Effects of Different Laser Times Exposure on Skin Structural

T.L.I.R. Mahmood, M.Z. Matjafri

Abstract—Lasers play an increasing role in the medical field for diagnostic and therapeutic applications as well as surgery applications. However, several important parameter need to be considered before using it in medical practice. Therefore, laser parameter such as wavelength and time exposure shall be selected carefully to optimize efficacy while minimizing unwanted side effects and surrounding tissue damage. This fundamental understanding of how the laser parameter effects the tissue composition will help the physician and dermatologist to choose the most appropriate parameter for a clinical situation and maybe we able to use the same laser for many purpose of cosmetic treatment.

Keywords—Continuous wave carbon dioxide (CO₂), fibre coagulation, lasers, photothermal effects

I. INTRODUCTION

For over 30 years, laser technology has continuously developed due to the industrial and medical demand [1]. This is due to the unique characteristic of the laser light that differentiates it from other conventional light source. Unlike conventional light source which spread lights in all directions, laser light is collimated which means that the radiation beam is almost parallel without significant divergence. Another characteristic of laser light is coherent, means that the wave of lights is exactly in phase, in time as well as in space. Moreover, the light emitted by a laser have the same energy, wavelength and frequency, thus it is said to be monochromatic [2]. These properties make the laser light as a main choice for a range of applications. In medical applications, the wavelength of laser mostly used is in ultraviolet, visible and infrared range [3]. Each wavelength of laser interacts differently with living tissue [4].

One of the most important lasers that widely used today is the carbon dioxide (CO₂) laser [4]. This is due to the high power output making it suitable for industrial as well as in medical applications. The CO₂ laser is a continuous wave (CW) laser that emits far infrared light at 10,600 nm and has tissue water as its major target. However, CO₂ laser only penetrates superficially owing to the high absorption coefficient of tissue water that allows minimal thermal damage and enough to cause tissue vaporization. The dermatology applications such as skin resurfacing and tissue removal made use of these featured [3].

Superficial CO₂ laser only produces thermal damage depth approximately 30 μm per pass and the underlying zone of thermal damage from heat diffusion can goes up from 250 μm until 400 μm in the skin [5]. This is due to the thermal relaxation times of the 30 μm tissue layer heated by the laser is less than 1 microsecond [2].

Researchers also found that others factors also plays a vital role in the reactions of laser when expose to the skin. First, the amount of damage that produce by a laser pulse is due to the amount of pigmentation or color and the amount of substance keratin present in the skin [1]. Second factor is the power per unit area delivered by the laser and the duration of the laser pulses. If the pulse energy is below the level of tissue vaporization, the heat will be transferred to the surrounding tissue. Under these conditions, the pulse duration will become much more important factor [5], [6].

Therefore, this study aimed to investigate the effects of tissue composition due to the different laser time exposure when expose to the CO₂ laser. To evaluate the laser effects on skins composition, the light microscopes were used in this research.

II. MATERIALS AND METHOD

A. Animal preparations

Two male Sprague Dawley rats weight between 400 g to 500 g were used in this research. The rats were 14 to 18 weeks old. Animals were supplied by Animal House of the Universiti Sains Malaysia, Penang, Malaysia. Firstly, the rats were anesthetized with a mixture of ketamine (75 mg/kg) and Xylazine (10 mg/kg). Then, the hairs on the side of the rats were shavies gently using a sharp razor blade and the target skin were marked for laser irradiation.

B. Infrared irradiation

Firstly, the distance between the target skin and laser aperture measured is 5 cm and this distance remained constant from the beginning until the end of experiment. The size of spot diameter used is 0.35 cm. Next, the target skins were exposed to the continuous wave carbon dioxide (CO₂) laser at 17.5 W/cm² (power output) with different time exposure (15s, 30s, 45s and 60s). The laser radiation with the same power

Tengku Lina Izzati Raja Mahmood from School of Physics, 11800 Universiti Sains Malaysia, Pulau Pinang, Malaysia (019-3794233; tilm10_phy003@student.usm.my). Mat Zubir Mat Jafri from School of Physics, 11800, Universiti Sains Malaysia, Pulang Pinang, Malaysia (mjafri@usm.my)
output and four irradiation time is repeated on second rats in order to get duplicate samples.

Any effect on the skin observes macroscopically to check the change in pattern of skin after each run of irradiation. Four different sections for each of times exposure were obtained for histology examination. The unexposed area were taken as a control samples and processed under the same conditions.

C. Histological investigations

After the experiment complete, the rats were euthanized according to the rules of Animal Ethics Committee, USM. The 1cm x 1cm cross section of the rats skin is going to cut from each spot of exposed area and immediately transferred to the well labeled bottle contained 10% neutral buffered formalin for fixation (15s, 30s, 45s and 60s). This include the complete cross section of unexposed area (control sample).

