Comparison of histologic and mechanical properties of rat skin treated with CO_2, Er: YAG, and Diode 808-nm lasers

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Abstract

The aim of this study was to compare the effects of different pulses and fluences of CO_2, Er: YAG, and Diode 808-nm lasers treatments on rat skin in vivo. Skin strength measurements were carried out, and skin tissue samples were taken for histological study. The CO_2 laser effects resulted more laser pulses produced more ablative removal of the epidermal layer. Deep dermal collagen coagulation with low CO_2 and Er:YAG laser fluences was increased. The histological results indicated that the low fluence of Er:YAG laser with different pulses targeted not only the epidermis layer, but could also create heat deposition in the upper dermis layer. While the diode 808-nm laser demonstrated that, no observed damage to skin layers but most of hair follicle destroyed with the low-level 808-nm laser. Our results demonstrated that the CO_2 laser was more effective than Er:YAG laser in ablative treatments and LLT diode laser more effective in hair removal, but our results needed to be confirmed in human. It appeared that strength of treated skin with CO_2, and Er:YAG lasers were increased with laser pulses which caused by the photothermal effect

Keywords: ablation, collagen, hair follicle, thermal damage, tensile test.

Introduction

In past few years, the use of lasers in dermatologic disorders has increased significantly. The Erbium : YAG (Er:YAG) laser with wavelength 2940 nm represents a chance to decrease the thermal damage to surrounding area observed with the CO_2 laser. This wavelength is strongly absorbed by water(absorption coefficient 12 000 vs 800 $cm^{-1}$for CO_2 laser) [1] , which resulted that the thermal damage has been shown to be less than about 50μm vs the 150 μm observed after multiple pulses of pulsed CO_2 laser exposure. Some studies estimated that the Er:YAG laser wounds reepitherialize earlier than CO2 laser wounds 2. The ablation threshold for Er:YAG laser observed at 1.7 J/cm2 for pulses of about 100-microsecond duration [3-4]. In contrast, the CO_2 laser

has a wavelength of 10600 nm, which is within the infrared portion of the electromagnetic spectrum. Since the CO_2 laser has a high absorption coefficient of water, it penetrates superficially through the skin, as skin contains a substantial quantity of water: thus radiation at CO_2 laser wavelength is very well suited for use in skin treatments [5]. Clinically, there was immediate visible skin contraction after second and third CO_2 pulses; in contrast, there was no need to wipe debris between subsequent pulses using the Er:YAG. The Er:YAG laser's affinity for water is 10-16 times greater than the CO_2 laser, which makes it a suitable resurfacing laser because of the reduced heat injury and deeper control the different properties of these two laser systems produce different clinical effects. The carbon dioxide ablates and penetrates deeper than Er:YAG laser, it also causes greater thermal damage zone of the skin tissue. Otherwise, with Er:YAG laser skin treatment, skin epithelialization is faster within 2-7 days and erythema surgery is shorter within 2 to 4 weeks. In clinical applications, the deeper tissue penetration which is produced by carbon dioxide is very important since it is able to create dermal remodeling which necessary for achieving the youthful esthetic result [6]. In a recently publish research 7 using an animal model in vivo, it demonstrated that deep collagen coagulation can be achieved up to 260 μm by rapidly stacking of Er:YAG laser pulses without complete epidermal ablation were observed at single-pulse fluences near to the ablation threshold. Mordon et al [8-9] recently studies utilizing an animal model to estimated that a repetitive of Er:YAG glass laser pulses at (3 Hz) with the help a cooling device can demonstrate zone of thermal damage at depth of 200-500 μm with a completely epidermal ablation. The number of studies has been demonstrated that dermal collagen coagulation of 70 μm or deeper is required to optimize the clinical outcome for laser skin resurfacing [10-11].Majaron et al. estimated that the dermal coagulation depth can be achieved up to 250 μm below the epidermal-dermal junction with repetitive irradiation of strongly absorbed Er:YAG laser [12]. These results matched well the prediction of the numerical model of thermo-mechanical laser ablation of skin [13-14]. The CO_2 laser is used for a treatment of photoaging appearance, removing wrinkles and leading to a recreation of the dermis via the transmission of the thermal effect [15-16]. Treatment using the CO_2 laser is focused on treating only the target area partially since the treated area can be regenerated quickly from surrounding skin tissue though the injured epidermis by laser. The temperature of skin tissue can reach up to 400 C during CO_2 laser irradiation with as few as three stacking pulses, which leads to greater coagulation of tissue [17]. The photothermal effects caused by CO_2 laser irradiation commonly produce different morphological changes on the skin surface, such as carbonization,

