Abdullahi Ibrahim UBA

CLOSURE OF SKIN INCISION BY DUAL WAVELENGTH (980 AND 1064 nm) LASER APPLICATION

M.S.
2014

FATİH UNIVERSITY

Institute of Biomedical Engineering

Master of Science in
Biomedical Engineering

CLOSURE OF SKIN INCISION BY DUAL WAVELENGTH (980 AND 1064 nm) LASER APPLICATION

By

Abdullahi Ibrahim UBA

ISTANBUL/JANUARY 2014

T.C.
FATİH UNIVERSITY
INSTITUTE OF BIOMEDICAL ENGINEERING

CLOSURE OF SKIN INCISION BY DUAL WAVELENGTH (980 AND 1064 nm) LASER APPLICATION

ABDULLAHI IBRAHIM UBA

MSc THESIS
BIOMEDICAL ENGINEERING PROGRAMME

THESIS ADVISOR
ASST. PROF. DR. HAŞİM ÖZGÜR TABAKOĞLU

İSTANBUL/JANUARY 2014

T.C.
FATİH ÜNİVESİTESİ
BİYOMEDİKAL MÜHENDİSLİK ENSTİTÜSÜ

DERİ KESİLERİNİN CİFT DALGA BOYLU 980 VE 1064nm LAZER UYGULAMASİ İLE KAPATILMASI

ABDULLAHI IBRAHIM UBA

YÜKSEK LİSANS TEZİ
BİYOMEDİKAL MÜHENDİSLİĞİ PROGRAMI

DANIŞMAN
YRD. DOÇ. DR. HAŞIM ÖZGUR TABAKOĞLU

İSTANBUL/ OCAK 2014

T.C.

FATIH UNIVERSITY

INSTITUTE OF BIOMEDICAL ENGINEERING

Abdullahi Ibrahim Uba, a MSc. student of Fatih University **Institute of Biomedical Engineering** student ID 520112027**, successfully defended the** thesis **entitled "CLOSURE OF SKIN INCISION BY DUAL WAVELENGTH (980 AND 1064 nm) LASER APPLICATION",** which he prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below**:**

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

Jury Members : Asst. Prof. Dr. Haşim Özgur TABAKOĞLU.....
Fatih University

Asst. Prof. Dr. Sukru Okkesim.....
Fatih University

Prof. Dr. Ata AKIN .....
Bilgi University

Date of Submission: 26th December, 2013

Date of Defence : 20th January, 2014

To my families: Malam Abdullahi Maisaje's trend (my father's) and that of Alhaji Sani spikin (my mother's), both emanated from Makwarari , Kano

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

ACKNOWLEDGEMENTS

I have the necessity to praise and thank Almighty ALLAH for everything he makes possible throughout my life. My parents, Alhaji Ibrahim Uba Abdullahi and Hajiya Halima Sani Spikin remain the people I have always been short of words to express my gratitude to, for the proper up-bringing they have ensured, guidance, disciplines, supports and loves they gave and continue giving to me throughout my life and for their patience with me over a long period I was way during the course of my studies. I really feel and therefore appreciate the love and supports received from all my relatives during the period with special support from siblings, Mubasshir Ibrahim Uba, Isah Ibrahim Uba, Rabi'u Ibrahim Uba, Najashi Ibrahim Uba, Hassan Ibrahim Uba and Hussain Ibrahim Uba.

The inspirational caring and indeed, warmest motivation received from H. S. Bichi during the course of the program is felt and highly appreciated , thus deserves a to be acknowledged

The countless academic supports and research grooming provided by my highly respected and inspiring teacher, my humble thesis advisor, Asst. Prof. Dr. Haşim Özgur Tabakoğlu, would continue to be felt forever. He spared his time, energy and resources for ensuring that this thesis research was successful and completed at the right time. I am therefore very much indebted to him.

The Institute of Biomedical Engineering has done a lot to me academically; exposed me deeply to proper understanding of multidisciplinary field of science, Biomedical Engineering which is why I deem it necessary to offer many thanks humbly, especially to the head of the institute, Prof. Dr. Sadik Kara and my motivating teachers Asst. Prof. Dr Şukru Ökkesym, Asst. Prof Dr. Saime Akar and Halil Ibrahin Çakar.

Special regard goes to Mahir Balik, Gradutae Institute of Science and Engineering for taking time to format this dissertation to the standard.

I would like to acknowledge the technical contribution of Boğazici University Vivarium section staffs, especially that of the head of the section, Dr Arzu Temizyüret and Ersin Eruz and for provision and preparation of the study animals and for looking after them during the healing period following laser irradiation and also to the institute of Biomedical Engineering Biophotonic laborotory of the same university for allowing me access to their devices for Histological examination, right from tissue processing steps up to staining and microscopic examination. I appreciate the guidance of Mr Hakim Solmaz and Nermin Tapaloğlu for that complicated task.

I would like to offer a special gratitude to my brothers and friends as well , K. A. Madatai, S. I. Abdullahi and U. B. Isah as well as very important personalalities, D.A. Alhassan, Muhammad Abba, Mustapha Dandawaki, B.A Aliyu, and Mukhtar Auwal.

My academic colleagues, U. A. Abdullahi and M. M. Sani are central to the success of this research work by virture of their physical participation right from the start up to the end of the work; thus remain my partners in progress. A. A. Wamakko and N. I. Nuhu, undergraduate students of Banking and Finance and Business administration respectively served as my housemates in Istanbul, their advices, support and encouragements are highly recognized and duly appreciated.

Finally, thanks to all family members, friends, well-wishers, home and abroad whose list of names is too long to fit into this limited number of pages, nevertheless, their words of inspirations are being felt and well registered.

January 2014 Abdullahi Ibrahim

TABLE OF CONTENTS

Page

LIST OF SYMBOLS

C	Specific heat capacity
Cp	Heat capacity
D	Optical diffusion distance
Ea	The activation energy,
g	Anisotropy factor incorporating the effects of directionally dependent
h	Convection coefficient
k	Thermal conductivity of tissue
$\vec{n}$	Direction of the heat flux
R	The universal gas constant,
r	Radial distance from the source
S	Surface of the interface
T	Temperature
t	Time
P	Power of the light source
λ	Wavelength
Ω	Omega
ρ	Density of tissue
μa	Absorption coefficient in tissue
μ_{eff}	Effective attenuation coefficient on the tissue
μs	Scattering coefficient
μ's	Reduced scattering coefficient
ΔT	Difference between the inner and the outer temperature
δ	Distance
$\emptyset_{Conv}$	Heat flux through the surface
∑	Sum
±	plus or minus
^{o}C	Degree celceus

ABBREVIATIONS

AKT : Ali Kaan Tabakoğlu
CUR : Current
CW : Continuous Wave
DUO : Dual wavelength mode
Er:YAG:Erbium Ywittrium Aluminium Garnet
IgA : Immunoglobulin A
IgG : Immunoglobulin G
OfT : Off Time
OnT : On Time
Nd:YAG: Neodymium Ywittrium Aluminium Garnet
TTA : Thermally Altered Area
UV : Ultraviolet

LIST OF FIGURES

LIST OF TABLES

SUMMARY

CLOSURE OF SKIN INCISION BY DUAL WAVELENGTH (980 & 1064nm) LASER APPLICATION

Abdullahi Ibrahim UBA

Biomedical Engineering Programme

MSc Thesis

Advisor: Asst. Prof. Dr. Haşim Özgür TABAKOĞLU

Laser welding has proven to be effective in clinical applications as a potential alternative technique for closure of tissue incisions.Thermal effect of dual wavelength (980 & 1064nm) laser application in skin incision closure was assessed on 12 male and female Wister rats. Incisions (0.5cm-long) were made along vertical plane on the dorsal region of shaved 220-250g Wister rats. The incisions closed by laser irradiation at 1W and exposure time, 5 seconds, irradiance level, 31.83W/cm^2 in Continuous wave mode (CW) and 1W and exposure time, 10 seconds in pulsed mode to deliver a total energies, 5J and 10J per spot onto the incision respectively.. Animals from each group were sacrificed at 0th, 4th and 7th day and the sample excised for histological analysis using H&E stain. The thermal effect assessed by measuring the most extensive thermally altered area in the adjacent region to the irradiated site on either side and by closure capability. Mean thermally altered area (TTA) (μm^2) of CW and pulsed mode treated groups was found to differ significantly ($p>0.05$) at 0th and 4th days with higher mean value from CW mode group than pulsed mode group in both periods while no significant difference ($p<0.05$) was statistically found at 7th day post-irradiation. Moreover, tighter closure was observed with CW group at 7th day. In conclusion,1W, 5 seconds and 5J CW mode application of 980 & 1064nm combined beam form in skin incision closure was found to have minimal thermal alteration which appeared histologically reversed at late healing period and absolute wound healing capability.

Keywords: Dual wavelength laser, CW mode , pulsed mode, thermal alteration

ÖZET

DERİ KESİLERİNİN CİFT DALGA BOYLU 980 VE 1064nm LAZER UYGULAMASİ İLE KAPATILMASI

Abdullahi Ibrahim UBA

Biyomedikal Mühendisliği Programı

Yüksek Lisans

Danışman: Yrd. Doç. Dr. Haşim Özgür TABAKOĞLU

Lazer kaynak doku yarıkların kapatılması için potansiyel alternatif bir teknik olarak, klinik uygulamalarda etkili olduğu kanıtlanmıştır. Cilt kesi kapatılması çift dalga boyu termik etkisi (980 ve 1064 nm) lazer uygulaması, 12 erkek ve dişi wistar sıçanlar üzerinde değerlendirildi. Kesikler (0.5 cm uzunluğunda) tıraş 220 - 250g wister sıçanların dorsal bölge üzerine dikey bir düzlem boyunca yapılmıştır. 1W lazer ışıması tarafından kapatıldı kesiler ve maruz kalma süresi , 5 Sürekli dalga modunda (CW) saniyede ve 1W ve maruz kalma süresi , sırasıylakesi üzerine toplam enerjileri , 5J'ye ve nokta başına 10J teslim darbeli modda 10 saniye .. Her bir gruptan Hayvanlar 0 , 4 ve 7 gün ve H & E leke kullanılarak histolojik analiz için çıkarıldıörnek feda edildi. Termal etkisi her bir tarafında ve kapatma yeteneği ile ışınlanmış siteye bitişik bölgenin en geniş termal olarak değiştirilmiş alanı ölçülerek değerlendirildi . Termal değişmiş alanı CW ve titreşlik modu ve tedavi grupları (TTA) ((mm 2 den) ortalama iki dönemde de titreşlik grubuna göre CW modu gruptan daha yüksek ortalama değere sahip 0 ve 4. günlerde ($p > 0.05$) anlamlı farklılık bulundu anlamlı iken fark ($p < 0.05$) istatistiksel olarak 7 gün sonrası ışınlama bulunmuştur . Ayrıca, sıkı bir kapatma 7. günde CW grubu ile gözlenmiştir. Sonuç olarak , cilt kesi kapatılması 980 ve 1064nm kombine ışın formu 1W , 5 saniye ve 5J CW modu uygulama histolojik geç iyileşme periyodu ve mutlak yara iyileşmesi özelliği de ters çıktı minimal termal değişiklik olduğu tespit edildi .

Anahtar kelimeler: Çift dalga boyu lazer , CW modu , darbeli modu , termal değişikli

CHAPTER 1

INTRODUCTION

1.1 Lasers and Medical Applications

It seems hard to imagine that a narrow, monochromatic, coherent, straight line moving, amplified beam of light fired by excited atoms is powerful enough to slice through a material like steel. Albert Einstein, in 1917, noted that under certain conditions atoms could absorb light and consequently be stimulated to shed the energy they have borrowed. The term laser (light amplification by stimulated emission of radiation) was coined by Charles Townes in 1951 while the glare of a flash lamp in a rod of synthetic ruby was investigated by Theodore Maiman, leading to the creation of the first human-made laser in 1960. The laser action mechanism involves exciting atoms and passing them through a medium such as 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 reflected back and forth, leading to gaining of energy to produce a high wattage beam of light.Medical application of lasers, among other areas, is the most ideal and therefore requires much attention. As a surgical tool the laser is capable of three fundamental functions. When focused on a point it has the power to cauterize deeply as it cuts.Thus reduces the surgical trauma caused by a traditional knife. It can vaporize the surface of a tissue.Laser can also be used through optical fibers permit a doctor to see inside the body. Lasers have become an indispensable tool in biological applications from high-resolution microscopy to subcellular nanosurgery level. Undoubtedly, medical lasers are a prime example of how the movement of an idea can developmessntally change the medical world. Various applications of lasers in medicine are now being practiced and many more emerging [1].

1.2 Tissue welding

Laser welding has proven to be effective in clinical applications as a potential alternative technique for closure of tissue incisions [2-4]. The major advantages of the laser tissue welding are immediate water-tight closure of wounds, less or in some cases no scar formation, and the foreign-body reactions against the suture materials is completely eliminated. For the laser tissue welding to be successful wavelength of laser light, optical and thermal properties of tissues, exposure time, spot size, laser power, pulse duration and repetition rate are carefully chosen and noted [5, 6].

Lasers of infrared class have been and are being applied for tissue welding for decades because of their distinct capability to bring about the thermal apposition of biological tissues [7-13]. 1064nm laser was used for skin welding to achieve the deep heating at the weld site, an effort to produce strong, full-thickness skin welds [2-4].The skin welding ability of a 980-nm diode laser was investigated because of its relatively higher absorption by water than other near infrared lasers, and good wound sealing ability was reported in the early days of the healing period. [15-17].

Laser light application as a means to weld tissues, alternative to suture is a surgical technique based on the activation of thermal or photochemical bonds by irradiating the tissue using appropriate laser wavelength [18,1].

It is believed that the absorbed energy alters the molecular structure of tissues, forming new bonds and the act of intertwining determined constituents of the tissue. The collagen protein in particular, is generally believed to play a major role in the tissue fusion mechanism, with its helical structure being the most important aspect. The activation of collagen molecules is the basis of a thermally induced incision closure. The structural uncoiling of the collagen molecule by thermal processes and the subsequent formation of new bonds between these molecules (crosslinks), proven to

occur by scientific studies [19], is thought to be the micro-mechanism responsible for the progressive closure of an incision [18].

Laser welding of tissues has lots of advantages over traditional closure methods, such that it leaves little or no scar and reduces bleeding, and forming an immediate watertight seal, and thus enabling faster wound healing and reduced operative time [19]. As it does

not require the insertion of foreign material in the tissue, problems related to undesirable reactions are avoided, or eliminated [18].

Laser heating can be applied to bond tissues. The exact mechanism of such laser welding or soldering is not fully understood, but it has been understood and believed that it is critically dependent on the temperature of the tissue to be bond [20-23].

The main disadvantages of the laser procedure are the possible thermal damage, determining the end-point of the procedure and lack of reproducibility [20].

980nm and 1064nm lasers among other lasers have been shown by many researchers to be good candidates for skin wound closure. Tabakoglu *et. al.,* in 2006, closed the skin incisions by 980nm-diode laser welding and suggested that diode laser could be a good candidate for skin wound closure [15]. Ahmad in 2012, achieved Human skin wound closure using 980 Diode laser [24]. . Liming *et al.,* work in 2011, which raises contention and motivates our current study performed welding of skin incisions with a combination of two near-infrared diode lasers which was a preliminary study for the determination of optimal parameters [25]. Tensile strength and wound healing results in vivo with Nd:YAG laser ws studied by Nathaniel and his team to indicate the effectiveness of the laser in skin wound closure [26, 27] . According to the studies by Abergel *et al.,* in 1986, skin wound could be effectively closed by Nd:YAG laser with reduced healing duration.

Many of the previous works, however, pay a good deal of attention to the skin welding ability of a single wavelength laser, and little was paid to skin welding with more than one laser, as far as it is known.The safety for the use of dual wavelength laser to achieve skin incision closure needs to be well understood [25].

