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Full Length Research Paper

The effects of different laser doses on skin

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The laser-skin interaction was studied using the laboratory albino rat skin as an experimental sample and 10.6 µm wavelength CO_2 laser as a source of irradiation. This study aimed to determine the effect of different laser doses on the skin structure as a trial to understand how laser exerts its medical effects in treating skin problems. It also aimed to determine the relationship between the laser dose and biological effects and thus determine the lowest dose that had highest medical effects with lowest skin damage. Briefly, the rat skin was exposed to CW CO_2 laser at 12.5, 14.1, 15.6 and 17.2 W/cm^2 for 15 s. Directly after the exposure, biopsies of normal and exposed skin were preserved and fixed for histological studies. The images obtained from the compound light and electron microscopes exerted changes contributed to the interaction of the skin cells to the heat and energy produced by the continuous wave carbon dioxide (CW CO_2) laser during the exposure time. Basically, the tissue damage caused by the laser was mainly due to photothermal effect and increased gradually as the irradiation dose increased. Epidermal loss along with coagulation, homogenous hyalinization, lost of hair associated with shrinkage and collapse of hair follicle structures of varying depths at the-burn sites were detected in the histologic sections. Damage-power density (DPD) relationship was confirmed by measuring the damage depth using the software provided in the light microscope. On the other hand, Scanning electron microscopy (SEM) showed detailed images of the extensive epidermal epithelial cells damage which also increased by increasing the laser dose. Rough surface, partial destruction of intercellular junctions giving rise to loss of adherence between squamous cells and formation of narrow spaces between these cells were the most evident changes detected. The findings may help specialists to choose the best laser parameters for certain applications.

Key words: Lasers, laser-tissue interaction, continuous wave carbon dioxide (CW CO_2) laser, photothermal effect.

INTRODUCTION

The lasers have varied applications in nearly every part of modern life, including consumer electronics, information technology, science, medicine, industry, law enforcement, entertainment, and the military. Since the early period of laser history, laser research has yielded a variety of improved and specialized laser types, optimized for different performance goals, and this research research continues until nowadays.

Laser is usually emitted as a beam which can propagate over long distances with low divergence and can be concentrated in very small spots. It has a very narrow bandwidth, and emitted continuously or alternatively in the form of short or ultrashort pulses, with durations from microseconds down to a few femto seconds. These properties makes laser light very interesting for a range of applications (Paschotta, 2010). Various light therapies have been employed in clinical practice. Advances of these treatment modalities, such as various types of laser, intense pulsed light (IPL), and light emitting diodes

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(LED), open a good opportunity for multiple clinical applications in treating cutaneous diseases and performing cosmetic procedures. In dermatologic treatment with laser, the continuous wave carbon dioxide (CW CO_2 laser) is the most widely used today (Omar et al., 2009). It emits spectral energy in the far Infrared (IR) portion of the electromagnetic spectrum at 10,600 nm. This wavelength is heavily absorbed by water, which is the primary constituent and chromophore of cells in living tissue. Thus, the energy produced by this laser can be used for cutting or volume ablation by tissue vaporization (Lee, 2008). Hair removal, skin resurfacing and tattoo removal are the most common applications where CW CO_2 laser plays a vital role (Williams et al., 1999; Stratigos et al., 1998). The results of laser treatment depend on the immediate interaction of incident light with tissues as well as the subsequent tissue regeneration and repairing. Researchers found that the reaction of the skin to laser radiation depends on many factors. First, the color or amount of pigmentation in the skin is very important in determining the amount of damage that will be produced by a given laser pulse. Second, the amount of the substance keratin present in the irradiated region appears to play a role. Third, the output characteristics of the laser, particularly the wavelength, are also important. Furthermore, the reactions of the skin to laser radiation vary so widely, depending on the exact circumstances and the type of skin (Bailin et al., 1990).

Since the role of lasers in dermatology has increased dramatically over the past three decades. A fundamental understanding of laser-tissue interactions is crucial for the proper and appropriate used in clinical practice. Several excellent review articles have been published addressing principles of laser physics (Paschotta, 2010; Bailin et al., 1990; Welch and Torres, 1989). This study focused on the biological effects of the carbon dioxide laser, since in many areas of medicine, histological examinations are necessary to evaluate the biological reaction of epidermal, dermal, vascular, and appendage components of skin after laser therapy. Therefore, this study aimed to investigate the structural effects of different doses of CW CO_2 laser on the normal skin when irradiated at the wavelength of 10.6 µm. Both light and electron microscopes were used to evaluate the laser effects on the rats' skin.

