HISTOLOGICAL AND MORPHOLOGICAL CHARACTERIZATION OF RAT SKIN AFTER CW CO_2 LASER IRRADIATION

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UNIVERSITI SAINS MALAYSIA

2013

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By

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Thesis submitted in fulfillment of the requirements
for the degree of
Master of Science

July 2013

ACKNOWLEDGEMENTS

“All praises and thanks to ALLAH”

I would like to express my deepest appreciation to my supervisor, Professor Mohd Zubir Mat Jafri for the valuable guidance, encouragement and advice he has provided throughout my research work. I also wish to acknowledge my former supervisor, Dr. Khalid Mutashar Omar for his guidance and cooperation in the past.

I also want to take this opportunity to thank the staff of the Histology and Electron Microscope Laboratories, School of Biological Sciences and staff of the Advanced Medical and Dental Institute for their technical assistance and excellent hospitality during the period of my research work. My gratitude also goes to the staff of Medical and Biophysics laboratories, School of Physics for their assistance. In addition, I would like to convey special thanks to Universiti Sains Malaysia for providing the financial means under the PGRS-grant scheme no: 1001/PFIZIK/835002.

I would like to express my eternal appreciation towards my parents (Raja Mahmood Bin Raja Omar and Nik Radzihah Bt Wan Hussain) and siblings for all their love, patience and tremendous moral supports all the way till the completion of my research. Lastly, I would like to thank my dear friends and colleagues for their support and it has been great to know all of you during my time here at USM.

Tengku Lina Izzati Binti Raja Mahmood

School of Physics, USM. July 2013

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LIST OF ABBREVIATIONS

C	Carbon
CO_2	Carbon Dioxide
CW	Continuous Wave
DPX	Distrene Plasticiser Xylene
ECM	Extracellular Matrix
EM	Electron Microscope
Er:YAG	Erbium: Yttrium Aluminium Garnet
FES	Field Emission Source
FESEM	Field Emission Scanning Electron Microscope
H&E	Hematoxylin and Eosin
HMDS	Hexamethyldisilizane
LM	Light Microscope
Nd:YAG	Neodymium: Yttrium Aluminium Garnet
O	Oxygen
PWM	Pulse Width Mode
PWS	Port Wine Stain
UV	Ultraviolet

LIST OF SYMBOLS AND UNITS

π	Pi
α	Specific absorption coefficient
μ_a	Absorption coefficient
µm	Micrometre
ºC	Degree celsius
c	Chromophore
I	Transmitted intensity
I_0	Incident intensity
cm	Centimetre
cm^2	Square centimetre
g	Gram
J/cm^2	Joules per square centimetre
kg	Kilogram
L	Skin thickness
M	Molar
mg	Miligram
mL	Mililitre
mm	Milimetre
mm^2	Square milimetre
nm	Nanometre
pH	Power of hydrogen
s	Second
W	Watts
W/cm^2	Watts per square centimetre

PENCIRIAN HISTOLOGI DAN MORFOLOGI KULIT TIKUS SELEPAS PENYINARAN LASER CW CO_2

ABSTRAK

Dalam kajian ini, interaksi laser-kulit menggunakan tikus jenis Sprague Dawley sebagai sampel eksperimen dan laser selanjar karbon dioksida (CW CO_2) sebagai sumber sinaran. Mikroskop cahaya dan bidang pelepasan mikroskop imbasan elektron (FESEM) digunakan untuk menilai permukaan dan struktur kulit bagi kedua-dua sampel normal dan yang terdedah dengan sinaran. Ringkasnya, tikus telah dibahagikan kepada dua kumpulan dengan set pendedahan yang berbeza. Bagi kumpulan pertama, kulit tikus didedahkan kepada laser CW CO_2 dengan ketumpatan kuasa yang berbeza-beza (15.63 W/cm^2, 17.19 W/cm^2, 18.75 W/cm^2 dan 20.31 W/cm^2). Setiap ketumpatan kuasa pula dipancarkan pada empat tempoh sinaran yang berlainan (15 s, 30 s, 45 s dan 60 s). Keputusan kajian telah menunjukkan peningkatan pada kedalaman kerosakan terma dan pengurangan pada ketebalan epidermis dengan peningkatan pada kedua-dua tempoh sinaran dan ketumpatan kuasa. Walau bagaimanapun, sampel yang didedahkan pada 20.31 W/cm^2 menunjukkan ciri-ciri yang berbeza. Kedalaman kerosakan telah meningkat pada 15 s dan 30 s dan kemudian kedalaman kerosakan terma telah menurun pada 45 s dan 60 s disebabkan ablasi laser. Pemerhatian ini menunjukkan bahawa kesan ablasi amat bergantung kepada tempoh sinaran yang akan mengurangkan kedalaman kerosakan terma. Dalam imej FESEM, kemusnahan *corneocytes* dan pembentukan ruang yang berselang antara *corneocytes* mempunyai hubungan yang baik dengan peningkatan dalam tempoh sinaran dan ketumpatan kuasa. Malah, perubahan dramatik telah diperhatikan di permukaan kulit yang disinari dengan laser pada 20.31 W/cm^2

dengan beberapa lubang dalam sepertimana yang dapat dilihat dalam imej FESEM. Bagi kumpulan kedua pula, ketumpatan kuasa 20.31 W/cm^2 disinarkan pada kawasan kulit yang berbeza (sisi, dada, perut dan kaki). Bunyi “pop” yang didengari merupakan tempoh sinaran yang direkodkan bagi setiap kawasan kulit. Hasil kajian menunjukkan bahawa ketebalan kulit boleh dikaitkan dengan masa permulaan ablasi, di mana kulit tebal memerlukan masa yang lebih lama untuk pengewapan letupan berlaku berbanding kulit nipis. Tambahan pula, kehilangan stratum korneum dengan epidermis masih melekat atau sepenuhnya terpisah daripada lapisan dermis, ketebalan pembekuan kolagen, kerosakan separa pada granul melanin dalam mentol rambut serta di sepanjang batang rambut, pengecutan lapisan dermis bersama dengan pengembangan ruang kosong antara folikel rambut dan tisu sekitarnya telah terbukti dalam setiap sampel histologi. Semua perubahan ini mencerminkan kerosakan terma manakala kesan fototerma memainkan peranan yang dominan semasa proses interaksi antara laser CW CO_2 dengan kulit tikus.