1.3 Effect of Non-ionizing Laser Radiation on Tissue

Lasers have been and are currently being used widely in medical applications for more than three decades. The majority of those applications involve thermal effects. For example, in laser hyperthermia, the temperature inside a pathological tissue is often elevated up to 42-45 ^{0}C so that the growth of malignant tumor can be retarded and eventually combatted. In laser coagulation, as the heat rise inside tissue is higher, laser beams can cause immediate irreversible damage of pathological cells by heating them

up to above 60^{o}C. In the case of laser surgery, a laser beam can vaporize and cut tissues like a scalpel when tissue temperature is heated to as high as 100^{o}C. It is clear that no matter which medical treatment is performed, a thorough understanding of the damage distribution within both pathological tissue and the surrounding healthy tissue is imperative [28].

There are mainly five different important laser-tissue interaction mechanisms observed on tissues. These are *photothermal, photochemical, photoablation, plasma-induced ablation, and photodisruption* all of whose main mechanisms' concern is the deposition of delivered laser energy which is determined by *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,, etc. [29-32].

It is indeed, hard to elucidate the thermal damages caused by the laser beam as the principle tissue damage mechanism for repetitively pulsed or scanned laser exposures is still in question.With progressive research in the area, current evidences indicate that the major mechanism is a thermal process wherein the effects of the individual pulses may be additive. There appears to be a different damage process for repetitively pulsed laser exposures when the individual pulses are shorter than 10 microseconds than when the pulses are longer [33]. Both acute and chronic exposures to all forms of optical radiation can produce skin damage of varying degrees [34].

Several types of lasers have been explored extensively for the treatment of skin disorders. For the common laser sources in the 0.3 to 1.0 μm range, almost 99% of the radiation penetrating the skin is absorbed in at least the outer 5 mm of tissue. In most cases, the absorption occurs in tissue thicknesses less than 5mm. For those wavelengths greater than 350 nm, the reaction of the skin to the absorbed optical radiation is essentially that of a thermal coagulation necrosis [34]. Such type of injury can be produced by any optical radiation source of similar parameters and is, therefore, not a reaction specific to laser radiation. This, is very much similar in causality and clinical appearance to the tissues reaction of the deep electrical burn. In the case of pulsed laser irradiation, including exposures of the picoseconds domain, there are likely to be other secondary reactions in the tissue. Studies have revealed that the volume of vaporized tissues produced by high-level irradiation with laser pulses in the millisecond domain

can backscatter a significant portion of the incident energy and thus effectively reduces the amount of absorbed radiation in the tissues [34].

Thermal effects of laser exporsure, pricipally depend upon the following factors:

- Absorption and scattering coefficients of the tissues at the given laser wavelength
- Radiant exposure of the laser beam
- Duration of the exposure and pulse repetition characteristics, where applicable
- The extent of the local vascular flow inside tissue
- Size of the area irradiated. [34 & 35]

Laser welding was shown by many researchers to be associated with thermal damages. Chen et al., 2008 performed histological and modeling study of skin thermal injury up to 2.0 micron laser irradiation, thus confirming the laser's potential damages [36]. As studied by Margaret et al., 2008, 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 [37]. Zhang and his team in 2013 have performed a study with 1,064-nm to compare laser-induced skin burn with thermal skin burn and found similarity in thermal damage patterns [38]. 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 demonstrated that the average size of individual lesions depends on the number of passes [39]. A bioheat transfer approach is proposed by Jianhua *et al*., 2007, to study thermal damage in biological tissues caused by laser radiation. Their approach was that, the laser light propagation in the tissue is first solved by using a robust seven-flux model in cylindrical coordinate system. The resulting energy spatial distribution of the following absorption is then incorporated into the bioheat transfer equation for solving temperature response. Thermal damage to the tissue is assessed by the extent of denatured protein using a rate process equation. It is found that for that tissue studied, a significant protein denaturation process would take place when temperature exceeds about 53 ◦C [1].

The accuracy of diagnosis of laser-induced thermal effects (both wound closure capability and thermal changes) on the histopathologically, requires thorough

understanding of histologic and anatomic arrangement of different layers of the skin. Knowledge of the function of the skin in general, and each of it numerous components is requisite to the understanding of the effects of laser –induced thermal effects on skin.The potential damages caused by the laser irradiance when used for skin wound closure can be detected according to individual components of the skin, namely, epidermis, , structures of the dermoepithelial interface, blood vessels and lymphatics, collagen, nerves and muscles. *The strategy is to consider how the skin looks by examination grossly and by inspection using conventional microscopy* following the irradiation during the course of healing . The important clue is to bear in mind that under normal condition a healthy skin tissue has a standard appearance and thus any abnormal appearance indicates morphological changes induced by light energy [36].

1.4 Purpose of the Thesis (Background and Objectives of the Study)

Most of the previous studies performed with 980 and 1064nm, single laser wavelength was applied to achieve incision closure. Consequently, 980nm wavelength does not reach deeper lying targets (such as blood vessels or hair follicles), and can result in excessive damage to the epidermis and other healthy skin structures and 1064nmwavelength heating not superficial leading to delayed healing period due to incomplete wound brindging and severe thermal side effects and even those that simultaneously applied the two wavelengths to achieve wound closure have not been able to perform such studies in all respective such that the potential thermal effects on both irradiated and neighbouring tissue has not been given proper attention and lack proper illucidations of the extent of tissue thermal alterations [25] .

This study is aimed at evaluating the thermal effects of dual wavelength (980 & 1064nm) laser application in skin incision closure at laser optimum therapeutic dosage.

The specific objectives are:

1)To determine possible best therapeutic mode of dual wavelength application of 980 and 1064nm lasers

2)To evaluate tissue thermal changes or damages if any by applying H &E staining method

3)To quantify tissue thermal changes by determining thermal areas occurring postoperatively on different wound healing days

1.5 Hypothesis

Due to the fact that 980nm is absorbed by the skin surface tissues and hence causes superficial heating seen as coagulation and 1064nm absorbed by the deep skin layers' tissue, synergetic welding effects is expected with reduced thermal damages and faster healing period at optimum therapeutic dosage

CHAPTER 2

LITERATURE REVIEW

2.1 Skin Structures and Their Orderly Arrangement

All constituents of human skin are derived from two of the three primary germ layers, either ectoderm or mesoderm. The epithelial structures, i.e., epidermis , sebaceous units and hair follicles, apocrine units, eccrine units, and nail units, are said to be derived from ectoderm. Melanocytes, and specialized sensory receptors as well as nerves develop from neuroectoderm. The other elements in skin , i.e., Langerhans' cells, macrophages, mast cells, from mesoderm [40].

The generative cells of the **epidermis**, nail and follicles units all mature to dead cornified cells which contain large quantities of keratin. The filament of cornified cells that takes the form of hair is similar to the layer of cornified cells that makes up the stratum corneum and the plate of coenocytes that constitutes the nail [41].

Below the epidermis is the layer called the **dermis**. The top layer of the dermis — the one directly below the epidermis — has many ridges called papillae.So the ridges are not on the outermost layer of skin, as it might appear.The dermis contains a variable amount of fat, and also collagen as well as elastic fibers which provide strength and lexibility to the skin. *The* basement membrane was defined first by conventional microscopy as a thin band that is situated immediately beneath basal keratocytes, a strip that stained magenta by the periodic acid-Schiff method. It came to be recognized with the aid of electron microscopy, as a specialized structure which is formed at the junction of epithelial cells and adjacent connective tissue to them [42].The vasculature and Schwann cells of nerves also are surrounded by a basement membrane.

In the skin, **smooth muscle** occurs in two different unrelated settings. One is in fascicles that form muscles of hair erection and the other is in the wall of arterioles and venules. The fascicles that make up the arrector pili muscle originate from bulges of the isthmus and the upper part of the stem of a follicle and seem to insert at the base of epidermal rete ridges [40,42] .

Blood vessels in the skin of an embryo come into being when mesenchymal cells arranged in an intricate network arborize and encircle conduits that are seen through a microscope as mere spaces. The vasculature in dermis is such that it consists of a superficial and a deep plexus of both arterioles and venules, those plexuses are being connected to one another by communicating vessels that arise from arteries and lead eventually to veins that reside within septa of the subcutaneous fat. The deep plexus of arterioles and venules is situated in the lower part of the reticular dermis, and the superficial plexus, which is positioned in the upper part of the reticular dermis, beneath the papillary dermis.

The skin consists of an elaborate network of **lymphatic vessels** that parallel the major blood vascular plexuses but independent of them. From a superficial plexus of lymphatic capillaries thicker-walled lymphatic vessels whose valves constitute a one-way drainage system that directs its contents into the venous circulation progressively arise. The superficial plexus of lymphatic capillaries is situated in the upper part of the reticular dermis, just beneath the superficial plexus of blood vessels.The Cutaneous lymphatics structure enables clearance from the dermis of fluidsand macromolecules, cells and foreign material. The twin roles played by lymphatic network as a conduit for Langerhans' cells and as an exit for macromolecules such as proteins, lipids, and immune complexes make it indispensable for maintenance of homeostasis [40].

The subcutaneous fat, like the dermis, is embryologically derived from mesenchyme. Primitive mesenchymal cells give rise to fibrocytes and to adipocytes. An adipocyte produces so much fat within its cytoplasm that the lipid displaces and flattens the nucleus against the periphery of the cell, and thus causing an adipocyte to appear as a large clear cell whose nucleus either is a dark speck at its border or not visualizable at all in a random section [40-43].

2.1.1 Collagen

Normal dermis is estimated to consist of more than 65% of the dry weight of the collagen [44]. The major collagens in the skin are Type I and Type III . The molecular mass of Type I collagen is about 290 Kda, accounting for approximately 80% of the total amount of collagen in the dermis. Type I collagen is arranged in a dense orthogonal meshwork of bundles inside the reticular dermis. Type III collagen makes up 10% of dermal collagen.It is also called fetal collagen because it is noted first in embryonic dermis where it serves as a framework on which Type I collagen is subsequently manufactured. Type III collagen appears basically as loosely arranged argyrophilic fibers inside the adventitial dermis. Although Type III collagen, known, also, as reticulin, is found in the adventitial dermis especially, it is present throughout the entire dermis, associated with Type I collagen. Types I and III collagen, together, form the extracellular fibers responsible to a greater extent, for the tensile strength of the dermis. Mutations in Types I and III collagen genes can lead to abnormalities of connective tissue both in the skin and in joints, as well as in other tissues, in different expressions of a complication, Ehlers-Danlos syndrome, and in fragility of bone in osteogenesis imperfect[40, 45] .

Another collagen of Type II is found in cartilage. Type IV collagen is a constituent of basement membrane and located especially in the lamina densa. Type V collagen accounts for less than 5% of all collagen in the dermis. Type VI collagen may be found in a variety of tissues, including skin where it is assembled into relatively thin microfibrils which form a network that acts as an anchor for stabilizing the broad assembly of collagen fibers, the same time serving as a basement membrane [46] .

Type VII collagen is produced mostly by keratocytes, from which it is transported across the basement membrane at the dermoepithelial junction to its final destination, where it anchors fibrils in the papillary dermis. In the papillary dermis, Type VII collagen interacts with anchoring plaques at one end and then with Type IV collagen and/or laminin five components of the basement membrane at the dermoepidermal junction at the other end, thereby stabilizing the attachment of the basement membrane to the dermis. *Any undesirable alteration in the expression, structure, or molecular interactions of Type VII collagen with other components of the basement membrane may cause fragility of the skin* [47].

A transmembrane protein, Type XIII collagen whose expression has been detected in normal skin and in cultured keratocytes is localized to the dermoepidermal junction and to the periphery of keratocytes which reside at all layers of the epidermis,hence it is being identified as a cell adhesion molecule that may partly play a role in enabling keratocytes to adhere to one another. Type XVI collagen, one member of the fibril-associated collagens with interrupted triple helices, is known to be produced not only by fibrocytes, but also by keratocytes. It is a component of the extracellular matrix and confined to a narrow zone within the papillary dermis near the basement membrane, which is in close proximity to Type VII collagen and to collagen of blood vessels. Type XVII collagen is also a transmembrane protein characterized by alternating segments,can be both collagenous and noncollagenous and was recognized initially as the 180 KDa bullous pemphigoid antigen 2, a constituent of anchoring filaments at the basement membrane, and now is known to be one of the targets of circulating IgG auto-antibodies in the serum of some patients with bullous pemphigoid and linear IgA dermatosis, and cicatricial pemphigoid [40,45].

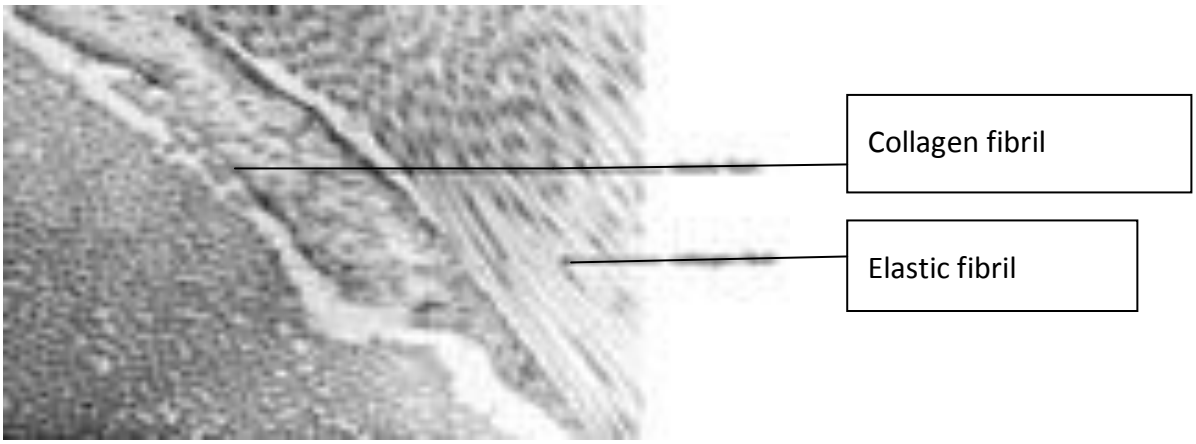

Figure 2.1 Collagen fibrils cut longitudinally have a structure distinctly different from that of the elastic fibril , the latter consisting of noncollagenous microfibrils embedded in homogenous elastin. (x 55,000)(Picture adapted from [42-45]).

Fibrocytes increase in number in a process called Fibroplasia during the active phase and manufactured collagen organized abnormally. This is an expected finding in some kinds of inflammatory diseases of the skin, e.g., those that occur secondary to trauma, such as keloids, scars, and dermatofibromas; those marked by extensive suppuration

and subsequent degeneration of collagen, such as ruptured epidermal and follicular cysts, deep fungal infections and atypical mycobacterial infections and in the case of pyoderma gangrenosum [42-45].

2.1.1.1 Effects Of Heat Energy On Collagen And Neocollagenesis

Collagen is believed to play a pivotal role in tissue wound closure as immediately collagen denaturation is followed by neocollagenesis after laser irradiation of the tissue. Increased small collagen fiber formation- evidence of neocollagenesis has been noted at 30 days post heat treatment [48]. A second study which aimed at tracking tissue changes after heating to denaturation range found collagenesis, elastogenesis as well as deposition of new hyaluronic acid at 10 weeks post treatment[49]. *Hayashi et al., assessed the effects of a wide range of temperature (37, 55, 60, 65, 70 and 80^{o}C). Among the higher temperature tested (70, 75 and 80 ^{o}C) histological analysis showed no significant difference, suggesting that additional heat has no effect on collagen [50].Temperatures below 60 ^{o}C have minimal effect on collagen structure and thus are unlikely to have significant effects on collagenesis*. Lin et al., 2007 noted that while collagen fibers begin to curve at 52 to 55 ^{o}C, structural changes were not seen at lower temperature (25 and 40 ^{o}C) [50] .

Hambleton and Shakespeare studied the effect of temperature on the solubility of frozen skin collagen in vitro and its susceptibility to digestion by proteolytic enzymes .Both of these parameters are increased with temperature. Above 55 ^{o}C, there is a sudden increase in both the solubility of collagen and its susceptibility to digestion, suggesting that this temperature is associated with changes in the structure of the skin collagen.*Consequential increase in susceptibility to digestion may have an influence on the nature of the healing process in the burn wound* [51]

2.2 Wound Physiology and Healing

Wounds include cuts, scrapes, scratches and punctured skin. They often occur as a result of an accident or a form of injury, but surgical incisions, sutures, and stitches also cause wounds. Minor wounds usually aren't serious, but even cuts and scrapes need immediate care [52].

