Research article

The Effects of Multiple Power Densities of Carbon Dioxide Laser on Photothermal Damage in Rat Skin Tissue

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Abstract

A CO_2 laser produces infrared photons that are largely absorbed by the skin and cause morphological alterations. Twenty-four (Wistar) rats weighing 290-380 g and ranging in age from 8 months to a year were chosen at random and divided into sixteen rats for histological examination and eight rats for tensile testing to determine the extent of injury caused by photothermal damage induced by multiple doses of a CO_2 laser. Anesthesia was achieved with intramuscular doses of 10 mg/kg ketamine and 60 mg/kg Xylazine. Two equal 0.5 cm surgical incisions of rat dorsal skin were performed on the left and right sides. One was utilized as a control while the other was subjected to a 10600 nm CO_2 laser at various power levels (12.5, 14.1, 15.6, and 17.2) W/cm^2. According to the histological analysis, the non-irradiated skin appeared to be flawless, and normal skin layers were observed. The amount of radiation in the irradiated skin samples was closely related to tissue damage. Higher dosages of irradiation resulted in the most severe cellular mutilation. Tissue injury manifested as epidermal obliteration, coagulation, homogeneous hyalinization, and hair loss. The effects of CO_2 laser interaction with the skin were explored in-depth in this study. Exposure to the CO_2 laser resulted in severe burns and coagulation.

1. Introduction

The thermal effects of high-power density lasers on tissue and blood have gotten a lot of attention in medical uses of such lasers in surgery and, more especially, in dermatology. The majority of studies that use high-power density lasers were focused on thermal therapy in order to achieve optimal irradiation conditions. Absorption and radiation-less transitions turn the laser power density into heat within the front section of the tissue during CO_2 laser exposure [1, 2]. Rapid

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localized heating generates substantial thermal transients and shock waves, which can propagate into the blood vessels of the dermal tissue layers. Data showed that structural proteins such as collagens underwent denaturation at temperatures exceeding 60-70^{o}C [3, 4].

The most common cause of laser-induced skin damage is thermal burn. Thermal damage is commonly associated with lasers with exposure times of more than 10 ms and wavelength ranges ranging from near ultraviolet to infrared [5]. Tissue absorption and scattering coefficients, laser wavelength, time of exposure, degree of vascular flow, and size of the irradiated area determine the primary thermal effects of laser exposure [6]. It penetrates deeply through the skin which includes a significant amount of water, due to its high optical absorption, the CO_2 laser is ideal for skin treatments. A reduced amount of lateral thermal transmission results in less heat in the tissues because the heat is already absorbed during the tissue processing phase [7, 8].

Other characteristics that allow the laser to minimize patient discomfort are the short irradiation time and smaller spot size on top of the skin. On the other hand, the CO_2 laser has a number of disadvantages, for instance, it generates burning feeling that extends across the surrounding area. *In vivo* animal models have lately been employed to study the tissue consequences of CO_2 and Er: YAG lasers [9].

The temperature effect is, of course, influenced not only by the beam characteristics but also by the type of skin pigmentation. The efficiency of skin pigmentation protection is determined by the color of the skin. To illustrate the importance of melanin and hemoglobin in the absorption process, 5 to 10 Joule of energy, for instance, has minimal effect on white skin but burns pigmented skin [10]. The skin's constituent layers have different levels of radiation protection. The dense hyperkeratotic layers are resistant, whereas the thinner dermis layers closer to the surface are more sensitive [11]. The temperature of skin tissue can reach 400°C throughout CO_2 laser irradiation by as few as three pulses, resulting in increased tissue coagulation. CO_2 laser irradiation can cause various kinds of deformation on the skin's exterior, including carbonization, in addition to vaporization, as well as protein denaturation [12].

This research utilized a CO_2 laser to perform a tensile test on rats' skin. Expressions of thermally damaged epidermal layer depth and the effect of laser at different power densities on the rats' skin are also investigated.

2. Materials and Methods

2.1 Animals

The animal house at Baghdad University provided twenty-four adult male (Wistar) rats weighing 290-380 g and ranging in age from 8 months to a year. The procedures were done in according with the Baghdad University/Animal Iraq's Ethics Committee's guiding principles for the care and use of experimental animals.

For histology analysis, sixteen rats were employed, and for the tensile test, eight rats were used. Every day, they were given the same meals and kept in the same surroundings, with 12 h of light and then 12 h of darkness. Intramuscular dosages of 10 mg/kg ketamine and 60 mg/kg Xylazine were used to anesthetize the rats. Their back hair was neatly shaved with a razor blade and an electric shaver to prevent infection, and then cleaned with alcohol.