vaporization and protein denaturation, which lead to the reduction of both pain and inflammation [18-19]. Hawkins et al [20] have reported that the visible red and infrared wavelength revealed to have highly absorbent and unique therapeutic effects in vivo tissues. In the visible to near-infrared wavelength, melanin is the main chromosphere for targeting hair follicles, where the diode 808 –nm laser is absorbed by melanin with deep penetration into the dermis layer [21]. The use of lasers in hair removal allows selective targeting of the hair bulb and can diminish regrowth for at least three months.[22-27] .The mechanisms for laser hair removal is the specific targeting of melanin in the hair bulb. Melanin absorbs the light emitted by the laser at a specific wavelength. The energy of the laser converts into heat, causing the selective destruction of the hair bulb. However, melanin in the surrounding epidermis can also be targeted. In addition, animal studies may only be approximated to the human situation in a guarded and limited fashion. Differences in the behavior of hair follicles between species have been well documented28.

Material and methods

Experimental animals

Male rats (n=50) with an average weight of 200g were obtained from the animal house of Universiti Teknologi Malaysia. These rats were 12 weeks old and randomly allocated to four groups (n=10 each group). In Group 1 and 2 were exposure with Er:YAG on one side of rats back and left another side as control and use G1 for histology examination and G2 for the tensile test. In Groups 3 and 4 was exposure to CO2 laser with different number of pulses where used the G3 for histological examination and G4 for tensile test and G5 were exposure to diode (CW) 808-nm laser with different density for 10s. They were fed unvarying meals and were kept for 12 hours in light followed by 12 hours in the dark in a constant environment. The hair on their backs was shaved gently using a razor blade and an electric shaver. The anesthetic was utilized during this treatment, followed by cleaning using alcohol to prevent infection. The full thickness of the rats' skin from the dorsal area was irradiated with laser and prepared for histological examination. The samples were collected immediately and after the first, third and sixth day after treatment.

Two lasers were implemented in this study. One of them was the HY CO2 laser; model MTO4, manufactured in China. The laser was operated in two modes: the continuous mode (CW) wavelength of 10600nm. The laser beam was modulated to the pulse laser with the fluence 16.9

J/cm2. The second laser was the Er:YAG laser, manufactured in the USA. The laser was operated in pulse mode and could be triggered either internally or externally. The internal trigger operated in repetitive mode with a frequency of 20Hz. The external trigger was designed to operate with a single pulse. However, with a single pulse we used with fluence 3.25 J/cm2. Once these were set, both laser energies were fired various times over each treatment spot to get the equivalent of 1, 3, 6 and 10 pulses for each. 808 nm diode lasers in continuous wave mode, the spot size of the beam at focus point were a 0.47 mm2. To keep from direct thermal damage and to cover a wide irradiated area, the target tissue is placed 5 mm away from the focal point. The rats were treated with power 100 W, 200 W, 400 W, and 500W.

The groups rats were exposed to the Er:YAG and CO2 lasers with 1, 3, 6 and 10 pulses in the marked area without moving the target to create the required number of pulses. Control samples were obtained from the unexposed side of the skin and processed under the same conditions as the exposed samples. Each exposure was done on the back of the rat in order to get duplicate samples for histology studies. Control samples were obtained from the unexposed skin and processed under the same conditions as exposed samples.