HISTOLOGICAL AND MORPHOLOGICAL CHARACTERIZATION OF RAT SKIN AFTER CW CO_2 LASER IRRADIATION

ABSTRACT

The laser-skin interaction was studied by using the Sprague Dawley rats as experimental samples and continuous wave carbon dioxide laser (CW CO_2) as a source of irradiation. Light microscope and field emission scanning electron microscope (FESEM) were used to evaluate the skin surface and underlying structures for both exposed and control samples. Briefly, the rats were divided into two groups with different setting exposures. In the first group, the rat skin was exposed to CW CO_2 laser at different power densities (15.63 W/cm^2, 17.19 W/cm^2, 18.75 W/cm^2 and 20.31 W/cm^2). Each power density was exposed in four different exposure durations (15 s, 30 s, 45 s and 60 s). The results showed an increase on thermal damage depth and a decrease on epidermal thickness with the increase in both exposure duration and power density. However, the sample exposed at 20.31 W/cm^2 showed different features. The damage depth was increased at 15 s and 30 s and then the thermal damage depths were decreased at 45 s and 60 s due to the laser ablation. These observations showed that the ablation effect strongly depended on the exposure duration which then decreased the depth of thermal damage. In the FESEM images, the disruption of corneocytes and the formation of the intervening spaces between corneocytes correlated well with the increase in exposure duration and power density. In fact, dramatic changes were noted in the skin surface, which was treated by the laser at 20.31 W/cm^2 with several deep holes that could be seen in the FESEM image. As for the second group, the power density of 20.31 W/cm^2 was irradiated at different areas of the skin (side, chest, stomach and leg). The time duration when the distinct “popping” sound was heard was the explosion duration recorded for each area of the skins. The results showed that the skin thickness could be associated with the ablation onset time, in which the thick skin needed more time for the explosive vaporisation to occur than the thin skin. Furthermore, loss of the stratum corneum with the epidermis still attached or completely ablated from the dermis layer, thick collagen coagulation, partial damage on melanin granules in the hair bulb and along the hair shaft, dermal shrinkage with expanded empty spaces between hair follicles and surrounding tissue was evident in every histologic section. All these changes resembled the thermal injuries whereas the photothermal effect played a dominant role during the interaction process between the CW CO_2 laser with the rat skin.

CHAPTER 1

INTRODUCTION

1.1 Background and Significance

The CO_2 surgical laser was the first laser to be introduced to the medical field during last four decades and it is still one of the most useful and efficient medical laser available in the market. Since its invention by Dr. Kumar Patel in the early 1960s, the CO_2 laser has evolved into one of the most extensively manipulated lasers in medicine. The first documented use of the CO_2 laser dates back to the early 1970s, when otolaryngologists used it on cadaver larynxes (Shapshay and Beamis, 1989). It was then adapted to the laparascope and colposcope, which allowed for the treatment of various gynecologic diseases. The successful precision of the laser-tissue interaction of the CO_2 laser in gynecologic studies has led to the laser introduction to dermatology, where it was originally applied to aesthetically improve the appearance of congenital vascular lesions such as port wine stains (PWS) and hemangioma (Oh and Kim, 2012).

Effective CO_2 laser procedures are achieved by tailoring the laser parameters to the physical characteristics of the target tissue. Moreover, laser parameters are selected to optimize efficacy while minimizing unwanted side effects and tissue damage. From a phenomenological standpoint, it is known that laser-induced skin tissue injury occurs via different reaction mechanisms. Unfortunately, the specific cellular and molecular pathways which initiate and govern these mechanisms are poorly understood. Besides that, current research in laser-skin tissue interaction has just focused on the damage that is generated in the collateral tissue. In that case, only little data has been carried out to examine the histological dose response of human skin on CO_2 laser irradiance and exposure duration which these two factors may lead to the disappointing results or serious complications.

A common objective in medical laser applicator is coagulation of a desired volume of tissue with minimal or controlled thermal effect in the surrounding healthy tissue. Essentially, the theoretical model and analysis may be used to understand the complex process involved in optimize light dosimetry and treatment parameter (Zhu et al., 2002). It is because the thermally generated tissue damage that accompanies CO_2 laser ablation of skin tissue is a great interest considering of its role in inducing hemostasis and interfering with the healing process. Apart from that, the thermal damage or coagulation of collagen below the skin surface is generally believed to be beneficial, because it leads to skin tightening or sculpting and formation of new collagen. However, most models describing CO_2 laser-induced coagulation of skin tissue has not accounted fully for the dynamics of irradiation parameter and thermal damage-dependent mechanical tissue properties (Shibib, 2010; LeCarpentier, 1993; Frenz et al., 1991). These factors were in part because laser-skin interaction is a dynamic thermal event that governed by the penetration of CO_2 laser radiation into the skin tissue and the heat generated by the absorption of the incident laser light.

Generally, regarding the use of CO_2 laser in skin treatment, numerous additional scientific articles further heralded the ability of a CO_2 laser to produce char-free tissue ablation with minimal residual thermal damage. Emitting infrared light at 10,600 nm, the CO_2 laser was used for tissue vaporisation and destruction of various epidermal and dermal lesions. However, the occurrence of scarring that was mostly dependent on the depth of thermal necrosis and on the anatomic location, for example thin skin such as eyelids and upper lips that was more prone to scarring than the other facial area render the CO_2 laser as a difficult prospect for many to consider.

As more skin treatments have been performed, the number of reports of scarring and erythema has increased significantly throughout the year (Metelitsa and Alster, 2010; Alster and Tanzi, 2008).

Therefore, it is believed that a better understanding of the thermal response of skin to CO_2 laser radiation will improve the understanding of clinical results. Furthermore, the relationship between the laser operating parameters and the biomolecules effects can be carefully defined, leading to the rational selection of CO_2 laser parameters for improved outcomes in the clinical procedures. Ultimately, this research may improve current clinical laser procedures and may guide the development of novel applications for the laser in dermatology.