On both sides of their backs, two identical 0.5 cm sections of rat dorsal skin were created. One was used as a control, and the other was exposed to CO_2 laser radiation. The laser was directed at the target rat skin for 15 s. We computed the spot's size on the target rat's skin using the spot's 0.35 cm diameter and determined that it should have a circular shape. Therefore, the area was 3.14x $(0.35/2)^2$, which equals 0.096 cm^2. The power density was calculated (as shown in Table 1)

Table 1. The results of power density were calculated using various power values, and the output power values were measured based on the PMW duty cycle.

PWM Duty Cycle (%)	Power Values (W)	Power Density (W/cm^2)
4	1.2	12.5
4.5	1.35	14.1
5	1.5	15.6
5.5	1.65	17.2

utilizing information from the predicted power using an increasing PWM (Pulse-Width Modulation) duty cycle, from 4.0% to 5.5% of the maximum power of 30 W.

2.2 Examination of the histology

Following laser irradiation, ten full-thickness (2x2 mm) pieces of skin were removed from the rats' backs and placed in bottles right away. After 48 h, the skin pieces were fixed in 10% formalin. Each sample was dehydrated in increasing ethanol concentrations for 1.5 h each, starting with 50%, and then 70%, 90%, and 100%. The tissue lost its humidity as a result of this gradient. xylene was used to clean the samples for 1 h before being infiltrated into paraffin wax at 60ºC for 3 h [13]. Following the processing of the tissue, the samples were carefully positioned in a paraffin wax mold, cooled at the embedding station's cooling unit, and prepared for sectioning.

The tissue samples were then serially cut into 5 to 10 µm thick sections in the sagittal plane using a microtome, and each resulting ribbon was transferred into a flotation bath to allow any creases in the sections to disappear. A microscope slide with labels was used to carefully lift 3 to 4 slices, which were then dried on a hot plate to remove any excess moisture. The slides were then put in an oven set to 30°C for an overnight wax meltdown, which fused the portions to the slide. The slides were then mounted with a transparent DPX medium after being stained with hematoxylin and eosin to improve contrast in the microscope image. The slides were then seen under a light microscope. The sections of the exposed and non-exposed samples were looked at, and detailed comparisons of the structural skin of the exposed and non-exposed samples were done.

2.3 Tensile test

Firstly, eight rats were given varying doses of CO_2 laser. After 3 h of irradiation, the rats were anesthetized and killed in accordance with international animal welfare standards. The specimens were cut with equal dimensions, totaling 5 cm in length and 2 mm in width A skin strip was subsequently put in between the tensiometer's two clamps, and the clamps were tightened to prevent the specimen from moving near both clamps, where subcutaneous tissue had been sacrificed [14]. All specimens were examined in a tensiometer with a crosshead speed of 30 mm/min until skin breaking was observed.

3. Results and Discussion

The specific photodamage generated by the CO_2 laser system was examined as part of the basic examination of laser-tissue interaction. Our research established the existence of physiological changes caused by laser contact, including photodamage. Figure 1, which shows the frames in ascending order of laser power densities, illustrates the changes inside the skin surface that are

visible to the naked eyes. Despite the skin being agitated, it did not burn, and protein denaturalization was suggested by the photodamage's white hue. The photodamage appeared as an oval with a black border and an oily pond in the center. The water in the skin evaporated as a result of the laser irradiation, carbonizing the skin. This resulted from the laser's stimulation and disintegration as it reached the skin [15, 16].

Histological analysis of the control rat skin tissue indicated a normal skin structure, with the epidermis, dermis, and subcutaneous layers all seeming normal. Keratinized epithelium cells formed in the outer layer, resembling flattened entities with large nuclei. Interior strength was provided by a web of bushed, uneven tendons, while suppleness is created by a thin layer of collagen fibers under this layer as shown in Figure 1(a).

The changes in the skin's surface can be seen in Figures 1(b), (c), (d), and (e), which are shown in increasing order of laser power density. Figure 1(b) depicts the outcome of an exposure to the skin of 12.5 W/cm^2. The skin was warmed but not burned. The photodamage appeared white and was caused by denaturing proteins. The photodamage was shaped like an oval with a black rim as well as an oily pond in the middle. The skin's surface was heated and melted, allowing the laser to pass through it. Due to the water within target tissue absorbing the laser energy, this skin became carbonized following exposure. The largest cell abnormalities along with epidermal ablation were detected within specimens that were irradiated with 17.2 W/cm^2. The epidermis was gradually thinned and the stratum corneum was completely gone. The decrease of epidermal thickness in skin specimens treated with CO_2 laser increased over time. In general, the epidermal loss was dependent on and enlarged with rising power density for all histological sections irradiated, as shown in Figures 1(c), (d), and (e).