The goals of wound management are simply to avoid wound infection, assist in homeostasis, and favor the formation an esthetically pleasing scar [53]. The majority of current studies focus on the esthetic nature of wound healing rather than infection rates,

because infection rates remain low, regardless of management and that is where laser comes into play.

The epidermis, dermis, subcutaneous layer, and deep fascia are the tissue layers of complete concern in wound closure [54].The two close layers, epidermis and dermis are tightly adhered and clinically indistinguishable, and together constitute the skin. Dermal approximation is thought to provide the strength and alignment of skin closure.The subcutaneous layer is mainly comprised of adipose tissue. Nerve fibers, blood vessels,

and hair follicles are located in this layer.The deep fascial layer is intermixed with muscle and occasionally need to be repaired in deep lacerations [55].

The healing process of skin occurs in several stages:

- ***Coagulation*** starts immediately following the injury. Vasopasm, platelet aggregation and fibrous clot formation occur. During the *inflammatory phase*, the proteolytic enzymes released by neutrophils and macrophages break down damaged tissue.
- ***Epithelialization*** occurs in the epidermis, as it is the only layer capable of regeneration. Complete bridging of the wound occurs within 48 hours after suturing.
- New blood vessels growth peaks four days after the injury.
- ***Collagen formation*** is necessary for restoration of tensile strength to the wound. The process begins within 48 hours following injury and peaks in the first week. Collagen production and remodeling continue for up to a year.
- ***Wound contraction*** occurs three to four days following the injury, and the process is yet, poorly understood. The full wound thickness moves toward the center of the wound, which may affect the final appearance of the wound [56].

2.3 Laser-Tissue Interaction

laser light interacts by different mechanisms with human tissue depending on various parameters, such as wavelength, power density or time of exposure. These mechanisms of interaction are basically, of five kinds: thermal mechanisms, photochemical,

electromechanical (photo-disruption) , and plasma-induced ablation photo-ablation mechanisms [29-31].

2.3.1 Thermal mechanisms: These mechanisms are of primary importance to surgical procedures, and tissue welding is known to be the procedure that falls under this category.It is based on three distinct phenomena, starting with heat production in the tissue by absorbing the laser light, and then heat transport , culminating in a tissue reaction. The absorption of laser light is largely dependent on the laser parameters and the kind of tissue being radiated (the presence of an adequate chromophor greatly increases the absorption).The effects of this mechanism seen ranging from ***hyperthermia*** (increase in tissue temperature to about 41^{o} C resulting in bond destruction and membrane alterations) to ***vaporisation*** or ***ablation*** at temperature higher than 100 ^{o} C). Halfway between these two extremes are ***protein denaturation***, ***coagulation*** (between 50 and 100 ^{o} C) ***collagen denaturation***- an effect that plays a significant role in tissue repair and welding [29] .

2.3.2 Photochemical mechanisms

In these, the laser light induces a chemical reaction in a tissue, like molecule formation or oxidation, utilising irradiance light sources of low intensity. Photodynamic therapy (PTD) is an example of procedure that uses this mechanism [29].

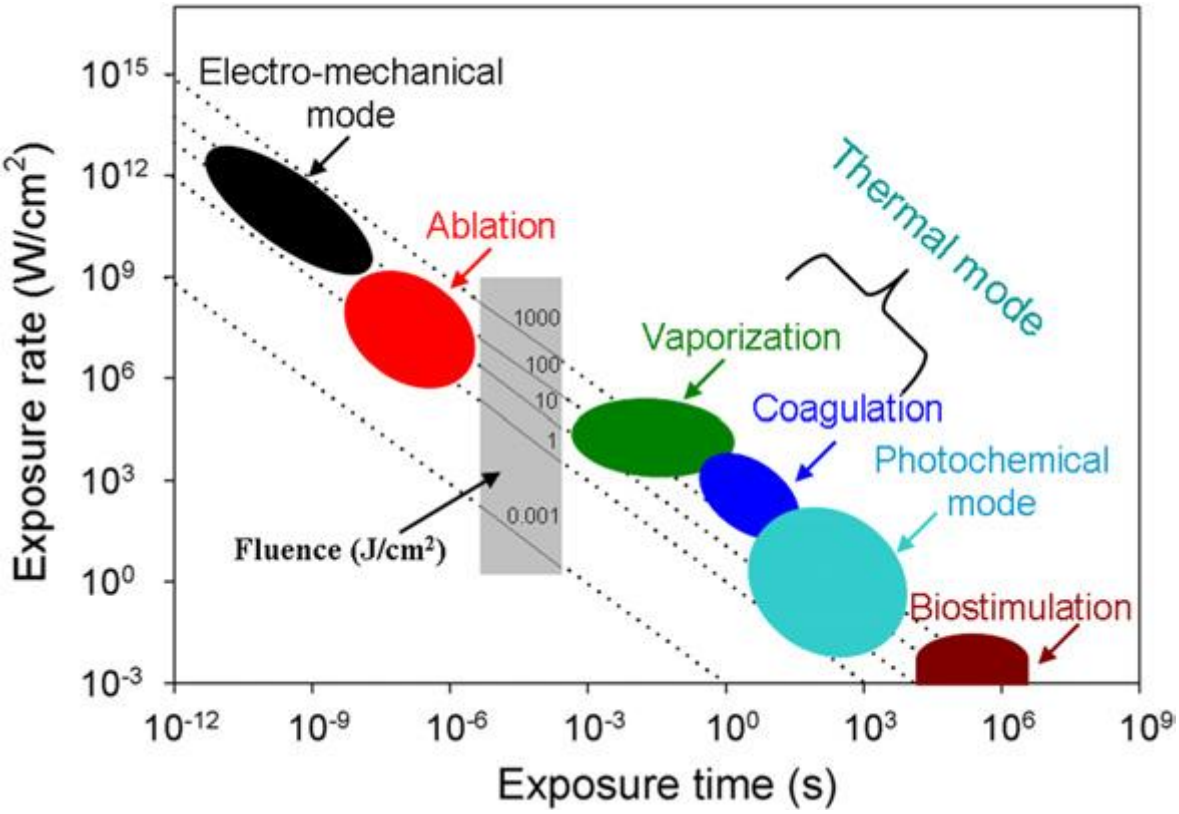

Figure 2.2: (a) Different types of laser–tissue interaction are dependent on total exposure and exposure time. (b) Different types of laser–tissue interaction function of exposure rate and interaction time (figure is adapted from [30]).

2.3.3 Photo-disruption or photo-mechanical interaction

These are mechanisms by which tissues are destroyed through the formation of shock waves, produced by the quick expansion of plasma formed as a consequence of the ionization of atoms in the tissue. The mechanism requires high irradiation (in the infrared or visible range of the spectrum) and short laser pulses in the microsecond range to get the required effect [29, 30].

2.3.4 Photo-ablation mechanism

This is the highest level of interaction leading to tissue decomposition by exposing it to high intensity laser irradiation in order to destroy chemical bonds of its molecular constituents, without temperature rise in the surrounding tissues. The characteristic wavelengths used in this procedure are usually in the UV range with nanosecond pulse duration. This mechanism allows for a very precise and predictable tissue ablation.

2.3.5 Welding Method

When a laser system is applied directly onto the tissues without the use of any other means to achieve the required interaction – coagulation – namely the use of solders or dyes, then welding is said to be done. The laser can be applied directly onto the incision while holding the extremities together, to sufficiently heat the tissue to obtain a viable weld but not to cause necrosis to cells [21].

By carefully controlling the parameters of the laser such as wavelength , power,duration of exposure, and spot diameter it is possible to control the localized temperature increase that leads to ***collagen denaturation*** *[21] .*

The subsequent cooling of the tissue in the two interfaces allows for the formation of collagen crosslinks, to effectively form a watertight welded seal. Weld depth and post

weld strength is also controlled for a more effective closure, while avoiding undesirable effects on the collagen crosslinking process, which can result in an inefficient healing [20]

The initial formation of crosslinks between the collagen molecules, when the tissue is cooled, forms a matrix wich serves as the basis for subsequent tissue rebuilding. Post-weld strength and long-term stability of laser-welded tissue affected as a result of the effects on existing collagen crosslinks as well as from new crosslinks synthesized as part of the wound healing process. With the formation of matrix by the laser exposure, it is logical to assume that the healing time will be relatively shorter, when compared to the classic methods which merely join the tissues. Several works support this assumption, Gülsoy *et al.*, performed a comparative study of epidermal thickness and granulation area of incisions on a large number of mice using sutures and laser techniques. Epidermal thickness measurements made 1, 4, 7, 14, 21 days post-irradiation and their results implied that, although epidermal thickness was the same after 21 days, at the 14^{th} day there was already a significant (30%) reduction of this parameter on the laser welded incisions, while the incisions closed by suture remained unchanged since the 1^{st} day. The same article also states that at the 7^{th} day after the laser closure procedure, the incision was completely closed, while on the suture closed wounds there were still visible openings at the same day [15].

The main disadvantage of the method is that the mechanism responsible for the tissue fusion can to cause cell necrosis, by thermal effects. This reaction is proportional to

exposure time to the laser, and exponential with temperature [2]. As such, the control of heat accumulation in the tissue is the prime factor for controlling the extent of this undesirable reaction.

To avoid significant thermal damage the precise control of laser parameters and/or of the tissue surface temperature is of critical importance, and because of it being a difficult thing to achieve, this method is only now seen as a viable alternative, with the advancement of knowledge in the laser-tissue interaction.

A compromise has to be considered between radiation penetration depth and radiation absorption.Using higher wavelength results in high absorption causing undesirable tissue damages. To reduce the absorption of radiation, lower wavelength lasers are preferably used with this method, namely lasers that emit at 1064nm, or high power diode lasers [23, 24]. The use of UV lasers is of course completely excluded due to the extremely high absorption.

Alternatively, post-procedure weld strength is improved and thermal induced cellular necrosis is also reduced by a method called Soldering [29]. Soldering method improves weld strength and maintain tissue alignment. Materials are added over the incision area, to act as weak glue that hold the tissue together, partly shielding the underlying tissue from the radiation and improving the post-weld healing. Examples of some of such materials are *albumin or collagen*, in liquid or colloidal form [22].

Wavelength sensitive dyes or chromophores are added to those soldering materials to reduce thermal damage in the underlying and lateral tissues in the method.In this form of wound closure the absorption of the radiation occurs preferentially in the dyed area and limiting the extent of thermal damage. The chromophors must to be compatible to the used laser, to absorb the monochromatic radiation, and the concentration pre-determined to control optical penetration. Example of such dyes used are indocyanine green for use with 808nm diode lasers and fluorescein dye for argon lasers [29].

2.3.6 Cryogenic Cooling in Laser Welding

CW or Pulsed laser application to tissues, with the appropriate power density and wavelength, the tissue temperature gradually increases with the exposure time. Thus, a method for localized cooling of the tissue was devised, to prevent the negative thermal effects. Cryogen cooling during laser tissue welding is explored as another way of reducing thermal damage near the tissue surface [29].

Several studies report the appearance of denaturation zones in the tissue surrounding the weld site, an undesirable side effect, although thermal denaturation of tissue is necessary to produce a strong weld. Further studies referred tissue cooling as an efficient means to minimize epidermal injury during laser treatment [15].

The weld site need to cool to approximately its initial temperature between successive laser pulses in order to prevent a build-up in the baseline temperature over time. On the other hand, it is argued that the present welding techniques are currently inefficient because of large operative times with a majority of this time (99%) spent waiting for the weld site to cool to its original temperature, below the threshold temperature of collagen denaturation, between successive pulses of radiation [18] .

Cryogen cooling system is introduced to minimizes thermal denaturation zone, preventing thermal build-up by the laser irradiation. It also allows for the production of stronger welds, when applied between successive laser pulses, without interfering with the collagen anneling process [15, 18] .

2.3.7 Mathematical model of Light distribution in tissue

The light emitted from the tip of the fiber directed onto the tissue surface was modeled as an isotropically radiating point source. Lizuka et al, had previously proposed spatial distribution to have been considered dominated by scattering processes [58]. The light irradiance rate ($W.mm^{-2}$) of an isotropic point source emitting laser power P_{laser} (W) within an infinite homogeneous medium can be expressed as

$$\varphi(r, t) = P_{Laser} \exp(-\mu_{eff}) 4\pi Dr \quad (2.1)$$

where: P_{Laser} (W): power of the light source

μ_{eff} (mm^{-1}): effective attenuation coefficient on the tissue

r (mm): radial distance from the source

D (mm): optical diffusion distance

μ_{eff} is determined by the following equation

$$\mu_{eff} = 3\mu a(\mu a + \mu' s) \quad (2.2)$$

where μa (mm^{-1}): absorption coefficient in tissue

μ's (mm^{-1}): reduced scattering coefficient: $\mu's=\mu s(1-g)$

μs (mm^{-1}): scattering coefficient

g: anisotropy factor incorporating the effects of directionally dependent scattering.

D (mm) is determined by the following equation:

$$D=13(\mu a+\mu's)=\mu a\mu 2eff \quad (2.3)$$

r is defined by the following equation

$r=(x^2+y^2+z^2)$

Where: x, y (mm): transverse dimensions

z (mm): depth

2.3.7.1 Estimation of temperature rise inside tissue

Absorption of light in tissue causes a local elevation in temperature inside tissue. Tissue heat transfer due to that deposited light is described by the following bioheat transfer equation

$$\nabla\cdot k\cdot\nabla T(r,t)+Pabs(r,t)-\omega bCp\cdot[T(r,t)-Tart]=Cp\partial T(r,t)\partial t \quad (2.4)$$

Where

T (r, t): temperature (°K)

ρ: density of tissue (g mm^{-3})

C: specific heat of tissue ($J.g^{-1}.°K^{-1}$)

$Cp = C\cdot\rho$: heat capacity ($J.mm^{-3}.°K^{-1}$)

k = thermal conductivity of tissue ($W. mm^{-1}.°K^{-1}$)

r = radial distance (mm)

t = time (s)

Convection of the skin surface can be calculated using the following equation

$$\emptyset_{Conv} = hS\Delta T \quad (2.5)$$

$\emptyset_{Conv}$: heat flux through the surface (W)

h: convection coefficient ($W.m^{-2}.°K^{-1}$)

S: Surface of the interface (m^2)

ΔT: difference between the inner and the outer temperature (°K)

Boundary conditions for the other surfaces were

$$\vec{n} . k\nabla T=0 \quad (2.6)$$

$\vec{n}$: direction of the heat flux

k: thermal conductivity

2.3.7.2 Thermal Damage function

Thermal damage in cells and tissue can be described mathematically by a first-order thermal-chemical rate equation, in which temperature history determines damage. *Damage is considered to be a unimolecular process, where native molecules transform into a denatured/coagulated state through an activated state leading to cell death*. Damage can be quantified using a single parameter Ω, which ranges on the entire positive real axis and is therefore calculated from the Arrhenius Law [59]. *Damage Ω is dimensionless, exponentially dependent on temperature, and linearly dependent on time of exposure.*

$$\log(\Omega)=\log(A)+\log(\int\infty 0\exp(-EaRT(r,t))dt) \quad (2.7)$$

Where A (s^{-1}) is the frequency factor,

T (°K) is the temperature.

Damage Ω parameter is reflective of the extent of damage. A is a frequency factor and describes how often a change in configuration actually occurs when such a reaction is energetically possible .It is also very dependent on molecular structure.

The equation clearly shows that the measure of damage describes the probability of tissue being destroyed. It is the logarithm of the ratio of the initial concentration of undamaged tissue to the new concentration once damage has accumulated, for the time interval t = 0 to t = τ. Therefore, Ω = 1 corresponds to an irreversible damage of 100% of the affected cells.