. 1.2 Problem Statement

The interaction of CW CO_2 laser with the skin in the previous studies were more focused on the effects of laser onto the epidermal and dermal damage depth. Only a little data has been published on this field that is occurring directly to the other structure of skin cells. Thus, the experiment has to be conducted to observe the effects of CW CO_2 laser on the some compositions of dermal tissue for instance, hair follicles and melanin. Furthermore, by using basic parameters that are power densities and exposure times, the histological changes in tissue may or may not show the similar results and the types of reactions occurred will mostly depend on the conditions and settings that were used in the experiment.

In addition, the relationship between the thicknesses at different areas of the skin with the explosive event has not been systematically studied. It is critical to understand that, because difference in skin thickness has a different mechanical strength and properties which were varied from site to site. Other than basic parameters, this factor must be considered which could also be the reason for unwanted thermal injury during the CW CO_2 laser treatment.

1.3 Research Objectives

The principle objectives of this research can be summarized in the following points:

a) To investigate the irradiation effects of CW CO_2 laser on skin surface and underlying structures.
b) To identify the types of reaction in CW CO_2 laser-skin interaction when different laser parameters applied to it.
c) To study the connection between the tissue thickness at different areas of the skin with the ablation initiation time.

1.4 Scope of Research

The aim of this research is to assess the histopathologic effects and cellular profiles of the skin tissue after a CW CO_2 laser was applied. Laser-skin tissue damage was investigated using a CO_2 laser wavelength of 10.6 µm in a continuous mode with a different exposure duration and power density. An albino rat model was used to address the complications associated with the use of CW CO_2 laser for medical application. Additionally, the maximum power density was used as to see the correlation between the skin thickness and the onset of ablation. Then, the comparisons of structural in intact with the exposed rat skin was examined by using the histological methods. The observations continued with the FESEM images of the rat skin surface with and without exposure of the CW CO_2 laser.

1.5 Outline of the Thesis

Chapter 1 provides a brief introduction to the problem addressed in this research, establishing the significance of the work. The specific aims of the research are also outlined and summarized. The critical points in the previous studies of laser-skin interaction including with the substantive findings as well as theoretical and methodological used will be explained in Chapter 2. The brief theories and general aspects of laser and skin tissue, including with the factors that determined the interaction mechanisms are covered in Chapter 3. As in Chapter 4, the methodology and instrumentation involved in this research are described in this chapter.

The obtained results from this research work were analysed and discussed in details in Chapter 5. Chapter 5 also discussed the results of CW CO_2 laser-tissue interaction when different parameters were used; power densities and exposure times. It also includes the experimental result when CW CO_2 laser exposed at different areas of the rat skin. Finally, Chapter 6 presents the conclusions of the research works and suggestion for further studies in laser-tissue interactions.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter provides a review of the literature on the interaction between laser and skin tissue. Accordingly, it starts with the discussion on CO_2 laser techniques in comparison with other method used in clinical application. Then, proceed with the explanation of a CO_2 laser system that is currently in used which are CW and superpulsed mode. Next, is the analysis of laser-tissue interaction which has been studied by using animal as an experimental model. Finally, there will be a short analysis of the benefits and challenges of implementing the CO_2 laser system in medical practice today.

2.2 CO_2 Laser for Clinical Applications

Skin resurfacing is a technique which can be used to correct significant abnormalities in the epidermis and superficial dermis layer. Several modalities have been implicated of evenly wounding these layers to produce the desired result of a new epidermis from surrounding intact epidermis and the appendage structures of a regenerated dermis. These methods include the dermabrasion, chemical peels and CO_2 laser therapy or abrasion.

The limiting depth of tissue destruction was not always easy when method such as dermabrasion or chemical peeling were used. Stegman (1980) design an animal model for comparing the histology injuries resulting from both dermabrasion and chemical peel techniques. Both of these methods used in resurfacing the skin does cause a deep dermal and subcutaneous injuries with the correction of actinically damaged skin whereas wrinkling are most often seen after dermabrasion treatment. Due to this reason, the CO_2 laser becomes a gold standard for the treatment of photoaging and acne scars for nearly 40 years. The mechanism of skin resurfacing by using the ultrapulse CO_2 laser on rabbit skin was investigated by Wei-Wei et al. (1997). The results obtained from light and electron microscope shows that almost all cell layers of the epidermis were ablated and excellent wrinkle smoothing were also reported. Similarly, Fujimura et al. (2001) also recorded the healing process in photodamaged or wrinkle skin after resurfacing with a CO_2 laser, following by the increases in the thickness of the newly synthesized dermal collagen layer.

The reduction in healing time, less side effects and avoiding complications to the increase patient acceptance with the treatments are the reason for the development of non-ablative laser treatments. However, these devices are unable to stimulate significant dermal coagulation and are not as effective as CO_2 laser resurfacing. These findings are iterated by Levy et al. (2001) who found that almost all patients treated with the non-ablative 1320 nm Nd:YAG laser failed to show any improvement in wrinkle removals even though side effects were minimal with this treatment. Only 16 cases (53.3%) showed good to excellent result in the treatment of post acne scars using low level energy of 1450 nm diode laser with a relatively high incidence of post inflammatory hyperpigmentation as reported in Rasheed (2005) research work. Improvements in rhytides and atrophic scars with minimal morbidity achieves with non-ablative laser have been discussed in several previous papers, however the results are not yet comparable with those of ablative laser system (Dayan et al., 2003; Alster and Lupton, 2002).

The short pulsed 2940 nm Er:YAG laser was also developed as an alternative to the CO_2 laser in an attempt to imitate some of its beneficial effects while limiting its side-effect profile. Skin treated with multiple pass of Er:YAG laser does cause the improvements in wrinkles and scar as seen in Kunzi-Rapp et al. (2006) and Fitzpatrick et al. (2000) studies with production of new collagen bundles. In fact, pulse stacking does not seem to increase the depth of thermal damage in comparing with CO_2 laser, however due to the reduction in collateral thermal damage, minimal vascular coagulation is affected, leading to poor intra-operative hemostasis as reported by these authors. Furthermore, Ross et al. (2008) and Tanzi et al. (2003) demonstrated that one-pass CO_2 laser treatment could emulate multiple-pass of Er:YAG laser treatments in the reduction of wrinkles. Despite the prolonged recovery and side effect profile, the high energy, pulsed and scanned CO_2 lasers still considered the gold standard for facial rejuvenation in compared with other treatments.