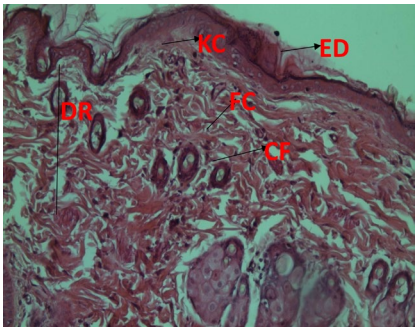
Using the free program ImageJ, the photodamaged areas were measured, and the results are shown in Figures 1(b), (c), (d), and (e). Figure 2 displays the estimation findings. Due to heat diffusion through the skin layers [17, 18], it was discovered that the photodamaged area grew linearly with laser power density. At 17.2 W/cm^2, the highest loss in thickness was 2.4±1.9 µm. The epidermal thickness of the untreated skin tissue samples was 60±1.33 µm. A paired t-test revealed that the magnitude of epidermal thickness loss was substantially different from the control samples ($p=0.005$). Altogether, there were symptoms of epidermal damage and the creation of cellular infiltrates, and also dramatic destruction of connective tissue including nucleus cells in the dermis layer, fiber swelling, and heat damage in the forms of collagen coagulation without degradation.

All measurements were made at a magnification of (1000 X) using SEM, and thermal damage distance down to the deepest point was determined. The power density affects the CO_2 laser wavelength's amount of damage, with a larger power density producing deeper damage, as seen in Figure 3. In irradiated tissue, photothermal damage was confirmed and measured using conventional histological techniques.

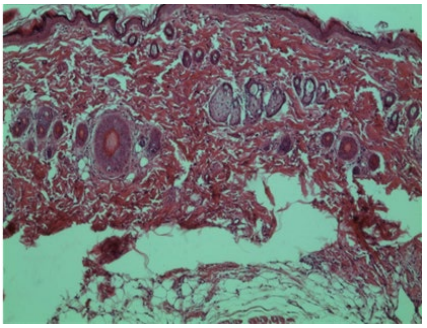
Furthermore, the extent of the damage—represented by the black areas—was estimated and the images were scaled. Figure 4 shows a plot of CO_2 power density against the depth of damage. Figure 3 shows that photodamage depth was greatest at 17.2 W/cm^2. This was because a significant amount of water inside the target tissue was vaporized at this power density, which led to greater penetration [19, 20].

The laser's thermal effects resulted in tissue damage that included epidermal destruction with coagulation, hyalinization, hair loss with volume reduction, and disintegration of hair follicle compositions of various intensities at the burn positions. This damage continued to progress with increase in power density [21, 22].

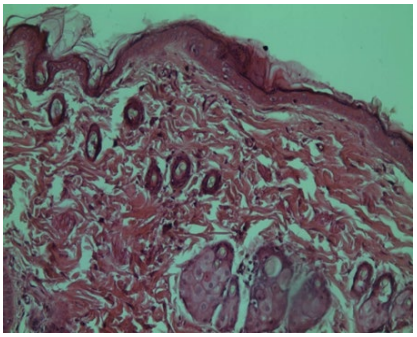
Based on histological observations, we assigned scores to assess the degree of heat damage. A value of (zero) indicates that there was no heat damage, and (1) indicates thermal damage, with 5 to 25% of cells being edematous as well as swollen, in addition to the tissue appearing coagulated. (2) indicates reasonable harm, with 40-60% of cells exhibiting indications of photo thermal damage, and (3) indicates harsh photo thermal damage, with symptoms of photothermal damage in the majority of cells.



