Dzana KATANA

TISSUE WELDING WITH 980nm AND 1064nm LASERS:
EFFECT OF PULSE MODULATION

M.S.
2014

FATIH UNIVERSITY

Institute of Biomedical Engineering

Master of Science in
Biomedical Engineering

TISSUE WELDING WITH 980nm AND 1064nm LASERS: EFFECT OF PULSE MODULATION

By

Dzana KATANA

ISTANBUL/JUNE 2014

T.C.
FATIH UNIVERSITY
INSTITUTE OF BIOMEDICAL ENGINEERING

TISSUE WELDING WITH 980nm AND 1064nm LASERS: EFFECT OF PULSE MODULATION

DZANA KATANA

MSc THESIS
BIOMEDICAL ENGINEERING PROGRAMME

ISTANBUL/JUNE 2014

T.C.
FATIH UNIVERSITY
INSTITUTE OF BIOMEDICAL ENGINEERING

TISSUE WELDING WITH 980nm AND 1064nm LASERS: EFFECT OF PULSE MODULATİON

DZANA KATANA

MSc THESIS
BIOMEDICAL ENGINEERING PROGRAMME

THESIS ADVISOR
ASST. PROF. DR. HAŞİM ÖZGÜR TABAKOĞLU

ISTANBUL/JUNE 2014

T.C.
FATİH ÜNİVESİTESİ
BİYOMEDİKAL MÜHENDİSLİK ENSTİTÜSÜ

980nm ve 1064nm LAZERLER İLE DOKU KAYNAĞI: ATIMLI MODÜLASYONUN ETKİSİ

DZANA KATANA

YÜKSEK LİSANS TEZİ
BİYOMEDİKAL MÜHENDİSLİĞİ PROGRAMI

DANIŞMAN
YRD. DOÇ. DR. HAŞIM ÖZGUR TABAKOĞLU

İSTANBUL/ HAZİRAN 2014

T.C.

FATIH UNIVERSITY

INSTITUTE OF BIOMEDICAL ENGINEERING

Dzana Katana, **a MSc. student of Fatih University** Institute of Biomedical Engineering **student ID 520112010**, successfully defended the **thesis** entitled "Tissue welding with 980nm and 1064nm lasers: Effect of pulse modulation", **which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below**:

Committee Members

Thesis Advisor : Asst. Prof. Dr. Haşim Özgür TABAKOĞLU.....
Fatih University

Asst. Prof. Dr. Şükrü OKKESİM.....
Fatih University

Asst. Prof. Dr. Mehmet ŞENEL.....
Fatih University

It is approved that this thesis has been written in compliance with the formatting rules laid down by the Institute of Biomedical Engineering.

……

……

……

.....Prof. Dr. Sadık KARA

Director

Date of Submission: 23.05.2014

Date of Defence : 23.06.2014

I lovingly dedicate this thesis to my parents.
For their endless love, support and encouragement.

This study was supported by Fatih University Research and Development Management Office

ACKNOWLEDGEMENTS

I would like to acknowledge all the people without whom this thesis might not have been written. I will be always greatly indebted and greatful to all of you, for your help, love and support.

First of all, I would like to offer a special gratitude and thanks to my advisor Asst. Prof. Dr. Haşim Özgur Tabakoğlu, who has been an immense mentor for me. I would like to thank him for all his patience, motivation, enthusiasm, immense knowledge and his unwavering support. His guidance helped me in all the time of research and writing of this thesis.

My sincere appreciation goes to my committee members, professor Sadık Kara, professor Mehmet Şenel, professor Şükrü Okkesim for serving as my committee members.

Furthermore, special regard goes to the staff of Boğazici University, The Institute of Biomedical Engineering Biophotonic laborotory for allowing me to use their equipment, devices and materials and for making this research possible.

A special big, heartfelt thank you goes to my loving parents, Nevzeta and Reuf Katana for their words of encouragement, love and support throughout my life. Thank You both for giving me strenghth to reach for the stars and chase my dreams. I am so appreciative of everything You have done for me.

I wıll be grateful forever for the love and supports received from all my relatives during this period.

Finally, I would like to thank all of my friends and colleagues for their support and encouragement throughout this period of research and for making me believe that dreams can come true. I would also specially like to thank Mustafa Acar for listening, offering me advice and being with me through this entire process.

June 2014 Dzana Katana

TABLE OF CONTENTS

Page

TABLE OF CONTENTS vi

LIST OF SYMBOLS viii

LIST OF ABBREVIATIONS ix

LIST OF FIGURES x

LIST OF TABLES xii

CHAPTER 1 15

INTRODUCTION 15

1.1 Lasers: Applications for therapeutic use 15

1.2 Laser Modes of Operation 16

1.2.1 Continuous Wave Operation 17

1.2.2 Pulsed Operation 18

1.2.3 Pulsing 19

1.3 Tissue welding 20

1.4 Interaction Mechanisms and Effects of Laser Radiation 24

1.5 980nm Laser application in Tissue Welding 29

1.6 1064nm Laser application in Tissue Welding 30

1.7 Motivation and Objectives 31

1.8 Hypothesis 32

CHAPTER 2 33

MATERIALS AND METHODS 33

2.1 AKT-Epidermis Dual Wavelength Laser System 33

2.2 Methodology 35
2.2.1 Animals 35
2.2.2 Surgery 35
2.2.3 Post-surgery 35
2.2.4 Closure method (Laser irradiation) 36
2.4 Histology Examination 38
2.4.1 Tissue processing 38
CHAPTER 3 45
RESULTS 45
CHAPTER 4 55
DISCUSSION 55
CHAPTER 5 58
CONCLUSIONS AND RECOMMENDATIONS 58
REFERENCES 59

LIST OF SYMBOLS

J: Joule

nm: Nanometer

T: Temperature

t: Time

W: Watt

λ: Wavelength

ρ: Density of tissue

±: Plus or minus

^{o}C: Degree celceus

LIST OF ABBREVIATIONS

A-C: Coagulation Area

A-G: Granulation area

A-HT: Hypothermal Area

AKT: Brand Name of the LASER

CO2: Carbon Dioxide

CW: Continuous Wave

Er:Cr:YSGG:Ebrium, Chromium doped Yttrium Scandium Gallium Garnet

Er:YAG:Erbium Ywittrium Aluminium Garnet

H&E: Hematoxylin and Eosin Staining

HO:YAG:Holmium YAG

L: Incision line's length

LTW: Laser Tissue Welding

Nd:YAG:Neodymium Ywittrium Aluminium Garnet

OfT: Off Time

OnT: On Time

LIST OF FIGURES

Page

Figure 1. 1 **Graph A** presents Continuous wave mode (CW) which seems to be continuosly on (OnT) **Graph B and C** shows modulated lasers which produce light that is half of the time on and half of the time off. As light is 50% on and 50% of, this cycle is also knows as 50% duty cycle. **Graph D** representes a cycle which is 25% time on, or one fourt of the time and the rest of the time is off (OfT). [The figure taken from (42)] 17

Figure 1. 2 Laser tissue welding is a photothermal process [The figure taken from (12)] 23

Figure 1. 3 Map of laser- tissue interactions [The figure taken from (7)]…… 25

Figure 1.4 Thermal effects in biological tissue[The figure adopted from (7)]……27

Figure 2. 1 AKT Epidermis dual wavelength sytem parts..... 34

Figure 2. 2 AKT Dual wavelength Laser System..... 34

Figure 2. 3 Incision irradiation 37

Figure 2. 4 Wound excision..... 37

Figure 2. 5 Microtome 41

Figure 2. 6 Paraffin Embedded Tissue Sectioning Method [The figure ataken from (12)] 42

Figure 2. 7 H&E staining method [The figure taken from (12)] 44

Figure 3. 1 Graphical representation of Arithmetic Mean for the incision line's length at 4th post-irradiation day for 980nm and 1064nm laser.....48

Figure 3. 2 Graphical representation of Arithmetic Mean for the incision line's length at 7th post-irradiation day for 980nm and 1064nm laser..... 48

Figure 3. 3 Graphical representation of Arithmetic Mean for the A-C, A-G and A-HT at 4th post-irradiation day for 980nm and 1064nm laser..... 49

Figure 3. 4 Graphical representation of Arithmetic Mean for the A-C, A-G and A-HT at 7th post-irradiation day for 980nm and 1064nm laser..... 49

Figure 3.5a The incision line appereance at 4th day post-irradiation……..…51

Figure 3.5b The incision line appereance at 7th day post-irradiation..... 52

Figure 3.6a The A-C (1), A-G (2) and A-HT (3) appereance at 4th day post-irradiation ……53

Figure 3.6b The A-C (1), A-G (2) and A-HT (3) appereance at 7th day post-irradiation 54

LIST OF TABLES

Page

Table 1. 1 The approaches for Tissue Bonding [The table adopted from (1)] 21
Table 1. 2 Penetration depth of some Medical Lasers in water and in Tissue [The table taken from (7)] 22
Table 1. 3 Thermal effects of laser radiation [The table taken from (7)] 27
Table 2. 1 Tissue dehydration procedure……39
Table 3.1 980nm and 1064nm side effects indicators on 4th day after irradiation.....46
Table 3. 2 980nm and 1064nm side effects indicators on 7th day after irradiation..... 46
Table 3. 3 Arithmetic Mean; Standard Deviation; Student T-Test for 980nm and 1064nm for 4^{th} day after irradiation 47
Table 3. 4 Arithmetic Mean; Standard Deviation; Student T-Test for 980nm and 1064nm for 7^{th} day after irradiation 47

SUMMARY

TISSUE WELDING WITH 980nm AND 1064nm LASERS: EFFECT OF PULSE MODULATION

Dzana Katana

Biomedical Engineering Programme

MSc Thesis

Advisor: Asst. Prof. Dr. Haşim Özgür TABAKOĞLU

Lasers have alreday become irreplacable tools in biological applications and in modern medicine as it has the power to cauterizd as it cuts, vaporize the tissue and reduce the surgical trauma. Laser tissue welding is a process that utilizes use of laser energy to join or bond tissues and has proven to be effective in clinical applications, such as tissue incision closure. Study was performed on 18 Wister rats (randomly selected) weighing 200-220g in order to study side effects of 980nm and 1064nm laser pulse modulation for which is known to cause milder thermal damages when compared to CW mode. Incisions were made on dorsal region and closed by laser irradiation at 1W for 10 seconds (exposure time) and 5J per spot. Post surgically animals were divided into two groups and sacrificed at 4th and 7th day. Once samples were taken and tissue processed, histological analysis was performed by quantifying thermal changes on tissues by determining and analysing hypothermal area, granulation and coagulation area as well as the length of the incision line. It was found that closure of incision line was better and thighter at 7th day post-irradiation than at 4th. The incision line length and hypothermal area were less in samples irradiated with 980nm laser than those irradiated with 1064nm laser yet the difference was not significant. Granulation area differed significantly at 4th day where the same pattern was not observed at 7th day. Coagulation area was present in samples of both groups with no statistical difference.

Keywords: Pulse modulation; 980nm & 1064nm laser; Side effects;

FATIH UNIVERSITY - INSTITUTE OF BIOMEDICAL ENGINEERING

ÖZET

980nm ve 1064nm LAZERLER ILE DOKU KAYNAĞI: ATIMLI MODÜLASYONUN ETKİSİ

Dzana Katana

Biyomedikal Mühendisliği Programı

Yüksek Lisans

Danışman: Yrd. Doç. Dr. Haşim Özgür TABAKOĞLU

Lazerler dokuyu buharlaştırabilme, kesi yaparken aynı zamanda kanamayı durdurabilme ve cerrahi endikasyonları en aza indirme gibi çeşitli biyolojik etkileri ile çağımız modern tıbbının vazgeçilmez ürünleri haline gelmişlerdir. Lazer ile doku kaynağı uygulaması lazer enerjisinin dokuları birleştirmek için kullanıldığı ve klinik uygulamalarda da özellikle kesilerin kapatılmasında etkili olduğu kanıtlanmış bir yöntemdir. Bu çalışmada sürekli uygulamaya nazaran daha az termal zarar oluşturduğu bilinen atımlı lazer modunun farklı iki dalga boyundaki 980 nm ve 1064 nm etkileri 18 Wistar cinsi sıçan (rasgele seçilmiş, 200-220 g arası, dişi) üzerinde araştırılmıştır. 3 çift kesi omurga ortada kalacak şekilde paralel olarak açılmış ve daha sonra her bir noktasal atış için 1 Watt' lık optik güç 10 saniye (500 ms açık – 500 ms kapalı) süre ile kesi üzerine aktarılmıştır; böylelikle her bir noktasal atışta dokuya aktarılan toplam enerji 5 Joule' dür. Lazer cerrahisinden sonra denekler iki gruba ayrılmış ve bulunduğu gruba göre iyileşme sürecinin 4. ve 7. günlerinde hayatlarına son verilmiştir. Deri örnekleri toplandıktan ve doku takibi yapıldıktan sonra histolojik termal değişim analizleri belli kriterlere bakılarak yapılmıştır. Bu kriterler: Hipertermal alan tespiti ve sayısallaştırılması, granülasyon ve koagülasyon alanlarının tespiti ve sayısallaştırılması, ve kesinin iyileşme sürecindeki açıklık kapalılık boyutlarının sayısallaştırılmasıdır. 7. Günde kesilerin genel kapanma durumları 4. Gündeki kapanma durumlarından daha iyi olduğu gözlemlenmiştir. Kesinin derinlemesine kapanma miktarı ve hipertermal alan 980 nm ile kapatılan kesilerde 1064 nm ile kapatılan kesilere göre daha azdır. Koagülasyon alanı 1064 nm ile kapatılan kesilerde her iki günde de yüksek olduğu gözlemlenmiştir. Yine her iki grupta granülasyon alanlarının bulunduğu ve özellikle 1064 nm lazer ile kapatılan kesilerde granülasyonun çok daha fazla artış gösterdiği tespit edilmiştir.

Anahtar kelimeler: Atımlı modülasyonu; 980 & 1064 nm lazer; Yan etkisi;

CHAPTER 1

INTRODUCTION

1.1 Lasers: Applications for therapeutic use

The name **LASER** was coined by Charles Townes in 1951 and it stands for **L**ight **A**mplification by the **S**timulated **E**mission of **R**adiation. LASER is a device that creates and amplifies a very narrow intense beam of light whose photons are coherent and which can be focused on a tiny spot. The basic tent of laser's operating is pumping atoms or moleculs from the medium so that more of them end up in a higher energy levels than at the lower energy levels. This process is know as stimulated emission. The very first idea of this innovation was suggested by Albert Einstain in 1917 and the whole concept was based on the quantum theory of radiation. Therefore, we can say with a certainty that Albert Einstain was the first one who theorized the process which makes lasers possible, previously mentioned, stimulated emission.