Due to the fact that lipid bilayer components of the cell membranes are held together only by forces of hydratation, *the lipid bilayer is the most vulnerable to heat damage. Even at temperatures of only 6°C above normal (i.e. 43°C), the structural integrity of the lipid bilayer is thus lost [60].*

2.4 980nm Laser and Skin Welding Application

Although near IR light has demonstrated clinical value, yet their effects at the cellular level have not been examined. To determine whethernear IR can improve cell growth and recovery, Mark *et al*., utilized a clinical 980nm laser in a cellular model of wound healing. Their results reveal that limited doses of the light can increase the rate of cell growth within hours of light exposure [61&62]. The results confirm clinical observations that low-level exposure to 980 nm laser light can accelerate healing of superficial wounds. However, these results also demonstrate the need for appropriate supervision of laser therapy sessions to prevent over exposure to laser light that may reverse increases in cell growth rates observed in response to lower levels of laser exposure [63]

The photothermal interaction of 980nm wavelength diode laser with the tissue is thought to be attributed to the moderate absorption of the tissue components to that wavelength. This wavelength is well absorbed by the water and also by the hemoglobin and melanin, in comparison with 810 and 1064nm which caused its importance in the coagulation and hemostasis procedures Figure (3) [64].

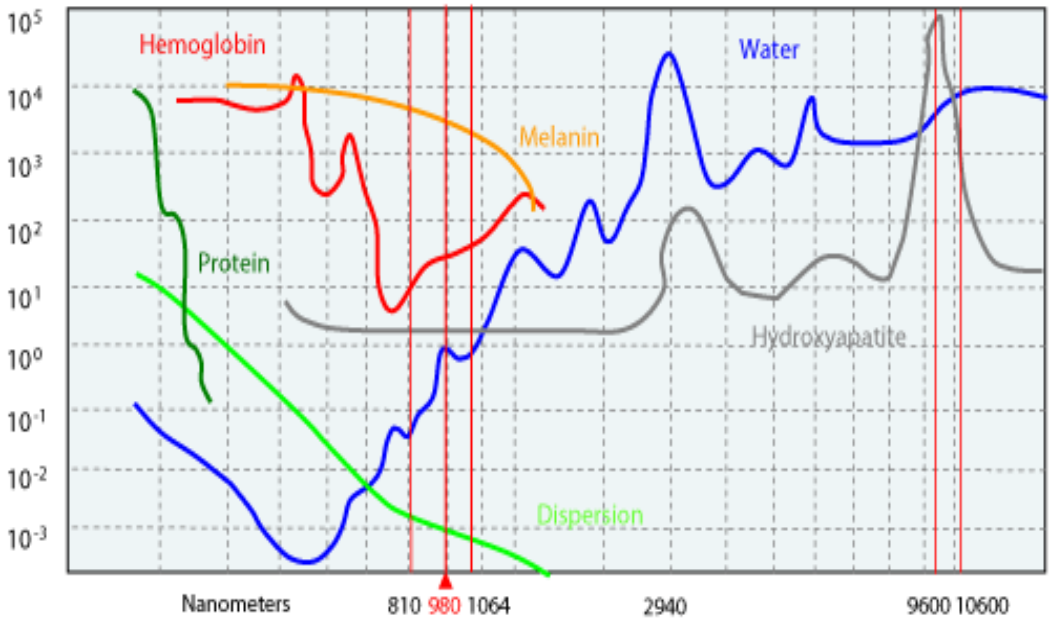

Figure 2.3: Absorption of 980 nm wavelength by water in comparison with 810 nm and1064 nm wavelengths [The figure adopted from 64].

980nm Diode laser application in wound healing was demonstrated by Tabakoglu et al., 2005 and as a continuation to their study, they conducted another research which aimed to compare 2 different application methods by histological and mechanical tests. 1-cm long, 6 incisions were welded with 980-nm diode laser by two different applications: high power (6W-400ms) and low power (0.5W-5s). Throughout 21-day healing period, incision were removed from dorsal skin of Wistar rats under anesthesia on control days (1st, 4th, 7th, 14th and 21st) for histology and mechanical tests. Closure index, thermally altered areas, epidermal thickness and granulation areas of H&E stained samples were calculated.In this study, effects of high (6W-400ms) and low power (0.5W-5s) applications of same energy level (2.5J) 980-nm diode laser irradiation on skin tissue was shown to be more effective [17].

2.5 1064nm Laser and Skin Welding Application

The laser wavelength should match the highest absorption of the targeted structure relative to the surrounding tissue if the clinical objective is to cause selective modifications of a specific tissue structure.This approach is called "selective photothermolysis" [65]. *Typically, however, the wavelengths that are highly absorbed in skin imperfections are also highly absorbed by non-target structures, for example, melanosomes [66] or hemoglobin-containing RBC [67]. Consequently, these wavelengths do not reach deeper lying targets (such as blood vessels or hair follicles), and can result in excessive damage to the epidermis and other healthy skin structures.* The choice of wavelength is thus dictated not only by the need for good absorption of the laser in the treated skin structure, but also by the need to avoid unnecessary damage to the epidermis. For this reason, it is often better to select a laser wavelength that penetrates more deeply into the tissue, and then achieve selective tissue modification by adjusting the laser pulse duration to the thermal relaxation time of the targeted imperfection [68].

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 (Fig. 2.4) [68,69].

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 [70]. 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 [71]. The potential undesirable effects associated with their widespread use comes the increased risk of injury from their use and the need for methods to ameliorate these injuries. 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 study 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 [72–74]. 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 [75]. In this study, a heated copper brass bar attached to an HQ soldering iron is used to induce thermal burns on skin, as a control to laser. This study aims to understand the interaction between laser and the skin, and to investigate the differences if at all there are, between 1,064-nm laser-induced burns and thermal burns.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 [76].

2.6 Laser Modes of Operation

A laser can be classified as operating in either continuous or pulsed mode, depending on whether the power output is essentially continuous over time or whether its output takes the form of pulses of light on one or another time scale. (Figure) [77].

- Continuous Wave mode

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

2.6.1 Continuous Wave Operation

Continuous wave (CW) emission has no interruption during the delivery of their energy. CW mode is like a light that is constantly "on". Some applications of lasers depend on a beam whose output power is constant over time and thus make use of such a laser known as CW. Many types of lasers can be made to operate in this mode to satisfy such an application. Many of these lasers actually lase in several longitudinal modes at the same time, 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 that is spacing between modes), typically a few nanoseconds or less [77]. For lasers to be able to operate in continuous wave mode it is required for the population inversion of the gain medium to be continually replenished by a steady pump source. In some lasing media this is quite impossible. In some other lasers it would require pumping the laser at a very high continuous power level which seems impractical or destroy the laser by producing excessive heat. Such lasers cannot be run in CW mode [79].

2.6.2 Pulsed Operation

Pulsed operation of lasers refers to any laser not classified as continuous wave, so that the optical power is delivered in pulses of some duration at some repetition rate producing light that is "on" half the time and "off" half the time. The duty cycle is 50%, because the light is "on" half the time and "off" half the time. Another definition of duty cycle is the duration of the pulse divided by the period, or the time from the start of one pulse to the beginning of next one.

Some lasers are pulsed because they cannot be run in continuous mode.

In other cases the application requires the production of pulses having as large energy as possible. Since the pulse energy is equal to the average power divided by the repetition rate, a goal which can sometimes be satisfied by lowering the rate of pulses so that more energy can be built up in between the pulses.

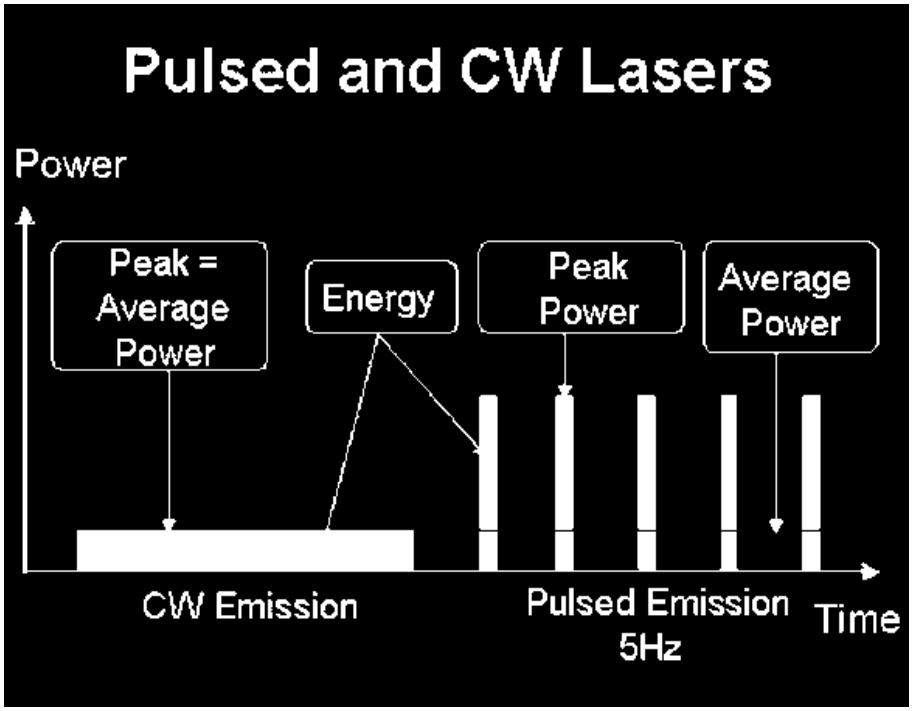

Figure 2.5 Graph of laser power vs CW and Pulsed lasers respectively (the picture adapted from [79])

2.6.2.1 Pulsing and Frequency

The words pulsing and frequency are used interchangeably to describe the same concept which mean the interruption of energy flow on a predetermined basis.

There are two types of pulsing in laser photo therapy

- chopped (switched) or
- superpulsed

A chopped beam is a continuous beam that is electronically (or mechanically) being switched between on and off. And during the moments when it is on it has typically the same output power as in continuous mode. But when it is not on in the whole time, the average output power is , of course, less than when it is continuous.

The average power is a function of the cntnous mode power and duty cycle (the ratio of the “on” time of the beam to the total emission (“on” + “off”) time, usually expressed as a percentage) [77]

CHAPTER 3

MATERIAL AND METHOD

3.1 AKT-Epidermis Dual Wavelength Laser System

AKT Dual Wavelength Laser System for skin Applications is a Laser system with two wavelengths, 980nm, 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. Decreased to minimum mechanical, and thermal effects. 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 and and in a combined beam form.

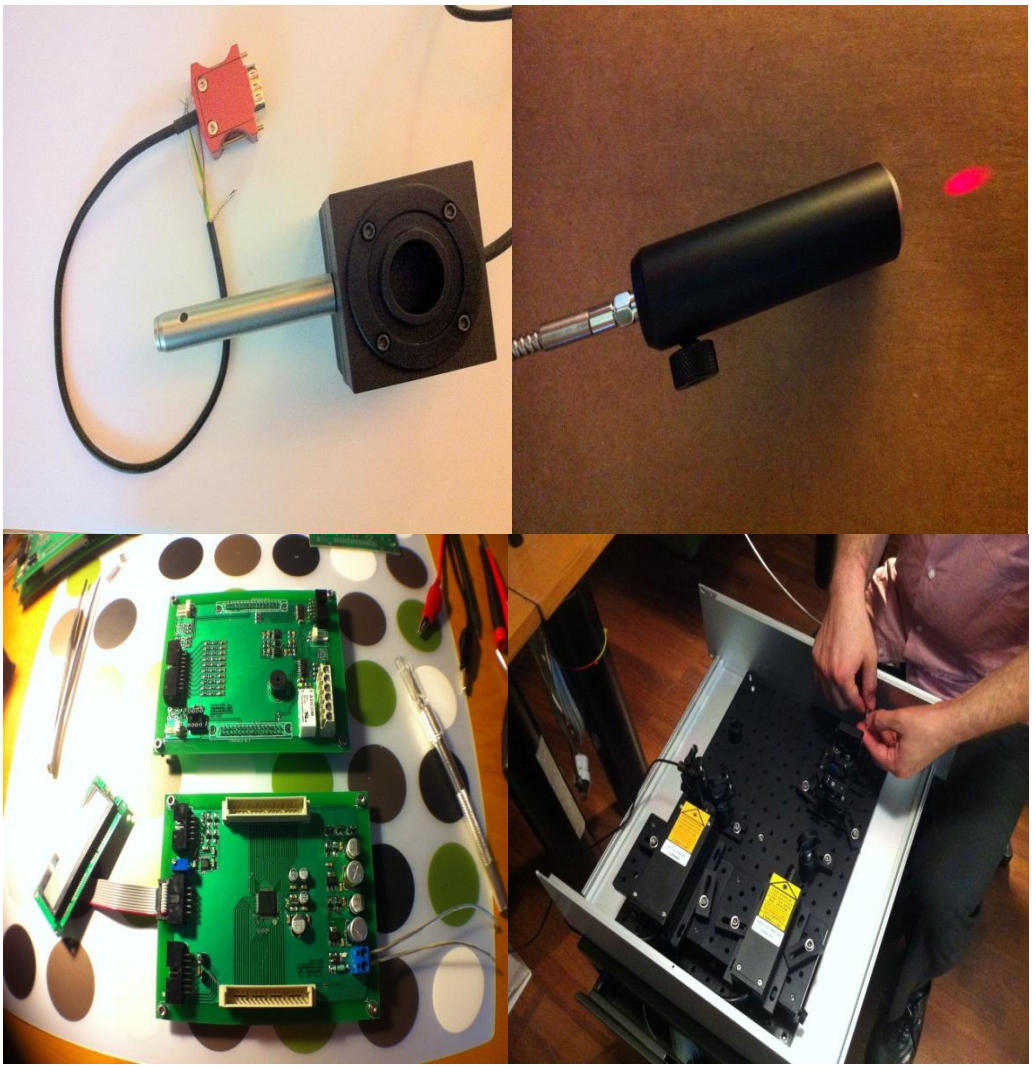

Figure 3.1 Bit by bit assemblies of the dual wavelength sytem parts

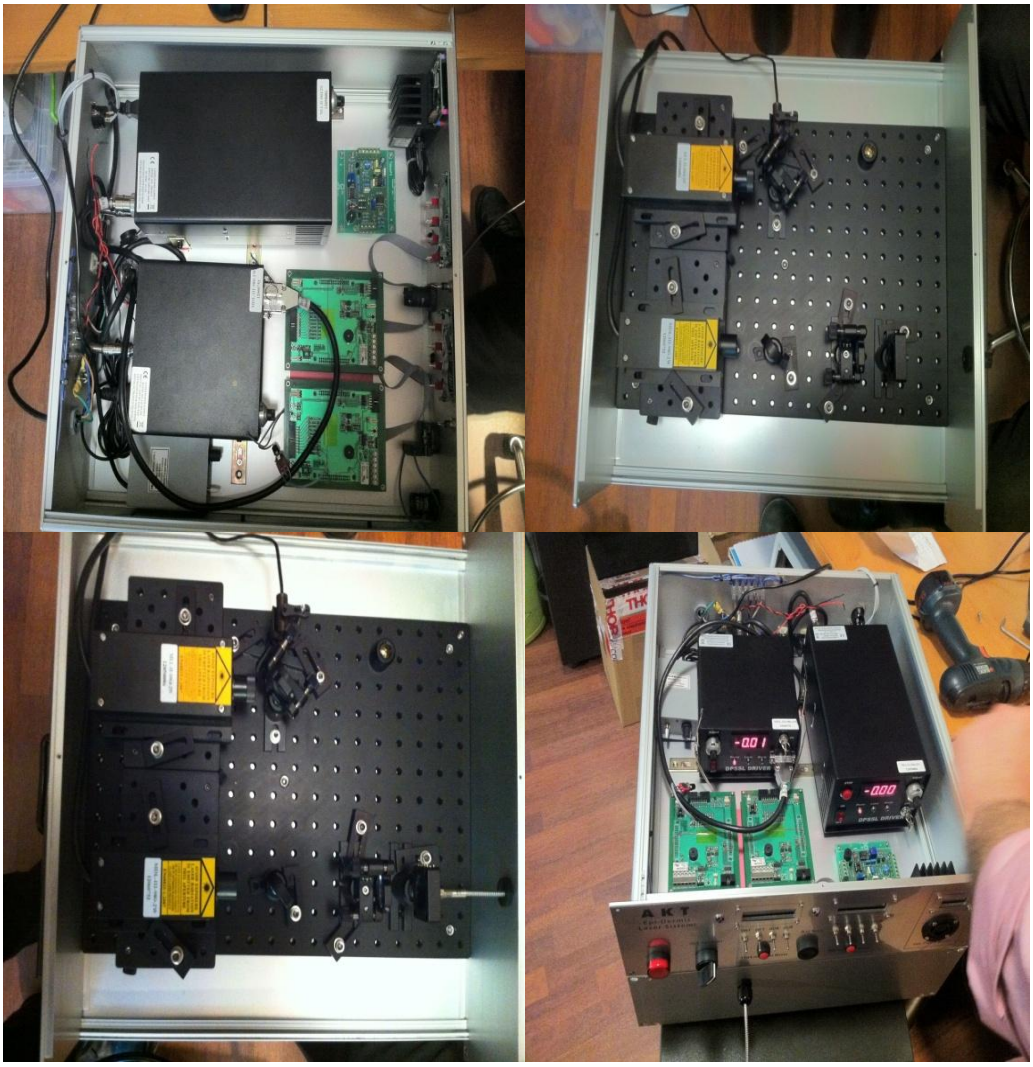

Figure 3.1 (cont.)