2.3 CO_2 Laser System: Continuous and Pulsed Delivery

Though the CO_2 laser is often referred as the workhorse (Brightman and Geronemus, 2011; Chi, Wang and Huang, 2005; Fitzpatrick and Goldman, 1995; Lanzafame et al., 1988) of dermatology lasers, many practitioners in dermatology and other surgical specialties become disappointed with it because of the realization that thermal damage is difficult to control, hence leading to unsatisfied clinical results and unacceptable side effects. For that reason, the laser manufacturers responded to this situation by developing the pulsed CO_2 laser systems. The energy necessary for tissue vaporisation needs to be delivered by a laser with high irradiance and short exposure time which can only be achieved with superpulsed CO_2 laser. The theoretical advantages of the pulsed CO_2 laser which ablates tissue precisely and leaves a minimal zone of thermal damage have been supported by the Cotton et al.

(1996) research work. The histologic findings following laser irradiation with a Surgipulse XJ-150 laser system for one to two laser passes revealed the removal of the epidermis with a formation of a superficial collagen repair zone. Further studies by Ross et al. (1997) which compared the histologic tissue effects between the scanned and pulsed system showed that both laser systems produce the similar result with a formation of new collagen in wrinkle removal treatment although the result obtained by scanned system produce more immediate thermal damage.

For most superpulsed CO_2 laser, each individual pulse contains inadequate energy per single pulse to ablate the tissue. Hence, the repetition rate needed to accumulate enough heat to reach the tissue vaporisation threshold thus creates a situation that may allow thermal diffusion beyond the target area. A report by Smith et al. (1997) correlated well with that assumption. These investigators evaluate the depth of tissue damage and viability in weanling pig skin after one, two and three passes of the CO_2 laser and found that after two and three passes, there were progressive increases in collagen denaturation with the damage extended into the papillary dermis layer. The other previous studies were also encouraging about this finding. Ruiz-Esparza and Gomez (1999) evaluate the long term effects of skin tightening from CO_2 laser irradiation for both single and multiple passes. As a result, the immediate dermal tightening after multiple pass was noticeable but it is the result of edema that can be considered as thermal damage, in comparison with a more natural look and faster recovery period achieved with one passes of a CO_2 laser. Hence, complications can occur in an attempt to achieve desirable results although with the use of superpulsed CO_2 laser which required multiple passes for adequate energy in cosmetic treatment. Either pulse or CW mode, the attempt to limit the depth of thermal injury could be achieved if the parameter setting in a free-running mode is adjusted as to produce the same effects as obtained with the pulsed laser.

2.4 CO_2 Laser-Tissue Interaction: Animal Study

The successful laser treatments depend on the understanding of interactions between laser irradiation and biological tissue, and also depend on the adjustment of the parameter used for a particular application. Thus, several studies have been performed to investigate the laser induced skin changes by using basic parameters for instance power density, pulse duration, spot sizes and wavelength of laser light. A study by Abuarra et al. (2012) has examined the relationship between the CW CO_2 laser doses and changes in composition of the rat skin tissues. The result obtained from Abuarra et al. shows that cell distortion is increased gradually as the radiation dose increased. Meanwhile, studies on the effects of CO_2 laser fluence and pass number on thermal damage depth and tissue shrinkage has been performed by Ross et al. (1999). The author found that the depth of thermal damage increased with pass numbers for low and moderate fluence groups, however in high fluence groups, the thermal damage depth remained consistent with an increasing in number of passes. Other than that, the histological studies on effect of tissue type with thermal injury were also extensively studies. The simple thermal model in Walsh et al. (1988) research work shows that the effects of short-duration pulses of CO_2 laser radiation on different types of the tissues were varied. Primarily, the thermal damage evidence in Walsh et al. histological result was attributed to the different element of the tissue. Additionally, Walsh and Deutsch (1989) further the investigations by conducting the experiment of ablation rate on pig skin and bovine aorta which ablation efficiency was found to be strongly dependent upon the ultimate tensile strength, thus mechanical properties of specific tissue types are important in the ablation rate.

As indicated previously, the thermal wounding depend on the types of tissue, thus if the damage were too deep, scarring will occur. Similarly with the factor of skin thickness which varies in different facial area, therefore, the amount of thermal damage varied according to the anatomic location with the same laser setting and frequency of passes. Freeman (1996) applies this concept by indicating the comparative safety of laser resurfacing in different area of the body based on the relative thickness of the dermis and not the entire dermal-epidermal dimension. As might be expected, the results show that the large dermal breadth is a safer area to expose than the thin skin area. In this context, the results from the previous research can be extrapolated to be used on human for different clinical applications.

2.5 CO_2 Medical Laser: Benefits and Challenges

The success of high energy CO_2 laser in the improvement of photodamaged facial skin, photoinduced facial rhytides and atrophic scars are well documented. These clinical systems have been proved by multiple studies in animal experiments or human trial which shows that tremendous result could be obtained by using CO_2 laser in skin resurfacing treatment. However, the thermal necrosis, scarring and the unpleasant postoperative recovery period resulted from the CW CO_2 laser resurfacing does limited the use of this technique in this kind of treatment. The difficulty in accomplishing a specific result as well as high side-effect profile in the CO_2 laser system resulted in the chemical peel and dermabrasion as the ultimate choice for the treatment of facial scarring. Non-ablative laser system in rejuvenating the skin was also developed even though the results do not approximate the improvement typically seen after the CO_2 laser treatment. Other than that, the efficacies of these methods are limited and there is currently no gold standard as reported by previous studies (Friedman and Lippitz, 2009; Langsdon and Armstrong, 2008). They had found no histologic changes following dermabrasion, chemical peeling and non-ablative laser, but merely a removal of a portion of the dermis layer that did not regenerate.