MATERIALS AND METHODS

Sample preparation for laser irradiation

Two male Swiss albino rats with an average weight of 300 g were obtained from the animal house of Universiti Sains Malaysia, main campus, Penang, Malaysia. Firstly, the rat will be anesthetized with ketamine (75 mg/kg) and xylazine (10 mg/kg). Then, the rat was fixed onto a rectangular wooden block by using the tie tape on its hands and legs. Next, the hairs at the back skin surface were shaved gently by using a razor blade. After that, the target skin was marked for laser radiation. The laser light was focused directly onto the marked area of the rat back skin. Four different laser powers were applied for both rats (12.5, 14.1, 15.6 and 17.2 W/cm^2) for 15 s. The distance from laser aperture to target skin measured is 10 cm. The distance remained constant from the beginning to the end of the experiment. The spot diameter used was 0.35 cm. Each exposure was done in quadruplicate in order to get duplicate samples for histology and SEM studies from each rat. Eight different sections were obtained for each laser power and were divided into four for histology work and four for electron microscopy. Control samples were obtained from the unexposed skin and processed under the same conditions.

Histological examination (light microscopy examination)

After irradiation, the rats were sacrificed according to international rules of animal care ethics (animal ethics approval reference #: PPSG/07 (A)/ 044/ (2010)(59)) and ten 1×1mm full thickness skin sections were cut from the rat back and immediately transferred into well labeled Bijou bottles (control, 12.5, 14.1, 15.6 and 17.2 W/cm^2). Two spots were taken for each sample. These included a complete cross section of the irradiated region and adjacent normal skin. Skin samples were fixed in 4% formalin for 24 h, and then dehydrated in ascending concentrations of ethanol, starting from 50, 70, 80, 90, 95 and 100% for one to two hours each. This gradient ensures that the tissue loses its humidity gradually so it would not be shocked and distorted. After that, tissue samples were cleared with xylene for half an hour followed by Infiltration with melted wax. Next, the skin samples were embedded in paraffin wax and cooled down on the cooling unit of embedding station. 3 to 10 microns cross sections are cut on stainless steel knives mounted in the microtome, the resultant ribbons are transferred to the flotation bath to expand, then 1 to 2 sections are taken on to labeled slides and let dry for overnight . Finally, the slides are stained with Hematoxylin and Eosin (H&E) stains, then mounted with transparent DPX medium and examined under compound light microscope. The surface morphology of the rats' skin samples was studied using light microscopy (OLYMPUS BX50). The magnifications were set at 40, 100, 200 and 400 times. The images were seen clearly through the CCD Camera. Skin layer measurements were done at various magnifications. The thickness of the dermis is measured automatically at 40 times magnification by the software provided with the compound light microscope, however, epidermis layer thickness was measured at 100X, using the same software. Finally, the comparisons between the exposed and non-exposed samples were studied in detail.

Scanning electron microscopy

The tissue sections were fixed immediately after removal in a MC Dowell-Trump fixative prepared in 0.1 M phosphate buffer (pH 7.2) for 2 h. Fixed samples were stored overnight at 4°C. Tissue sections were washed twice with 0.1 M phosphate buffer, then post fixed in osmium tetroxide solution for 1.5 h. This step was done in fume hood, since osmium tetroxide is classified to be carcinogenic. Next, tissues were post washed twice with distilled water and dehydrated in an increasing series of ethanol (50, 75, 95 and 100%), then covered with HMDS media for 10 min. Following dehydration, samples were left to air dry at room temperature, and then kept in the desiccators until mounted on stubs using an adhesive tape and were coated with gold palladium alloy using a fine coat ion sputter coater. Finally, skin sections were examined under a scanning electron microscope (LEO SUPRA 50 VP Field Emission SEM, Germany). Images were captured at different magnifications (50, 100, 200 and 1000X).