In 1960 at Hughes Laboratory in Malibu, California, the first functional laser was operated by Theodore H. Maiman. This laser is known as Ruby Laser as it uses a solid state flashlamp-pumped synthetic ruby crystal to produce red laser light. In a ruby laser light from the flash lamp excites the molecules in the ruby rod and they bounce backward and forward between two mirrors until coherent light escapes from the cavity. First ruby laser was able to produce only pulsed modulation.

Laser light is usually characterized as a coherent, monochromatic and collimated light. Coherent means that all wavelengths are in phase, monochromatic means the light is consisted of one wavelength or color and collimated means all the rays are narrow, parallel and with minimal spreading. Therefore, they all travel in the same direction and can be easily focused on small spots.

Briefly, mechanism of laser action includes excitation of atoms and their passing through different medium (crystal, gas or liquid).

The production of laser light is achieved through generating a cascade of photon energy to sweep through the medium, while bouncing off mirrors, which is then being reflected backward and forrward, leading to receivement of energy to produce an intense beam of light.

Various kinds of lasers have alreday become irreplacable tools of modern medicine as it has the power to cauterize as it cuts, vaporize the tissue and reduce the surgical trauma. Although clinical applications were firstly limited to opthamology, since the eye and its interior belong to the easiest accessible organs because of their high transparency, laser found its application in dentistry, gynecology, urology, neurosurgery, dermatology, angioplasty and cardiology too. Furthermore, lasers have alreday become a necessary instrument in different biological applications, for instance, imagining tools and optical microscopy which are used for clinical and medical diagnosis, especially in histopathology, enabling scientists to investigate and analyse tissues and molecular structures so as eventual changes on cellular and molecular level in diseases such as infection, cancer, inflammation and many others.

Thus, it is not hard to conclude that laser applicatin in medicine and research is promptly growing and slowly transitioning from an 'alternative technique' to main method for various medical applications.

1.2 Laser Modes of Operation

Lasers are tolls which create monochromatic, coherent and collimated intensive beam of light. Employing various aspects and cosiderations, lasers can be sorted into few groups [1]. Based on their power output which can be continuous over a certain period of time or pulsed which is measured on specific time scale , lasers can be classified into two groups, continuous mode lasers and pulse mode lasers [42].

Laser modes of operation are as follow:

- Continuous Wave
- Modulated Continuous Wave
- Q Switched (nanosecond pulses)
- Long pulsed (micro or millisecond pulses)

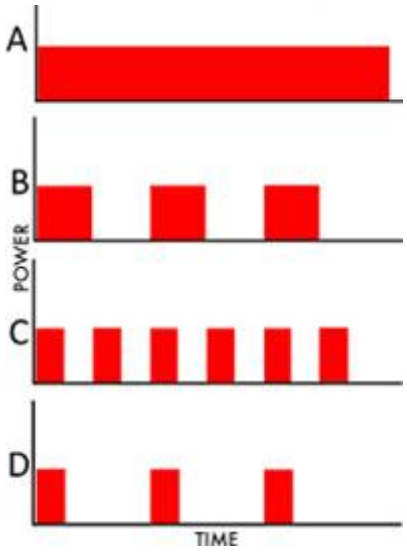


Figure 1. 1 **Graph A** presents Continuous wave mode (CW) which seems to be continuosly on (OnT)

Graph B and C shows modulated lasers which produce light that is half of the time on and half of the time off. As light is 50% on and 50% of, this cycle is also knows as 50% duty cycle.

Graph D representes a cycle which is 25% time on, or one fourt of the time and the rest of the time is off (OfT).

[The figure taken from (42)]

1.2.1 Continuous Wave Operation

Continuous wave (CW) operation mode of a laser, as its name implies, is a mode of laser which is constantly being pumped and which continually emits light [8]. During CW mode of irradiation there is no any disruption while the energy is being delivered. This mode of laser irradiation is like a light that is constantly 'on' [42].

Helium neon laser was the first laser operated in a continuous wave mode at 1153nm wavelength. Shortly after this laser was presented, new versions of lasers were introduced which were operated at 632.8nm wavelength. This emission wavelength is nowdays very frequent and usual in CW mode operated lasers [8]. Today there are many types of lasers that can be made to operate in this mode, such as: gas lasers, different kinds of solid state lasers (for example semiconductor lasers) and dye lasers [8].

CW mode operated lasers in fact lase in several longitudinal modes simultaneously and beats between the slightly different optical frequencies of those oscillations will in fact produce amplitude variations on time scales shorter than the round-trip time (the reciprocal of the frequency spacing between modes), a few nanoseconds or less [42].

For lasers to be able to function in continuous wave mode of operation it is necessary for the population inversion of the gain medium to be constantly charged by a regular and constant pump source. But in certain medias this process doesn't seem possible. To make some lasers operate in CW mode it is required to pump lasers at a constant and high power level. The process itself is hard to be achieved and it may lead to laser destruction due to high quantity production of heat. These kinds of lasers can not be operated in continuous wave mode [42].

For some types of lasers such as those with low gain transitions, it is hard, but not impossible to operate them in CW mode, even though operating them in pulsed mode is easier to achieve. To obtain CW mode sometimes it requires the use of fiber lasers as its geometry can highly enhance the gain effectiveness [8].

1.2.2 Pulsed Operation

Lasres operated in pulsed mode are all the lasers which are not classified and operated in a CW mode. Optical power of these lasers is in a pulse form measured on specific time scale at certain repetition rate [42]. The outcome is a laser beam which is 'on' half of the time and 'off' half of the time. This cycle is also knows as a 50% duty cycle as the light is 'on' 50% of the time and 'off' 50% of the time. Because of the large population inversion which occurs in a short time, lasers operated in pulsed modulation are characterized by high gain [1].

As some lasers can not be operated in CW mode, they are just run in pulsed mode. For some other lasers to achieve pulse modulation it is required to generate as much an energy as possible. This aim sometimes can be accomplished just by lowering the pulse rate in order to generate more energy between pulses as the pulse energy is equal to the average power divided by the repetition rate [42].

If a surface of a certain material is heated in a short period of time, laser ablatation can be achived and an evaporation of the heated area will take place.

On the contrary, when applying energy progressively, bit by bit, there is a chance that the heat will be absorbed by the material itself which will not result in achiving adequately high temperature at a certain spot. In case of gaining nonlinear optical effects, it is recommended to lean on the peak pulse power, over the energy in the pulse. If the pulse energy is known all that is necessary is creating a short duration pulses by applying so called Q switching technique [42].

One of the main advantages of pulsed operated lasers is their capacity of producing very short pulses with a very high intensity whose duration can range from couple of milliseconds to several femtoseconds [1].

1.2.3 Pulsing

Pulsing and frequency are terms both used to describe the very identical notion, the interruption of energy flow on a predetermined basis

There are principally two kinds of pulsing:

- Chopped (switched):

A beam that is continuous and which is being swiched mechanically or electronically between ‘on-s’ and ‘off-s’ is called a chopped beam. While the beam is on, it usually has an output power equal to CW mode output power. But as the beam has its ‘on-s’ and ‘off-s’ periods and therefore is not active all the while, its average output power is less than if the beam would be operated in continuous mode. The average power of chopped beam can be defined as a function of CW power and duty cycle. Duty cycle is a ration of the period when beam is active, ‘on-s’, to the total period of irradiation, ‘on-s’ plus ‘off-s’. The ratio is usually given in a percentage. Some of the lasers operated in chopped pulsing mode would be gas lasers, for instance helium neaon laser and most of the semiconductor diode lasers [42].

-Superpulsed:

Superpulsed beam’s peak power is usually very high. Howsoever, as electrical current is being led throughtout material, heat is also being produced. As a high current is required for the proces to happen, a side effect such as direct combustion of a crystal may occur. The only way of preventing crystal’s burning is by setting a very short current conduction time.

The maximum pulse time of lasers operated as superpulsed is between 100 and 200ns. It is also important to mention that after every pulse application, a cooling period is necessary. This usually lasts 1000 times longer than the pulse duration do. Even though the peak power is really high, the average output of lasers operated as a superpulsed laserss is low [42].

1.3 Tissue welding

Lasers are vigorous devices used for a variety of purposes, including tissue engineering. Tissue engineering can be defined as a growing multidisciplinary area that utilizes various types of light-tissue interactions. Based on these ligh-tissue interactions, there are 3 major types of tissue engineering:

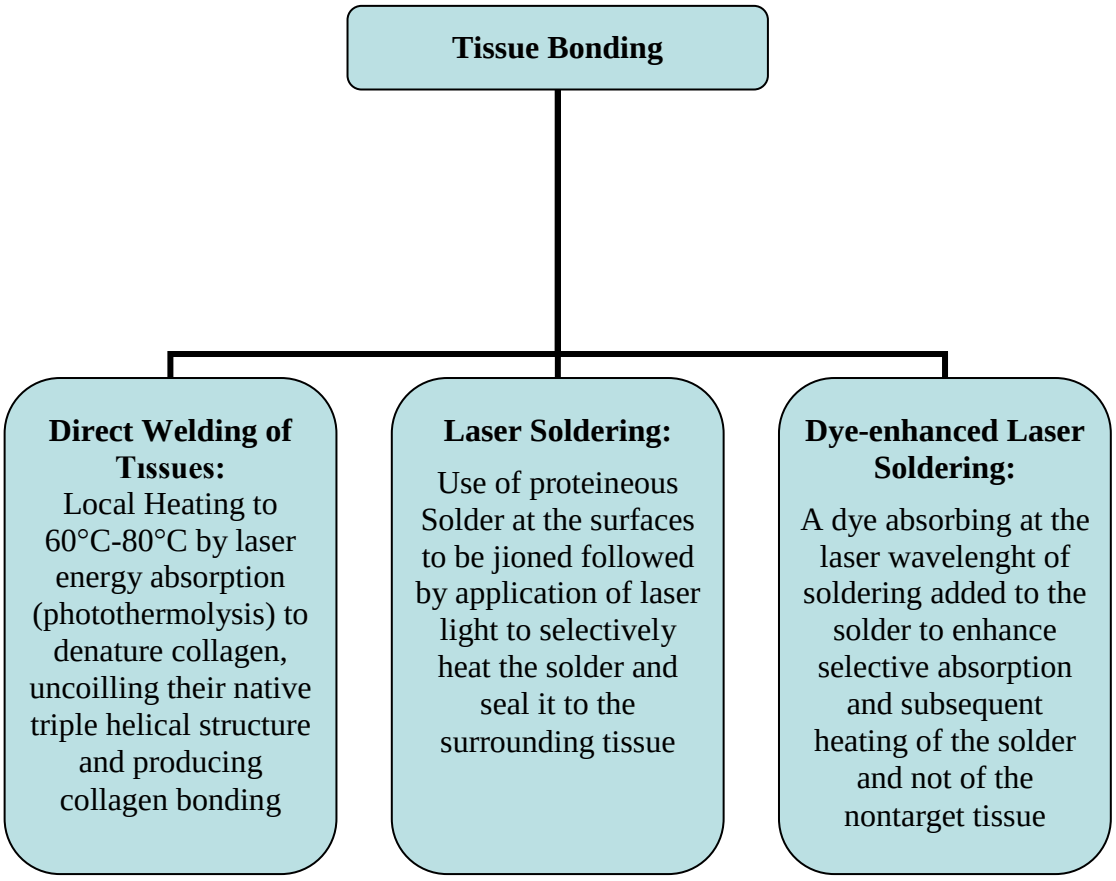
- Tissue Contouring and restructuring
- Tissue Welding
- Tissue Regeneration

Laser tissue welding is a process which utilizes use of laser energy in order to bond or join tissue. There are 3 major types of LTW:

- Direct Welding
- Laser Soldering
- Dye-enhanced Laser Soldering [1].

Table 1. 1 The approaches for Tissue Bonding

[The table adopted from (1)]



Laser tissue welding has shown promises in clinical applications as a possible alternative method for tissue incisions' closure. The technique is most applicable on soft tissues.

In 1979 Jain and Gorisch revealed the use of Laser Tissue Welding for the first time. They used Nd:YAG laser light in order to seal rat arteries.

Furter studies showed that laser light besides being used as a 'glue', when heated enough could denaturate collagen in tissue and thus initiate forming of new connections. First researchs of laser tissue welding included use of CO2 laser as its use depends on water, a major component in the body and it also absorbs firmly at its 10.6mm wavelength which causes shorter optical penetration depth (~13mm) thus restricting its use to supremely tenuous tissues [1].

Besides CO2 laser, Nd:YAG and argon-ion laser are also utilized for LTW. These lasers when compared to CO2 laser have deeper and more homogenized tissue heating.

When performing Laser Tissue Welding its important to take into consideration that CW lasers causes severe thermal side effects where pulsed lasers have the appeal that they can minimize collateral thermal damage.

The choice of an optimal laser wavelength and exposure parameters (energy, pulse duration, etc.) depends on the tissue absorption, optical penetration depth, and the thermal relaxation time in the tissues to be welded. The optical penetration depth obviously has to be matched with the extent of the thickness to be welded to provide homogeneous heating [18].

Table 1. 2 Penetration depth of some Medical Lasers in water and in Tissue

[The table taken from (7)]

Laser	λ (nm)	δ_{water} [53, 54]	δ_{tissue} [54, 55]
Argon ion	488	23 m	0.8 mm
KTP/Nd:YAG	532	16 m	1.1 mm
HeNe	632.8	4.8 m	3.5 mm
GaAlAs	780	60 cm	7 mm
	820	46 cm	8 mm
	870	25 cm	7 mm
Nd:YAG	1064	4 cm	4 mm
Nd:YAP	1080	5 cm	4 mm
Ho:YAG	2100	0.2 mm	1 mm
Er:YAG	2940	1 μm	$<$1 μm
CO_2	10600	10 μm	20 μm

The major advantages of the laser tissue welding are: direct watertight incision closure, less scar formation, and none of foreign-body reactions against the suture materials.

It does not require the insertion of foreign material in the tissue, problems related to undesirable reactions are avoided or eliminated and thus enabling faster wound healing and reduced operative time [18, 19].

Successful laser tissue welding primarily relies on factors, such as: the wavelength of laser, optical and thermal properties of tissues, spot size, laser power, exposure time, pulse duration and repetition rate [3]. The main disadvantages of the laser procedure are the possible thermal damage, determining the end-point of the procedure and lack of reproducibility [18].