Figure 3.2 Completed AKT Dual wavelength Laser System

Table 3.1 Foot trigger connection table

Red	Middle
White	NOff
Black	Non

Cannon Connector: Male

1. White
2. Black
3. Red

Table 3.2 Optimized measurement values for the connection of fiber optic lasers with collimator

980 nm			
Current (A)	Fiber Input (W)	Fiber output (W)	Coupling efficiency
1.5	1.05	0.940	90%
1.5		0.900	OK
1064 nm			
Current (A)	Fiber Input (W)	Fiber output (W)	Coupling efficiency
2.5	1.27	1.09	86%
2.5		1.10	OK

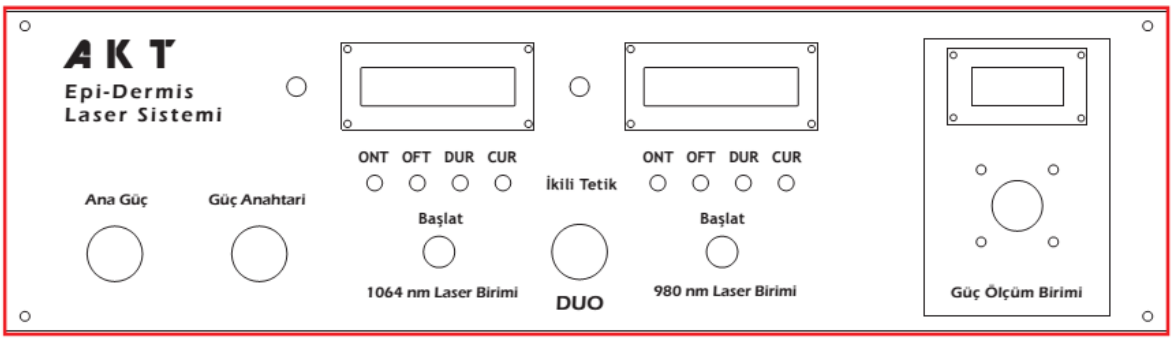

Figure 3.3 Laser system administrator user frontend box

Table 3.3 Microprocessor and electronic circuit connection list

NetName	F149 Port	Property	Application	Action	MSP430F149 Port	MSP430F149 Pin
Inc_Switch_Laser_Current	p1.0	ok			p1.0	15
Dec_Switch_Laser_Current	p1.1	ok			p1.1	15
Inc_Switch_OnTime	p1.2	p1.4 ok			p1.2	16
Dec_Switch_OnTime	p1.3	p1.5 low?			p1.3	14
Inc_Switch_OffTime	p1.4	p1.3 ok			p1.4	15
Dec_Switch_OffTime	p1.5	p1.2 ok			p1.5	16
Inc_Switch_Duration	p1.6	ok			p1.6	15
Dec_Switch_Duration	p1.7	ok			p1.7	16
&Sync	p3.0				p3.0	20
DIN	p3.1				p3.1	21
SCLK	p3.3				p3.3	23
LCD_D4	p5.5				p5.5	38
LCD_D5	p5.4				p5.4	39
LCD_D6	p5.3				p5.3	40
LCD_D7	p5.2				p5.2	41
ELCD	p5.6				p5.6	42
LCD_RS	p5.7				p5.7	43
TXUC	p3.6		RS232			
RXUC	p3.7		RS232			
Isolated_In	p2.2		Isolated Trigger			
Buzzer	p2.3		Amplified Buzzer			
RunProcess	p2.1	ok	Process Button			
Safety_Switch	p2.6	not wired yet	Safety Switch			
Safety_Out	p4.7		Safety Relay			
HeartBeat	p2.0	ok	Laser Out LED			
Test_Out	p5.1		LED2 Test Pin			

3.2 Methodology

3.2.1 Study site and subjects

The study was carried out on male and female Wistar rats weighing 200-220g as taken prior to the experiment with ethical approval by Ministry of science and Technology, Turkey. The animals were housed in Vivarium section of Boğaziçi University Istanbul , Main Campus and therefore the laser irradiation , post-irradiation animal care and wound excision as well as formalin fixation were carried out there with the approved permission of the university.

3.2.2 Animal preparation (Anaesthesia and incision)

Eighteen (18) male and female Wistar rats were divided into three groups (0 hour, 4^{th} day and 7^{th} day). The animals were anaesthetized with anesthesia solution containing Ketamine (75-100mg/kg) and Xylazine (10mg/kg) by intraperitoneal injection. Their back (dorsal region) was shaved and eight- 0.5cm full incisions were made along parallel but vertical axis on each rat's skin (0.75mm thick).

Laser irradiation

Each incision was irradiated with Dual wavelength (**980nm** and **1064nm) laser** with the following laser parameters: **laser power 1W for 5s exposure time to deliver a total energy of 5.0J and power density 31.83 per spot on the skin incision in CW** (**(980nm: On Time= 099 Off Time= 001 Duration= 005s Current= 62%) (1064nm: On Time= 099 Off Time= 001 Duration= 005s Current= 80%)) and 1W for 10 seconds and 10J 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%)** group .These parameters were chosen according to the previous studies in this area as presented in the literature [15, 25].

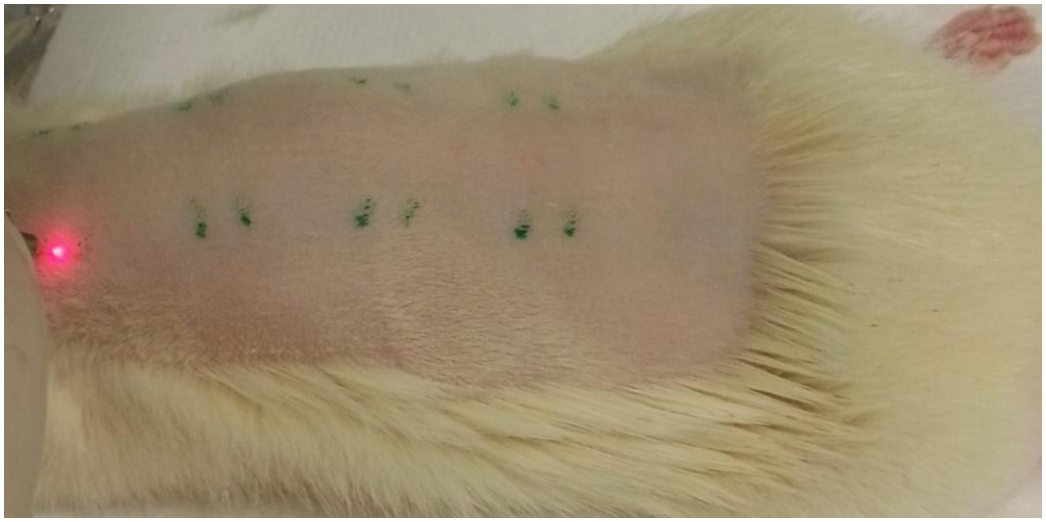

Figure 3.4 Incision irradiation

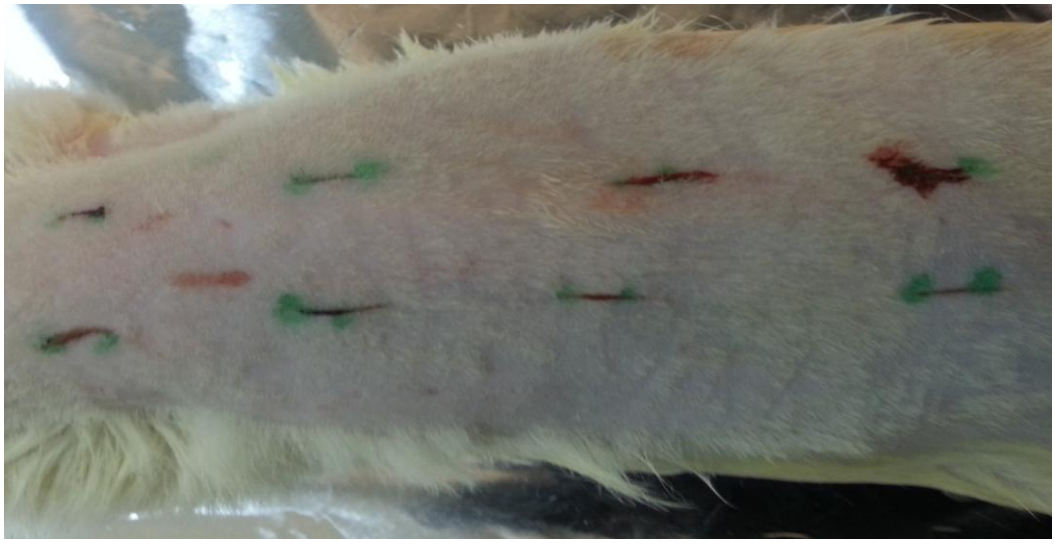

Figure 3.5 Weld incisions post-irradiation

Following irradiation closure of the incision, six (6) animals were sacrificed at 0 day, after four (4) days and then at seventh (7^{th}) day and the wound sites excised with at least 5mm margin and fixed in Formaldehyde Solution(37% Ph. Eur. Bp Usp, Mercck KGaA 64271 Dermsdart Germany). The tissue samples were stored in the solution at 4^{o} C.

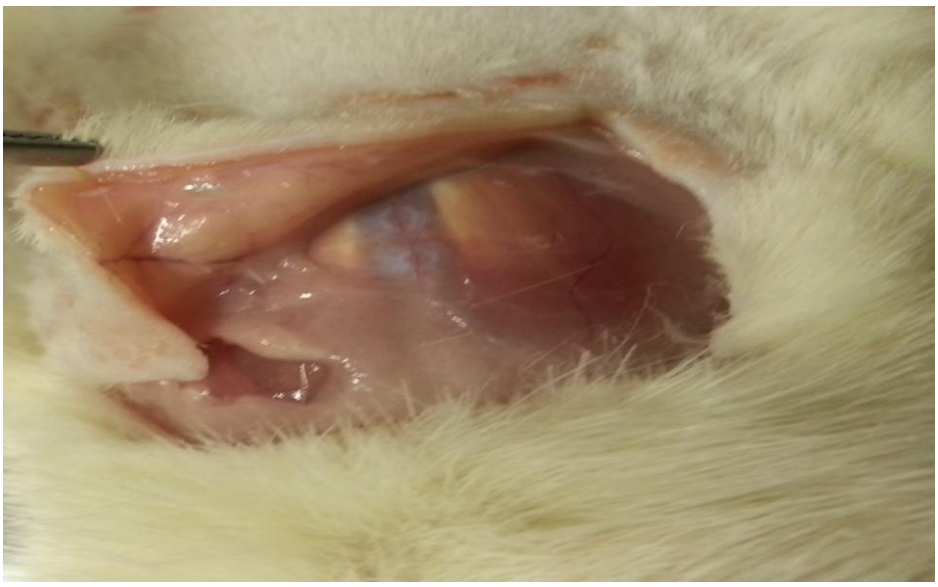

Figure 3.6 Wound excision

3.4 Histologic Evaluation

3.4.1 Principles of tissue processing

Tissue processing involves the diffusion of various substances into and out of stabilize porous tissues. The diffusion process results from the thermodynamic tendency of processing reagents to equalize concentrations inside and outside blocks of tissue, thus generally conforming 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 [80].

From the above stated law it can be seen that the significant variables in tissue processing are the operating conditions, particularly temperature, the characteristics and concentrations of the reagents and the properties of the tissue.

The first step in processing is dehydration of water present in tissues in free and bound (molecular) forms. Tissues are then processed to the embedding medium by removing some or all of the free water. During this procedure various cellular components are dissolved by dehydrating fluids. For example, certain lipids are extracted by anhydrous alcohols, and water soluble proteins are dissolved in the lower aqueous alcohols [81].

In the paraffin wax method, following any necessary post fixation treatment, dehydration from aqueous fixatives is usually initiated in 60%-70% ethanol, progressing

through 90%-95% ethanol, then two or three changes of absolute ethanol before proceeding to the clearing stage. While well fixed tissues can be transferred directly to 95% ethanol, incompletely fixed tissues may exhibit artifacts if placed directly in higher alcohols. The dehydrant concentration at which processing is initiated depends largely upon the fixative employed. Following fixation in anhydrous fixatives such as Carnoy's fluid, for example dehydration is initiated in 100% ethanol.

To minimize tissue distortion from diffusion currents, delicate specimens are dehydrated in a graded ethanol series from water through 10%-20%-50%-95%-100% ethanol [80].

Duration of dehydration should be kept to the minimum consistent with the tissues being processed. Tissue blocks 1 mm thick should receive up to 30 minutes in each alcohol, blocks 5 mm thick require up to 90 minutes or longer in each change. Tissues may be held and stored indefinitely in 70% ethanol without harm. Other dehydrants, including universal solvents, are used in a similar manner to that described for ethanol, though generally in different concentration increments.

Embedding is the process by which tissues are surrounded by a medium such as agar, gelatin, or wax which when solidified will provide sufficient external support during sectioning.

Infiltration is the saturation of tissue cavities and cells by a supporting substance which is generally, but not always, the medium in which they are finally embedded. Tissues are infiltrated by immersion in a substance such as a wax, which is fluid when hot and solid when cold. Alternatively, tissues can be infiltrated with a solution of a substance dissolved in a solvent, for example nitrocellulose in alcohol-ether, which solidifies on evaporation of the solvent to provide a firm mass suitable for sectioning [81]

Paraffin wax is a polycrystalline mixture of solid hydrocarbons produced during the refining of coal and mineral oils. It is about two thirds the density and slightly more elastic than dried protein [82].

Wax hardness (viscosity) depends upon the molecular weight of the components and the ambient temperature. High molecular weight mixtures melt at higher temperatures than waxes comprised of lower molecular weight fractions. Paraffin wax is traditionally marketed by its melting points which range from 39°C to 68°C.

Tissue-wax adhesion depends upon crystal morphology of the embedding medium. Small, uniform sized crystals provide better physical support for specimens through close packing [80]

Experimental Procedure

The formalin fixed tissue samples were transferred to Kandiili campus, Boğaziçi university for histologic investigation.

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

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

Sectioning: The praffin blocked sample were cut into slices in 9,12 and 14 micron size with Tissue Sectioning Machine RM 2255 and then collected on the slides, leveled and paced in the incubator for 24 hours to remove excess paraffin from the tissue awaiting staining.

Staining: The slides were stained according to the conventional H & E staining protocol as follows

Reagents: Alcohol - HPLC Fisher A995-4 or histological A962, FLAMMABLE store at room temp. in a flammable cabinet

Eosin Y, disodium salt (Sigma #E-6003, store at room temperature)

Harris Hematoxylin Stain, acidified (Lerner Laboratories #1931382)(R.T.)

