Thermal Effect Created by Diode 810 nm Laser with Methylene Blue Stain: *Histological an in Vitro Study*

Ali M. Sadeq[1], Balsam S. Abdulhameed[2], Ameer Dh. Hameedi[3]

[1]Institute of Laser for Post Graduate Studies, Department of Biomedical Applications, Baghdad University, Aljadiriy, Baghdad, Iraq, [2]Consultant Oral and Maxillofacial Surgery, Ministry of Health, Al-Kadhimyia Teaching Hospital, Department of Maxillofacial Surgery, Baghdad, Iraq, [3]Lecturer, College of Medicine, Department of Pathology, Baghdad University, Baghdad, Iraq

Abstract

Background: In dental practice the Diode laser is considered as a good tool for oral soft tissue surgeries due to its effectiveness in cutting soft tissue with minimal bleeding and post-operative pain and edema.

Aim behind this study is to evaluate histologically the thermal effect created by diode 810 nm diode laser with use of methylene blue stain. **Material and Methods**: 60 incisions with length of 2 cm made on 60 samples of dimensions (2,1.5,0.5) cm on the dorsal surface of three sheep tongues was divided into four groups. Group I, 10 incisions (1.5W), Group II, 20 incisions (1.5W), Group III 10 incisions (2.5W) and Group IV 20 incisions (2.5W). Groups (I,III) without stain and groups (II, IV) with adding of methylene blue stain 1%. 810 nm diode laser used in C.W, with initiated fiber optic.

Result: highly significant increase of incision depth for groups (II ,IV) with ($P \leq 0.01$), highly significant decrease in damage width for both (II ,IV) groups with ($P \leq 0.01$).

Conclusion: use methylene blue stain with diode 810 can result in decreasing the thermal damage produced by laser.

Key words: *810 nm diode laser, Methylene blue, Thermal effect.*

Introduction

The laser is a comparatively novel and contemporary tool developed in 1960 by Maiman [(1)]. The used of laser in dental practice with subsequent improvements and innovations over time Since first successful application in dentistry in 1977 [(2)].The unique characteristics of Laser which are collimation, coherence, and monochromatic radiation, render special application in medicine and surgical field, especially in oral and maxillofacial surgery [(3)]. Laser wavelengths like (2,780 nm, 2,940 nm) work on soft and hard tissue while diode laser 810 nm work on soft tissue only and has excellent incision performance, very good surgical and hemostatic action via sealing small lymphatic and blood vessels which result in minimize post-operative pain and edema [(4, 5)]. The initial tissue influence is determined by laser wavelength, optical and thermal tissue properties [(6)]. The interaction mechanism of laser on soft tissue is determined by degree of laser energy absorption by target tissue chromophores inside tissue components [(7)]. The well absorption of diode laser by melanin, hemoglobin and other chromophoers which are exist within tissue [(8)]. Which render diode laser cuts tissue precisely, coagulate, ablate and vaporize tissue with less trauma.[(9,10)]. The aim of this study is to evaluate histologically the surgical margin of incision made by 810 nm diode laser with adjunct of methylene blue stain.

Materials and Methods

Sample preparation: Three fresh sheep's tongues collected directly after animal scarification. Methylene blue stain used with concentration 1%.

Laser Device and Parameters: 810 nm diode laser (quick lase, UK) fig (1).The delivery of laser beam is through optical fiber, the power used are (1.5, 2.5) W,

in C.W mode.

Surgical procedure:

60 samples of dimensions (2,1.5,0.5) cm and length of 2cm, on the anterior two third of dorsal surface of the tongues divided into four groups, Group I, 10 incisions, power 1.5 W without stain, Group II, 20 incisions, power 1.5 W with application of methylene blue stain 1 % application on the surface, Group III, 10 incisions, power 2.5 W without stain, Group IV, 20 incisions, power 2.5 W with methylene blue stain 1%. For Group II and Group IV, the incisions made after application of methylene blue stain by micro-applicator. The fiber optic used perpendicularly on the dorsum of the tongue along the planned incisions as shown in fig (2). The samples transferred to container with 10% buffered formalin for histological examination. Double blind histological examination done with optical microscope, the samples are sliced and sectioned with microtome into thin cross section, fixed with 4% formaldehyde in phosphate – buffered saline to prevent tissue degradation, passed through more progressively ethanol bath to remove the water, After that through hydrophobic clearing agent, xylene for removing alcohol, Lastly the paraffin wax used as an infiltrating agent and epoxy resin as un embedding media.