Due to the dramatic improvements in the clinical and histologic appearance of photodamaged facial skin obtained by CO_2 laser, the new generation of high energy rapid-pulse machines of CO_2 laser were developed, produce a precision and visibility of resurfacing that not available with any other techniques (Alexiades-Armenakas, 2008; Alster and Lupton, 2002). However, the char can be presented even with the use of superpulse CO_2 laser. Lack of understanding of the fundamental mechanisms that govern CO_2 laser-tissue interactions and a lack of having the correct laser parameters could lead to this unnecessary thermal damage such as erythema and edema. Therefore, it is essential that basic laser irradiation parameters for instance power density, fluence, exposure time, wavelength and spot sizes were tailored according to the specific clinical treatment for maximal target destruction with minimal unwanted thermal damage. Nevertheless, in many cases, the laser parameters used are determined by physical limitations of conventional laser devices and empirical studies of laser-skin interaction. Furthermore, the knowledge of some basic concepts of temperature and heat deposition is necessary to be understood and to evaluate the effects of lasers on biological tissues. If both factors taken into consideration, it will then result in major enhancement of existing CO_2 laser resurfacing technology as well as novel clinical applications in the future.

CHAPTER 3

THEORY

3.1 Introduction

This chapter provides a brief overview of the general principles and theories of various aspects involved in this research. It begins with a short explanation of the CO_2 laser operation system, follow by the mechanism and mechanical properties that govern the interaction of laser with the skin tissue. Include in here is the explanation of lasers as an integral tool for a wide variety of dermatological applications. Then, proceed with the background information of cells that make up the skin tissue. Particular explanation is placed on the animal as an experimental model in this research. For the last part, it will be the theoretical background of the instruments that were used for the sample evaluation.

3.2 Properties of Laser Light

Laser is a powerful source of light that exhibits extraordinary characteristic which differentiates it from other normal light sources like tungsten lamp, mercury lamp and others. The unique properties of laser light are:

a) Monochromatic

Sunlight or ordinary bulb light is a composition of different colours, which means it contains more than one wavelength or in other terms it is polychromatic. In contrast, the emitted light produce by the laser light is one clearly defined single and discrete wavelength which is determined by the lasing medium. That makes laser energy more profitable to produce one wavelength with higher effect on the target and more power with the same energy. This remarkable feature of laser light is very important in clinical practice as molecules or chromophores selectively absorb different types of wavelength (Allemann and Kaufman, 2011).

b) Collimate

The laser beam propagates parallel to each other, so the significant divergence is very low. Therefore, the laser can propagate across long distance without loss of light cause by spreading compare to non-source laser which spread light in all directions, consequently leading too much lower irradiance than that of laser beam (Carroll and Humphreys, 2006).

c) Coherence

Coherence refers to the fact that the wave of light is in phase, in time as well as in space both spatially and temporally. As such, the beam diameter produced by laser light is fixed and can be focused to a far smaller spot size rather than incoherent light source. This coherence characteristic is due to the process of stimulated emission where the only light in the same phase and direction can be emitted. That also explains the power of laser beam of which photons hit the target together at the same moment that brings additive effect (Sener, 2012). Moreover, this feature of laser loads energy more easily at shorter exposure time and can be more destructive as well.

These three characteristics stated earlier will permit the ability of laser light to obtain high energy density for tissue ablation and precise surgical procedure.

3.3 Continuous Wave Carbon Dioxide (CW CO_2) Laser System

The carbon dioxide laser is a molecular laser and the most important of all the lasers from the point of technological applications points of view. In molecular lasers, the energy levels are provided by the quantization of the energy or vibrational and rotational motions of the constituent gas molecules. Usually, the radiations associated with vibrational rotational transitions are in the far infrared range and because of this reason, the molecular lasers have infrared outputs. The CO_2 molecules are basically linear arrangements of the two oxygen atoms and a central carbon atom, which can undergo three modes of vibration as shown in Figure 3.1.

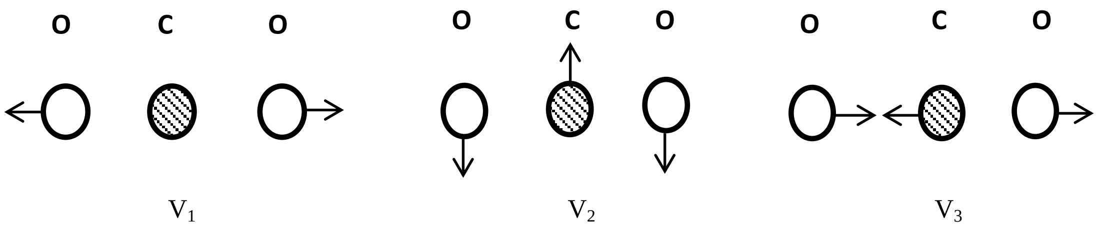

Figure 3.1: Schematic of CO_2 vibrational modes. (a) Symmetric stretch mode (V_1); (b) Bending mode (V_2); (c) Asymmetric stretch mode (V_3).

The molecule can vibrate in any linear combination of these fundamental modes. The modes of vibration are denoted by three quantum numbers (mnq) that represents the energy quanta or amount of energy associated with each mode of vibration.

The CO_2 laser system contains a mixture of three different gases, carbon dioxide, nitrogen and helium gases (as active medium) with the ratio of 1:4:5 in a glass tube. A mixture of nitrogen and CO_2 are placed inside a chamber. Typically an electric discharge is used as a pumping source to excite the CO_2 molecules. The nitrogen molecules are excited by the collisions with electron to their first excited vibrational state. The excited state of the nitrogen is long lived which means that a large portion of the nitrogen molecules will stay in their excited state for a long time before transferring it to the CO_2 molecules through collisions (Csele, 2004). The molecular collision with nitrogen thereby excite CO_2 molecules to a particular vibrational state. The energy level diagram of the CO_2 laser is shown in the Figure 3.2 along with the ground state and first excited state of vibrational modes of the nitrogen molecules.