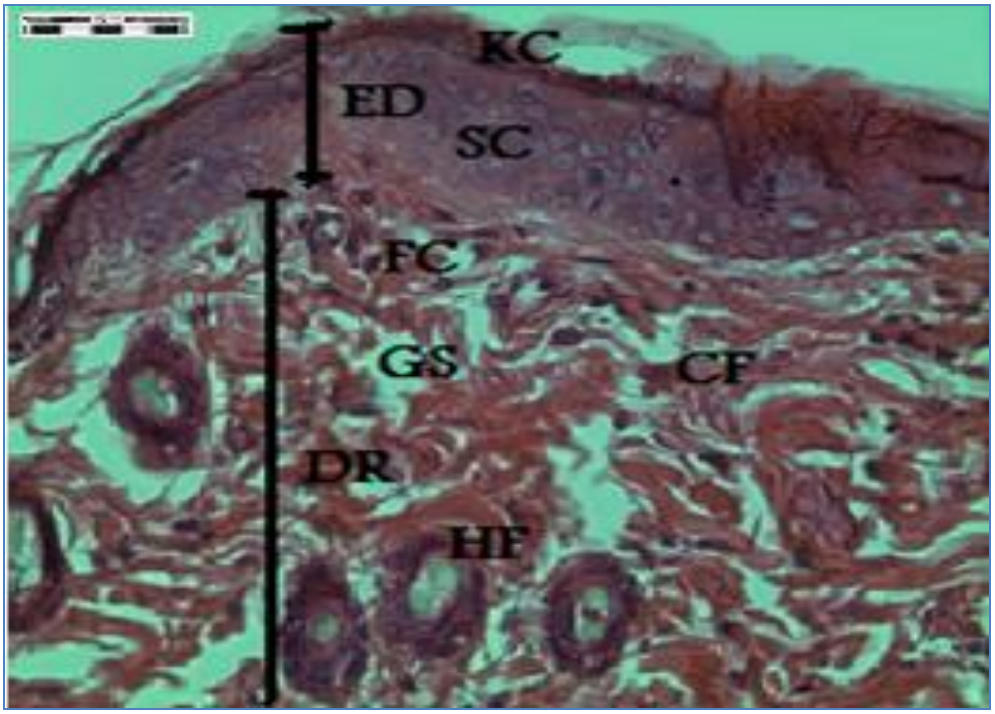

Figure 1. Histological images of normal rat skin taken at 40×. Epidermis EP (keratinocytes KC and stratified squamous epithelial cells SC), dermis DR (collagen fibers CF, fibrocyes/blasts FC and ground substance GS). Appendages (hair follicles HF, sebaceous glands SB and sweat glands SW). hypodermis layers (adipose cells AC).

RESULTS AND DISCUSSION

Normal rat skin (Control)

Examination of the histological changes in the skin samples stained by hematoxylin and eosin and viewed under the light microscope revealed that the unexposed skin section mimicked the normal skin where all the skin layers appeared normal and arranged in three layers, including (from surface downwards) the epidermis, the dermis (and its appendages), and the subcutaneous tissue (Ross and Pawlina, 2006; Swanson, 1996). The epidermis layer consisted of the keratinized squamous epithelium of the skin with a rough-appearing surface (undulated). The squamous epithelial cells appeared as flattened structures with large nuclei. Immediately below the surface epithelium there is a thick area of eosinophilic-staining stroma, composed of collagen and elastic fibers making up the mesh of dense irregular connective tissue that gives support and strength. Connective tissue is vascular exhibited by capillaries running through it. The fibroblasts/fibrocytes contain slender elongated nuclei. Seated on the fibers to maintain and repair them. The fibroblasts/cytes are sitting in the minute spaces between the fibers, hardly visible as anything more than elongated nuclei. Moreover, a variety of appendages, mainly hair follicles, sweat glands, sebaceous glands and blood vessels are also distributed within the dermis layer. Finally, subcutaneous layer (also known as subcutis or hypodermis) was mainly composed of adipose cells or fat cells that are grouped together in lobules separated by connective tissue as shown in Figure 1.