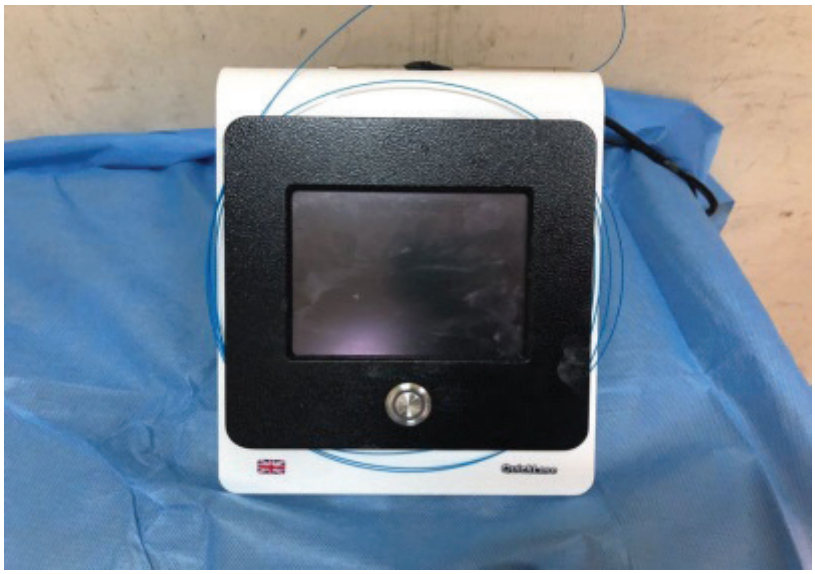

Figure 1: diode laser 810 nm

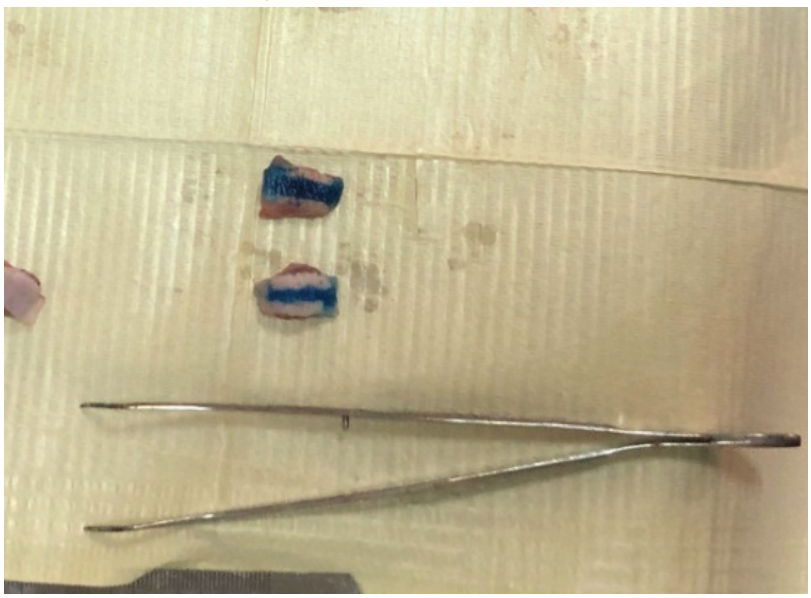

Figure 2: the planned incision with methylene blue stain

Statistical Analysis

The Statistical Analysis System- SAS (2012), program was used to detect the effect of difference factors in study parameters. T-test was used to significant compare between means. Chi-Square test was used to significant compare between percentage in this study [11].

Result

Histological evaluation:

1. incision depth, incision width, damage depth, damage width.

A. Group I and Group II :The result shows that there is a significant difference in Incision width (I.W) and Damage depth (D.D) with P-value≤0.05. And significant difference Incision depth (I.D) and Damage width (D.W) with P-value≤0.01 between group I and group II as shown in tab.(1).

Table 1: Comparison between group I and group II in I.D, I.W, D.D and D.W.