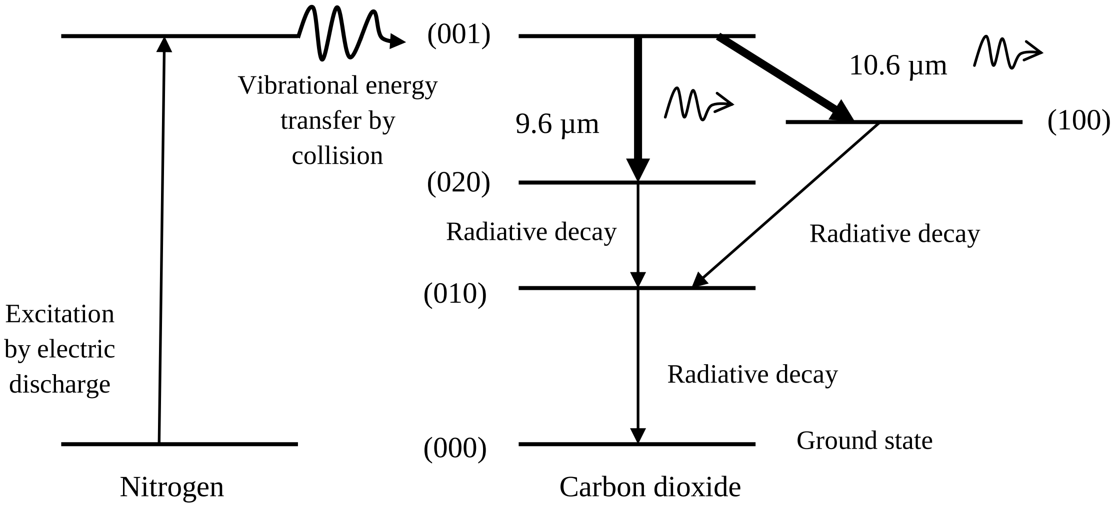

Figure 3.2: Energy level diagram of carbon dioxide laser.

The (100) and (020) levels of CO_2 have a low energy and cannot be populated this way, thus the population inversion is created between the (001) with (100) and (020). This will then result in stimulated emission at about 10.6 µm and 9.6 µm. If there is any excitation left in the CO_2 molecules after they decay and produce light, they will collide with the helium atom which then help to remove any of the excess energy by taking the energy from the CO_2 and moving to the gain tube wall and releasing the energy there through more collisions. Also, another role play by the helium atom is to increase the laser efficiency by speeding up transition from (100) level to ground state via collisions. In addition, the radiation of CO_2 laser cannot be transmitted by standard fibre-optic due to absorption rather than transmission by the quartz fibre. Another alternative to fibre-optics that can be used for delivery system is via an articulated mirror arm, hollow waveguides or fibres made of metal halides (Peng et al., 2008).

3.4 Laser-Tissue Interaction

3.4.1 Optical Properties of the Skin Tissue

There are four ways that light can interacts with the tissue, that is through transmission, reflection, absorption and scattering. Transmission refers to light pass through the tissue without producing any effect on that tissue. Reflection refers to the lights repel off the surface of the tissue without an entry into the tissue (Carroll and Humphreys, 2006). Specifically, the epidermis layer is responsible for most of the reflection from the skin tissue (Anderson and Parrish, 1981). The regular reflectance of a normally incident beam of light is approximately between 4% to 7% for the normal human skin (both white and black skins) as a result of the difference between the refractive index of air and skin tissue.

Scattering is due to the heterogeneous structure of skin tissue, such as molecules, organelles or cells and occurs after the light has entered the tissue. The amount of light scattered determined by the variations in particle size and index of refraction between different parts of the tissue. Hence, the penetration depth depends on the irradiated area where the scattering effect occurred. The penetration depth will be double if, for the same irradiance, the beam diameter increases from 1 to 5 mm. For the dermatological application, this effect must be taken into account. In the skin, the scattering effects greater in the dermis layer as a result of the presence of collagen fibrils which cause most of the tissue scattered. This is an important concept as scattering decreases the desired effect on the targeted chromophores. Moreover, the amount of scattering effects in the tissue is proportional decreases with the increasing of laser wavelengths.

The fundamental goal of medical lasers is laser light absorption by specific tissue targets or chromophores. According to the first law of photobiology, the Grothus-Draper law, light must be absorbed by the tissue to produce an effect in that tissue whereas transmitted or reflected light has no effect (Tanzi, Lupton and Alster, 2003). Longer wavelength thus penetrates tissue more deeply. However, for the laser light in the mid to upper infrared range, its only penetrate superficially owing to the high absorption coefficient of tissue water (Ee, 2011). A relationship between the absorption of light in a purely absorbing medium and the thickness of the medium can be approximated using Beer-Lambert law:

$$I = I_0 e^{-\mu_a L} \tag{3.1}$$

where L is the skin thickness, I is the transmitted intensity, I_0 is the incident intensity and μ_a is the absorption coefficient (Star, 2011). The absorption coefficient can thus be defined as the probability that a photon will be absorbed by the medium per length. The Beer-Lambert law states that the absorption into the tissue (homogenous medium) at a given wavelength is proportional to the concentration of chromophore c present.

$$\mu_a = \alpha c \tag{3.2}$$

where α known as the specific absorption coefficient.

Substituting for μ_a in the Beer-Lambert law gives:

$$I = I_0 e^{-\alpha c L} \tag{3.3}$$

The Beer-Lambert law is only valid under certain limited conditions, that is, the light entering the medium must be monochromatic and perfectly collimated, and the medium itself should be purely and uniformly absorbing. Specifically, the intensity of the laser energy decreases exponentially with the depth of the tissue as determined by the equation stated above.

3.4.2 Selective Photothermolysis

The major key to the successful laser treatment is greatly enhanced with Anderson and Parrish's theory of selective photothermolysis. The theory of selective photothermolysis refers to laser energy absorption by a target chromophore without significant thermal damage to surrounding tissue. To achieve selective photothermolysis, the laser must produce a beam of light with a wavelength preferentially absorbed by the chromophore in the lesion (Anderson and Parrish, 1983). Moreover, the energy or power density must be high enough to destroy the target chromophore within the time length laser exposes on it. Equally important, the duration of laser exposure must be shorter than chromophore thermal relaxation time as to limit the amount of energy deposited within the skin. The thermal relaxation time is defined as the time required for target chromophore to cool to one half of its peak temperature after laser irradiation, which is proportional to the square of the size of the chromophore.