Histology examination (light microscopy analysis)

After laser irradiation, the rats were killed according to international rules of animal care ethics and ten 2×2mm full thickness skin sections were cut out from the back of each rat and immediately transferred into bottles (control, 1, 3, 6 and 10 Er: YAG pulses with one pulse of the CO2 laser). Skin samples were fixed in 10% formalin for 48 hours and then dehydrated in ascending concentrations of ethanol, starting from 50%, 70%, 90% and 100% I and 100% II for one and a half hours each. This gradient ensures that the tissue loses its humidity gradually. The tissue samples were then cleaned with xylene for one hour, followed by infiltration in paraffin wax for three hours at 60 degrees. The samples were then cooled using the cooling function of the embedding machine and then a microtome machine was used to cut samples: 3µm cross sections of skin tissue were cut on stainless steel using very sharp blades. The resultant sections were transferred to a water bath to expand; then three to four sections were placed on labeled slides and put onto a warmer slide to dry and to allow the layer of water between the sections

and the glass slide to evaporate overnight at 50 ^0C. Finally, the slides were stained with Hematoxylin and Eosin (H&E) and Masson's Trichrome and then mounted with transparent glue medium using a glass cover slide. Both the unexposed and exposed samples were then examined under a light microscope. The magnifications were set at 4 and 40 times. The samples were taken from the rats one, three and six days after treatment to observe the collagen concentration and were histologically examined.

Tensile Test

Initially, ten rats were exposed to the Er:YAG laser at different numbers of pulses Furthermore, another 10 rats were exposed to CO2 laser. The rats were then killed three hours after irradiation according to international rules of animal care. The samples were cut out with a specific mold which has the same geometric dimension of 7mm in length, including the 1 cm clamp allowance at both ends to be exactly 5 cm between the clamps in length and 2mm in width. To get similar dimensions for all harvested specimens from the rat skins, we flattened them on a surface with 0.4N deed weights. The dimensions were perpendicular to the direction of the preload. The thickness of all samples was then measured with a digital caliper and taken at three different locations before getting the mean value which presented in Table 1. There was a slight but nevertheless significant effect in thickness with increasing laser pulses. The rats used were about the same age of 8 weeks to ensure the thickness of their skin were similar. The skin strip was placed between the two clamps of the tensiometer, which was a universal Testing Unite (USA) model and the clamps were secured to avoid any slippage of the sample. Subcutaneous tissue was scarified near both clamps. These samples were used directly for the tensile test at a loading rate of 30mm/min and sampling until they broke. The maximum stress recorded was defined as the tensile strength.

Results

We are using the change in the pulse number of the Er:YAG laser as a parameter in this part which lead to a range of results. This is shown in Figure 1, where the frames are arranged according to increasing number of laser pulses. The laser's effect looked like a strip of coagulation

in all exposed regions. After the laser irradiation, a layer of powdery white debris was seen in the irradiated regions. No char formation was visible on the skin surface. In frame 1a, we can see that, the skin was heated without damage to surrounding area, after exposure by a single Er:YAG pulse of 3.25 J/cm2. When the number of pulses increased, the damaged area increased. Additionally, the color of the photodamage became uniformly with brown desiccated tissue, representing more severe and deeper damage at 10 pulses which means that the skin layer vaporized due to water absorption. The CO2 laser effects was demonstrating the cone-shaped of a zone of vaporization, a zone of necrosis, and a zone of thermal damage which explained that the rat skin tissue which was irradiated with a CO2 laser pulse became damaged and interacted with the laser through absorption of the main chromorophe by water which leads to ablation of the skin layer. The photodamage was seen as a darker patch which increased as laser pulses light increased which meant that more of the water in the target was vaporized and the skin was carbonized as shown in Figure 1(a´´, b´´, c´´ and d´´). There was a linear increase in the photodamage area as the function of laser pulses numbers when the laser fluence increases, where the photodamage area was a maximum of 10 pulses. When the laser fluence increase, the most of the water in the skin target being vaporized and, when there was no water present, the skin became burnt and presented as a dark color in the damaged area. The most frames of damaged area has cone shapes with burning being seen at the damaging zone and there being an oily center due to epidermis slipping out, as well as penetration, and a small area of cavitation's zone around the configuration shapes due to heat diffusion. The tissue demonstrated large zones of collagen homogenization resulting heat damage, the vaporization zone was more dominated, reflecting vacuoles in the dermis with an accompanied basophilic zone of collagen damage.