Permount - Fisher SP15-100, FLAMMABLE HEALTH HAZARD

Xylenes (Fisher #HC700-1GAL, FLAMMABLE, store R.T. in flammable cabinet)

Solutions:

1. Eosin Y, 1 % aqueous (store at room temperature)

Eosin Y dye 1 g

Deionized water 100 ml

2. Harris Hematoxylin, acidified (store at room temperature)

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

3. Alcohol 90, 100%

Tap water l00ml

Staining Procedure:

1. The sections were immersed in Xylene for 3 minutes with dipping at about every 45 seconds.

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

3. The slides were dipped in tap water.

4. They were then immersed in Hematoxylene stain for 1.5 minutes

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

6. And then immersed in EOSIN stain for 2 minutes.

7. Rinsed with tap water gain.

8. The tap water was exchanged until the water is clear.

9. Final dehydration took place in ascending alcohol solutions (90% &100%).

10. And finally cleared with xylene (2).Coverslip was mounted onto a labeled glass slide with Permount.

Microscopic Examination was carried out using light microscope under 4x and 10x magnifications, the images captured and thermally altered areameasured using NIS-Elements D 2.30 program on Apple-Mac Computer connected to the microscope

Statistical Analysis: Analyis of Variance (ANOVA) and Paired comparison analysis followed T-test were employed to test for statistical differences in the set of data at p= 0.05 level of significance and under T_9 degree of freedom for CW and pulsed mode groups at different wound healing periods.

CHAPTER 4

4.1 RESULTS

The appearance of both CW and pulsed mode the laser treated incisions was quite smooth immediately following the irradiation. Less coagulation noted with pulsed wave mode than CW mode laser treated wounds (figure 4.1U & X). Speedy wound closure with less scaring was observed at both 4th and 7th days in the two laser modes groups. Tighter closure with CW mode laser closed wounds than pulsed mode at 7th day post-irradiation (figure 4.1V,W,Y & Z).

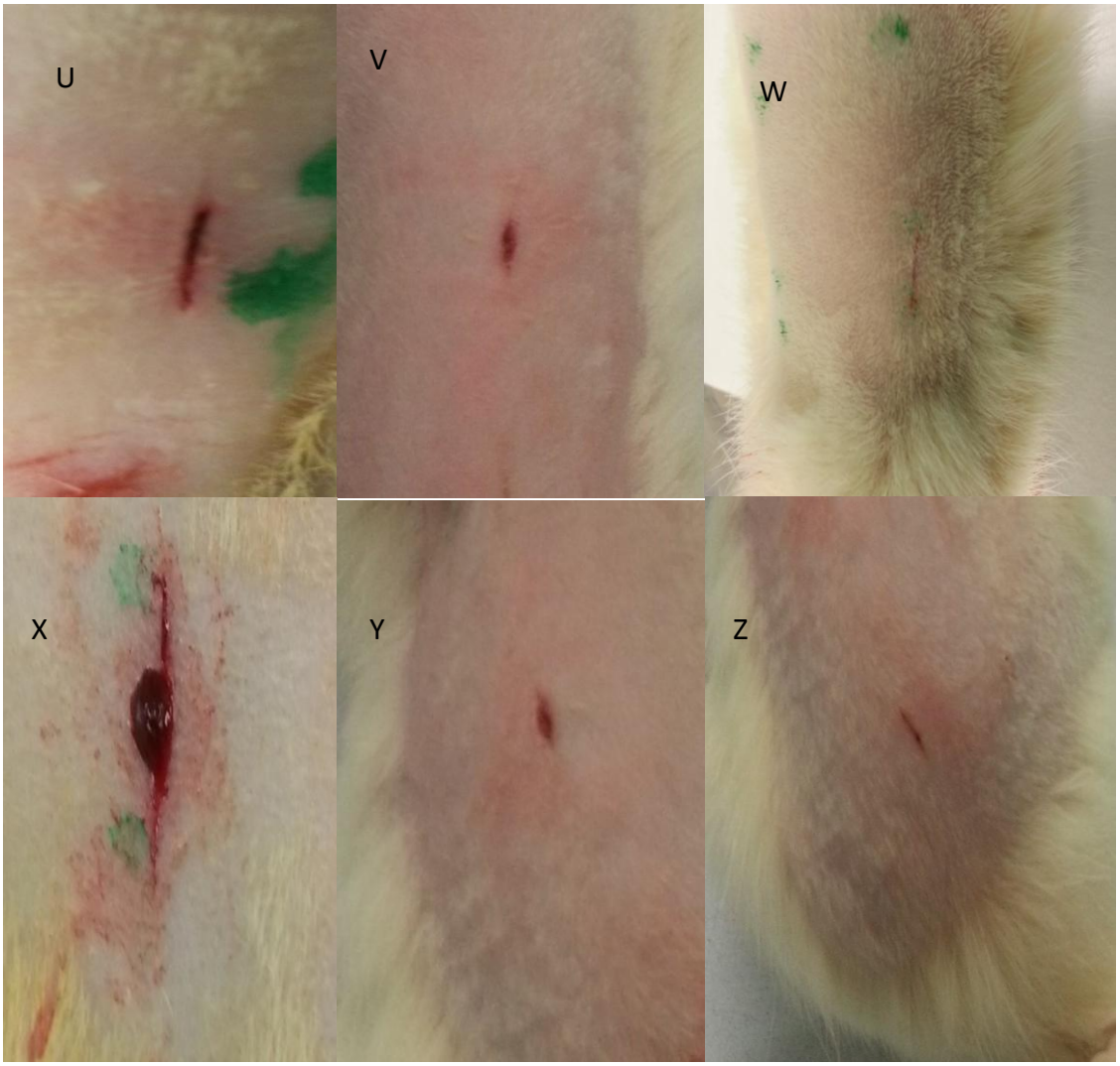

Figure 4.1 U, V & W= Appearance of CW mode laser weld incisions at 0th, 4th & 7th days post-irradiation respectively And X, Y & Z =Appearance of pulsed mode laser welded incisions at 0th, 4th & 7th days post-irradiation respectively

Thermal alteration in the skin tissue was histologically assessed by measuring the most extensive thermally altered area (TTA) in the region adjascent to the irradiated site from either side of the incision. Mean thermally altered area (μm^2) in continuos wave and pulsed wave mode treated incisions was found to differ signifcantly ($p<0.05$) at 0th and 4th days(figure 4.2.a&b) while no significant difference was found ($p<0.05$) 7th day post-irradiation (Figure 4.2c)

Table 4.1 Mean and standard deviation of Thermally Altered Area (TTA) in CW and pulsed wave mode welded groups at 0th, 4th and 7th day post-irradiation

Group	**0th day TTA (μm^2)**		**4th day TTA(μm^2)**		**7th day TTA(μm^2)**	
Laser Application mode	**CW mode**	**Pulsed mode**	**CW Mode**	**Pulsed mode**	**Cw mode**	**Pulsed Mode**
Mean	**5.31**	**3.70**	**2.62**	**2.26**	**0.64**	**0.63**
STD	**2.06**	**1.98**	**1.76**	**0.80**	**0.34**	**0.28**

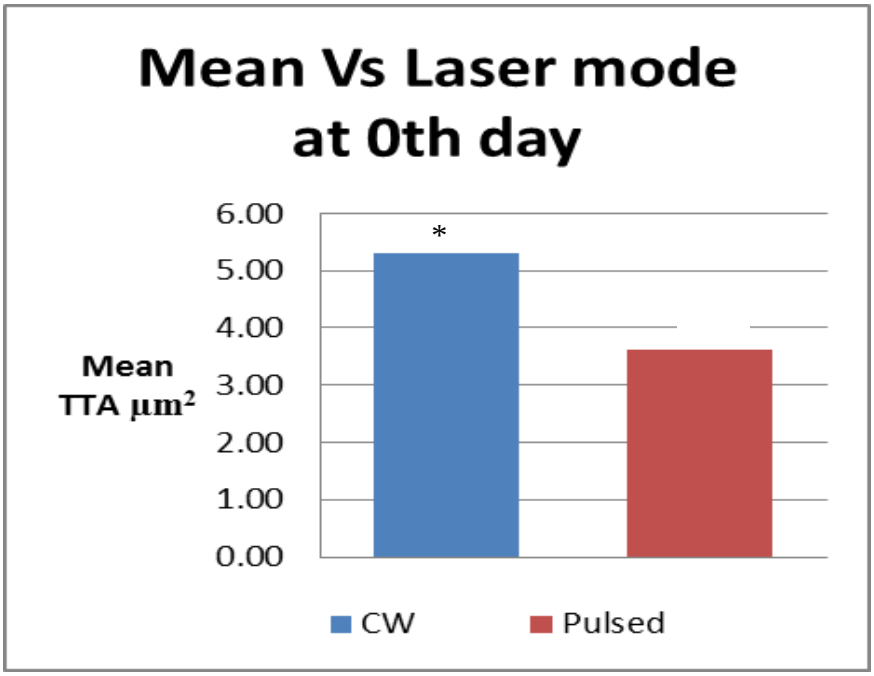

Figure 4.2.1 Graphical representation of Mean thermally altered area value Vs CW and pulsed modes laser treated wounds at 0^{th} post-irradiation. (*) indicates a significant difference in mean value

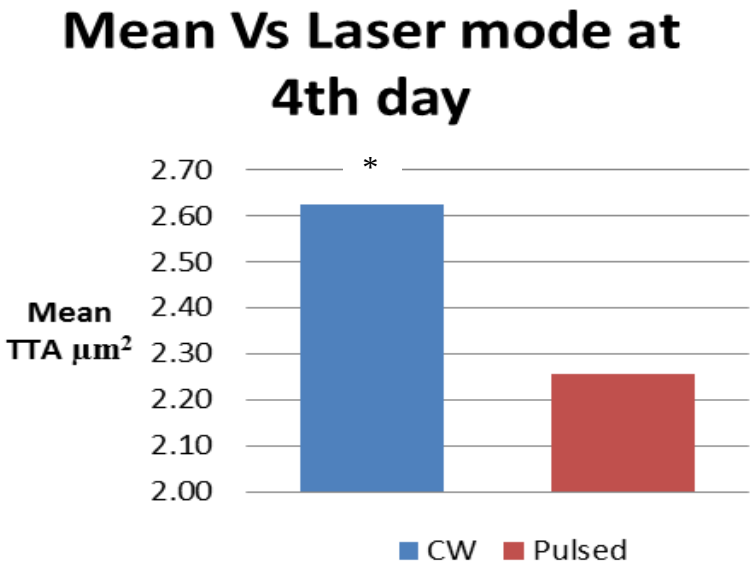

Figure 4.2.2: Graphical representation of Mean thermally altered area value Vs CW and pulsed modes laser treated wounds at 4th. (*) indicates a significant difference in mean Value

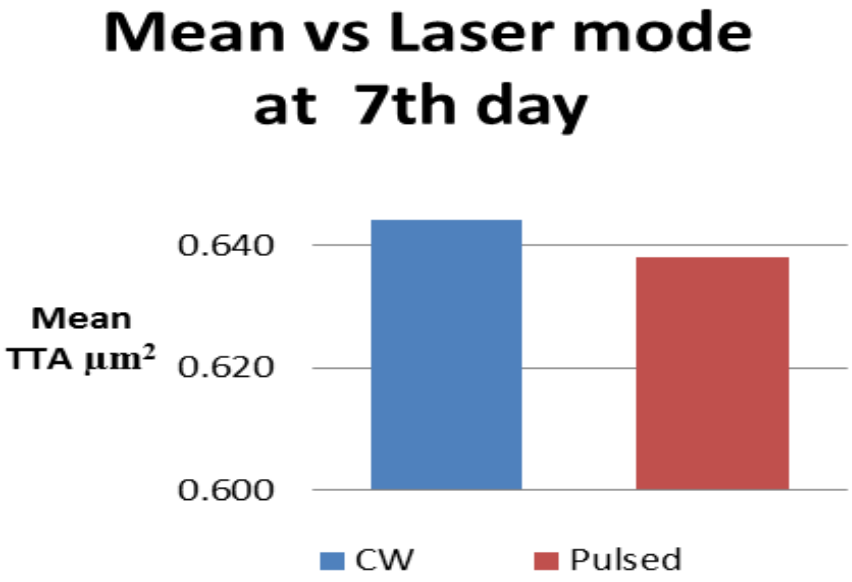

Figure 4.2.3: Graphical representation of Mean thermally altered area value Vs CW and pulsed modes laser treated wounds at 7th post-irradiation.

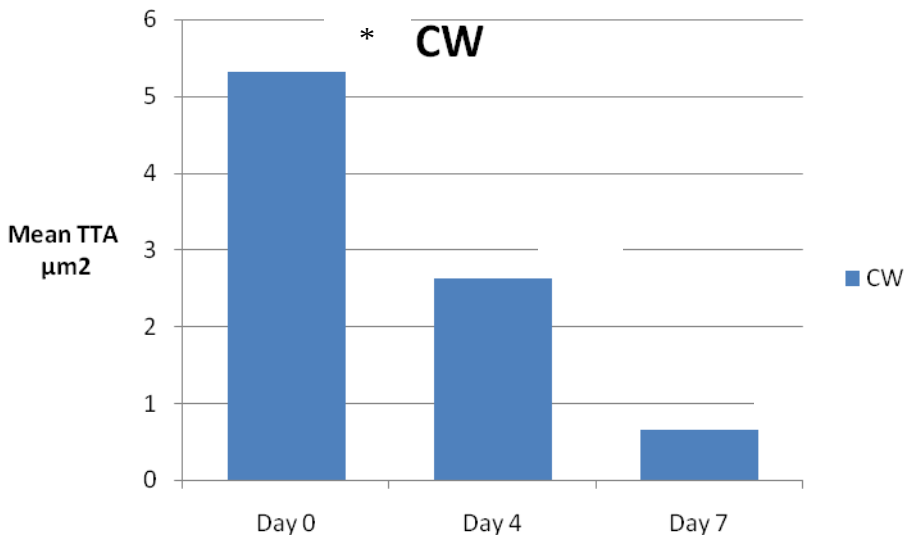

Figure 4.3 Representation of Mean Thermally Altered Area of CW mode treated group at 0th ,4th and 7th days post-irradiation. (*) indicates a significant difference in mean value

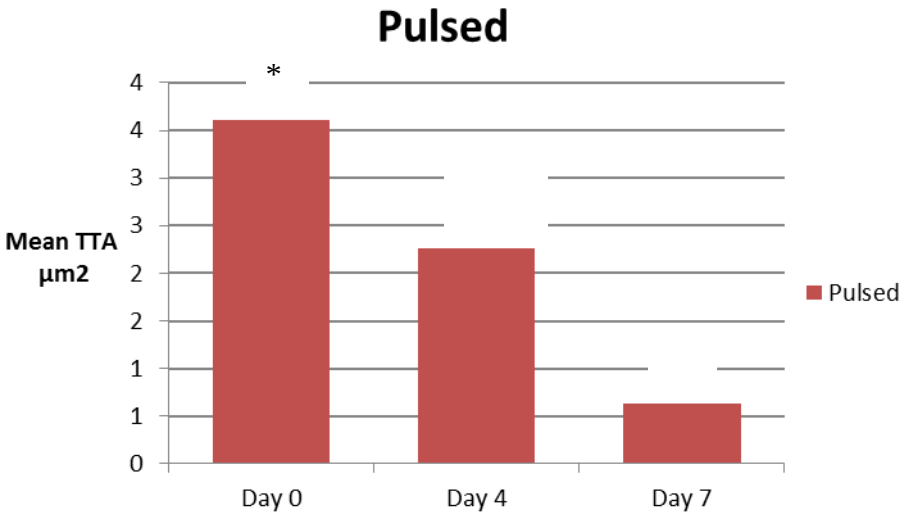

Figure 4.4 Representation of Mean Thermally Altered Area of pulsed mode treated group at 7th days post-irradiation (*) indicates a significant difference in mean value

Histologic investigation using H&E stain revealed a significant tissue alteration in the region adjascent to the irradiated wound in both continuous wave and pulsed mode laser treated groups with little wound seal when compared with control, a skin tissue sample excised from a region other than the irradiated site at 0th day (figure 4.6.1,2& 3) . Tighter closure of the incision was observed in CW mode laser treated group than in pulsed mode laser treated one at both 4th and 7th days post-irradiation . The thermal changes were reversed gradually at 4th day and more reduced at 7th days in both CW and pulsed mode group (figure 4.3D, E, F & G).

.