Laser tissue welding is a photothermal process. Photothermal interaction occurs in a way that, laser (photon) energy, applied directly to the tissue surface is converted to the heat energy by molecular vibration of tissue chromophores such as water, melanin, hemoglobin by absorption (Figure 1.2). The rate of heat generation depends on the rate of absorption of photons within the tissue [31].

Scattered light that is absorbed may cause heating outside the laser beam. Increase in temperature to a certain degrees causes structural changes (interdigitation) in tissue proteins such as collagen and fibrinogen, in a way that they can bond each other in their open sites at the cooling phase can cause irreversible damage of the proteins of the tissue [4].

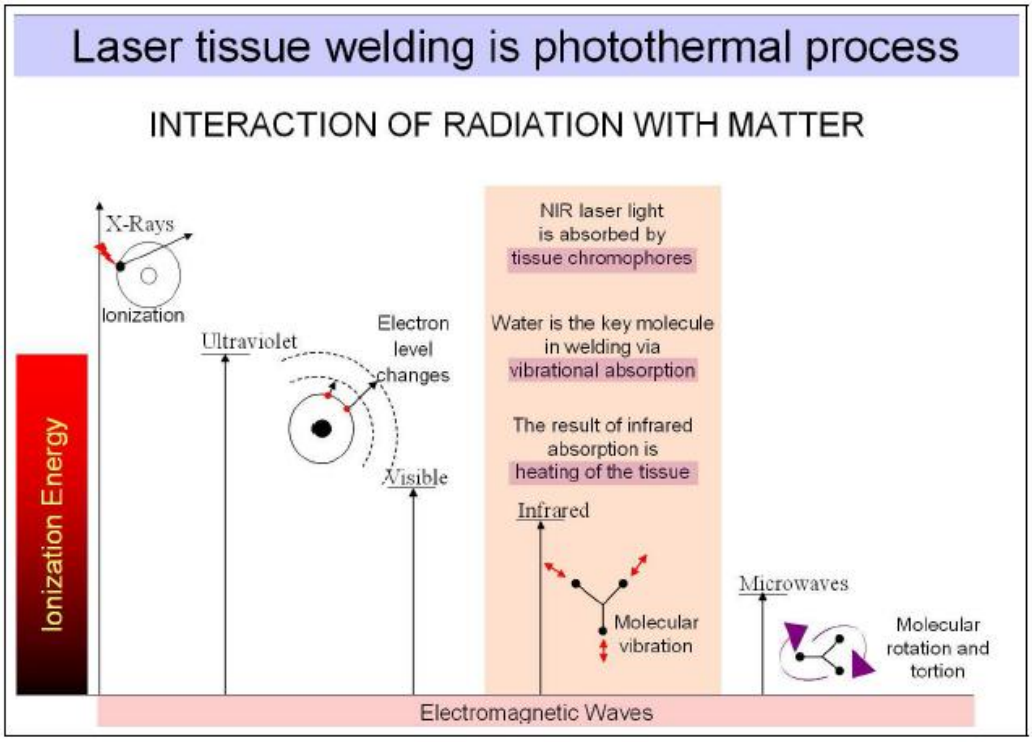


Figure 1. 2 Laser tissue welding is a photothermal process

[The figure taken from (12)]

IR or infrared lasers have frequency somewhere between visible light and microwave light. Thers lasers have adapted well and found use in different areas including tissue engineering because of their unique ability of bringing thermal apposition of tissue [17,13].

Longer high power irradiation of some lasers such as CO2, Ho:YAG, and Er:YAG laser, which are absorbed within 2-20mm at the tissue surface, can cause irreversible thermal damage of tissue. On the other hand, lasers which irradiate around 800nm and 1064nm have ability to penetrate deeper into tissue layers. Because of its optimal penetration in tissue and a good wound sealing as well as high absorption by water, 980nm wavelength is found to be mostly convenient for LTW [6].

980nm and 1064nm lasers among other lasers have been shown by many researchers to be good candidates for skin wound closure.

A 980nm diode laser is used as an alternative welding tool in dermatology becase of its, as priviously mentioned, good penetration in tissue and a fine wound sealing [14, 17]. A positive side of this laser is that is has high absorption rate by water in near infrared region [5].

On the other hand, 1064nm laser is used for skin welding to achieve deeper heating at the welding spot, an effort to create tight, full-thickness skin welds [2-4]. 1064nm laser can be used with or without albumin soldering [5].

Many studies have shown that 980nm and 1064 nm lasers are a good candiadates for skin wound closure. According to the studies done by Abergel *et al.*, back in 1986, skin wound could be effectively closed by Nd:YAG laser with reduced healing duration. In 2006, Tabakoglu *et. al.,* closed the skin incisions by 980nm diode laser and suggested that diode laser could be a good nominee for skin wound closure [5]. In 2009, Tabakoglu *et. al.,* showed that strong closure in skin tissue over joints can be obtained by 980 nm laser application. According to Tabakoglu *et. al.,in 2010,* low irradiance of a 980-nm laser was found reliable and strong with a minimum thermal damage. In 2011, Liming *et al.*, did a preliminary study to show how skin welding with a combination of two near-infrared diode lasers (980nm and 1064nm) can be a good candidate for incision closure [3]. Ahmad in 2012, performed human skin wound closure with 980nm diode laser.

.

1.4 Interaction Mechanisms and Effects of Laser Radiation

Laser tissue welding as a process based on irradiation can result in destruction and denaturation of three dimensional structure of a protein and thus cause an irreversible cell necrosis due to heat deposition and temperature increment [6].

One of the ways of preventing these unwanted reactions is constant control of accumulated heat by optimizing and frequent checking of laser parameters as well as the temperature of the irradiated tissue spot. How this process is hard to accomplish, laser tissue welding is stil not so present and used by medical institututions and its seen as an alternative technique.

A number of various laser-tissue interactions may happen when tissue is being irradiated with laser light. Different laser parameters as well as the various tissue properties increase the number of possible interactions. Some of the major optical tissue characteristisc which define the total tissue transmission at a specified wavelength are: absorption, coeddicient of reflection and scattering. Another properties of the tissue, such as thermal properties (heat capacity and conduction) also play an important role and contribute to the diversity of laser-tissue interactions. On the contrary, some of the main laser irradiation parameters are: wavelength, exposure time, applied energy, focal spot size, energy density, and power density [7].

There are mainly five different important laser-tissue interaction mechanisms observed on tissues: photothermal, photochemical, photoablation, plasma-induced ablation, and photo disruption (Figure 1.3).

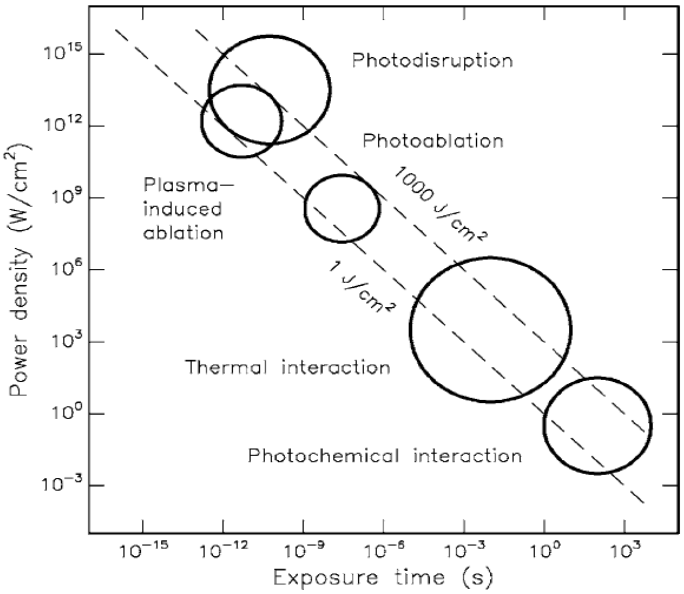


Figure 1. 3 Map of laser- tissue interactions

[The figure taken from (7)]

All of the laser-tissue interaction mechanisms are concerned on the deposition of delivered laser energy which is determined by, previously mentioned, *laser parameters* such as wavelength, spot size, pulse duration, exposure time and repetition rate, relaxation time etc., *optical tissue properties* such as anisotropy factor, absorption and scattering coefficients of a given tissue and, *thermal tissue properties* such as tissue density heat capacity, heat conduction [22-25]. Even though it seems how all of the mentioned interactions are very different, they all have one factor in common, their specific energy density, which is ranging between 1 J/cm2 and 1000 J/cm2. The only factor which actually separates these interactions from each other and controls them at the same time is the duration of laser irradiation [7].

Most of the laser applications involve thermal effects. One of the first notable thermal effects of irradiation on the biological tissue is the change of molecular conformation followed by bond collapse and membrane change. This process happens at 42◦C to 50◦C and is called hyperthermia. In case hyperthermia takes place for couple of minutes, the effect can be fatal, leading to serious tissue damage and irreversible tissue necrosis which is appliable in pathological tissue where growth of tumor can be retarded and eventually combatted. Above 50◦C the rate of enzyme action decreases whereas at 60◦C denaturation of three dimensional structure of a protein and collagen starts, leading to the tissue coagulation and irreversible cell necrosis. Water is the most abundant element in the human body thus present in all tissues and cells. At temperature of 100◦C water molecules in most of the tissues and cells start vaporizing and lead to the enhance of volume. This phase is also known as a transition phase where the moving energy of water breaks the hydrogen bonds and the water molecules become free and in the form of gas bubbles because of thermal and mechanical distruction of the tissue. If the temperature increases above 100◦C, carbonization will be notable and above 300◦C melting will take place (Table 1.3)

To make leaser surgery possible, one of the condition is to heat the biological tissue up to 100◦C. Once the tissue is heated, lasers are used to vaporize and cut tissues very accurately. Before any laser medical treatment is performed, it is required to be aware of possible damage distribution not just to the pathological tissue but also surrounding non-pathological tissue [21].

Table 1. 3 Thermal effects of laser radiation

[The table taken from (7)]

Temperature	Biological effect
37°C	Normal
45°C	Hyperthermia
50°C	Reduction in enzyme activity, cell immobility
60°C	Denaturation of proteins and collagen, coagulation
80°C	Permeabilization of membranes
100°C	Vaporization, thermal decomposition (ablation)
> 100°C	Carbonization
> 300°C	Melting

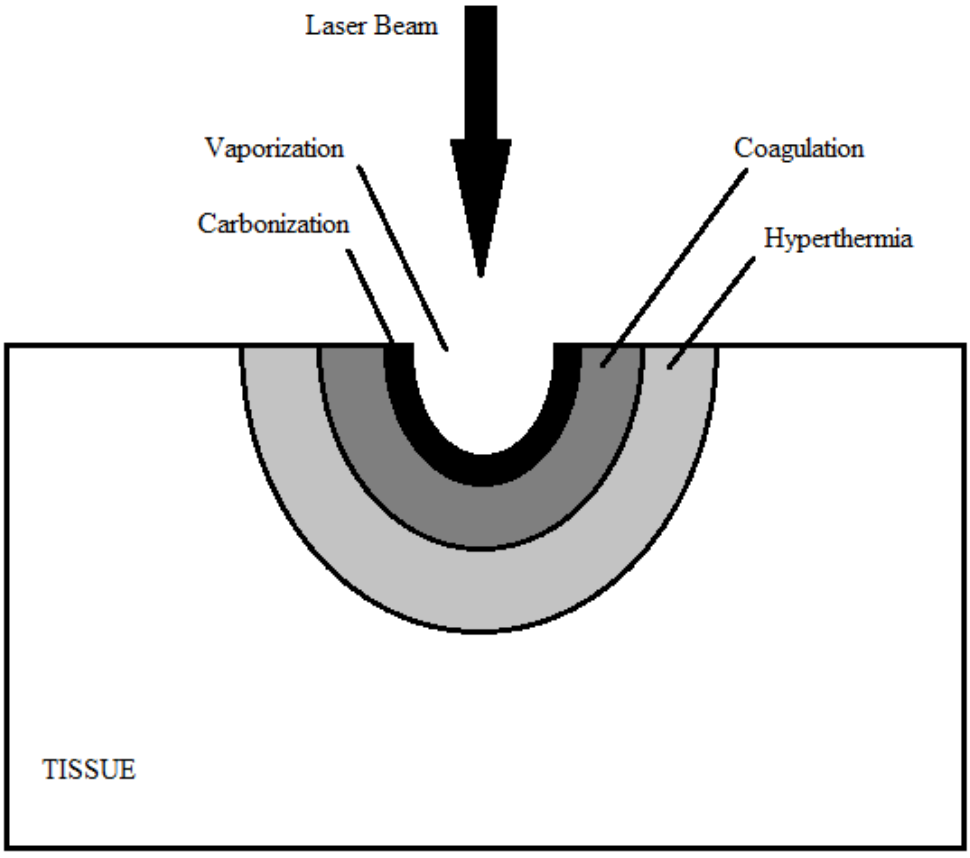


Figure 1.4 Thermal effects in biological tissue

[The figure adopted from (7)]

Many studies have revealed possible side effects of the lasers and tissue welding which are associated with thermal damages. For instance, pulsed modulations, which include exposure of the picoseconds domain, is likely to be another side reaction in the biological tissue. Some studies have also revealed that millisecond pulsed laser irradiation to the tissue will result in a tissue vaporization which further can backscatter a notable amount of the incident energy. Thus, the portion of tissue absorbed irradiation will be diminished [26]. Ho:YAG and Er:YAG which are used as dental lasers, are only used in a pulsed mode under application of a cooling water spray onto the location of treatment on the tooth because of the danger of overheating the pulp [7].

When it comes to the effects of tissue welding, Chen *et al.,* 2008 performed histological and modeling study of skin thermal injuries up to 2.0 micron laser irradiation confirming the laser's potential damages [28]. Studies by Margaret et al., in 2008, showed that the extent of thermal damages to skin could be determined by improved staining techniques. Intradermally focused laser pulses were applied by Khan and colleagues in 2005 to study thermal effects at defined tissue depth [28]. In 2013 Zhang and his team performed a study with 1064nm laser to compare laser-induced skin burn with thermal skin burn and they found similarity in thermal damage patterns [29].