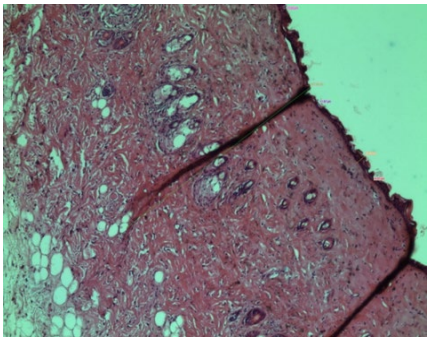
a) Normal rat skin tissue; (DR) dermis, (ED) epidermis, (KC) keratinocytes, (FC) fibrocytes, (CF) collagen fiber



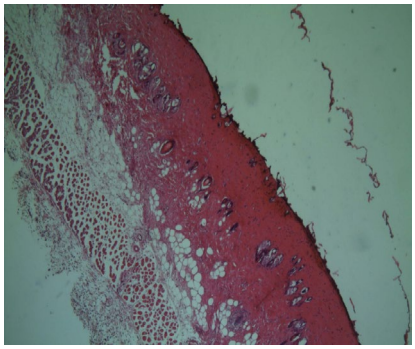
b) Irradiated rat skin tissue with 12.5W/cm^2



c) Irradiated rat skin tissue with 14.1W/cm^2



d) Irradiated rat skin tissue with 15.6W/cm^2



e) Irradiated rat skin tissue with 17.2W/cm^2

Figure 1. Histological changes under a light microscope at (40 X) magnification for: a) normal rat skin tissue; b), c), d) and e) irradiated rat skin tissue to a CO_2 laser at different doses

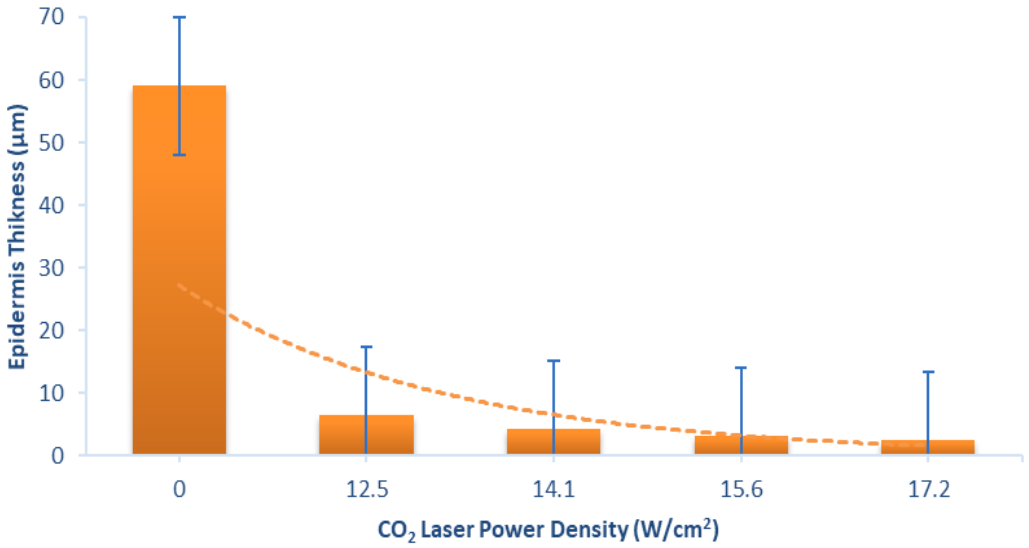
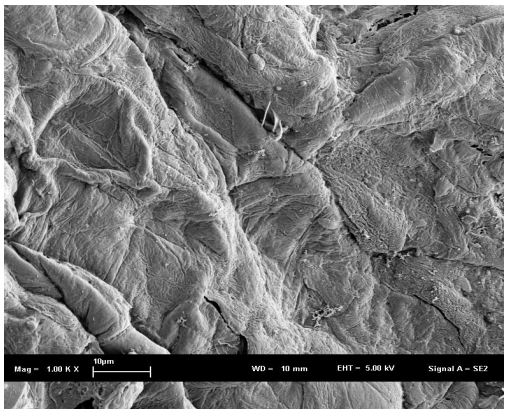


Figure 2. The remaining epidermis layer thickness for normal and irradiated rat skin tissue using a CO_2 laser at various power densities