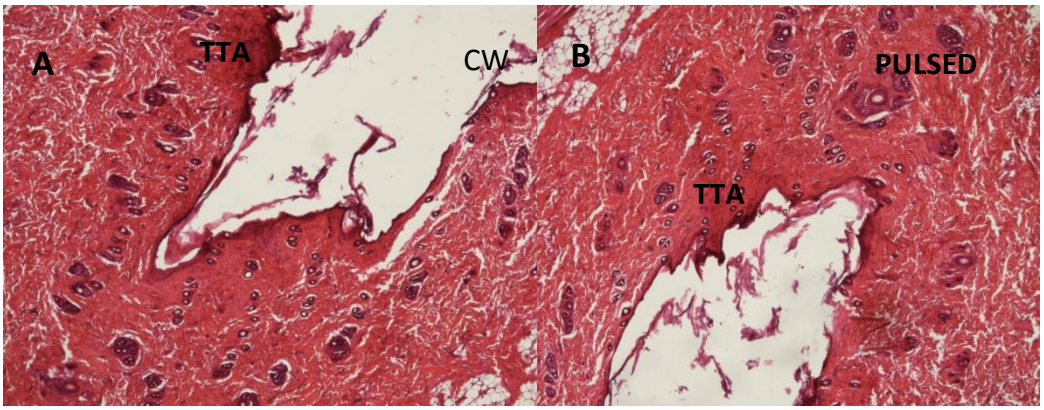

Figure 3.5.1-3 : A= CW mode laser treated incision and thermal alteration at 0th day. B= Pulsed mode laser treated incision and thermal alteration pattern at 0th day

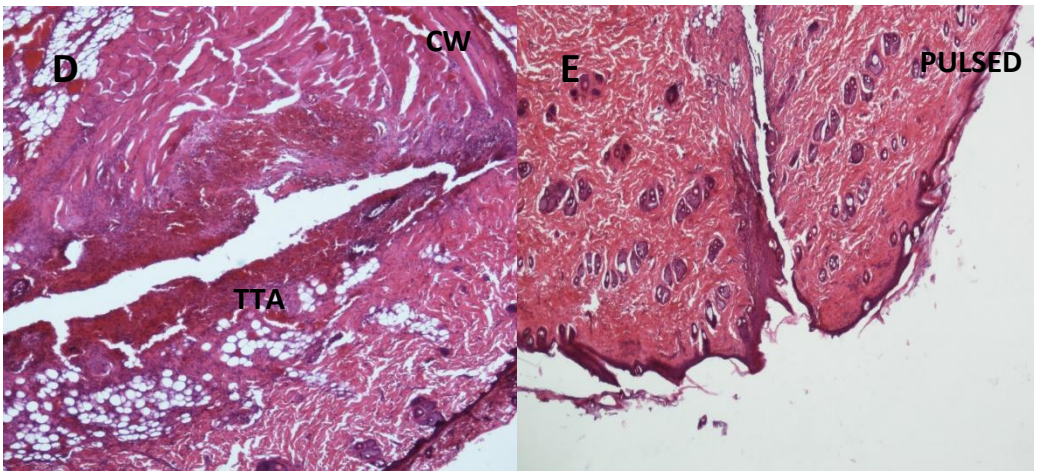

Figure 3.5.4-5 : D = CW mode laser treated incision appearance and thermal changes pattern at 4th day. E = Pulsed wave mode laser welded incision appearance and tissue thermal alteration pattern at 7th day.

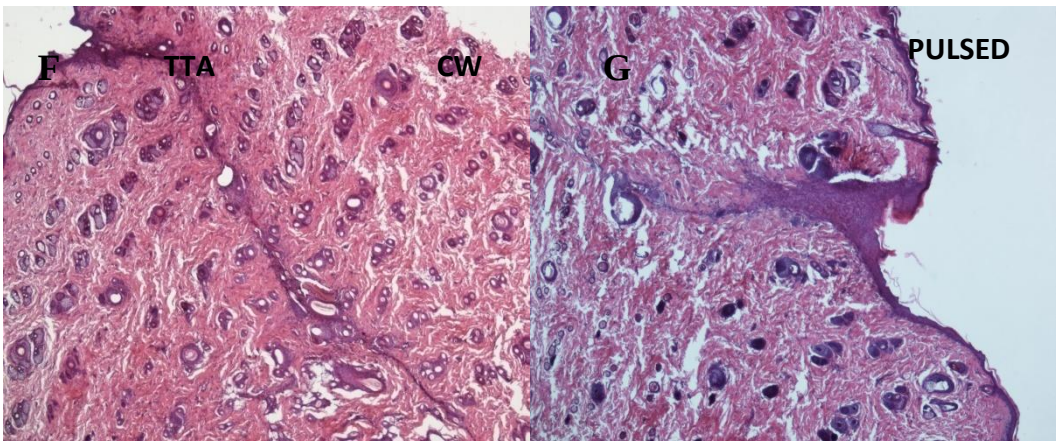

Figure 3.5.6-7: F = CW mode laser welded incision and thermal changes appearance And G = Pulsed mode laser welded incision and thermal changes appearance at 7th day post-irradiation.

DISCUSSION

Closure of skin incision with 980 and 1064nm laser was performed by many researchers with the most recent work by Liming *et al.*,attempted to close skin incision on rats by laser welding with a combination of these two lasers which was a preliminary study for the determination of optimum parameters as already described in the literature. As the two wavelengths have varying penetration power their individual effects have been studied and their combined thermal effects on the skin tissue remain a subject under investigation. The debate put forward by this study is that laser welding should be achieved with the combined beam form of these two wavelengths and a combination of optimal laser parameters in order not to cause a significant thermal damage while ensuring that the healing capability is not compromised.

Thermal alteration in the skin tissue was assessed in this study by measuring the most extensive thermally altered area (TTA) in the region adjacent to the irradiated site from either side of the incision. Mean thermally altered area (μm^2) in continuous wave and pulsed mode closed incisions was found to differ signifcantly ($p>0.05$) at 0th and 4th days with higher mean value from CW mode laser closed group in both periods (figure 4.1a&b) while insignificant ($p<0.05$) at 7th day post-irradiation (Figure 4.3) .The less thermal changes detected at 0th day and their gradual disappearance post-irradiation might have resulted from the precaution taken to select optimum exporsure time (5 seconds in CW and 10 seconds in pulsed mode) considering the recent finding by Yi-Ming *et al.*2013 who have performed a study 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 and whose results reveal that the laser-induced burn injury intensified significantly in both horizontal dimension and in vertical depth with the prolongation of exporsure time [72–74].Contrary to their finding , the damage pattern observed in this study at 0th day is more horizontal than vertical with CW mode irradiated incision having more extensive themally altered area deep inside dermis than pulsed mode irradiated incision (figure 4.5a&b) as seen histologically to be confined to the middle of dermis.Compared with those studies of skin incision closure performed with 980nm laser alone [15, 16], this study observed much less coagulation in both CW and pulsed mode closed incisions following irradiation.This could be thought of as the resultant combined beam effect of dual wavelength noted during CW mode irradiation of some slightly bleeding incisions as the laser light struck the incision without intensily coagulating or carbonizing the surface blood. This observation could be best explained as the modication of superficial effect seen when 980nm wavelength alone was used to achieve welding in previous studies [16].

Moreover, precise arrangement of newly synthesized collagen was noted in the depeest sub-layer of dermis which appear to be thermally unaltered histologically at 7th day post-irradiation (figure 4.6N&M). This is in line with the finding of Balasubramanian in 2001 who noted increased small collagen fiber formation- evidence of neocollagenesis at 30 days post heat treatment confirming the ability of laser light to stimulate collagenesis and neocollagenesis [48]. This study also took into consideration experimentally, the suggestion by Lin et al., 2007 who noted that while collagen fibers begin to curve at 52 to 55 ^{o}C, structural changes were not seen at lower temperature (25 and 40 ^{o}C) [50]. Thus, controlled laser parameters were carefully selected in accordance with the findings of these studies to ensure the highest temperature reached (48-57 ^{o}C) was at optimum range for collagen denaturation and their subsequent cross-linkages following cooling in-between spot irradition to reduce the consequential increase in susceptibility to digestion which could have an influence on the nature of the healing process in the burn wound [51].

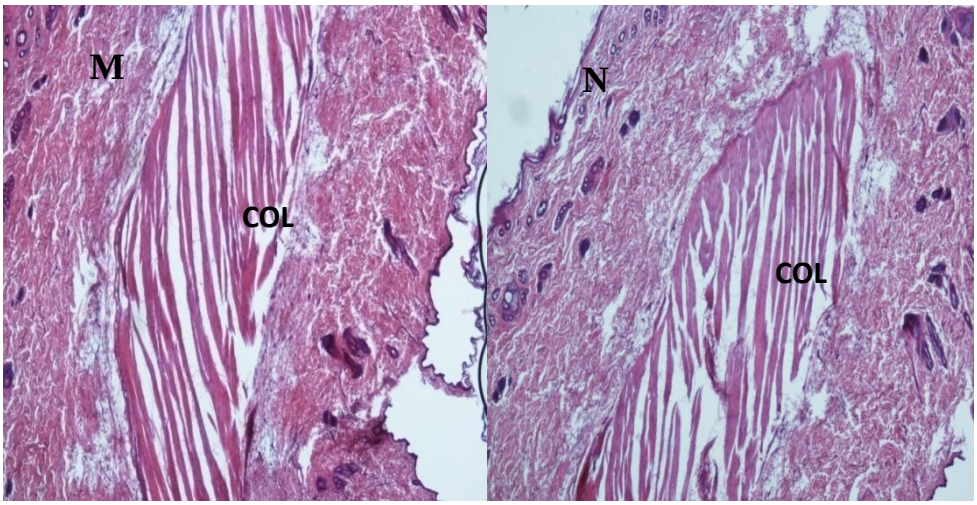

Figure 4.6.1-2: M &N =the collagen arrangements in the dermis at 7th day post-irradiation in CW and pulsed mode laser welded incisions respectively

Complete wound bridging was noted at and 7th days after wound closure and the thermally affected region in the neighbouring tissues greatly reduced in both the two laser welding modes groups (figure 4.5h&i) with CW mode group having tighter seal.This observation can be traced at the 4th day following wound closure when histologic examination revealed the presence of much more granulation tissues on the CW mode irradiated incision than on pulsed mode irradiated ones (figure 4.5.4D).The whole study suggests that skin wound closure can be achieved with the combined beam form of 980 and 1064nm at optimum laser parameters without causing significant thermal damages to the sorrounding tissues.

CONCLUSIONS AND RECOMMENDATIONS

Dual wavelength (980 & 1064nm) laser wound closure was found to be more effective in CW mode at power; 1W, energy; 5J per spot and exporsure time; 5 seconds. Thermal alteration of the wound sorrounding tissue was noted at early hours of irradiation in both the two laser mode groups but histologically appeared reversed at late healing period. Thus, laser wound closure could be achieved in CW mode with appropriate combination of laser parameters to avoid a significant tissue thermal alteration.

Further studies with dual wavelength (980 & 1064nm) laser are recommended to elucidate the following:

Thermal effects on the tissue at molecular level

Capability of internal organs (such as liver, kidney, spleen, intestine, and ruptured blood vessels) closure after surgery.

REFERENCE

[1] Jianhua Zhou , J.K.Chen and Yuwen Zhang. "Theoretical Analysis of Thermal Damage in Biological Tissues Caused by laser irradiation". *MCB*, vol.4, no.1, pp.27-39, ((2008))

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

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

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

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

[6] Welch A. J., , Gemert M. J. C., , and Star W. M., " Definitions and overview of tissue optics. ," in Optical-thermal response of laser-irradiated tissue. , Welch A. J., and Gemert M. J. C., Eds., pp. 15–46 , New York , Plenum Press ((1995)).

[7] Simhon D., , Brosh T., , Halpern M., , Ravid A., , Vasilyev T., , Katzir N., ,Katzir A., , and Nevo Z., " Closure of skin incisions in rabbits by laser soldering I: wound healing pattern. ," Lasers Surg. Med.. 35, (1), 1–11 ((2004)

[8] Brosh T., , Simhon D., , Halpern M., , Ravid A., , Vasilyev T., , Katzir N., ,Nevo Z., , and Katzir A., " Closure of skin incisions in rabbits by laser soldering II: tensile strength. ." Lasers Surg. Med.. 35, (1), 12–17 ((2004)).

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

[10] Lobel B., , Eyal O., , Kariv N., , and Katzir A., " Temperature controlled CO_2 laser welding of soft tissues: Urinary bladder welding in different animal models (rats, rabbits, and cats). ," Lasers Surg. Med.. 26, (1), 4–12 ((2000)).

[11] McNally K. M., , Sorg B. S., , Chan E. K., , Welch A. J., , Dawes J. M., , and Owen E. R., " Optimal parameters for laser tissue soldering. Part I: tensile strength and scanning electron microscopy analysis. ," Lasers Surg. Med.. 24, (5), 319–331 ((1999)).

[12] Athiraman H., , Wolf R. F., , Bartels K. E., , Shivakoti S., , Liu H., , and Chen W. R., " Selective photothermal tissue interaction using 805 nm laser and indocyanine green in tissue welding. ," J. X-Ray Sci. Technol.. 12, (2), 117–126 ((2004)).

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

[14] Fried N. M., and Walsh J. T., " Laser skin welding: *in vivo* tensile strength and wound healing results. ," Lasers Surg. Med.. 27, (1), 55–65 ((2000)).

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

[16] Tabakoglu H. O., , Topaloglu N., , and Gulsoy M., " The effect of irradiance level in 980-nm diode laser skin welding. ," Photomed. Laser Surg.. 28, (4), 453–458 ((2010)).

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

[18] Nathaniel M Fried, Joseph T Walsh Jr, (1999), Cryogen spray cooling during laser tissue welding

[19] Lynette M. B. “Welding of Skin using Nd:YAG Laser with Bipolar Contact Applicators” ((2003))

[20] Kyunghan K., and Zhixiong G. “Ultrafast Radiation Heat Transfer in Laser Tissue Welding and Soldering”. ((2004))

[21] Small W. “Thermal and Molecular Investigation of Laser Tissue Welding”. *PhD thesis.* 1998

[22] Karen, M. M.,, Brian, S. S., Ashley, J. W., Judith M. D., Earl R. O. “Photothermal effects of laser tissue soldering”. ((1998))

[23] Seigman, Anthony E., *Lasers*, University Science Books, 1986

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

[25] Liming Hu ; Zhihua Lu ; Biao Wang ; Junsheng Cao ; Xiaobo Ma ; Zhenhua Tian ; Zhijian Gao ; Li Qin ; Xiaodong Wu ; Yun Liu ; Lijun Wang. “Closure of skin incisions by laser welding with a combination of two near-infrared diode lasers:Preminary study for determination of optimal parameters J. Biomed. Opt. 16(3), 038001 (March 24, 2011). doi:10.1117/1.3552648

[26] Nathaniel M. F., Vincent, C. H., Joseph T. Walsh, J. R.. “ Laser Tissue Welding: Laser Spot Size and Beam Profile Studies” ((1999))

[27] Nathaniel M. F., Joseph, T. Walsh J. R. “Cryogen spray cooling during laser tissue welding”. ((1999))

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

[29] Goldman, L. “Application of the Laser” *CRC Press*, ((1977))

[30] Videocassette: High Powered Lasers in the Lab. *Interactive Media Communications*, ((1994))

[31] Goldman, L. “Application of the Laser” *CRC Press* ((1977))

[32] Pressley, R. J. CRC Handbook of Lasers *CRC Press*. ((1971))

[33] ANSI Standard Z136.1-2007, *American National Standard for the Safe Use of Lasers*, ((2007))

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

[35] Mallow, Alex and Leon Chabot, *Laser Safety Handbook*, Van Nostrand Reinhold, ((1978))

[36] Chen B, Thomsen SL, Thomas RJ, Oliver J, Welch AJ."Histological and modeling study of skin thermal injury to 2.0 microm laser irradiation".Lasers Surg Med. ((2008)) Jul;40(5):358-70. doi: 10.1002/lsm.20630.