Group	Mean ± SE			
	I.D (mm)	I.W (mm)	D.D (mm)	D.W (mm)
Group I: Power 1.5 w, without stain	1.71 ± 0.05	0.730 ± 0.03	1.19 ± 0.04	0.980 ± 0.03
Group II: Power 1.5 w, methylene blue 1%	1.855 ± 0.02	0.870 ± 0.04	1.095 ± 0.02	0.795 ± 0.03
T-test	0.1059 **	0.1221 *	0.0922 *	0.0906 **
P-value	0.009	0.0261	0.044	0.0003
* (P≤0.05) - Sig., ** (P≤0.01) -Highly Sig.				

B. Group III & group IV: The result shows that there is no significant difference between group III and group IV in incision width. There is a significant difference between group III and group IV in incision depth, damage depth and damage width with P-value≤0.01, as shown in tab. (2).

Table 2: Comparison between group III and group IV in I.D, I.W, D.D and D.W.

Group	Mean ± SE			
	I.D (mm)	I.W (mm)	D.D (mm)	D.W (mm)
Group III: Power 2.5 w, without stain	1.76 ± 0.05	1.09 ± 0.02	1.42 ± 0.02	1.30 ± 0.03
Group IV: Power 2.5 w, methylene blue 1%	1.99 ± 0.03	1.17 ± 0.03	1.14 ± 0.02	1.06 ± 0.02
T-test	0.130 **	0.1007 NS	0.0087 **	0.0749 **
P-value	0.0009	0.1148	0.0001	0.0001
** (P≤0.01) -Highly Sig. , NS: Non-Significant.				

2. Regularity and quality scale:

A. Group I & group II: The highest quality of incisions distribution is for group II with (P≤0.01). as shown in tab .(3).

Table 3: Distribution of samples according to Regularity and quality in group I and group II.

Regularity and quality	Power 1.5 w, without stain No. (%)	Power 1.5 w, methylene blue 1%	P-value
0	0 (0.00%)	0 (0.00%)	NS
<2	5 (50.00%)	3 (15.00%)	0.0001 **
≥2	5 (50.00%)	7 (35.00%)	0.0294 *
4	0 (0.00%)	10 (50.00%)	0.0001 **
Total No.	10	20	--
** (P≤0.01)-Highly Sig.			

B. Group III & group IV; The highest quality of incisions distribution is for group IV with (P≤0.01). as shown in tab.(4).

Table 4: Distribution of sample according to Regularity and quality in group III and study group IV.

Regularity and quality	Power 2.5 w, without stain No. (%)	Power 2.5 w, methylene blue 1%	P-value
0	0 (0.00%)	0 (0.00%)	NS
<2	4 (40.00%)	2 (10.00%)	0.0001 **
≥2	5 (50.00%)	6 (30.00%)	0.0074 **
4	1 (10.00%)	12 (60.00%)	0.0001 **
Total No.	10	20	--
** (P≤0.01)-Highly Sig.			

Discussion

This study determined the effectiveness of using methylene blue stain with diode laser 810 nm for cutting soft tissue utilizing an in vitro model. The procedure for histological evaluation used in this study is commonly applied for examination of incision properties and thermal effect on oral tissue. The in vitro model utilize the tongue tissue which considered the criterion for examine laser cutting efficiency, as this model used by Wilder Smith et al [12]. The histological evaluation

determined the incision properties and both lateral and deep damage of cutting epithelium through the basement membrane by laser along with using methylene blue stain as an external pigments. Each type of laser creates a degree of thermal damage by photothermal effect on target tissue. The thermal energy that transmitted by laser beam increase the tissue temperature at incidence point above the 100 c. The vaporization effect is the basis for laser action, that how laser cut tissue. The side effect of thermal reaction is due to increase the temperature of adjacent tissue which result in permanent or reversible damage. This damage is related tissue properties and laser wavelength [13]. The presence of increased level of melanin pigments in the periodontal diseases increased the absorption of laser energy result in better cutting efficiency of laser [14]. This study use the methylene blue stain as external pigments to act as target chromophores for laser beam which result in increase the absorption as the study of Agrwal *et al* 2018 [15]. The results show that there was increase in I.D and I.W along with decrease in D.D and D.W in both II and IV which use methylene blue stain 1% using different powers which agree with result of Agrwal *et al* 2018. When target chromophoers are matching or near the applied laser wavelength, the laser beam will be absorbed with high percentage rather than scattered [16]. The methylene blue stain (pigments) act as photoreceptors (chromophores) for laser energy that exist on the tissue surface along with hemoglobin and melanin which are the photoreceptors exist inside the oral tissues [16]. The result of incisions margin regularity show that the incisions made with use of methylene blue in both II and IV groups were with heist quality and smooth regular surface than other I and III groups without stain, which measured according [17] **.** The scale of incision regularity was from 0 – 4. The worst quality of incision is scored with 0, the rough and uneven edge scored with (<2), the smooth linear border of incisions with score (≥2), the highest incision quality is scored as 4. [18]. When the selected wavelength absorbed with high degree from water based chromophores, result in sharp, well defined, smooth incision with minimal sub surface disturbances [19]. For future studies to utilize an in vivo model.