The mechanical properties of rat skin tissue were examined using tensile tests. We estimated the increase in the tensile strength of skin in rats irradiated with different number of pulses. The skin tissue irradiated with Er:YAG laser has different values: the highest value of 7.036 MPa for a force not exceeding 70 N was collected for samples treated with ten pulses with a single –pulse fluence 3.25 J/cm², and the lowest value was at 1.569 MPa at a force of about 16 N, obtained in

samples irradiated with a single pulse. The strength and elasticity of irradiated skin in control samples versus those exposed to laser pulses is plotted in Figure 2. This increase in skin elasticity is correlated to the process of the inflammatory phase running after irradiation with the function of fibroblasts. The skin tissues was exposure to CO2 laser tissue has different values: the highest value of 9.85 MPa for a force not exceeding 99 N was collected for samples treated with ten pulses, and the lowest value was at 1.003 MPa at a force of about 10 N, obtained in samples irradiated with a single pulse. We can see clearly how much the increase in the strength of skin is correlated with exposure to laser pulses as laser radiant energy influences creation of collagen in the dermis layer.

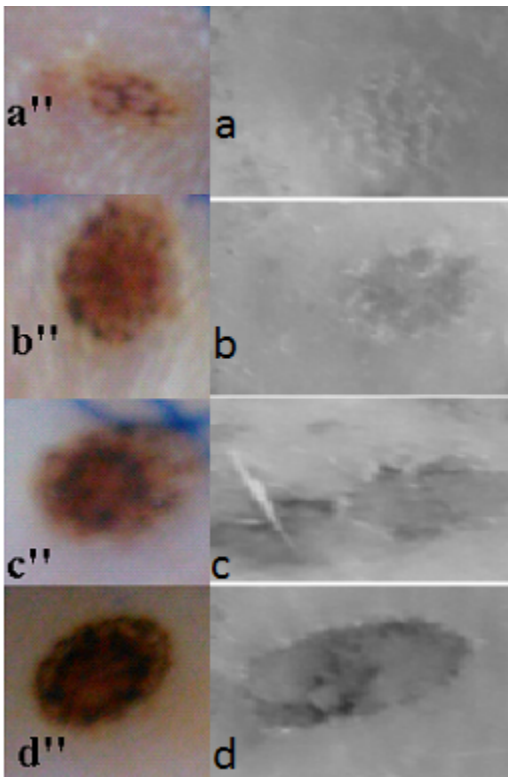

Fig. 1- The effect of Er:YAG and CO_2 lasers resurfacing on rat skin tissue.

The histological examinations revealed that after one pass of the Er: YAG with single –pulse fluence of 3.25 J/cm², there little change in the skin layer aside from superficial ablation of the epidermis layer down to the granular layer demonstrating collagen coagulation below the

epidermal-dermis junction. The extent of the damage is evidenced by strongly hyalinized appearance and tectorial change in this area. After three passes of the laser, in which there was ablation in the epidermis layer down to the basal cell layer where thermal damage could be identified. After six pulses of the laser, the epidermal damage became more severe where the epidermis layer almost ablated down the dermis layer with a reactive papillary dermis loss of collagen fiber. The epidermis layer was ablated totally after 10 pulses as showed in Figure (3-b). This damage will have affected the papillary dermis layer and some thermal necrosis will appear. In general, epidermal ablation and coagulation zone (dark area) were present in all histological sections, as shown in Figure (3-c) with six pulses. There was loss of epidermis cells