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

[38] Zhang Y. M., Ruan J., Xiao R., Zhang Q., Huang Y. S. "Comparative Study of 1,064-nm Laser-Induced Skin Burn and Thermal Skin Burn". Cell Biochem Biophys. ((2013))

[39. Manstein D, Zurakowski D, Thongsima S, Laubach H, Chan HH. "The effects of multiple passes on the epidermal thermal damage pattern in nonablative fractional resurfacing". Lasers Surg Med. ((2009)) Feb;41(2):149-53. doi: 10.1002/lsm.20711.

[40] Carlson BM. Integumentary, skeletal, and muscular systems. In: *Human Embryology and Developmental Biology*. St. Louis, Mo: Mosby; ((1994)):153-81

[41] Burns DA, Breathnach SM, Cox N, Griffiths CE, eds. *Rook's Textbook of Dermatology*. 7th ed. Malden, Mass: Blackwell Science; ((2008))

[42] Poblet E, Jiménez F, Ortega F. The contribution of the arrector pili muscle and sebaceous glands to the follicular unit structure. *J Am Acad Dermatol*. ((2004));51(2):217-22

[43] Prost-Squarcioni C. [Histology of skin and hair follicle]. *Med Sci (Paris)*. Feb ((2006));22(2):131-7

[44] Taylor GI, Pan WR. Angiosomes of the leg: anatomic study and clinical implications. *Plast Reconstr Surg*. ((1998));102(3):599-616; discussion 617-8

[45] Lamberty BG, Cormack GC. Fasciocutaneous flaps. *Clin Plast Surg*. Oct ((1990));17(4):713-26.

[46] McGregor I A, Morgan G. Axial and random pattern flaps. *Br J Plast Surg*. ((1963));26:202.

[47] Clark, G.; Parker, E.; Schaad, J. & Warren, W. J. . "New measurements of previously unknown large interplanar spacings in natural materials". *J. Amer. Chem. Soc* **57** (8): 1509. ((2009)) doi:10.1021/ja01311a504.

[48] Balasubramanian, D . "GNR — A Tribute". *Indian Academy of Sciences*.((2001))

[49] Leonidas, Demetres D.; Jardine, A. M.; Li, S.; Shapiro, R.; Acharya, K.R. "Binding of Phosphate and pyrophosphate ions at the active site of human angiogenin as revealed by X-ray crystallography". *Protein Science* **10** (8): 1669–1676. doi:10.1110/ps.13601. PMC 2374093.PMID 11468363. ((2001)).

[50] Subramanian, E. "Obituary: G.N. Ramachandran". *Nature Structural & Molecular Biology* **8**(6): 489–491. ((2001)) doi:10.1038/88544. PMID 11373614.

[51] Fraser, R. D.; MacRae, T. P. & Suzuki, E. "Chain conformation in the collagen molecule". *J Mol Biol* **129** (3): 463–481. ((1979)). doi:10.1016/0022-2836(79)90507-2.PMID 458854.

[52] Rahbar MH, del Junco DJ, Huang H, Ning J, Fox EE, Zhang X, Schreiber MA, Brasel KJ, Bulger EM, Wade CE, Cotton BA, Phelan HA, Cohen MJ, Myers JG,Alarcon LH, Muskat P, Holcomb JB; PROMMTT Study Group. A latent class model for defining severe hemorrhage: experience from the PROMMTT study. J Trauma Acute Care Surg. ((2013) 75(1 Suppl 1):S82-8. doi: 10.1097/TA.0b013e31828fa3d3.

[53] Hollander J. E., Singer A .J., "Laceration management". *Ann Emerg Med* ((1999)); 34:356.

[54] Kanegaye J. T., "A rational approach to the outpatient management of lacerations in pediatric patients". *Curr Probl Pediatr* ((1998)); 28:205.

[55] McNamara, R. N., Loiselle, J. "Laceration repair": Textbook of pediatric emergency procedures, Henretig, F, King, C (Eds), Williams and Wilkins, Baltimore ((1997)). p.1141.

[56] Robson M. C. "Disturbances of wound healing". *Ann Emerg Med* ((1988)) 17:1274.

[57] Trott, A. T., "Special anatomic sites": Wounds And Lacerations: Emergency Care and Closure, 3rd edition, Elsevier Mosby, Philadelphia ((2005)). p.153.

[58] Iizuka, M. N., Vitkin I. A., Kolios, M. C., Sherar, M. D. "The effects of dynamic optical properties during interstitial laser photocoagulation".*Phys Med Biol* 2000, **45**(5)**:**13351357. ((2009))

[59] Mohammed, Y., Verhey J. F.,: "A finite element method model to simulate laser interstitial thermo therapy in anatomical inhomogeneous regions". *Biomed Eng Online* ((2005)), 4(1):2.

[60] Moussa, N. A., Tell, E. N., Cravalho, E. G. "Time progression of hemolysis or erythrocyte populations exposed to supraphysiologic temperatures". *J Biomech Eng* ((1979)) **101:**213-217

[61] Dierickx C. C.. 1The role of deep heating for noninvasive skin rejuvenation. Lasers Surg Med ((2006)); 38:799-807.

[62] Mark D. S. and Scott C. "Effects of Infrared laser exporsure in a cellular model of wound healing Lasers "*Surg Med* 38:699-703.

[63] Cecchetti, C W., Guazzieri, A. Tasca, M. Dal B. F. And Zattoni, F. P. " 980 nm Diode Laser and Fiber Optic Resectoscope in Endourological Surgery". ((1996))

[64] Cecchetti, C. W., Guazzieri, A. Tasca, M. Dal B. F. and Zattoni, F. P.980nm Diode Laser and Fiber Optic Resectoscope in Pagano .*Endourological Surgery 2(3) 22-3.* ((1997))

[65] Anderson R. R. and Parrish J. A. "Selective Photothermolysis-precise Microsurgery by Selective Absorption of Pulsed Radiation. Science", ((1983)); 220(4596):524-7.

[66] Svaasand I. O., Milner T. E., Anvari B., Norvang L.T., Tanenbaum B.S., Kimel S, Berns M. W. and Nelson J. S. "Epidermal Heating During Laser Induced Photothermolysis of Port Wine Stains": Modeling Melanosomal Heating after Dynamic Cooling the Skin Surface, Laser Interaction with Hard and Soft Tissue II. SPIE Proc., ((1995)); 2323:366-77.

[67] Nakagawa H, Tan OT, Parish JA. Ultrastructure Changes in Human Skin after Exposure to a Pulsed Laser. J Invest Dermatol., 1985; 84:396-400.

[68] Anderson R, Parrish J. The optics of human skin. J Invest Dermatol., 1981; 77:13-9.

[69] Lukac M, Grad L. Scanner Optimized Aesthetic Treatments with the VSP Nd:YAG Lasers. J Cosmet Laser Ther, June 008; Vol. 10, No. 2.

[70]. 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, 239–244.

[71] 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, AB198.

[72] Dang, Y. Y., Ren, Q. S., and Liu, H. X. "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. ((2005)).

[73] Babilas, P., Shafirstein, G., Baumler, W. Selective photothermolysis of blood vessels following flashlamp-pumped pulsed dye laser irradiation: in vivo results and mathematical modelling are in agreement. The Journal of Investigative Dermatology, 125, 343–352. ((2005))

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

[75] Coolen, N. A., Vlig, M., van den and Bogaerdt, A. J. Development of an in vitro burn wound model. Wound Repair and Regeneration, 16, 559–567. ((2008))

[76] Yi-Ming Zhang • Jing Ruan • Rong Xiao •Qiong Zhang • Yue-Sheng Huang.. "Comparative Study of 1,064-nm Laser-Induced Skin Burn and Thermal Skin Burn" Springer Science+Business Media New York ((2013))

[77] Karn J. H. "Laser operation mode". Laser Therapy Diowave. *American Society of Laser Medicine and Surgey*. ((2013))

[78] Jan Tunér, D. S. and Lars, H. "Pulsed and super-pulsed laser mode". *Swedish Laser Medical Society*. ((2013))

[79] John H. L. "Laser pulsing and frequenc: Laser Therapy Diowave". *North American Association For Laser Therapy* (((2013))).

[80] Rolls G. 101 Steps to Bet ter Histology. Melbourne: Leica Microsystems ((2008))

[81] Rolls GO, Farmer NJ, Hall JB. Ar tifacts in Histological and Cy tological Preparations. Melbourne: Leica Microsys terms, 0086 ((2010))

[82] Leica Microsystems. Instruction Manual Leica RM22 35 V1.3 Nussloch: Leica Microsystems, 2008[83] Thompson, Samuel W. SELECTED HISTOCHEMICAL AND HISTOPATHOLOGICAL METHODS, Charles C. Thomas, Springfield, IL, ((2006))

APPENDICES

APPENDIX A

Paired comparison analysis of CW and pulsed mode groups' thermally atered area at 0th day

THERMALLY ALTERED AREA μm^2									
0TH DAY									
	Mode		d	d^2	Mean=(∑d/n)	Variance (S^2d)	Standard Deviation (sd)	Test Stat(T9)	P Value(%)
	CW	Pulsed							
	3.47	0.86	2.61	6.81	1.70	5.68	1.80	9.45	0.05
	3.65	3.27	0.38	0.14					
	3.38	3.38	0.00	0.00					
	6.14	0.08	6.06	36.72					
	7.95	3.07	4.88	23.81					
	5.99	4.94	1.05	1.10					
	8.71	5.83	2.88	8.29					
	7.37	6.86	0.51	0.26					
	3.45	3.18	0.27	0.07					
	3.00	4.66	-1.66	2.76					
Count	**10.00**								
∑	**53.11**	**36.13**	**16.98**	**79.98**					
Mean	**5.31**	**3.61**							

Paired comparison analysis of CW and pulsed mode groups' thermally atered area at 4th day

THERMALLY ALTERED AREA µm2									
4TH DAY									
	Mode		d	d^2	Mean=(∑d/n)	Variance (S^2d)	Standard Deviation (sd)	Test Stat(T9)	P Value(%)
	CW	Pulsed							
	1.07	1.43	-0.36	0.13	0.37	3.12	0.99	3.73	0.05
	4.74	2.93	1.81	3.28					
	1.07	1.72	-0.65	0.42					
	4.74	2.52	2.22	4.93					
	2.64	1.68	0.96	0.92					
	5.45	1.67	3.78	14.29					
	3.35	3.73	-0.38	0.14					
	1.39	3.29	-1.90	3.61					
	0.58	1.27	-0.69	0.48					
	1.20	2.31	-1.11	1.23					
Count	**10.00**								
∑	**26.23**	**22.55**	**3.68**	**29.43**					
Mean	**2.62**	**2.26**							

Paired comparison analysis of CW and pulsed mode groups' thermally atered area at 7th day

THERMALLY ALTERED AREA µm2									
7TH DAY									
	Mode		d	d^2	Mean=(∑d/n)	Variance (S^2d)	Standard Deviation (sd)	Test Stat(T9)	P Value(%)
	CW	Pulsed							
	0.92	0.40	0.52	0.27	0.01	0.15	0.05	1.26	0.05
	0.67	0.80	-0.13	0.02					
	0.28	0.22	0.06	0.00					
	0.35	0.33	0.02	0.00					
	0.39	0.48	-0.09	0.01					
	0.32	1.11	-0.79	0.62					
	0.64	0.63	0.01	0.00					
	0.51	0.85	-0.34	0.12					
	1.36	0.97	0.39	0.15					
	1.00	0.59	0.41	0.17					
Count	**10.00**								
∑	**6.44**	**6.38**	**0.06**	**1.36**					
Mean	**0.644**	**0.638**							

APPENDIX B

Calculation of mean and standard deviation values of thermally altered area for CW and pulsed mode groups at 0th day

0TH Day GROUP THERMALLY ALTERED AREA (µm2)										
	CW X	**Pulsed Y**	**X-Mean**	**Y-Mean**	**(X-Mean)^2**	**(Y-Mean)^2**	**Variance X**	**Variance Y**	**√X=STD**	**√Y=STD**
	3.47	0.86	-1.84	-2.75	3.39	7.58	4.26	3.90	2.06	1.98
	3.65	3.27	-1.66	-0.34	2.76	0.12				
	3.38	3.38	-1.93	-0.23	3.73	0.05				
	6.14	0.08	0.83	-3.53	0.69	12.48				
	7.95	3.07	2.64	-0.54	6.96	0.29				
	5.99	4.94	0.68	1.33	0.46	1.76				
	8.71	5.83	3.40	2.22	11.55	4.92				
	7.37	6.86	2.06	3.25	4.24	10.54				
	3.45	3.18	-1.86	-0.43	3.46	0.19				
	3.00	4.66	-2.31	1.05	5.340721	1.10				
Count	**10.00**									
∑	**53.11**	**36.13**			**42.59**	**39.03**				
Mean	**5.31**	**3.61**								

Calculation of mean and standard deviation values of thermally altered area for CW and pulsed mode groups at 4th day

4TH Day GROUP THERMALLY ALTRED AREA (µm2)										
	CW	**Pulsed**	**CW-Mean**	**Pulsed-Mean**	**(CW-Mean)^2**	**(Pulsed-Mean)^2**	**Var(x)**	**Var(y)**	**√X=STD**	**√Y=STD**
	1.07	1.43	-1.55	-0.83	2.41	0.68	3.00	0.64	1.73	0.80
	4.74	2.93	2.12	0.68	4.48	0.46				
	1.07	1.72	-1.55	-0.54	2.41	0.29				
	4.74	2.52	2.12	0.27	4.48	0.07				
	2.64	1.68	0.02	-0.58	0.00	0.33				
	5.45	1.67	2.83	-0.59	7.99	0.34				
	3.35	3.73	0.73	1.48	0.53	2.18				
	1.39	3.29	-1.23	1.04	1.52	1.07				
	0.58	1.27	-2.04	-0.99	4.17	0.97				
	1.20	2.31	-1.42	0.06	2.02	0.00				
Count	**10.00**									
	26.23	**22.55**			**30.03**	**6.39**				
Mean	**2.62**	**2.26**								

Calculation of mean and standard deviation values of thermally altered area for CW and pulsed mode groups at 7th day

7TH Day GROUP THERMALLY ALTERED AREA (µm2)										
	CW	**Pulsed**	**CW-Mean**	**Pulsed-Mean**	**(CW-Mean)^2**	**(Pulsed-Mean)^2**	**Var(x)**	**Var(y)**	**√X=STD**	**√Y=STD**
	0.92	0.40	0.28	-0.24	0.08	0.06	0.11	0.08	0.34	0.28
	0.67	0.80	0.03	0.16	0.00	0.03				
	0.28	0.22	-0.36	-0.42	0.13	0.17				
	0.35	0.33	-0.29	-0.31	0.09	0.09				
	0.39	0.48	-0.25	-0.16	0.06	0.02				
	0.32	1.11	-0.32	0.47	0.10	0.22				
	0.64	0.63	0.00	-0.01	0.00	0.00				
	0.51	0.85	-0.13	0.21	0.02	0.04				
	1.36	0.97	0.72	0.33	0.51	0.11				
	1.00	0.59	0.36	-0.05	0.13	0.00				
Count	**10.00**									
∑	**6.44**	**6.38**			**1.12**	**0.76**				
Mean	**0.64**	**0.64**								

CURRICULUM VITAE

Name Surname: Abdullahi Ibrahim UBA

Place and Date of Birth: Born in Kunar Madatai Kano Munipal , Nigeria on 1st April

Address: 114, Kunar Madatai Kano Munipal, Nigeria

E-Mail: abdulsurgeon@yahoo.com, abdullahiibrahimuba@ymail.com

B.Sc.: Biochemistry

Professional Experience and Rewards:

Technical experience with laboratory diagnostic devices at Histopathology, Hematology and Chemical Phatology Departments, Aminu Kano Teaching Hospital Kano , Nigeria (2009)

Technical experience with academic research laborotory devices and Teaching experience from The Department of Science Laborotory Technology, The Polytechnic of Sokoto State , Nigeria (2012)

Certificate of Student Industrial Work Experience (SIWES) Awarded by Aminu Kano Teaching Hospital , Nigeria

Letter of Recommendation for Dedication to National Youth Service at Science Laborotory Technology Department Sokoto State Polytechnic awarded by The Polytechnic of Sokoto State, Nigeria