Manstein and colleagues in 2009 studied the effects of multiple passes on the epidermal thermal damage pattern in nonablative fractional resurfacing by incubating the epidermal sheet in nitroblue tetrazolium chloride and showed that the average size of single lesions is dependent on the number of passings [30]. Inaki *et al.,* in 2010 did a comperative research in order to histologically evalluate the thermal side effects of laser irradiated tissues with different lasers. CO2, Er,Cr:YSGG and diode lasers were used. Results have showed notable variations in side effects between the irradiated tissues. Diode laser irradiared tissues were the ones with the highest amount of thermal side effects. Less damaged were tissues irradiated with CO2 and those irradiated with Er,Cr:YSGG had minimum damage. Furher results showed that tissues irradiated with Er,Cr:YSGG laser and water had less damage than those irradiated just with Er,Cr:YSGG, without water. Carbonization area was noticable in all CO2 and diode lasers iradiated tissues while the carbonization area in tissues irradiated with Er,Cr:YSGG with the use of water was negligible.

Samples irradiated with 2W with no use of water showed similarity in their morphology to the CO2 irradiated tissues in a CW mode. Insignificant carbonization area in samples irradiated with 4W was noticeable too. Macroscopis carbonization was not evident in Er,Cr:YSGG irradiated samples (2W) with a use of water [8].

As its difficult to avoid thermal damage, the only way to reduce it is by using lower wavelength lasers such as 980nm and 1064 nm where as higher wavelength results in higher absorption causing tisse damage [19].

1.5 980nm Laser application in Tissue Welding

As previously mentioned, 980nm laser emits wavelength which has quite high absorption rate by water and hemoglobin. Its absorption peak by melanin is not as high as the absorption peak of 780nm and 815nm diode lasers, which gives this laser an advantage of better penetration into deeper tissue layers, approximately 3mm to 4mm [9]. *In vitro* study performed by *Abod et al.*,had an aim to define wether 980nm diode laser is suitable for human skin wound welding or not. Even though this laser has showed to be a suitable and effective tool for the human skin wound welding, welds were not firm and tight enough to handle preassure of patient's movement and activities [20].

Another study was performed by *Tabakoglu et al.,* on the Wistar rat's skin. Results indicate that 980nm diode laser is a possible nominee for the tissue welding [10]. One more *in vivo* study was completed by *Tabakoglu et al.*,a comperative study between suture and leaser tissue welding effectiveness in wound healing. 1 cm long incision lines were made on the dorsal side of animals and closed with laser welding technique or suture. At 1st, 4th, 7th and 21st days wounds were removed and samples were prepared for histological analysis.

As epidermal thickness of saturated wounds is greater than those which were welded, it can be concluded that wounds closed with laser welding recover faster. It is also noticed that granulation area in sutured wounds was greater than in welded ones. The sudy has proved that 980nm diode laser is a good nominee for the LTW [11].

Research performed by Mark et al., utilizes 980nm in a cellular model of wound healing. Results indicate that limited doses of the light can increase the rate of cell growth within hours of light exposure [32,33].

1.6 1064nm Laser application in Tissue Welding

The 1064nm laser among all non-ablative laser sources holds the most prominent position. This is so because of the wavelength, which in terms of absorption, lies in an optical window that allows light of this wavelength to penetrate deep into the skin, while its absorption in a target such as a blood vessel or a hair follicle is strong enough to affect the target [34,35]. The 1,064-nm laser is also known to mainly produce photothermal effect on the irradiated skin due its penetrativity and it is easily absorbed by melanocyte [36]. Photothermal effect is also reported to be related to the model of action of laser; hence, 1,064-nm lasers with different action models have been applied in different fields [37].

Studies have been performed with 1,064-nm as a source to create a wound tissue environment to understand laser tissue interactions. Yi-Ming *et al.,* 2013 have performed a research to compare 1,064nm laser-induced skin burn and thermal burn by adopting 1,064-nm laser to induce skin damage, and to investigate photothermal effect on the irradiated skin. Several studies on skin burn have been performed [38–39]. In these studies, pressing a heated electric iron directly to the skin to create burn wounds of varying sizes has been proven to be the most effective way to study cutaneous wound healing and the therapeutic effect of drugs on wound healing [40]. A heated copper brass bar attached to an HQ soldering iron was used to induce thermal burns on skin, as a control to laser. The results reveal that the laser-induced burn injury intensified significantly in both horizontal dimension and in vertical depth with the prolongation of exporsure time. In addition, the laser is likely to injure the deep-seated tissues. Until 10th day, the laser induced dermal injuries as noted, were progressively more severe. Compared with the laser-induced skin burns, the thermally burned skin injuries did not show a clear development trend, or turned progressively severe with the passage of time after injury. The above results provide us with data about the injuring pattern and turnover in the skin and other tissues by the laser. It also pointed to the differences between the laser-induced skin burns and the normal thermal skin burns, establishing a sound foundation for treatment of laser burn [41].

1.7 Motivation and Objectives

Although conventional mechanical techniques are reliable in clinical practice, neither suturing nor stapling can provide leakproof closure. On the other hand, laser tissue welding is alternative to conventional mechanical closure techniques as it has the power to cauterizd as it cuts, vaporize the tissue, reduce the surgical trauma, reduce foreign body reaction, reduce bleeding and immediate watertight anastomosis. 980nm and 1064nm lasers among other lasers have been shown by many researchers to be good candidates for skin wound closure. A 980-nm diode laser is proposed to be an alternative welding laser in dermatology because of its optimal penetration in tissue and a good wound sealing where as 1064nm laser is used for skin welding to achieve the deep heating at the welding spot, an effort to create thight, full-thickness skin welds.

On the contrary, many studies have revealed possible side effects of the lasers and tissue welding which are associated with thermal damages. 980nm wavelength does not reach deeper lying and can result in excessive damage to the epidermis and other healthy skin structures. 1064nm wavelength leads to delayed healing period due to incomplete wound brindging and causes severe thermal side effects.

The aim of this study was to study side effects of 980nm and 1064nm wavelength pulsed modulation for which is know that causes milder thermal damages when compared to CW mode.

Objectives of the study:

1. To detemine the effectivness of 980nm and 1064nm wavelength pulse modulation;

2. To evaluate side effects of 980nm and 1064 nm wavelength pulse modulation;

3. To quantify thermal changes on tissues by determining and analysing hypothermal area, granulation and coagulation area;

4. To follow up the 7 days recovery period by measuring the length of the incision line at 4th and 7th day of sacrification in order to study laser tissue closure effectivness.

1.8 Hypothesis

980nm laser has good absorption in water and hemoglobin, allowing controlled tissue ablation and providing a bloodless field for most surgical procedures compared to 1064nm laser light which readily penetrates through the epidermis and reaches the dermis but often damages and chars surrounding tissue causing excessive bleeding with bigger hypothermal area.

Pulsed mode coagulation gives less thermal damage to the tissue when compared to CW mode coagulation, and in the case of 980nm and 1064nm lasers, we may expect 980 nm laser to have minimal scarring and fine quality healing with less hypothermal area than 1064 nm.

CHAPTER 2

MATERIALS AND METHODS

2.1 AKT-Epidermis Dual Wavelength Laser System

AKT Dual Wavelength Laser System for skin applications is an ultimate laser system with two wavelengths, 980nm and 1064nm, designed to produce radiation with maximum output power of 2watts. Optical apparatus, two-wavelength laser light by combining co-axis, the optical lens, and focusing on life optomechanical mechanism that sends transmission. Laser modules and optical apparatus, in a separate box, is based on an optical table. In the metal box the laser power supplies, electronic microprocessor and control cards, power-measuring unit are all located in the user interface. These wavelengths can be used individually or in a combined beam form (Figure 2.1-2.2).

In this study wavelengths were used individually as we were interested in the effects of 980nm and 1064nm laser pulse modulation.

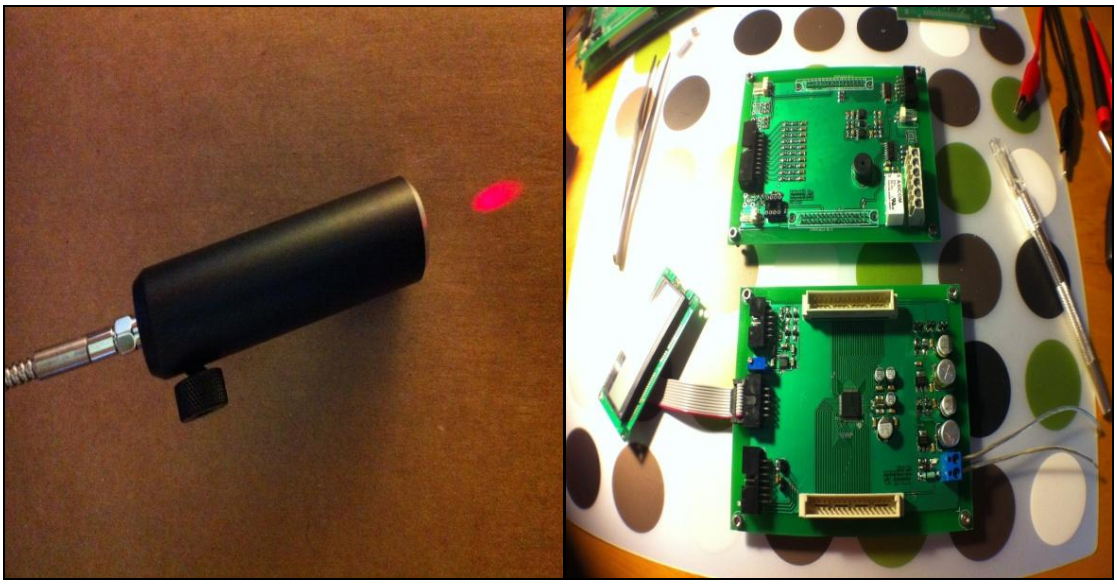


Figure 2. 1 AKT Epidermis dual wavelength system parts

Figure 2. 2 AKT Dual wavelength Laser System

2.2 Methodology

2.2.1 Animals

The research was carried out under a protocol approved by the Institutional Animal Research and Care Ethic Committee at Boğaziçi University, Istanbul. The study was conducted on eighteen (18) randomly selected, male and female Wistar rats, nan outbred albino rat, one of the most popular rats used for laboratory research. Animals were weighing 200-220g. Rats were housed in plastic cages in vivarium with the controlled temperature (22°±2°C).

The whole experimental procedure including the surgery and post-surgery procedure, irradiation, post-irradiation as well as wound excision and formalin fixation were performed in Boğaziçi University, Main Campus.

2.2.2 Surgery

18 Wistar rats, male and female were anesthetized with anesthesia solution containing Ketamine (75-100mg/kg) and Xylazine (10mg/kg) by intraperitoneal injection. Hair at the spot of application was shaved, more precisely dorsal region where 0.5cm full incisions were made along parallel but vertical axis on each rat's skin (0.75mm thick). As making incisions resulted in appereance of bleeding, the blood had to be removed and the incision spots had to be cleaned which was accomplished by compressing the site of incisions. This way potential light absorption by blood was prevented.

When the surgery was completed rats were divided into two groups (4^{th} day and 7^{th} day).

2.2.3 Post-surgery

Once the surgery was performed, the length and the thickness of the full incisions were checked by using digital calipe. Animals were not given any food or water for the next 24 hours. During 7 days, the healing progress of full incisions was observed where 4th and 7th day were chosen to be control days. Half of the animals were sacrificed at 4th and the other half at 7th day after the irradiation closure of the incisions.

On these days, tissue samples closed by AKT-Epidermis Dual Wavelength Lase System were collected and fixed in Formaldehyde Solutions (37% Ph.Eur.Bp Usp, Mercck KGaA 64271 Dermsdart Germany) for further histology testing.
At the end of the experimental procedure, all animlas were anesthetized again and killed.

2.2.4 Closure method (Laser irradiation)

Laser tissue welding or closure is a process that utilizes use of laser energy to join or bond tissues. As previously mentioned each incision was irradiated with AKT Dual Wavelength Laser System for skin applications which emits two wavelengths, 980nm and 1064nm.

Laser parameters are as follow:

1W for 10 seconds and 5J per spot for pulsed mode
(980nm) On Time= 050 Off Time= 050 Duration= 010s Current= 75%
(1064nm) On Time= 050 Off Time= 050 Duration= 010s Current= 80%.

These parameters were chosen according to the previous studies in this area as presented in the literature [14].

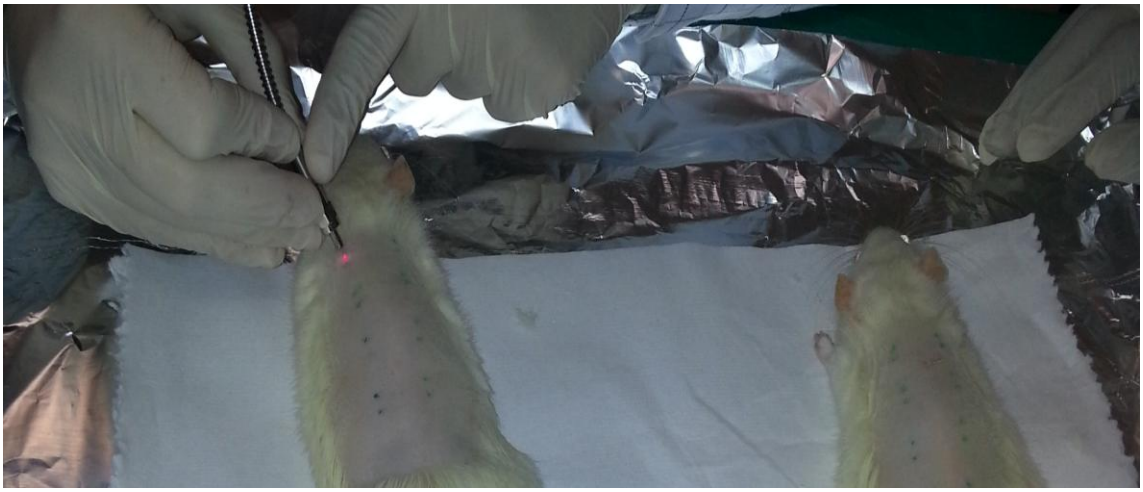


Figure 2. 3 Incision irradiation

Following irradiation closure of the incision, half of the animals were sacrificed at 4th day, and the rest at 7th day.

After, the wound sites excised with at least 5mm margin and fixed in Formaldehyde Solution (37% Ph. Eur. Bp Usp, Mercck KGaA 64271 Dermsdart Germany) and the tissue samples were stored in the solution at 4^{o} C

.