Effect of laser beam on skin

The irradiated samples showed that the tissue damage increased gradually as the radiation dose increased where the maximum cell distortions were observed in the fourth sample irradiated with the highest dose. There was thinning of the epidermis with loss of stratum corneum. The loss of epidermal cells and thinning of the squamous epithelium was minimal in 12.5 W/cm^2 as shown in Figure 2b. This became more evident as the radiation dose to the skin was increased as shown in Figure 2c to e and minor changes in dermal collagen and elastic fibers were also noted. Coagulation and homogenous hyalinization of varying depths at the burn area with complete loss or erosion of epithelium covering in all of the following samples was detected. When the collagen is totally coagulated, collagen bundles gradually became denser and darker and eosinophilic became homogenous, as shown in Figure 2c. Furthermore, tissue shrinkage was

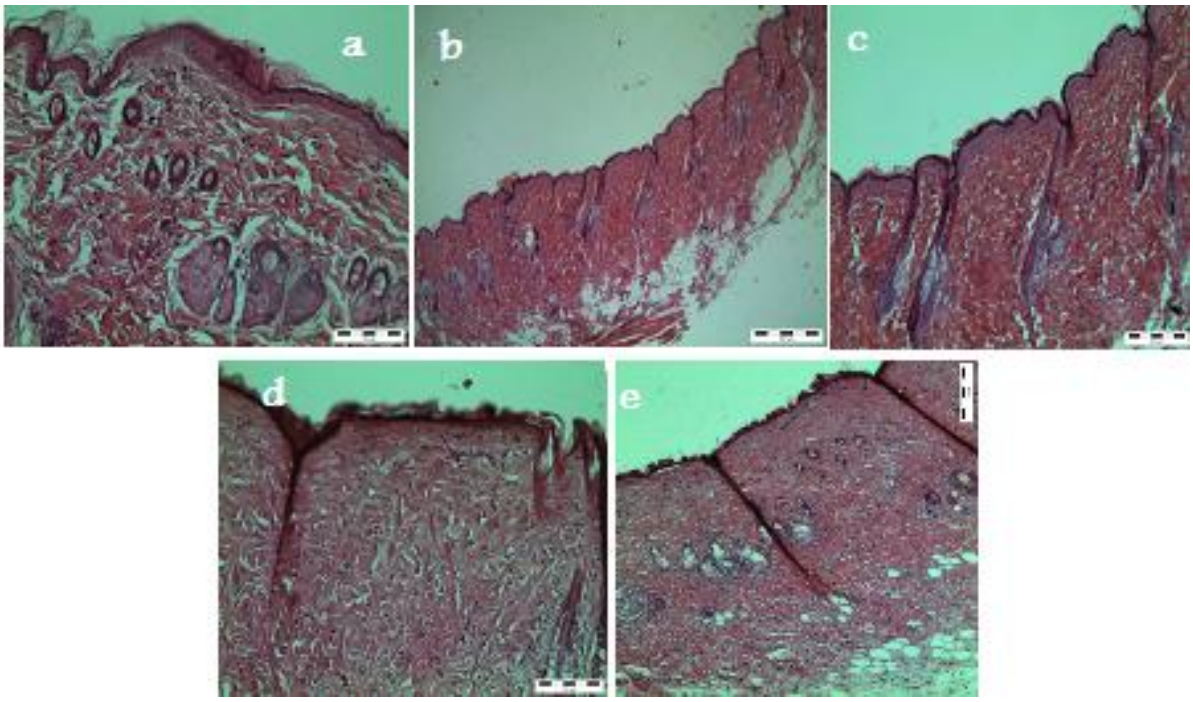

Figure 2. Examination of the histological changes in the skin samples stained by hematoxylin and eosin and viewed under the light microscope. (a) Control, (b) CW CO_2 laser irradiated skin at power density 12.5 W/cm^2 for 15 s at 4×, (c) 14.1 W/cm^2 at 40×, (d) 15.6 W/cm^2 at 40×, (e) 17.21W/cm^2 at 40×.

Table 1. The average depth of damage caused by laser irradiation on rat skin.