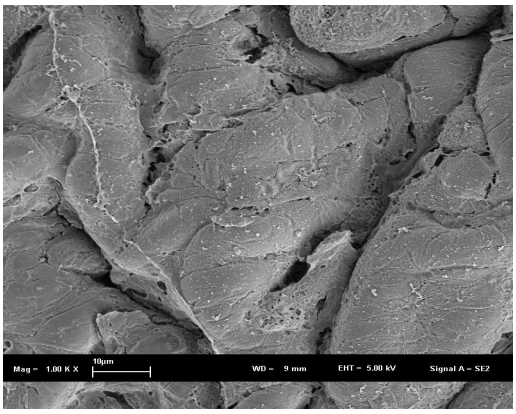
The histological evaluation showed that the values for thermal damage rose linearly with power density, and at the highest power density of 17.2W/cm^2, whole cells had been destroyed as can be seen in Figure 3. Epidermal cells that had been exposed to radiation died, and then left behind papilla-forming by-products that were thereafter exfoliated [23, 24]. This was because when a high-power density laser ablates the skin, layer, craters, or gaps emerge, and the skin substance actually extrudes, causing surfaces to dry out or deform. Previous research reported that adipose tissue effectively absorbed photons, resulting in photothermal or photodynamic effects that caused cell death [25, 26], a conclusion that backed up the thesis of this study.

The molecular structure of cells, which consists in type I collagen fibers at around 35% by volume, and water of about 65% by volume, deform or otherwise vaporize when a laser beam burns the skin tissue. Denaturation happens slowly at low temperatures and swiftly at high temperatures [27, 28]. The results showed that increases in laser density led to deeper heat damage and collagen coagulation in all histological sections examined.

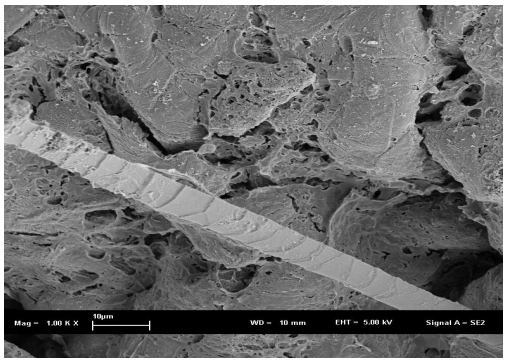
Tensile experiments were used to investigate the mechanical characteristics of skin tissues. The strength and flexibility of skin in the control and treated specimens were compared in Figure 5. The reason that the power density influenced the synthesis of collagen within the dermis layer, changes in skin strength were linked to it, and this was confirmed by histological investigation. The enhancement in skin flexibility had been linked to the inflammatory cascade that occurs after irradiation and includes the activities of fibroblasts. These values for tensile stretch are equivalent to those found by Ankersen *et al*. [29], who used tensile testing to demonstrate that rat skin and human tissue are identical. Collagen strands position themselves parallel to one another as the skin is stretched to its breaking point, causing friction and limiting further movement. The skin will break if the criteria strength is exceeded.



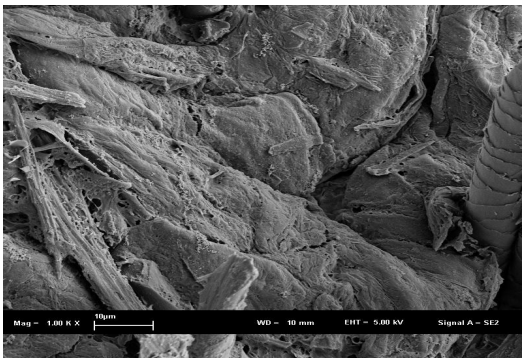
Normal rat skin tissue



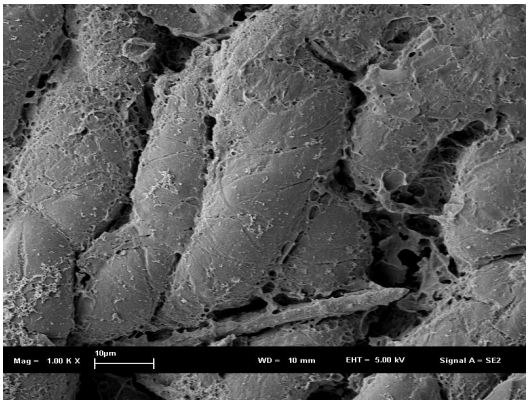
Irradiated rat skin tissue with 12.5W/cm^2



Irradiated rat skin tissue with 14.1W/cm^2



Irradiated rat skin tissue with 15.6W/cm^2



Irradiated rat skin tissue with 17.2W/cm^2

Figure 3. SEM images for normal and irradiated rat skin at (1000 X) with a CW CO_2 laser at various power densities