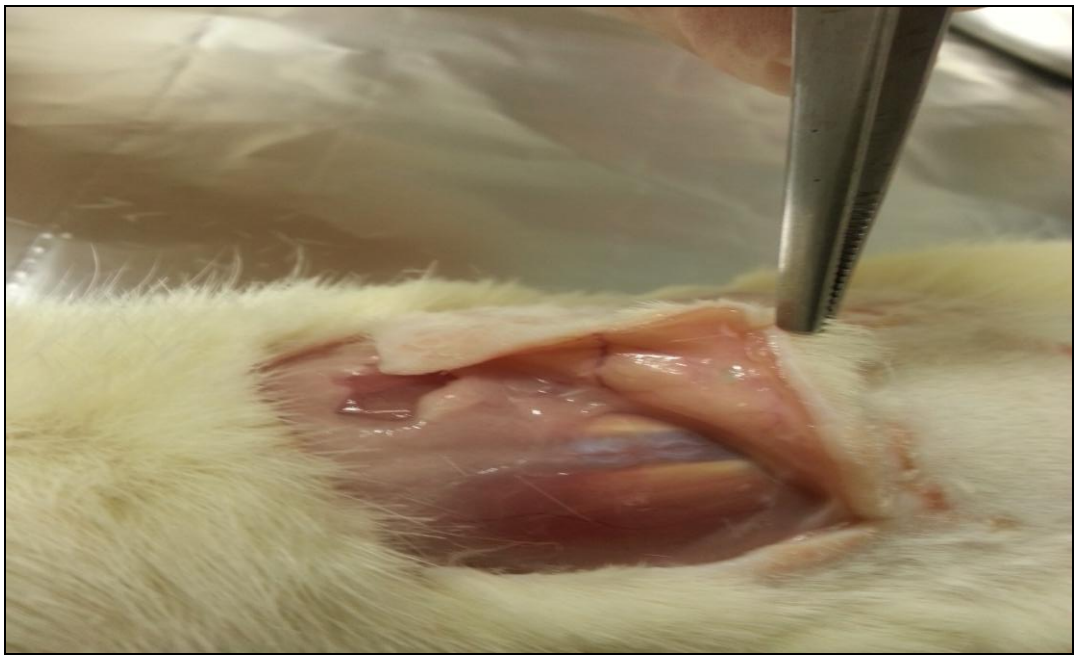


Figure 2. 4 Wound excision

2.4 Histology Examination

2.4.1 Tissue processing

Tissue processing involves the diffusion of different substances into and out of stabilize porous tissues. This way excess water is being removed from the samples and replaced with a substance that allows tissue cutting. Tissue samples have to be placed and supported by firm matrix that will make tissue cutting easier and which will allow cut of fine and thin sectiones, 5-10 μm thick.

Previously mentioned diffusion, is result of the thermodynamic tendency of processing reagents to assimilate concentrations inside and outide of the tissue blocks confirming to Fick's Law which states that the rate of solution diffusion through tissues is proportional to the concentration gradient (the difference between the concentrations of the fluids inside and outside the tissue) as a multiple of temperature dependant constants for specific substances.

From the above stated law it can be concluded that certain variables such as: operating conditions for instance temperature, the properties and concentrations of the reagents and the characteristics of the tissue play an important role in diffusion process [43].

As water is the most abundant element in the human body and thus present in all tissues and cells in different forms, free or molecular, the first thing in tissue processing that has to be done is dehydration. Some of the water is removed by blocking of tissue sections, the process known as embedding. As the process involves the use of different dehydrating substances for instance, alcohol, many cellular elements, such as proteins and some lipids, get dissolved.

Dehydration is a step required for more or less all infiltration techniques. Which dehydrant will be in use depends primarily on the task itself, technique and embedding medium. The ability of causing tissue shrinking is what separates and differes dehydrants. For instance in parafin wax method, samples are treated with 60%-70% ethanol, than prossesed through 90%-95% of the same dehydrant and at the end through an absolute ethanol after which the cleaning step starts. In case if tissue is not well fixed, it may be directly transfered into higher alcohols where the well fixed ones are placed into 95% ethanol.

The concentration of dehydrant is primarily dependent on the used fixative. If for instance, fixation is performed in a fixative like Carnoy's fluid, than dehydration will be performed in 100% ethanol.

General rule is that dehydration should not last for long, indeed, should be performed for a short period of time with the tissues being processed. Those blocks whose thickness is 1mm can stay up to 30 minutes in each alcohol, while those whose thickness is around 5 mm should stay for 90 minutes or even longer. If tissues are kept longer than required they may be harmed. The only exception is 70% ethanol in which samples may be kept indefinitely. Another dehydrants may be also used but in different concentrations.

Alcohols are the most common dehydrating agents. They are clear, without color and they are flammable. Besides their use in dehydration, they may be also used as a coagulation fixatives in tissue processing.

Table 2. 1 Tissue dehydration procedure

Dehydration Procedure		
ALCOHOL	70%	1-h
ALCOHOL	70%	1-h
ALCOHOL	80%	1-h
ALCOHOL	80%	1-h
ALCOHOL	90%	1-h
ALCOHOL	90%	1-h
ALCOHOL	100%	1-h
ALCOHOL	100%	1-h
XYLENE		1-h
XYLENE		1-h
PARAFFIN	60°C	1.5-h
PARAFFIN	60°C	1.5-h

Embedding is the process which includes tissue closure by different mediums, for instance gelatin, agar and wax. Once the medium soldifies, it will provide necesarry support for tissue cutting. The tissue is supported in a medium, allowing the technician to create even, accurate cuts without crushing or otherwise damaging the tissue. Samples of tissue can come from surgical biopsies, autopsies, and many other sources.

The choice of infiltrating medium depends on what kind of embedding media is in use. All tissue gaps should be filled with both medias, infiltrating and embedding. This way all tissue components will be provided with an adequate support. If a density of the medium is not approximetely equal to a density of tissue components, section deformity may occur. The matrix itself has to be elastic and sufficiently plastic in order to prevent and eventually recover some of the deformities caused during cuttin. Matrix's elasticity and plasticity also provides thin cutting.

The duration and temperature of the embedding process in partially determined by the liquefaction point of the medim and its viscosity.

Paraffin wax is a colorless, odorless and translucent polycrystalline mixture of alkanes with a raw form. It is produced during the refining of coal and mineral oils, especially crude oil and it was first time produced in 1867. Its hardness is primarily depedent on the molar mass of its components. At lower temperatures paraffin wex does not melt and it keeps its solid state. Paraffin wax's melting point may variate depending on molecular weight. Its typical melting point is between 39°C and 68 °C. Those with higher weights melt at higher temperatures, usually above 65°C. Paraffin wex whose melting poin is between 56°C and 58°C is usually used for histological studies.

This melting point makes parafin wax more viscous but as the temperature raises, its viscosity reduces.

It is generally suggested to use parafin wax 2°C above its melting point. If its necessry to reduce its viscosity or chnage infiltration of the tissue, it is recommended to increase the temperature as this will reduce the viscosity of the parafin wax.

Experimental Procedure

Thetissue samples which were previously formalin fixed were transferred to Kandiili campus, Boğaziçi University for the further histological examination.

The samples contained in plastic cassettes placed in tissue processing machine (Leica TP 1020) for 18h where dehydration, clearance and filling with paraffin took place through a series of processing in graded alcohol, xylene and praffin respectively.

The tissues were embedded in paraffin using paraffin embedding machine, Hot (Leica EG 1150 H) and Cold Plate (Leica EG 1150 C). The paraffin blocks were than stored in refrigerator for 24 hours to get well-solidified.

Sectioning: Once the excess water is removed and tissue samples placed and supported with a substance that will allow its cutting into thin sections, the process of sectioning may be performed. The device used for sectioning is called the microtome which is nothing but an extremly sharp knife which allows precise slicing (Figure 2.5).
The only important requirement for a fine and successful sectioning is a very sharp knife blade which has to be resharped to keep the sharpness. As the knife is incredibly sharp, it needs to be handled with caution.

Figure 2. 5 Microtome

The praffin blocked sample were cut into slices in 12 and 14 micron with Tissue Sectioning Machine RM 2255 and then collected on the slides and placed in the incubator for 24 hours to remove excess paraffin from the tissue.

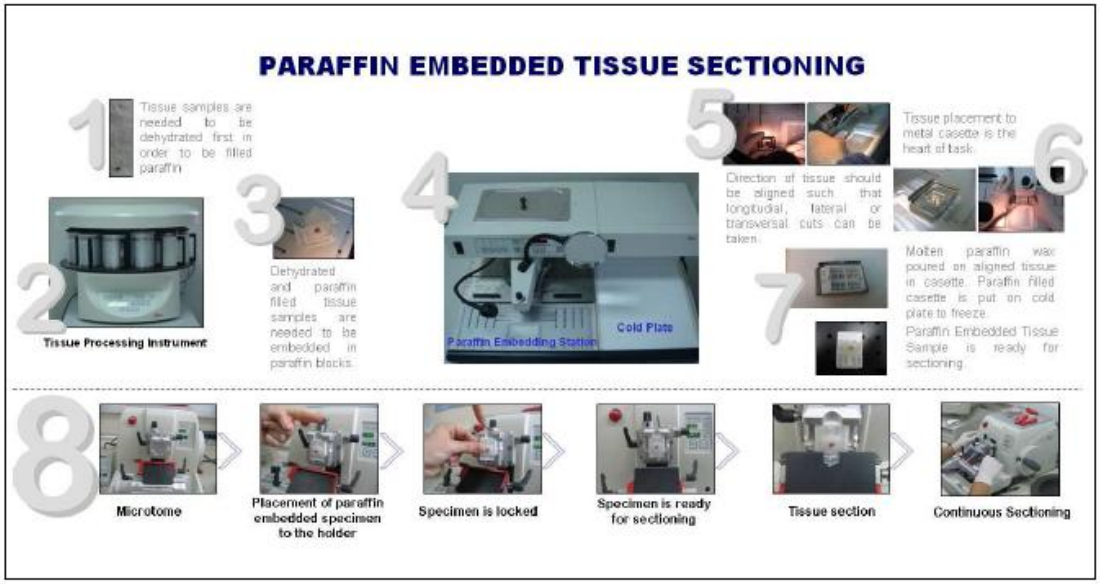


Figure 2. 6 Paraffin Embedded Tissue Sectioning Method

[The figure taken from (12)]

Staining: Staining is performed in order to to give contrast to the tissue. It may also be used to highlight certain areas and characteristics of the tissue. Hematoxylin and Eosin (H&E) staining was used for histological examinations. This stain is one of the main stains and it gives idea about general tissue structure. Hematoxylin stains nucle in blue and eosin stains other cellular components such as elastic and reticular fibers and cytoplasm in pink.

Protocol:

Reagents: Alcohol - HPLC Fisher A995-4 or histological A962

Eosin Y, disodium salt (Sigma #E-6003)

Harris Hematoxylin Stain, acidified (Lerner Laboratories #1931382)

Permount - Fisher SP15-100

Xylenes (Fisher #HC700-1GAL)

Solutions:

1. Eosin Y, 1 % aqueous

   Eosin Y dye 1 g

   Deionized water 100 ml

2. Harris Hematoxylin, acidified

   Filter (Baxter #F2217-150, Grade 363, Qualitative)

3. Tap water

90% and 100% Alcohol

Staining Procedure:

1. The sections were immersed in Xylene for 3 minutes where dipping was performed every 45 seconds.

2. The slides immersed in 100% alcohol for 2 minutes and then in 90% alcohol for 2 more minutes.

3. The slides were than dipped in tap water.

4. Then the slides were immersed in Hematoxylene stain for 1.5 minutes.

5. After removal from hematoxylene the slides were rinsed with tap water.

6. After rinsing, the slides were immersed in EOSIN stain for 2 minutes.

7. Slides were rinsed with tap water again.

8. The tap water continued to be exchanged until it became clear.

9. The very last dehydration took place in alcohol solutions (90% &100%).

10. At the end, slides were cleared with Xylene.

11. Coverslip was places onto a labeled glass.

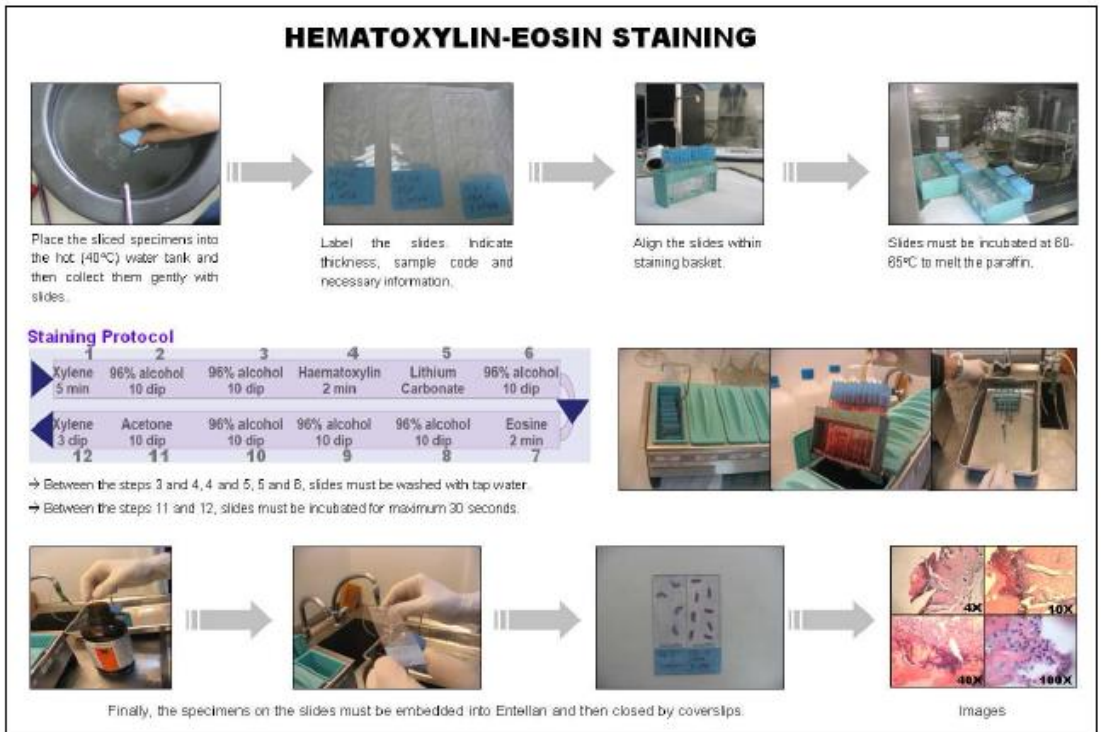


Figure 2.7 H&E staining method

[The figure taken from (12)]

Microscopic Examination was carried out under light microscope (Nikon), 4x magnifications and high resolution images were captured with Nikon camera and Imaging software (NIS Elements D 2.30) was used to quantify parameters which gave idea on how successfully an incision closed was by analysing thermal effects/hypothermal area, granulation and coagulation area and measuring the length of the incision line.

