evaluation, 20 patients with nasal obstruction due to hyperplasia of inferior nasal turbinates were included in trial. They reported that the healing process following non-contact diode laser application

revealed to be improved using 1470 nm diode laser compared to 940 nm. In our study, 940 nm and 810 nm continuous mode applications show faster granulation tissue formation.

CONCLUSION

Within the limitation of this study, the chopped mode of lasers accelerates the epithelisation regardless of wavelengths.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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