Sample	Control (EP:DR)	1	2	3	4
Average damage depth (µm)	45.6 : 925.3	26.8	245.3	331.6	776.7

clearly seen in the second sample as shown in Figure 2c to e. There are an appearance to show the loss of hair associated with shrinkage and collapse of hair follicle structures, which caused the surface epithelium to contract inwards. This was evident in Figure 2b to d where dense vertical dark bands were noted within the dermis. Inflammation was absent in all tissue samples, since all the biopsy sections were taken immediately after the laser irradiation.

The tissue damages encountered by the laser was expressed as epidermal loss along with coagulation, homogenous hyalinization, loss of hair associated with shrinkage and collapse of hair follicle structures of varying depths at the burn area were mainly affected due to photothermal effect that is increased gradually as the irradiation dose increased. The heat burned the layers of epidermis and caused its loss then heat passed through the hair follicle sites into the underlying dermis and coagulated the collagen proteins resulting in shrinkage of hair follicle and finally complete loss of the hair. This mechanism of action is the basis of laser hair removal, wrinkles and tattoo removal, since laser can be removed the layers due to photothermal effects. On the other hand, laser is absorbed highly by water and pigments of the skin that is why it is used for tattoo removal.

Measurement of the damage depth

The observed damage depth was measured to the deepest point as shown in Table 1. The damage depth was measured by software installed on the light microscope computer. Measurements were taken at 40× magnification. We found that the highest power (17.2 W/cm^2) exerted the deepest damage, while the lowest power (12.5 W/cm^2) exerted superficial damage only, as shown in Figure 1b. The normal skin layers thickness (µm) is given in the control column of Table 1. Measurements were taken as five values and the average depth was calculated (sum of values/5).

The most interesting thing is that the shrinkage occurred in the tissue sections at certain distance which is thought to be at the hair follicle site where the hair