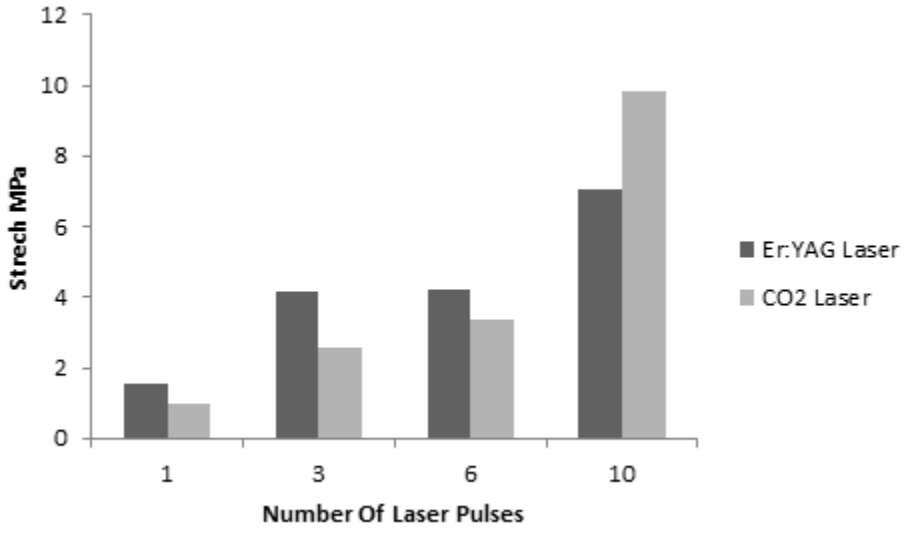

Fig.2- The stretch tensile test for rat skin irradiated with CO_2 and Er:YAG lasers.

and the thinning epidermis layer was smaller in the irradiated skin after a single CO2 laser pulse. Different depths of coagulation of the burn area and complete loss or erosion of epidermis sweeping were detected in skin tissue were irradiated with a CO2 laser.

There was loss of hair, disappearance of hair follicles and inflammation in all damaged areas because all the samples were collected immediately after the laser treatment. Most of the collagen fibers in the dermis were coagulated, which led to the collagen bundles becoming darker and slightly brushed, while the eosinophilia became homogenous. In all damaged areas, there were holes, marked with arrows, since the heat burned the epidermis layer, leading to its loss, and then passed through the hair follicles, which were also lost, presenting as holes, and coagulated the

collagen fibres, resulting in dark areas of thermal damage. The maximum cell distortions and total ablation of the epidermis were observed in samples irradiated with the maximum number of laser pulses – ten pulses. There was a total loss of the stratum corneum and gradual thinning of the epidermis. In the skin tissue samples were irradiated with LLT diode 808-nm (CW) revealed that there was no observed damage to the skin layers but most of hair follicle in epidermal laser, as well as deeper in dermis layer, was destroyed which was maximum with low laser fluence at 200W for 10 s as shown in Figure (3-d).