Statistical Analysis: Histological comparison between two groups (hypothermal area, granulation and coagulation area and the length of the incision line) followed by the arithmetic mean, standard deviation and Student T-test in order to test for statistical differences in the set of data at p= 0.05 level for 980nm and 1064nm wavelenght pulsed mode groups.

CHAPTER 3

RESULTS

As mentioned in the beginning animals were irradiated with 980nm and 1064nm laser individually and divided into two groups, 4^{th} day and 7^{th}. As the aim was to to study side effects of both, 980nm and 1064 nm wavelength pulsed modulation for which is known that it causes milder thermal damages when compared to CW mode, histological comparison between two groups was done by quantifying thermal changes on tissues by determining and analysing hypothermal area, granulation and coagulation area as well as the length of the incision line.

Histological analysis results indicate that closure of incision line was better and thighter at 7th day post-irradiation than at 4th. The incision line length and hypothermal area were less in samples irradiated with 980nm laser than those irradiated with 1064nm laser. The same case was with a coagulation area. Granulatiom area though present in samples of both groups, was more conspicuous in those irradiated with 1064nm laser.

Table 3. 1 980nm and 1064nm side effects indicators on 4th day after irradiation

(L-length of the incision line; A-C- coagulation area; A-G- granulation area; A-HT- hypothermal area)

4th day after irradiation								
LASER	980nm				1064nm			
Parameters	L(µm)	A-C(µm2)	A-G(nm2)	A-HT(µm2)	L(nm2)	A-C(µm2)	A-G(µm2)	A-HT(µm2)
Sample1	2599,58	213237,67	228711,74	84172,05	9552,18	107375,35	358088,62	207352,95
Sample2	2664,07	300237,43	207112,51	41868,11	3941,74	313599,22	496872,51	642910,27
Sample3	1912,55	140742,81	230595,49	5975,77	2954,3	213599,1	377876,05	214492,46
Sample4	2883,83	203327,09	342083,4	73510,99	3346,8	401722,15	519927,65	2212730,55

Table 3. 2 980nm and 1064nm side effects indicators on 7th day after irradiation

(L-length of the incision line; A-C- coagulation area; A-G- granulation area; A-HT- hypothermal area)

7th day after irradiation								
LASER	980nm				1064nm			
Parameters	L(µm)	A-C(µm2)	A-G(µm2)	A-HT(µm2)	L(µm2)	A-C(µm2)	A-G(µm2)	A-HT(µm2)
Sample1	1584,6	12589,4	184789,41	79214,39	3002,32	429602,64	478206,15	114294,2
Sample2	2146,39	111932,76	184280,44	57135,86	3425,4	299135,47	591345,56	1220303,22
Sample3	1821,75	80474,01	170955,8	49157,51	846,81	35055,83	45080,77	57358,88
Sample4	1607,52	269192,18	59978,07	0	1576,37	32911,31	277867,5	167341,53

Table 3. 3 Arithmetic Mean; Standard Deviation; Student T-Test for 980nm and 1064nm for 4th day after irradiation

4th day of irradiation								
LASER	980-nm				1064-nm			
Parameters	L(µm)	A-C(µm2)	A-G(µm2)	A-HT(µm2)	L(µm)	A-C(µm2)	A-G(µm2)	A-HT(µm2)
Arithmetic Mean	2515,008	214386,25	252125,785	51381,73	4948,755	259073,96	438191,208	819371,558
Standart Deviation	419,6654	65618,609	60910,6956	35199,9972	3095,6804	127019,61	82013,5843	950970,271
Student T-Test	0,206753	0,5310276	0,01403477	0,19988366				

Table 3. 4 Arithmetic Mean; Standard Deviation; Student T-Test for 980nm and 1064nm for 7th day after irradiation

7th day of irradiation								
LASER	980-nm				1064-nm			
Parameters	L(µm)	A-C(µm2)	A-G(µm2)	A-HT(µm2)	L(µm)	A-C(µm2)	A-G(µm2)	A-HT(µm2)
Arithmetic Mean	1790,065	118547,09	150000,93	46376,94	2212,725	199176,31	348124,995	389824,458
Standart Deviation	260,4547	108649,72	60356,0134	33429,5509	1206,1941	198046,89	240034,211	555470,945
Student T-Test	0,511351	0,6090785	0,18279364	0,30092096				

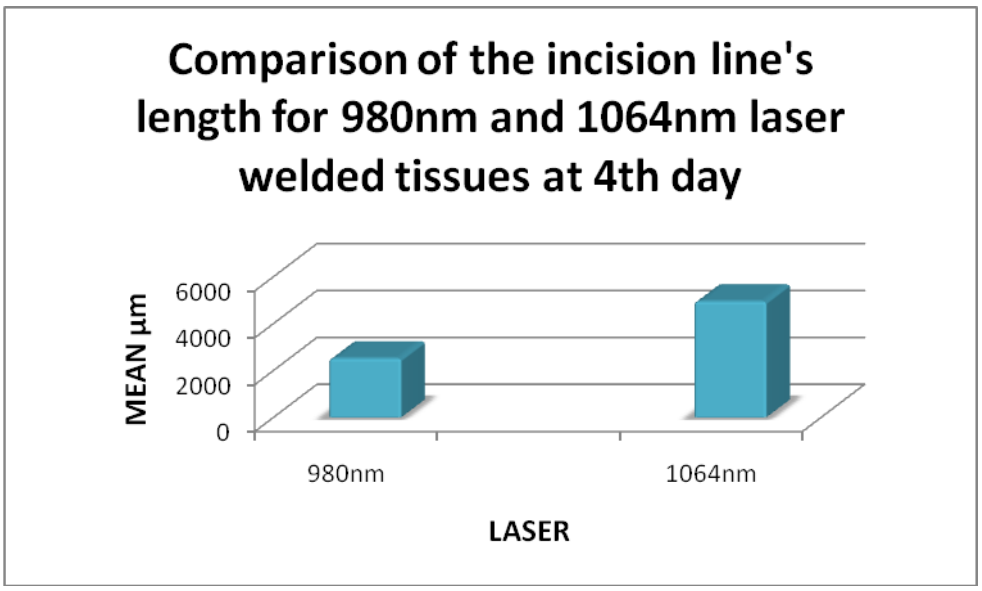


Figure 3. 1 Graphical representation of Arithmetic Mean for the incision line's length at 4th post-irradiation day for 980nm and 1064nm laser

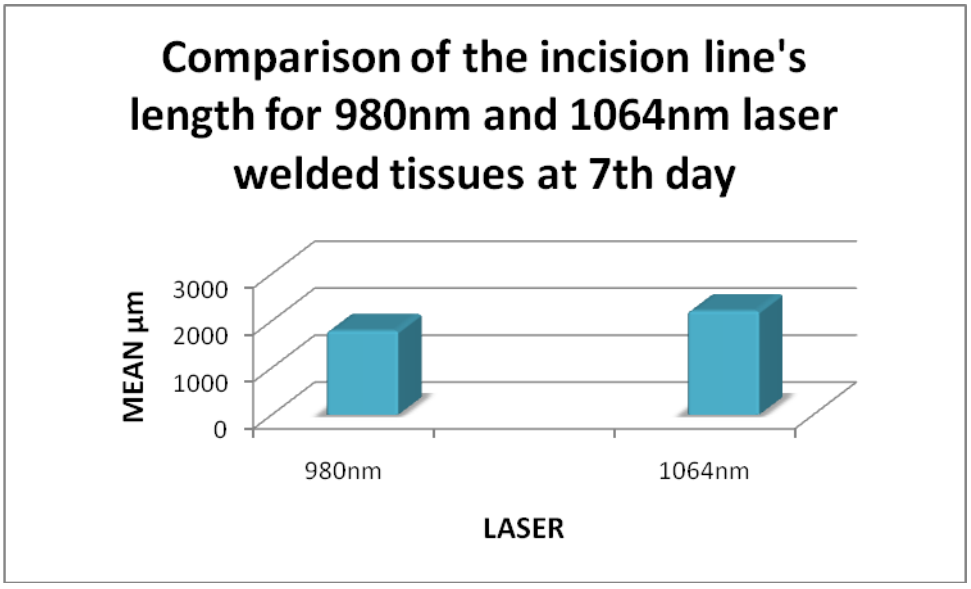


Figure 3. 2 Graphical representation of Arithmetic Mean for the incision line's length at 7th post-irradiation day for 980nm and 1064nm laser

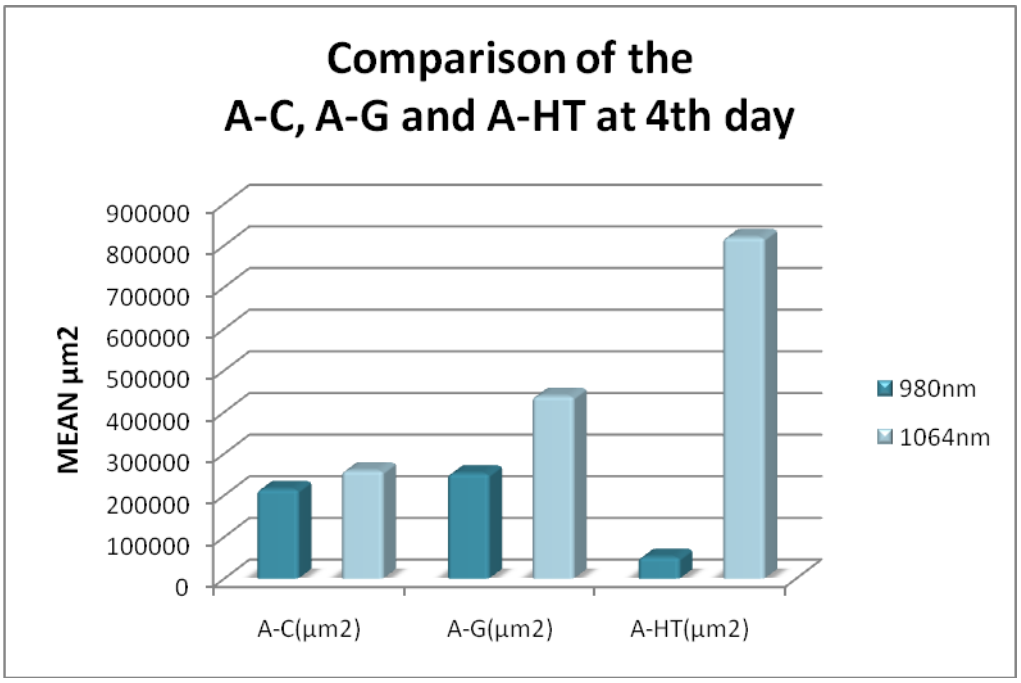


Figure 3. 3 Graphical representation of Arithmetic Mean for the A-C, A-G and A-HT at 4th post-irradiation day for 980nm and 1064nm laser

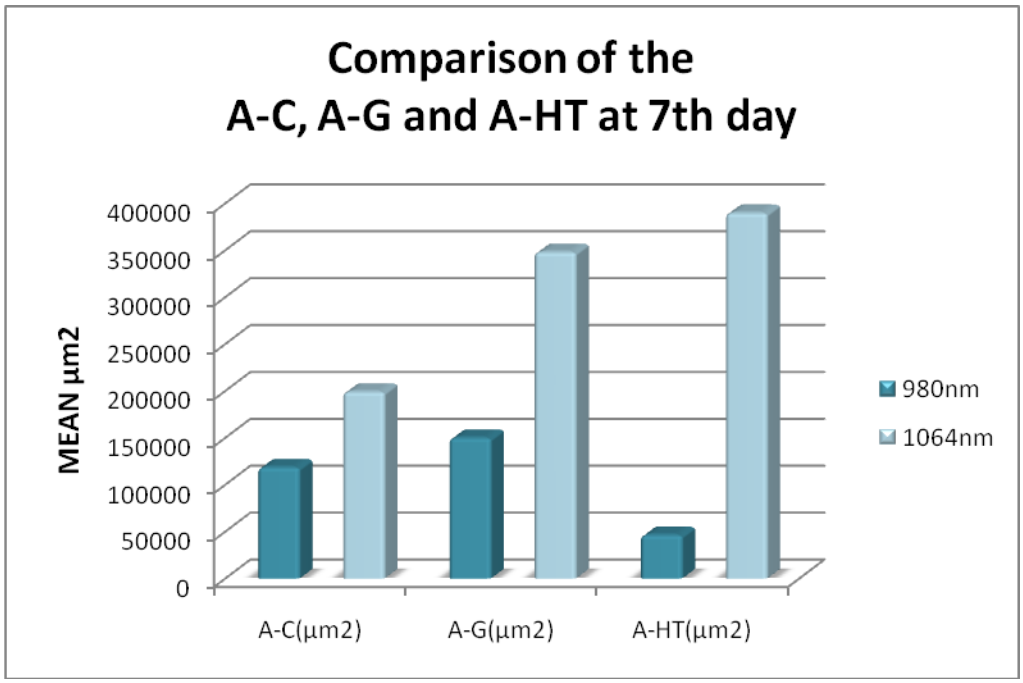


Figure 3. 4 Graphical representation of Arithmetic Mean for the A-C, A-G and A-HT at 7th post-irradiation day for 980nm and 1064nm laser

As the aim was studying the side effects of both, 980nm and 1064nm wavelength pulse modulation lasers, a histological comparative study was completed by quantifying thermal changes in tissues by determining and analysing hypothermal area, granulation and coagulation area as well as the length of the incision line. The areas of interest were photographed and compared.

Histological analysis showed that wound closure for both lasers at 4th day post-irradiation was not fully completed, in other words, there were stil some visable openings though the closure was tighter in samples treated with 980nm laser. The wound closure for both lasers was better at 7th day post-irradiation.

Arithmetic mean of the incision line's length at 7th day post-irradiation in both groups (980nm irradiated and 1064nm irradiated samples) was less than the mean at 4th day post-irradiation which indicates that wound closure is stronger and thighter at 7th day post-irradiation. The incision line's lenght in samples irradiated with 1064nm laser was longer than in ones irradiated with 980nm laser but statistically there was no significant difference between arithmetic mean of the incision line's lengths at 4th and 7th day bwtween these two lasers as $p>0.05$.

Thermal alterations were observed in samples treated with both lasers. They were histologically assessed by measuring the thermal area, hypothermal area and granulation area. Graphical representation of arithmetic mean for the A-C, A-G and A-HT at 4th and 7th post-irradiation day for 980nm and 1064nm laser is shown on figures 3.3 and 3.4. Histological investigation showed a notable tissue alteration in irradiated regions in both 980nm and 1064nm laser. Side effects were more noticeable at 4th day post-irradition for both lasers thus more present in samples irradiated with 1064nm laser.