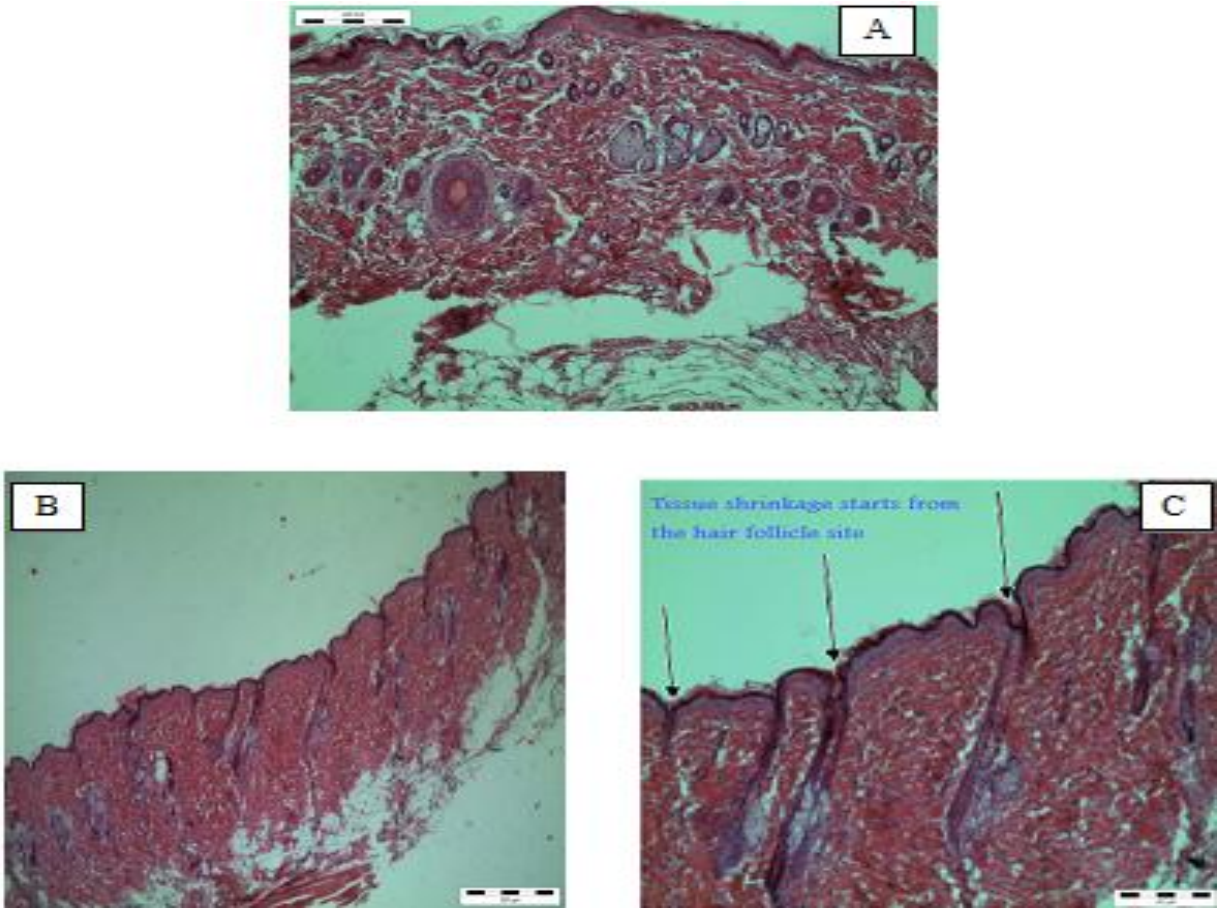

Figure 3. (A) Normal rat skin, (B&C) images showing that the tissue shrinkage appears to start from the hair follicle site (indicated by arrows in the third image (C)).

arises from the skin. The tissue surrounding hair follicle was shrunk; this may be due to the empty space around each hair follicle that can be an entrance for the laser beam into the skin (Figure 3).

Mechanism of tissue damage

The CO_2 laser emits infrared light which is perfectly absorbed by water as the skin is assumed to be homogeneous with thermal properties of water. Once laser energy is absorbed in the skin the basic effect possible is photothermal. Photothermal effects occur when tissue water absorbs the corresponding wavelength of energy and destruction of the target tissue results from the conversion of absorbed energy into heat. Then, the heat will transfer to the tissue target and also surrounding it. The epidermis which is normally protective loses its surface due to heat injury. The collagen denatures and coagulates forming dense dark hyalinization zone, this mechanism also causes tissue target to vaporize leaving the tissue shrunk as shown in Figure 4.

Surface morphology

Normal rat skin

SEM photomicrograph of control rat skin section (that was not irradiated), showed smooth skin surface with epidermal keratinocytes that are still intact and no significant change of stratified squamous epithelial cells as shown in Figure 5. The surface outer layer is the only area that is scanned, as SEM focuses on the surface and does not scan deeper into the tissue.

Laser effects on rat skin

The CO_2 laser irradiated surface sections appeared rough, and some vascular structures were present on the surface representing damage to the surface. By increasing the laser dose, the morphology of the squamous cells changed with partial destruction of intercellular junctions (indicated by arrow), giving rise to loss of adherence between squamous cells and formation