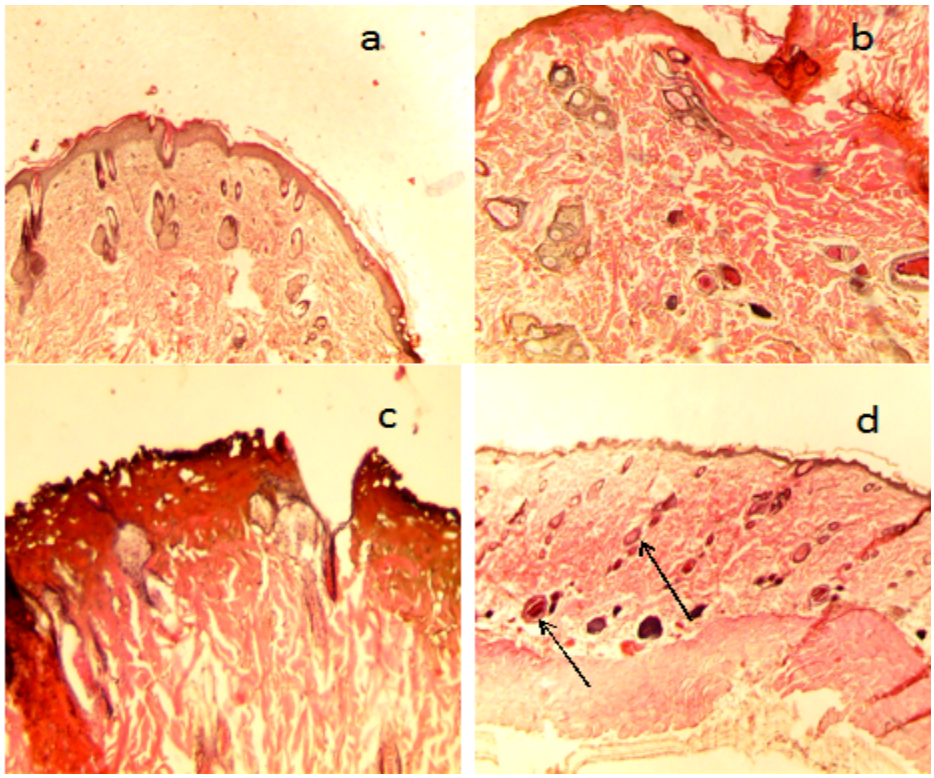

Fig.3- **a-** the unexposed rat skin tissue (control). **b**- Histological change of skin irradiated with 10 pulses of Er:YAG laser with 3.25 J/cm^2 .**C**- histological examination of rat skin irradiated with six pulses of CO_2 laser with fluence 16.9 J/cm^2 . **d**-histological changes using a 808nm (CW) diode laser, black arrows do mark a heat based destruction of hair bulbs indicating deep penetration.

Discussion

As a result, theoretical results have predicted that the deeper collagen coagulation up to 200-300 μm due to Er:YAG laser irradiation could appear without exceeding the ablation temperature at the skin surface. It was difficult to determine the depth of collagen coagulation unambiguously. The collagen coagulation depth decreases with increasing pulse fluence. With repetitive laser irradiation, the heat deposition at lower pulse fluencies is likely enhanced since to skin desiccation between repetitive Er:YAG laser pulses, which lead to removing the main absorber of the laser radiation. Resulting, the optical penetration depth increase, leading to further diminished ablation efficiency, enhanced deposition of heat, and increased the thermal effect, as evidenced also by epidermal damage data in Figure (3-b). In general, with the laser burn obtained and/or sectioned slightly off-center. When the skin samples were irradiated with the Er: YAG laser, this caused the dermal edema, and subsequent inflammatory changes which lead to the collagen fiber formation, to begin to increase more. Most reports arrived at similar results where the CO2 laser create the thermal injury zone deep to the ablation zone29-32, as well as a diminishing ablation depth increasing thermal necrosis zone with each successive laser pulses33-34. Ross et al35 recently estimated that, after three CO2 laser pulses on the same sites, tissue necrosis, and new collagen formation extended deeper than the acutely hyalinised collagen zone. The deepest thermal damage, which was 170.45 ±11.76 μm, was achieved by the CO2 laser at ten pulses and n=10, and 270 μm, due to the higher temperature created in skin tissue, which denatures and coagulates more collagen. This deep collagen coagulation formatted using CO2 laser can be achieved without complete ablation of the epidermis by rapidly Er:YAG laser pulses. The macroscopic results indicated that based on Figure 1 the CO2 laser interaction with skin tissue reflects three zones: the cavitation zone from the lateralization of the steam, (b) the heat damage zone and finally, (c) the vaporization zone which presented in bulb area35. Since the main and direct target is the water in tissue, this may lead to more dehydration of tissue, which also causes increased in collagen denaturation. The healing of treated skin tissue is a complicated process of overlapping biochemical and cellular events that lead to the repair of damaged tissue. All laser treatment leads to the loss of epidermal thickness after exposure, as shown in Figure 5. However, the loss of epidermis thickness increase as the laser radiant pulses increases as well, demonstrating that the CO2 laser can be considered as the most superior

ablative wavelength. Since its introduction in 1995, laser hair removal has estimated to be a superior and more permanent solution for hair removal compared to other techniques 36-38. Although a number of lasers and non-laser light sources have been developed for purposes of unwanted hair, and 800–810nm diode lasers remain common options for hair removal among individuals with Fitzpatrick skin types I– IV 39.The clinical study evaluated the long-term efficacy and safety of a newly developed diode laser (808 nm) system in comparison to a standard alexandrite 755nm scanning hair removal laser there have been few published studies illustrating the mechanism of action of these lasers, especially histological responses 40. Most studies to date have concentrated on the evaluation of efficacy of treatment by hair counts 41-42.Our histological investigation of laser effects on terminal hair-bearing rat skin explants showed comparable levels of thermal damage to the follicle while saving the epidermal compartment. These investigations highlight the safety of laser system tested. Based on our result, the laser power at 200 W has been stimulation the hair follicle to enhance the hair removal. However, our group and other have shown that LLLT 808-nm is capable of hair removal since of photostimulation.

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