The skin samples were placed in formalin for 24 to 48 hours. Next, the water in tissue samples must be removed by dehydration [7]. The samples are immersed in ascending concentrations of ethanol starting from 50%, 70%, 80%, 90%, 95% and 100% for one to two hours each. After that, the tissue samples were cleared with xylene for half an hour to remove the alcohol and finally followed by hot molten of paraffin wax which replaces the xylene and infiltrates the tissue [7].

Then, the tissue samples were embedded in paraffin wax and cooled down on the cooling unit of embedding station to hardened and ready to be sectioned. The tissue is then sliced into very thin cross section (5 to10 microns) using a machine call microtome. The resultant ribbons are transferred to the floatation bath to expand and 3 to 4 sections are taken onto labeled slides. The slides are heated on the hot plate to evaporate the layer of water between the sections and the glass slides. Next, put the slides into the oven to melt the wax as this process can improve adhesion between the samples and the slides.

Lastly, the slides are stain with Hematoxylin and Eosin (H&E) stains and then mounted with transparent DPX medium. The exposed and non exposed sample sections were examined using light microscopy and the comparison of structural skin between the exposed and non exposed samples were studied in detail.

III. RESULT AND DISCUSSION

A. Control (Unexposed Skin)

Histological investigations revealed that the unexposed rat skin show from Fig. 1(a) and Fig. 1(b) appears like a normal skin structure comprising three principle layers, the epidermis, dermis and hypodermis layer. The epidermis or outer layer predominantly populated by keratinocytes layers that appeared very rough and uneven. The dermis or inner layer composed of collagen fibres that support the hair shafts and sebaceous glands, blood vessels and cells. Finally, the subcutaneous tissue layer (hypodermis) is the innermost and thickest layer of the skin. It is made up of adipose cells or fat layers and loose connective tissue.

Fig.1 Histological images of unexposed rat skin taken at 40x Fig. 1(a) and 100x Fig.1(b) magnification. Epidermis (ER), Dermis (DR) Hypodermis (HD), Collagen fibres (CF), cells (CL), Hair follicle (HF), Melanin (ML)

B. Exposed skin

From the H&E stains biopsy section shown above, the depth of hyalinization varied from sections to sections. The damage depths were measured to the deepest damage point using the software install in the light microscope computer. The measurements were taken at 40x magnification and at four different places. The average damage depth is written in Table 1.

The maximum cell distortions were observed in the longest time exposure (60s) and for the minimum times exposure (15s), only at superficial papillary dermis were damaged [8]. The coagulation and homogeneous hyalinization clearly shown in the Fig. 2(a), 2(b), 2(c) and 2(d) as the coagulated area appear darker than other tissue with the increases in the laser pulsed duration. When the CO₂ laser interacts with the skin, the tissue consists of water will absorb the laser energy and converted it into thermal energy (heat) and diffuse into the tissue.
TABLE I
THE AVERAGE DAMAGE DEPTH CAUSED BY THE LASER–SKIN INTERACTION

<table>
<thead>
<tr>
<th>Times (s)</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average damage depth (μ)</td>
<td>200.98</td>
<td>249.36</td>
<td>299.06</td>
<td>355.11</td>
</tr>
</tbody>
</table>

The burning spot spreading into the bigger area due to the heat diffusion by thermal conduction and by the blood flow to the surrounding tissue. As the pulse duration increases, the temperature in the skin tissue also increases. The increases in tissue temperature will produce desiccation, blanching and a shrinking of the tissue by denaturation of proteins and collagen (dermis shrinkage). This evident shown in Fig. 2(b), 2(c) and 2(d) as the collagen fibres shrinks and coagulated. This fibres shrinkage is the characteristic that can be used in laser skin resurfacing [5]. Once all the water inside the tissue has boiled off (vaporized), the remaining organic material may carbonize (blackening).

Noted that the melanin inside the hair shaft partially damage as shown in the Fig. 2(d) mainly because of photothermal effects. Even though the melanin is not the target chromophores for the CO₂ laser, the heat that generated inside the tissue can also cause the destruction to the melanin in a high temperature.

These photothermal effects also cause the expanding of empty space between the hair follicles and its surrounding tissue [4]. Moreover, the interactions of laser onto the tissue also cause the thinning in the epidermis layer with loss of stratum corneum even with the lowest time exposure.

IV. CONCLUSION

The effects of CO₂ laser when irradiated onto the skin tissue can be concluded to cause fibre coagulation, thinning in epidermis layer, tissue shrinkage and also cause effects onto the melanin in the hair shaft due to the photothermal effects. From this research, the CO₂ laser expose on skin show the effects that good to be used in skin resurfacing if the parameter is choose wisely by physician and dermatologist.

ACKNOWLEDGMENT

The author wish to thank members of Medical Physics Lab, School of Physics, USM for technical assistance and Histology Lab, School of Biology, USM for histological staining. This research supported by USM RU grant 1001/PFIZIK/811153 and USM ST grant 304/PFIZIK/6311082.
REFERENCES