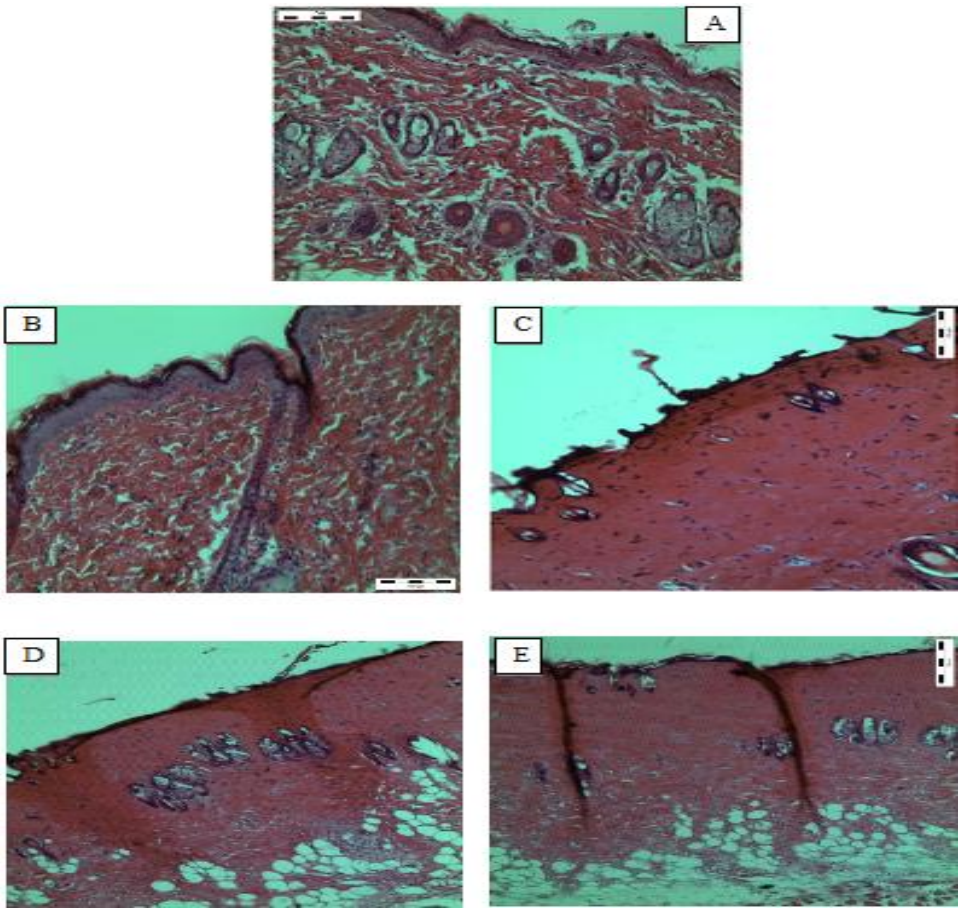

Figure 4. The mechanism of gradual tissue damage due to photothermal effect after CW CO_2 laser irradiation.

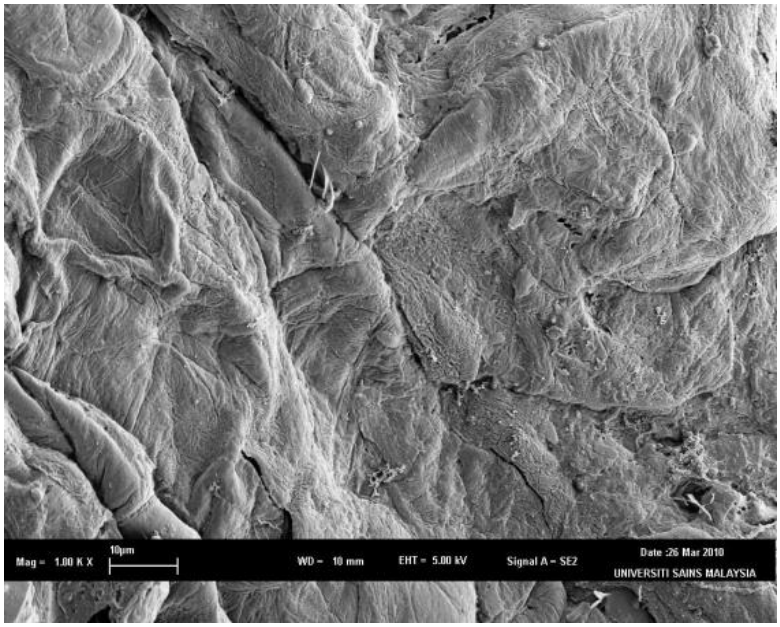

Figure 5. SEM image of unexposed normal rat skin (1000×) showing intact, homogeneous and smooth skin surface.