3.4.3 Chromophores in the Skin

There are many compounds in biological tissue which absorbs light radiation, collectively known as tissue chromophores and it can be either endogenous in the tissue or exogeneous. Each of these chromophores possesses its own absorption spectrum and absorption peaks, detailing their relative absorption for each wavelength (Figure 3.3). Essentially, tissue components can be chromatically target instead of just physically targeted. Usually, the targeted chromophores in the skin include melanin, hemoglobin and water as well as exogenous compounds like the different colours of tattoo inks that also act as chromophores.

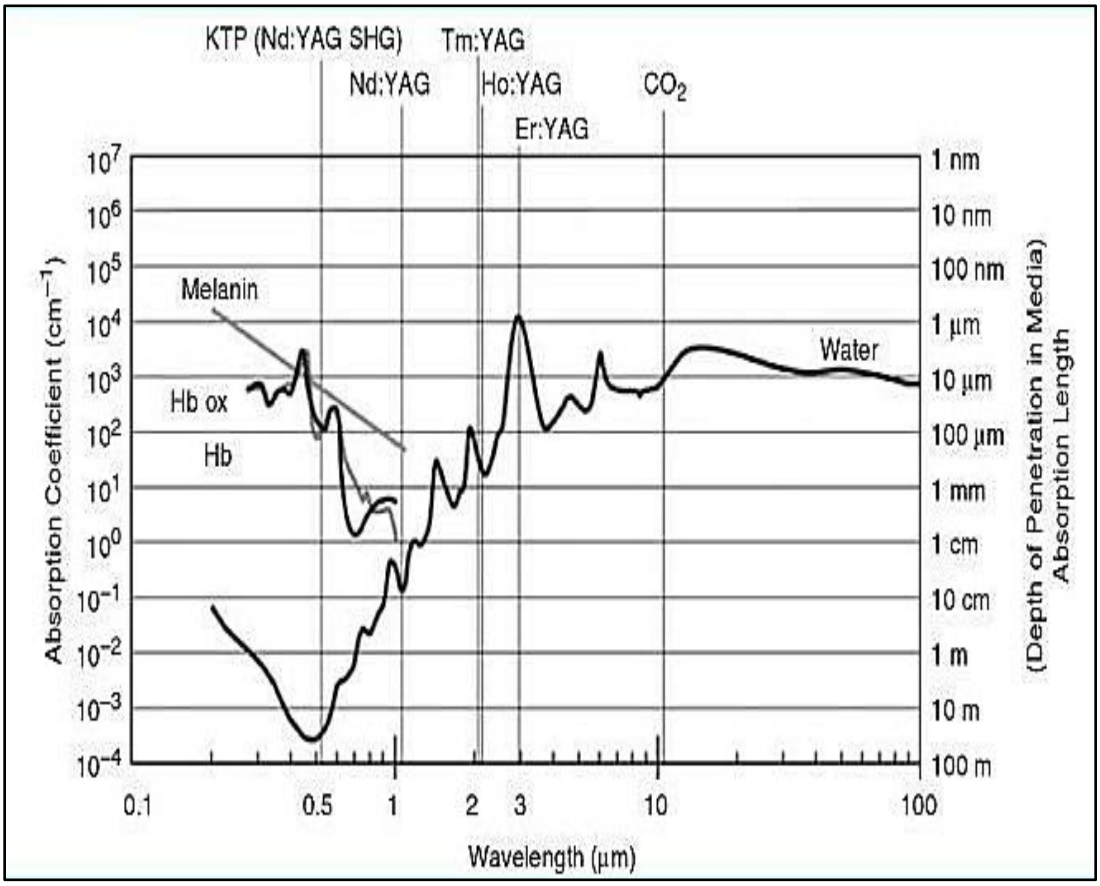

Figure 3.3: Absorption spectra of the three major chromophores: water, oxyhemoglobin and melanin in the skin (Teichmann, Herrmann and Bach, 2007).

3.4.3 (a) Water

Approximately 60% to 80% of total body mass is composed of water. Water is considered to be one of the most important chromophores in laser-tissue interactions because of its high concentrations in most biological tissue. The water content in different parts of the body varies which also depends on age and gender. Water absorbs light significantly in the infrared region such as Nd:YAG and CO_2 laser that owns long wavelength properties. With water as a target, high energy is needed to produce an effect because water is a large target as a percentage compares to any other chromophores. Moreover, the general rule when water as a target chromophore is the bulk heating of the tissue because water is not selectively located as a single target (non-specific heat diffusion). Cosmetic skin resurfacing and tissue coagulation for bloodless operative field use this concept to achieve an optimal result.

3.4.3 (b) Blood Vessel

Hemoglobin is carried in red blood cells and responsible for transporting oxygen from the lungs to the body tissue and eliminates the carbon dioxide and waste products. Blood vessels vary in size which is from 20 to 30 microns in normal skin but in port wine stains (PWS) and telangiectasia, the vessel diameter can reach until 500 microns. In treating all the vascular lesions, hemoglobin is a primary target. The laser wavelength of blue (418 nm), green (541 nm) and yellow (577 nm) of the electromagnetic spectrum is sufficient to cause thermal injury to the blood vessel wall. However, the depth of the targeted blood vessel must be considered, therefore longer wavelength such as Alexandria and Nd:YAG laser can be used to treat moderately deep, larger caliber spider and reticular veins (Chen et al., 2012).