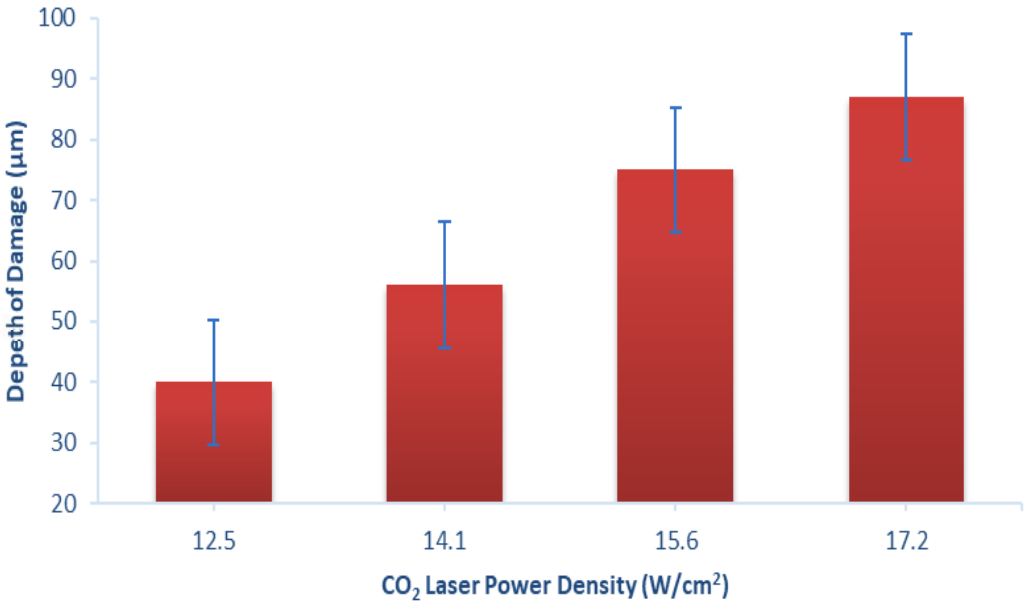


Figure 4. The depth of damage with a CO_2 laser at different power densities

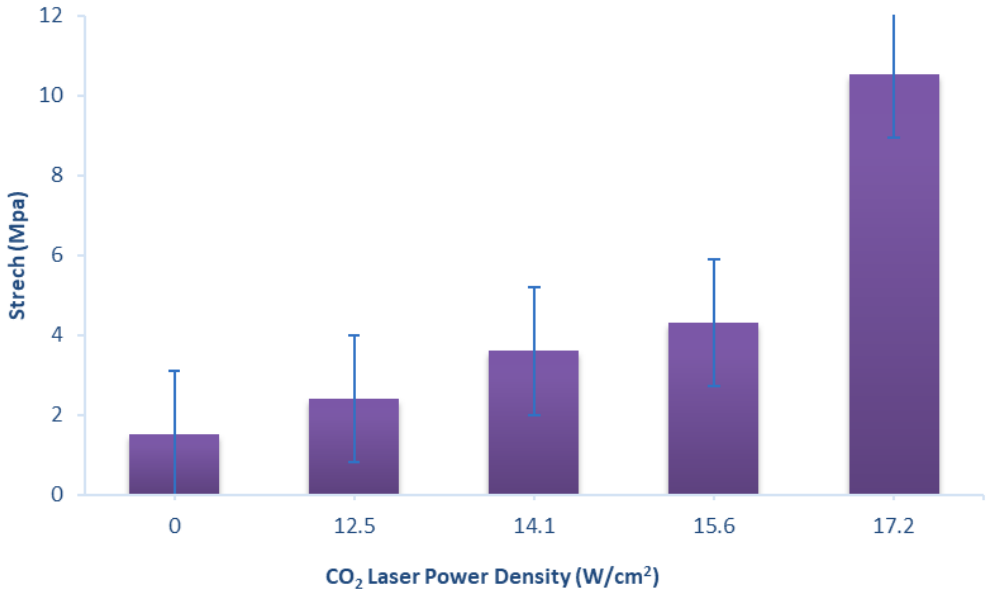


Figure 5. The stretch tensile test for normal and irradiated rat skin using various CO_2 laser power densities

A CO_2 laser is a useful tool for epidermal ablation in several medical and cosmetic applications. There is significant tissue heating and evaporation because intracellular water absorbs energy more effectively than extracellular water. The target chromophore's epidermis or thin top layer of the dermis receives practically all of the photons that are absorbed by the CO_2 laser since its wavelength of 10600 nm is close to the water absorption peak of 3000 nm. This leads to greater aesthetic ablation and less underlying thermal injury [30, 31]. The transmission of optical laser radiation in the skin is controlled by a number of variables that differ depending on the skin layer. A small amount of laser energy is absorbed into the skin, but the majority of the laser radiation penetrates into deeper layers beneath until it is scattered or absorbed in accordance with the optical qualities of the tissue [32].