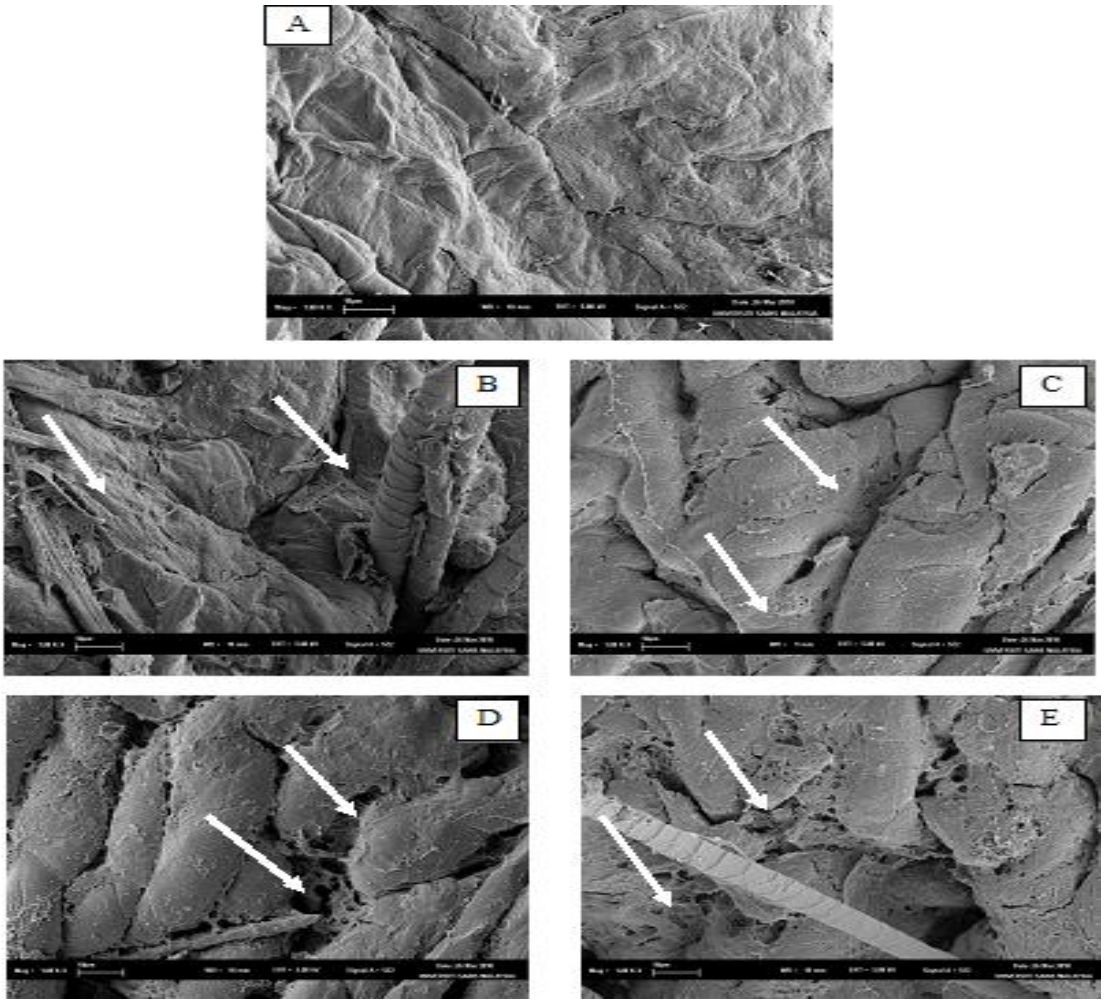

Figure 6. (A) SEM image of normal rat skin at 1000 ×. (B-E) CW CO_2 laser irradiated samples at power densities of 12.5, 14.1, 15.6, 17.2 W/cm^2, respectively. Cell distortion is increased gradually increasing laser dose. Tissue damage is indicated by arrows.

of narrow spaces between these cells as shown in Figure 6.

Conclusion

Lasers play an important role in the medical field for diagnostic and therapeutic applications. Therefore, the selected laser parameters can be used to optimize efficacy while minimizing unwanted side effects and surrounding tissue damage. By using CO_2 laser as the irradiation source, rat skin as a target sample and scanning electron microscopy was used with light microscopy to detect the skin morphological changes after laser irradiation. We found that the tissue damage encountered by the laser was mainly affected due to photothermal effect which was increased gradually as the irradiation dose increased. Epidermal loss along with coagulation, homogenous hyalinization and loss of hair associated with shrinkage and collapse of hair follicle structures at varying depths in burn area were noticed in the histological sections. Damage-power relationship was confirmed by measuring the damage depth using the software provided in the light microscope. On the other hand, SEM images showed that the extensive skin surface damage increased by increasing the laser dose. Further studies of laser-tissue interactions will result in the enhancement of existing technology as well as novel clinical applications. On the other hand, we found that the SEM images showed the same structure and effect for second rat. We can propose for further studies for side effects of laser interaction with the skin for long term effects using the biopsies. Furthermore, the studies on molecular level may be suggested for more understanding of laser-tissue interactions.

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