All the thermal changes reduced at 7th day in both groups. Again, there was no noticable significant statistical difference found in side effects mean at 4th and 7th day for both lasers except for the A-G (granulation area). A-G mean at 4th day in 980nm and 1064nm irradiated samples was found to differ significantly ($p<0.05$). The same patteren was not observed at 7th day.

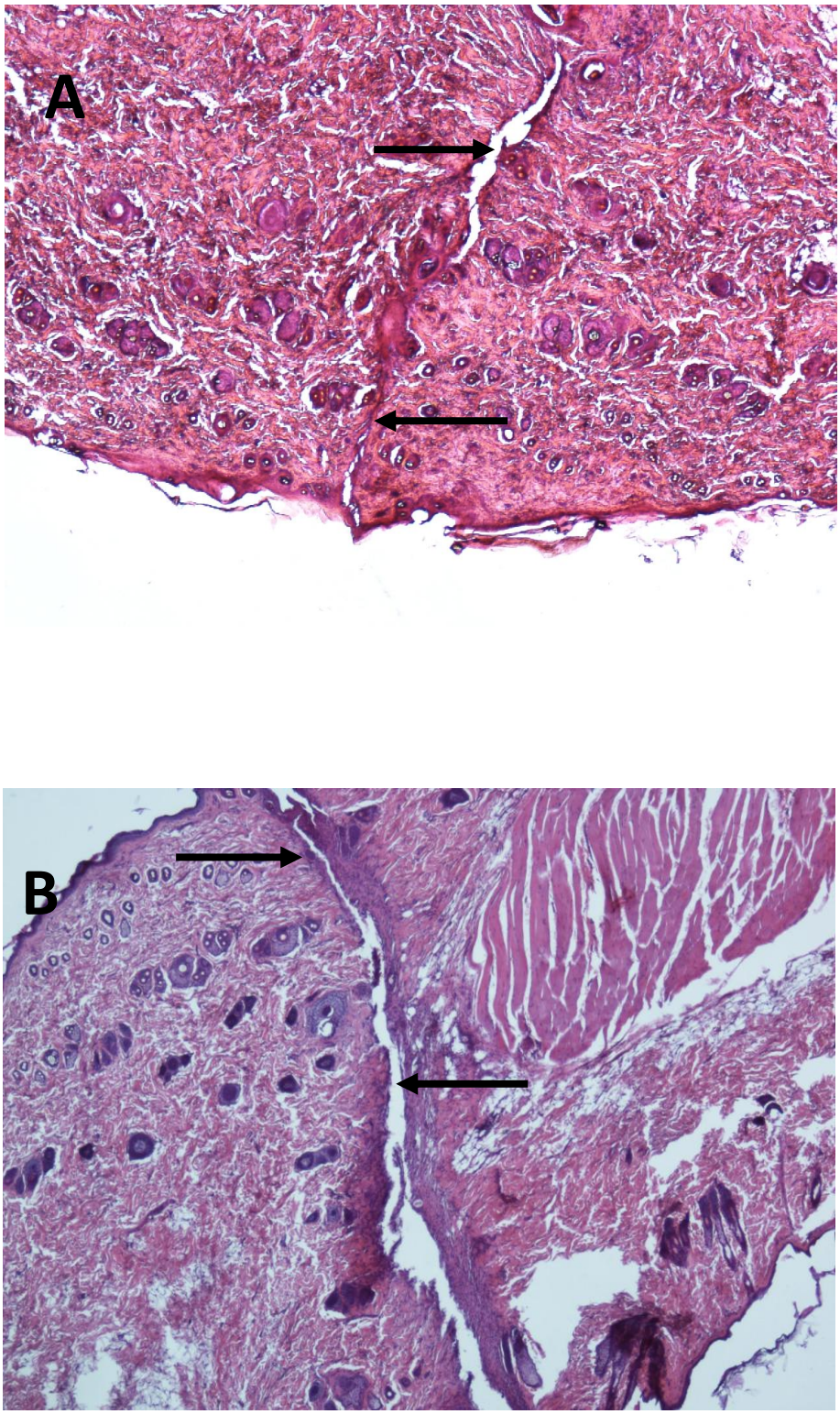


Figure 3.5 a The incision line appereance at 4th day post-irradiation

A-samples treated with 980nm; B-samples treated with 1064nm

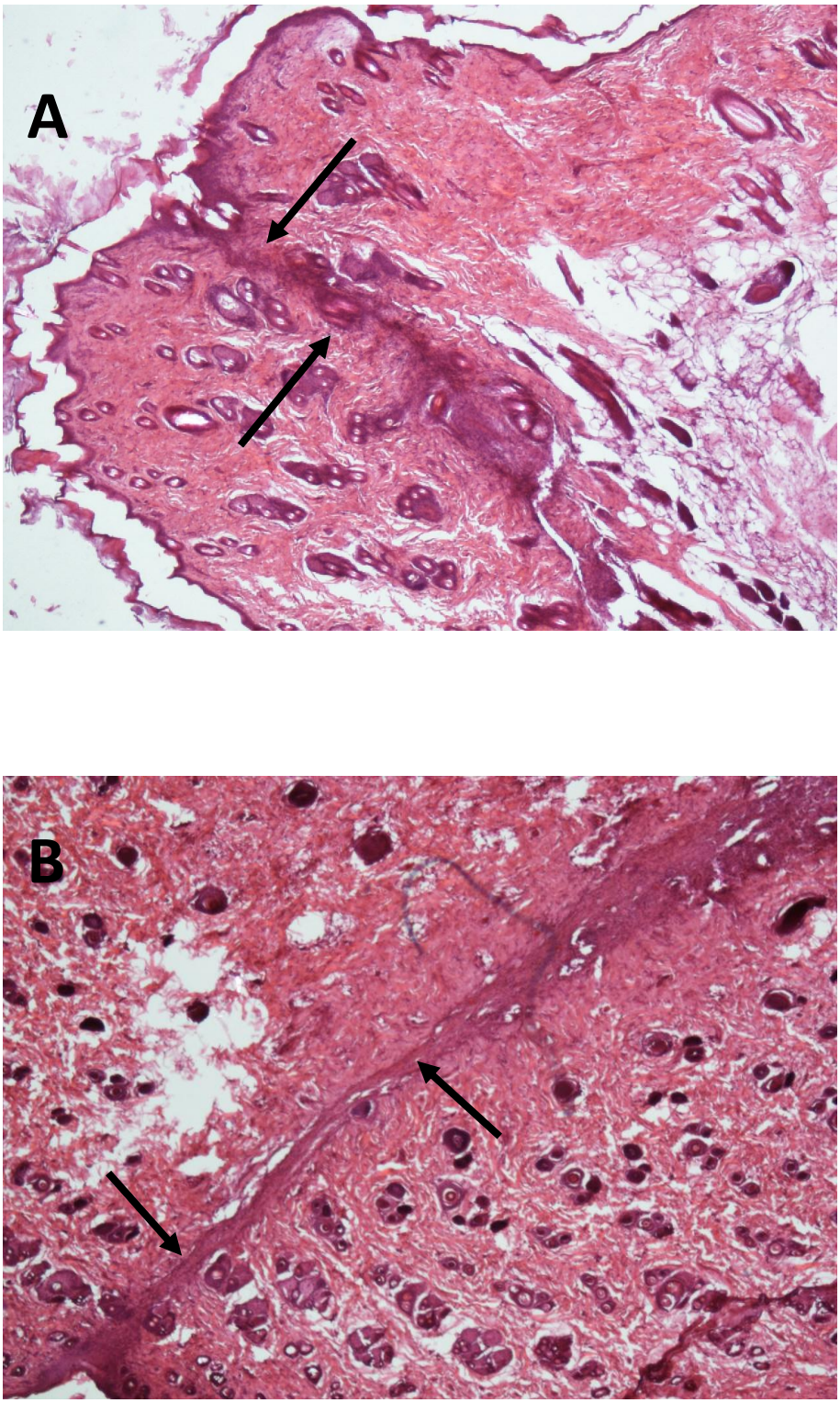


Figure 3.5 b The incision line appereance at 7th day post-irradiation

A-samples treated with 980nm; B-samples treated with 1064nm

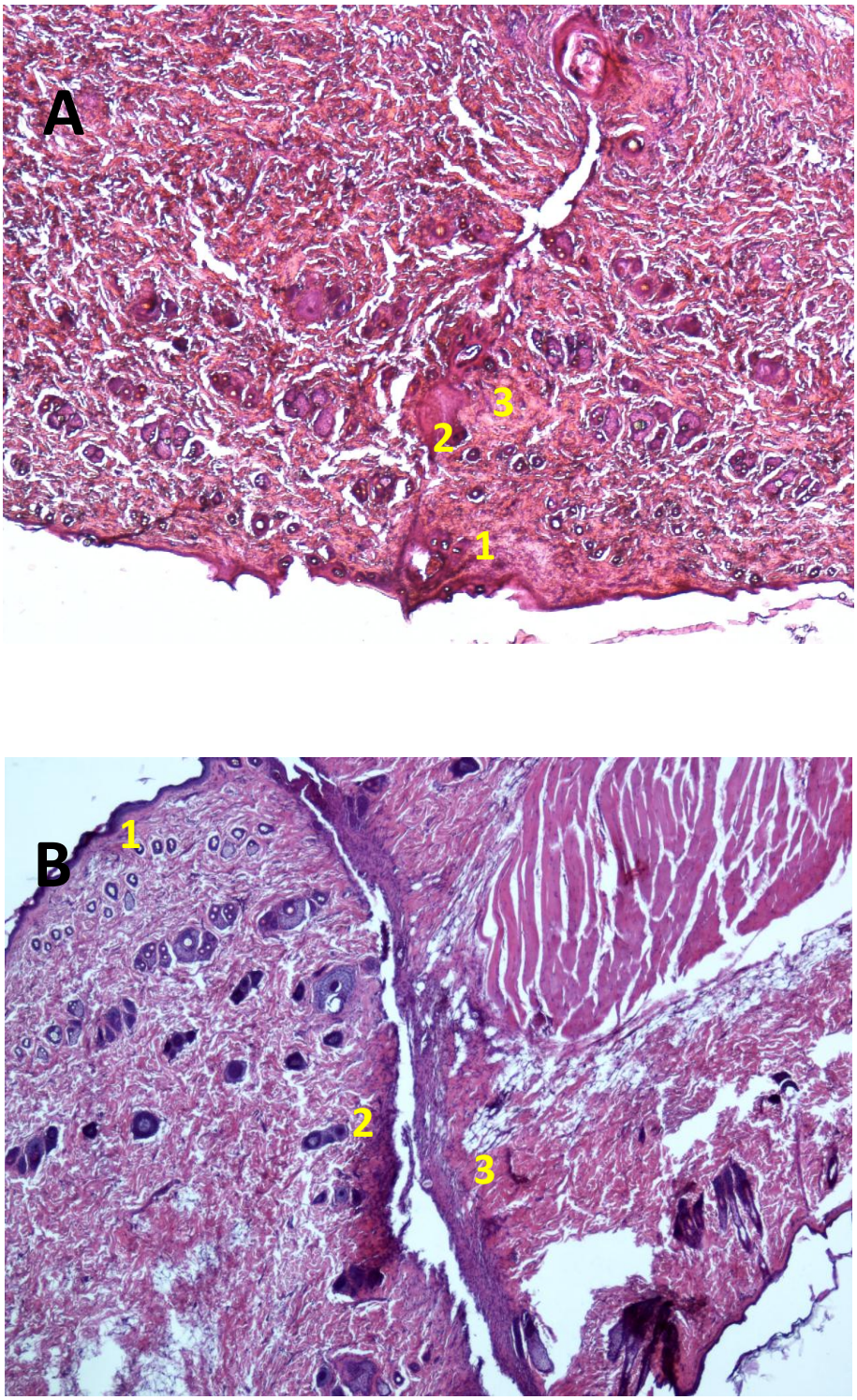


Figure 3.6 a The A-C (1), A-G (2) and A-HT (3) appereance at 4th day post-irradiation

A-samples treated with 980nm; B-samples treated with 1064nm

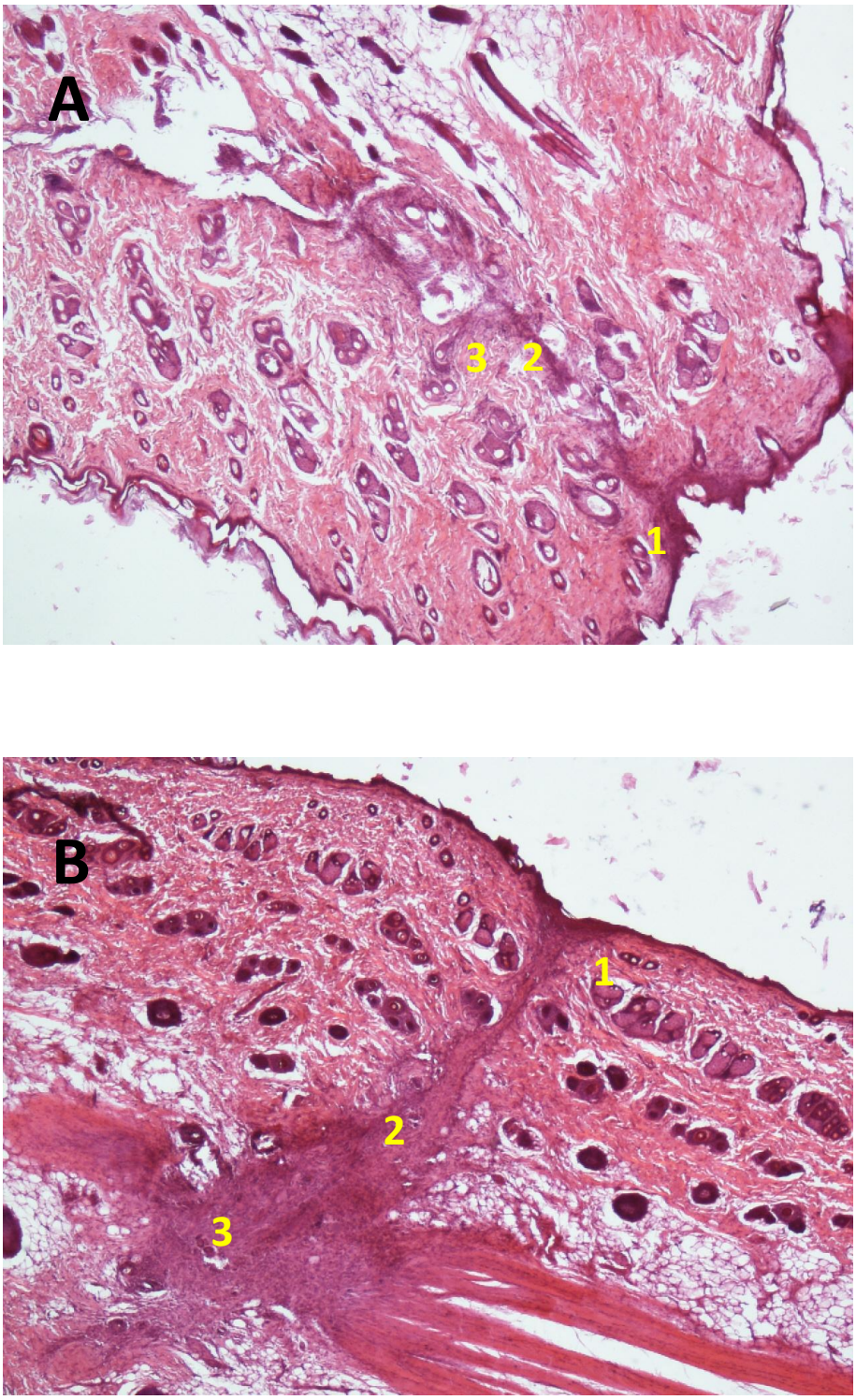


Figure 3.6 b The A-C (1), A-G (2) and A-HT (3) appereance at 7th day post-irradiation

A-samples treated with 980nm; B-samples treated with 1064nm

CHAPTER 4

DISCUSSION

Pulse mode coagulation gives less milder thermal damage to the tissue when compared to CW mode coagulation. One of the main advantages of pulsed operated lasers is their capacity of producing very short pulses with a very high intensity whose duration can range from couple of milliseconds to several femtoseconds [1]. Laser tissue welding (closure of skin incisions) with 980nm and 1064nm laser was performed by many researchers. The individual side effects, on the rat skin tissue, of these two wavelengths which have different penetration power have been studied.