Conclusion: using methylene blue stain 1 % with different powers of diode laser 810 nm can assist to decrease the thermal damage caused by laser heating.

Conflict of Interest: No

Source of Funding: Self funded

Ethical Clearance: Not Required

References

1. Mavrogiannis M, Thomason JM, Seymour RA. Lasers in Periodontology *Dent Update* 2004;31:535-38, 541-42, 545-47.
2. Stabholz A, Stabholz A, Leopold Y, Rosenburg Y, Moshonov J. Lasers in dentistry. Part ADevelopment, characteristics, structure, principles of operation and types of lasers. *Refuat Hapeh Vehashinayim* 2001;18:11-23, 61.
3. Deppe H, Horch G. Laser applications in oral surgery and implant dentistry. Laser Med Sci. 2007; 2: 217-21
4. Genovese MD, Olivi G. Use of laser technology in orthodontics: hard and soft tissue laser treatments. *Eur J Paediatr Dent.* 2010;1 1:44-48.
5. Stabholz A, Zeltser R, Sela M, et al. The use of lasers in dentistry: principles of operation and clinical applications. *Compend Contin Educ dent* 2003; 24:935-948
6. Douglas N, Dederich NH. Laser/tissue interaction. Algha Omegan. 1991; 84: 33-6.
7. Walsh LJ. The current status of laser applications in dentistry. Australian Dent J. 2003; 48: 146-55.
8. Raffetto N. Lasers for initial periodontal therapy. Dent Clin North Am. 2004;48:923–36
9. Goharkhay K, Moritz A, Wilder-Smith P, Schoop U, Kluger W, Jakolitsch S, et al. Effects on oral soft tissue produced by a diode laser *in vitro*. Laser Surg Med.1999;25:401–6.
10. Adams TC, Pang PK. Lasers in aesthetic dentistry. Dent Clin North Am. 2004;48:833–60.
11. **SAS.** Statistical Analysis System, User's Guide. Statistical. Version 2012, 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
12. Wilder-Smith P, Arrasita AMA, :Liaw LH, Berns M. Incision properties and thermal effects of three co2 laser in soft tissue. Oral surg Oral med Oral Pathol Oral Radiol Endod 1995;79(6):685-691.
13. Miyazaki H, Kato J, Watanabe H, Harada H, Kakizaki H, Tetsumura A, Sato A, Omura K Intralesional laser treatment of voluminous vascular lesions in the oral cavity. Oral Surg Oral Med Oral

Pathol Oral Radiol Endod 2009, 107(2):164-172.

14. Walinski C. Irritation fibroma removal: A comparison of two laser wavelengths. Gen Dent. 2004;52:236–8.
15. Agrawal AA, Prabhu R, Sankhe R, Wagle SV. Influence of external chromophore on cutting efficacy of 940 nm diode laser: An *In vitro* animal study. Contemp Clin Dent 2018; 9:S79-82.
16. Sankaran V., *et al*. "Comparative study of polarized light propagation in biologic tissues". *Journal of Biomedical Optics*. 7.3, 2002: 300-306
17. Azevedo AS, Monteiro LS, Ferreia F, et al. *In Vitro* histological evaluation of the surgical margin made by different laser wavelengths in tongue tissue. *J* Clin Exp Dent.2016;8 (4):e396. Published 2016 Oct 1. Doi:10.4317/jced.52830.
18. Baxter GD, Diamantopoulos C, O'Kane S, Shields T. D, Allen J. Therapeutic Lasers Theory and Practice. Harcourt Publishers Limited, first edition. 1999 pp. 56-64.
19. parker, s. verifiable cpd paper: laser-tissue interaction. british dental journal, 2007, 202 (2), 73-81