4. Conclusions

Macroscopic data revealed three zones of skin tissue contact with the CO_2 laser. The burnt region was divided into three zones: a heat-damaged zone, a vaporization zone, and a cavitation zone caused by the lateralization of steam. The primary and direct target is tissue water, greater tissue dehydration may happen, encouraging more collagen denaturation. The effects of laser skin interaction were examined using a CO_2 laser. In conclusion, it was discovered that the penetration depth of thermal damage was irradiation dose-dependent, with larger thermal injury reportedly occurring at the higher power density of 17.2 W/cm^2 and minimum harm occurring at the low power density of 12.5 W/cm^2. According to our findings, it is crucial that the doctor selects the right laser irradiation to prevent postoperative problems including scarring and erythema brought on by deep thermal injury.

5. Acknowledgements

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References

[1] Kim, H. J., Lee, G.M., Park, Y.L., Lee, J.S., Whang, K.U. and Lee, S.Y., 2014. Comparison of wound repair after irradiation of rat skin with 1064 nm Nd:YAG, CO_2, and Er:YAG lasers. *Korean Journal of Dermatology*, 52(4), 244-251.

[2] Shen, D., Wei, J., Chen, L., Shen, X. and Wang, L., 2019. Besides photothermal effects, low-level CO_2 laser irradiation can potentiate skin microcirculation through photobiomodulation mechanisms. *Photobiomodulation, Photomedicine, and Laser Surgery*, 37(3), 151-158.

[3] Luke, A.M., Mathew, S., Altawash, M.M. and Madan, B.M., 2019. Lasers: A review with their applications in oral medicine. *Journal of Lasers in Medical Sciences*, 10(4), 324-329, https://doi.org/10.15171/jlms.2019.52.

[4] Michalik, M., Szymańczyk, J., Stajnke, M., Ochrymiuk, T. and Cenian, A., 2021. Medical applications of diode lasers: Pulsed versus continuous wave (cw) regime. *Micromachines*, 12(6), https://doi.org/10.3390/mi12060710.

[5] Zahara, A.T., 2018. Clinical evaluation of scalpel Er: YAG laser 2940 nm and conventional surgery incisions wound after oral soft tissue biopsy. *Bangladesh Medical Research Council*