3.4.3 (c) Melanin and Other Pigments

Melanin is mostly found in the epidermis and hair follicle or may also be found in the dermis layer. Ultraviolet, visible and near infrared spectrum is the absorption band for treating melanin-pigment lesions because melanin highly absorbs light right across these wavelengths (Ee, 2011). However, red and near infrared spectrums like Nd:YAG laser is suitable for deeper penetration as to achieve optimal laser hair removal rather than shorter wavelengths. A longer exposure duration required when the aim is to destroy larger melanin-containing structures (hair shaft and bulb). In hair removal treatment, the reaction involves in the process is thermal and mechanical effects that could slow or destroy their ability to regrow (Goldberg and Hussain, 2005).

For the tattoo treatments, the choice of laser depends on the ink colours present within the tattoo. Particular tattoo pigments, such as yellow, green and fluorescent inks are more daunting to treat than darker blacks and blues. These pigments are more challenging to treat because they have absorption spectra that fall outside or on the edge of the emission spectra available in the respective tattoo removal laser. The predominant colours like blue and black absorbs well throughout the 532 nm to 1064 nm range. Meanwhile, the blue and green inks suitable for 600 nm to 800 nm range whereas red, orange and yellow inks specifically destroyed by green light with wavelength of 532 nm (Tanzi, Lupton and Alster, 2003). This wavelength of laser light must penetrate sufficiently with the adequate energy, deep into the skin as to reach the tattoo pigment in order to provide an effective treatment for tattoo removal.

3.5 Interaction of Laser Radiation with Biological Tissue

In 1986, Boulnois propose the first systematic interaction mechanisms included with laser-tissue mapping and time-scale distinction (Figure 3.4). Boulnois identifies the major interactions occur in laser-tissue interaction which leads to the alterations in tissue structure and composition. Therefore, once the energy (heat) absorbed by the correspond chromophores, four biological reactions were possible: photochemical, photomechanical, photothermal and photoablation effects.

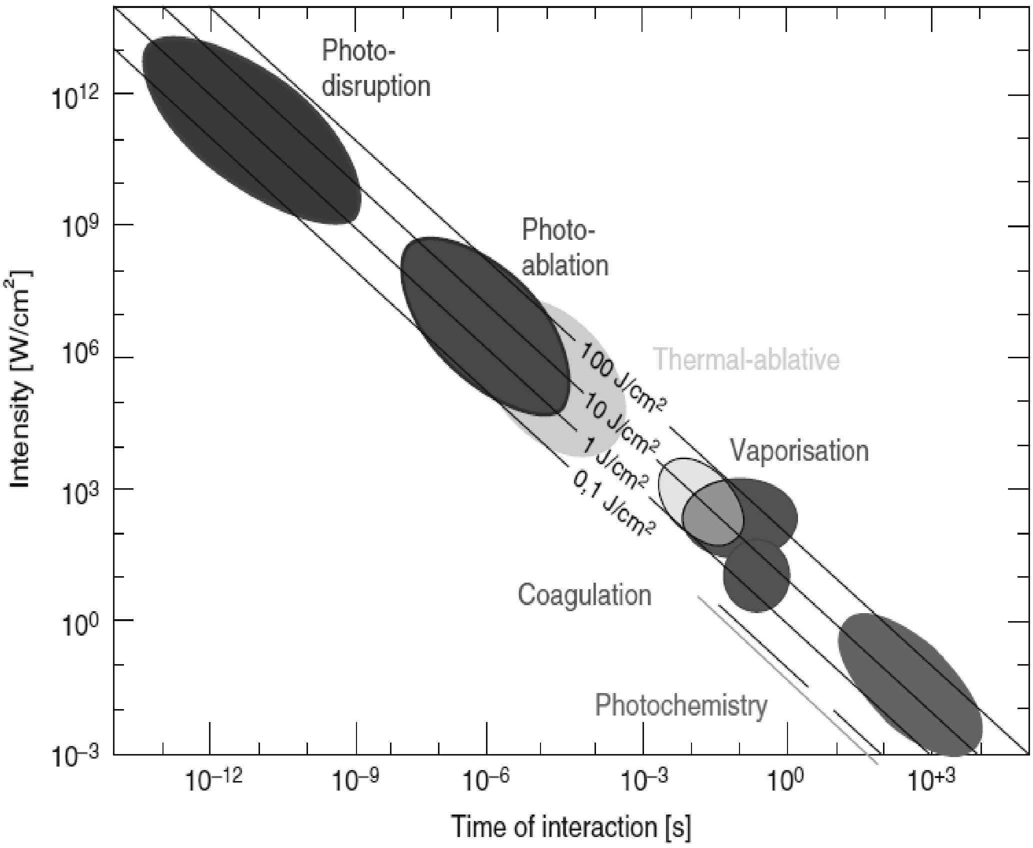

Figure 3.4: Map of laser-tissue interaction. The intensity-interaction time diagram presents all the various laser action on a diagonal. Modified from Boulnois J. -L. (Steiner, 2011).

Photochemical interactions occur when a wavelength used is absorbed by a molecule that can provide the necessary activation energy or effects to destroy the targeted tissue. For instance, the photodynamic therapy used a photosensitizing drug to cause necrosis (cell death) at the tumour cells. This interaction widely used in oncology to destroy cancerous tumours. For the photodisruption, the mechanism of interaction behind this effect is best described as plasma mediated ablation or also known as optical breakdown. It relies on the nonlinear absorption of laser energy in the target achieved when the material specific radiant exposure is exceeded (Vogel and Venugopalan, 2003). Basically, optical breakdown is characterized by three major events that is plasma formation, shock wave generation and cavitation. Mostly, these interactions are found in ophthalmology for cutting flaps of the cornea.

As for the thermal effects, it stands for a large group of interaction type and the significant parameter change is an increase in local temperature. Occurred when a chromophore absorbed the preference wavelength and the absorbed energy converts that energy into heat, causes the destruction in the target area. The photothermal application is included in the treatment of telangiectasia, tissue cutting and welding in laser surgery. As for the photoablation, it is defined as a clean and exact fashion of tissue removal without the appearance of thermal damage such as coagulation or vaporisation. With short laser wavelength and pulse duration, it causes small explosions and then the tissue was very precisely “etched” (Niemz, 2007). This effect mostly applied on the procedures to improve eyes refractive power by making radial cornea incisions to correct the difficulty of focusing onto the retina (Boulnois, 1986).