980nm laser has good absorption in water and hemoglobin, allowing controlled tissue ablation and providing a bloodless field for most surgical procedures compared to 1064nm laser light which readily penetrates through the epidermis and reaches the dermis but often damages and chars surrounding tissue causing excessive bleeding with bigger hypothermal area. The absorption peak of 980nm laser by melanin is not as high as the absorption peak of 780nm and 815nm diode lasers, which gives this laser an advantage of better penetration into deeper tissue layers, approximately 3mm to 4mm [9]. A comperative *in vivo* study between suture and leaser tissue welding effectiveness in wound healing was completed by *Tabakoglu et al,*. As epidermal thickness of saturated wounds is greater than those which were welded, it can be concluded that wounds closed with laser welding recover faster. It is also noticed that granulation area in sutured wounds was greater than in welded ones. The sudy has proved that 980nm diode laser is a good nominee for the LTW [11].

The 1064nm laser among all non-ablative laser sources holds the most prominent position. This laser is also known to mainly produce photothermal effect on the irradiated skin due its penetrativity and it is easily absorbed by melanocyte [36]. Photothermal effect is also reported to be related to the model of action of laser; hence, 1,064-nm lasers with different action models have been applied in different fields [37].

As the objective of the research was to evaluate side effects of 980nm and 1064 nm wavelength pulse modulation, a histological comparative study was completed by quantifying thermal changes in tissues by determining and analysing hypothermal area, granulation and coagulation area in the region adjacent to the irradiated site from either side of the incision line. Also, as the length of the incision line was measured.

The results have showed that arithmetic mean for coagulation area (A-C) and hypothermal area (A-HT) (μm2) does not differ significantly as $p>0.05$ for 4th and 7th days but with higher mean for 1064nm laser at 4th and 7th day. Reduction of previously mentioned areas was noticed at 7th day for both lasers but with higher arithmetic mean for 1064nm laser (figure 3.3&3.4). Thus it can be concluded that side effects diminish by time for both lasers.

Granulation area (A-G) at 4th day in 980nm and 1064nm irradiated samples was found to differ significantly ($p<0.05$). The same patteren was not observed at 7th day where the reduction of granulation area was noticed in both groups.

Moreover, histological analysis have showed that wound closure for both lasers at 4th day post-irradiation was not fully completed, in other words, there were stil some noticeable openings (figure 3.5a). The complete wound bridging for both lasers was noted at 7th day post-irradiation though the closure was tighter in samples irradiated with 980nm laser (figure 3.5b).

Arithmetic mean of the incision line's length at 7th day post-irradiation in both groups (980nm irradiated and 1064nm irradiated samples) was less than the mean at 4th day post-irradiation which indicates that wound closure is stronger and thighter at 7th day post-irradiation (figure 3.1-3.5b). The incision line's lenght in samples irradiated with 1064nm laser was longer than in ones irradiated with 980nm laser and but stll there was no significant statistical difference between arithmetic mean of the incision line's lengths at 4th and 7th day between two groups ($p>0.05$).

Though we had expected some statistical differences between samples irradiated with 980nm and 1064nm laser operated in pulse mode, T-test results did not confirm our assumptation. Histological analysis did show some visable differences between two groups yet these were not statistically sifnificant, except A-G at 4th day.

Many laser tissue welding studies were performed with these two lasers (980nm and 1064 nm) and proved that both of them are possible candiadates for a welding closure. As 980nm laser has better absorption in water than 1064nm, it allows controlled ablatation, it was expected for it to provide less damage of surrounding tissue as well. Our results did not confirm these facts, and there was no significant difference found between samples threated with these lasers, except A-G at 4th day.

The whole study suggests that skin wound closure can be achieved with both, 980nm and 1064nm laser with no significant statistical difference in side effects (A-C, A-G and A-HT) at 4th and 7th day post-irrasiation.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

980nm and 1064nm lasers operated in pulse mode have shown to be good nominees for skin wound closure. Side effects in both groups caused by the laser irradiation were noticed at 4th and 7th days after wound closure but more present at 4th day in samples irradiated with 1064nm laser. Reduction of A-C, A-G and A-HT was noticeable at 7th day in both groups. The reduction in incision line's length has also been noticed at 7th day. Therefore, it can be concluded that the side effects, results of 980nm and 1064 nm irradiation, get smaller with time. Everything being taken in account, we may conclude that both lasers were effective in wound closure with both of them causing side effects with no significant statistical difference between them at 4th and 7th day post-irradiation except A-G at 4th day.

For further studies it is recommended to:

- Perform reserch on the greater number of samples;
- Determine side effects at molecular level;
- Determine the Closure Index (CI);
- Determine the tensile strength of the welded wounds (welded with 980nm and 1064nm pulse mode laser);
- Determine the effectiveness of 980nm and 1964nm pulse mode laser in closure of human skin wounds as well as the side effects.

REFERENCES

[1] Parasan N.P., (2003). "Introduction to Biophotonics", First Edition, Wiley-Interscience., New Jersey.

[2] Scherr D. S., and Poppas D. P., (1998). " Laser tissue welding", Urol. Clin. North Am. 25, (1), 123–135.

[3] Bass L. S., and Treat M. R., (1995). " Laser tissue welding: A comprehensive review of current and future clinical applications", Lasers Surg. Med. 17, (4), 315–349.

[4] Scherr D. S., and Poppas D. P., (1998). " Laser tissue welding: a urological surgeon's perspective", Haemophilia. 4, (4), 456–462.

[5] Welch A. J., , Torres J. H., , and Cheong W. F., (1989). " Laser physics and laser tissue interaction. ," Tex. Heart Inst. J. 16, (3), 141–149.

[6] Hasim O., T., Nermin T., Murat G., (2010). "The effect of irradiance level in 980-nm diode laser skin welding", Photomedicine and Laser Surg. 28, (4), 453-458.

[7] Markholf H.N., (2007). "Laser-Tissue interactions", Second Edition, Springer., New York.

[8] Rudiger P., (2008). "Encyclopedia of laser physics and technology", First Edition., John Wiley and Sons., New Jersey.

[9] Cilesiz I., Thomsen S., , Welch A. J., , and Chan E. K., (1997). " Controlled temperaturetissue fusion: Ho:YAG laser welding of rat intestine *in vivo*, part two", Lasers Surg. Med. 21, (3), 278–286.

[10] Tabakoglu., Zeynep D., K., (2005). "Tissue welding with 980-nm diode laser system: preliminary study for determination of optimal parameters", Photonic Therapeutics and Diagnostics.

[11] Murat G., Zeynep D., Hasim O.T., Ozguncem B., (2006). " Closure of skin incisions by-nm diode laser welding", Lab. Med. Sci. 21, 5-10.

[12] Tabakoglu H.O., (2010). " Skin welding with near infrared lasers: Investigations of the optimal parameters", PhD thesis.

[13] Gobin A. M., , O'Neal D. P., , Watkins D. M., , Halas N. J., , Drezek R. A., , and West J. L., (2005). " Near infrared laser tissue welding using nanoshells as an exogenous absorber", Lasers Surg. Med. 37, (2), 123–129.

[14] Gulsoy M., , Dereli Z., , Tabakoglu H. O., , and Bozkulak O., (2006). " Closure of skin incisions by 980-nm diode laser welding", Lasers Med. Sci. 21, (1), 5–10.

[15] Tabakoglu H. O., and Gulsoy M., (2010). " *In vivo* comparison of near infrared lasers for skin welding", Lasers Med. Sci. 25, (3), 411–421.

[16] Nathaniel M. F., Joseph T W. Jr., (1999). "Cryogen spray cooling during laser tissue welding", Phys. Med. Bio. 45, (3), 111-119.

[17] Lynette M. B., (2003). "Welding of Skin using Nd:YAG Laser with Bipolar Contact Applicators", PhD thesis.

[18] Kyunghan K., and Zhixiong G., (2004). "Ultrafast Radiation Heat Transfer in Laser Tissue Welding and Soldering", Bioengineering Conference 20, (2), 185-168.

[19] Small W., (1998). "Thermal and Molecular Investigation of Laser Tissue Welding", PhD thesis.

[20] Ahmed A. A., (2012). " Human Skin Wound Welding Using 980 nm Diode Laser: an in Vitro experimental studies", Iraqi J. Laser, Part B, 11, 9-20.

[21] CFR Chapter I, Subpart J, Part 1040 - Performance Standard for Light Emitting Products., (2014). The Food and Drug Administration requirement document for light emitting products, USA.

[22] Goldman, L., (1997). "Application of the Laser", *CRC Press*.

[23] Videocassette., (1994). "High Powered Lasers in the Lab. Interactive Media Communications ".

[24] Goldman, L., (1997). "Application of the Laser", *CRC* Press.

[25] Pressley, R. J., (1997). "CRC Handbook of Lasers", *CRC* Press.

[26] CFR Chapter I, Subpart J, Part 1040 - Performance Standard for Light Emitting Products (2013). The Food and Drug Administration requirement document for light emitting products, USA.

[27] Chen B, Thomsen SL, Thomas RJ, Oliver J, Welch AJ., (2008). ”Histological and modeling study of skin thermal injury to 2.0 microm laser irradiation”, Lasers Surg Med. 40, (5), 358-70.

[28] Khan M. H., Anderson R. R., Sink R. K., Manstein D., and Eimerl D., (2005). ”Intradermally Focused Infrared Laser Pulses: Thermal Effects at Defined Tissue Depths”, Lasers in Surgery and Medicine 9999:1–11.

[29] Zhang Y. M., Ruan J., Xiao R., Zhang Q., Huang Y. S., (2013). “Comparative Study of 1,064-nm Laser-Induced Skin Burn and Thermal Skin Burn”, Cell Biochem Biophys 67, (3), 105-114.

[30] Manstein D, Zurakowski D, Thongsima S, Laubach H, Chan H., (2009). “The effects of multiple passes on the epidermal thermal damage pattern in nonablative fractional resurfacing”, Lasers Surg Med. 41, (2), 149-153.

[31] Clark, G., Parker, E., Schaad, J. & Warren, W. J., (2009). “New measurements of previously unknown large interplanar spacings in natural materials”, J. Amer. Chem. Soc. 57, (8), 25-32.

[32] Dierickx C. C., (2006). “The role of deep heating for noninvasive skin rejuvenation”, Lasers Surg. Med. 38, (9), 799-807.

[33] Mark D. S. and Scott C., (2006). “Effects of Infrared laser exporsure in a cellular model of wound healing Lasers ”, Lasers Surg. Med. 38, (9), 699-703.

[34] Anderson R. and Parrish J., (1981). “The optics of human skin”, J InvestDermatol 77, (2), 13-19.

[35] Lukac M. and Grad L., (2007). “Scanner Optimized Aesthetic Treatments with the VSP Nd:YAG Lasers”, J Cosmet Laser Ther 10, (2), 112-115.

[36] Rosenbach, A., Williams, C. M., Alster, T. S., (1997). “Comparison of the Q-switched alexandrite (755 Nm) and Q-switched Nd:Yag (1064 Nm) lasers in the treatment of benign melanocytic nevi”, Dermatologic Surgery 23, (4), 239–244.

[37] Parker, J., & Hamzavi, I., (2009). “Q-switched Nd:YAG 1064-nm laser for the treatment of acne-Induced postinflammatory hyperpigmentation”, J Am Acad Dermatol 60, (5), 198.

[38] Dang, Y. Y., Ren, Q. S., and Liu, H. X., (2005). “Comparison of histologic, biochemical, and mechanical properties of murine skin treated with the 1064-Nm and 1320-Nm Nd:Yag lasers”, Experimental Dermatology 14, 876–882.

[39] Chen, B., O’Dell, D. C., Thomsen, S. L., (2005). “Porcine skin Ed50 damage thresholds for 2,000 Nm laser irradiation”, Lasers in Surgery and Medicine 37, 373–381.

[40] Coolen, N. A., Vlig, M., van den and Bogaerdt, A. J., (2005). “Development of an in vitro burn wound model”, Wound Repair and Regeneration 16, 559–567.

[41] Yi-MingZ., Jing R., Rong X., Qiong Z., Yue-Sheng H., (2013). “Comparative Study of 1,064-nm Laser-Induced Skin Burn and Thermal Skin Burn”, First Edition, Springer., New York.

[42] Karn J. H., (2013). “Laser operation mode”. Laser Therapy Diowave, American Society of Laser Medicine and Surgey.

[43] Rolls G., (2008). “101 Steps to Better Histology”, Melbourne, Leica Microsystems., Wetzlar.