Bulletin, 43(3), https://doi.org/10.3329/bmrcb.v43i3.36429.
[6] Bellal, S., Feghali, R.E., Mehta, A., Namachivayam, A. and Benedicenti, S., 2022. Efficacy of near infrared dental lasers on dentinal hypersensitivity: a meta-analysis of randomized controlled clinical trials. *Lasers in Medical Science*, 37(2), 733-744, https://doi.org/10.1007/s10103-021-03391-1.
[7] Xu, F., Wen, T., Lu, T.J. and Seffen, K.A., 2008. Skin biothermomechanics for medical treatments. *Journal of the Mechanical Behavior of Biomedical Materials*, 1(2), 172-187, https://doi.org/10.1016/j.jmbbm.2007.09.001.
[8] Lee, J.Y., Cho, S.W., Park, C.S. and Kim, M.C., 2010. Comparison of wound healing in porcine skin with continuous-wave and pulsed mode CO_2 laser incisions. *Journal of Veterinary Clinics*, 27(6), 647-651.
[9] Jassim M., Al, T.Z., Saleem, I. and Alhabeel, M., 2019. Laser dental treatment techniques. In: S. Sundaresan, ed. *Prevention, Detection and Management of Oral Cancer*. London: IntechOpen, pp. 1-16.
[10] Borggreven, J.M.P.M., van Dijk, J.W.E. and Driessens, F.C.M., 1980 Effect of laser irradiation on the permeability of bovine dental enamel. *Archives of Oral Biology*, 25(11-12), 831-832, https://doi.org/10.1016/0003-9969(80)90142-9.
[11] Sramek, C., Paulus, Y., Nomoto, H., Huie, P., Brown, J. and Palanker, D., 2009. Dynamics of retinal photocoagulation and rupture. *Journal of Biomedical Pptics*, 14(3), https://doi.org/10.1117/1.3130282.
[12] Dalbosco, L., Zanini, G., D'Amato, E., Tessarolo, F., Boi, S., Bauer, P., Haase, A. and Antolini, R., 2015. Photodamage in deep tissue two-photon optical biopsy of human skin. *Journal of Biophotonics*, 8(10), 816-825, https://doi.org/10.1002/jbio.201400083.
[13] Singh, G. and Nayak, B., 2022. Processing of tissue specimen with special reference to fatty tissue. In: N. Kumar, V. Kumar, S. Shrivastava, A.K. Gangwar and S. Saxena, eds. *Tissue Scaffolds*. New York: Springer Humana, pp. 493-503.
[14] Cabezas, E.E. and Celentano, D.J., 2004. Experimental and numerical analysis of the tensile test using sheet specimens. *Finite Elements in Analysis and Design,* 40, 555-575.
[15] Al-Timimi, Z., 2022. Evaluation of the significance of constant laser therapy, 532 nm, in various exposure times on the healing process of wounds infected by *Acinetobacter baumannii*. *The International Journal of Lower Extremity Wounds*, 21(4), 640-646, https://doi.org/10.1177/1534734620984039.
[16] Zahra, A.T., 2019. A comparative study of determination the spectral characteristics of serum total protein among laser system and spectrophotometric: Advantage and limitation of suggested Methods. *Current Analytical Chemistry*, 15(5), 583-590, https://doi.org/10.2174/1573411014666180531092053.
[17] Coluzzi, D.J., Al Timimi, Z. and Saleem, M., 2021. Digitization and dental lasers. In: P. Jain and M. Gupta, eds. *Digitization in Dentistry*. Cham: Springer, pp. 141-167.
[18] Hamad, F., Jaafar, M., Hamid, A., Omar, A., Timimi, Z. and Houssein, H., 2009. Influences of different low level laser power at wavelength 635 nm for two types of skin; dark and light. *Proceedings of the 7th IMT-GT Uninet and the 3rd International PSU-UNS Conferences on Bioscienc*e, Hat Yai, Songkhla, Thailand, October 7-8, 2010, pp. 130-135.
[19] Bahrololoomi, Z. and Lotfian, M., 2015. Effect of diode laser irradiation combined with topical fluoride on enamel microhardness of primary teeth. *Journal of Dentistry (Tehran)*, 12(2), 85-89.
[20] Mathew, A., Reddy, N.V., Sugumaran, D.K., Peter, J., Shameer, M. and Dauravu, L.M., 2013. Acquired acid resistance of human enamel treated with laser (Er:YAG laser and CO2 laser) and acidulated phosphate fluoride treatment: An *in vitro* atomic emission spectrometry analysis. *Contemporary Clinical Dentistry*, 4(2), 170-175, https://doi.org/10.4103/0976-237X.114864.
[21] Alexiades-Armenakas, M.R, Dover, J.S. and Arndt, K.A., 2008 The spectrum of laser skin resurfacing: Nonablative, fractional, and ablative laser resurfacing. *Journal of the American Academy of Dermatology*, 58(5), 719-737, https://doi.org/10.1016/j.jaad.2008.01.003.
[22] Suhaimi, M. and Zahra, J., 2010. Therapeutic laser for chronic low back pain. *Bangladesh Journal of Medical Science*, 8(4), 118-128, https://doi.org/10.3329/bjms.v8i4.4709.

[23] Fornaini, C., Merigo, E., Vescovi, P., Lagori, G. and Rocca, J., 2013. Use of laser in orthodontics: applications and perspectives. *Laser Therapy*, 22(2), 115-124, https://doi.org/10.5978/islsm.13-OR-10.

[24] Baleg, S.M.A., Bidin, N., Suan, L.P., Ahmad, M.F.S., Krishnan, G., Johari, A.R. and Hamid, A., 2015. The effect of CO_2 laser treatment on skin tissue. *Journal of Cosmetic Dermatology*, 14(3), 246-253. https://doi.org/10.1111/jocd.12142.

[25] Eder, S.E., 2018. Early effect of fractional CO_2 laser treatment in post-menopausal women with vaginal atrophy. *Laser Therapy*, 27(1), 41-47, https://doi.org/10.5978/islsm.18-OR-04.

[26] Grema, H., Raulin, C. and Greve, B., 2002. "Skin rejuvenation" durch nichtablative laser- und Lichtsysteme Literaturrecherche und übersicht. *Hautarzt*, 53, 385-392.

[27] Rajamahmood, T.L.I., Mat Jafri, M.Z. and Omar, K.M., 2013. Role of tissue thickness on depth of morphologic skin damage. *AIP Conference Proceedings*, 1528, 400-405.

[28] Capon, A. and Mordon, S., 2003. Can thermal lasers promote skin wound healing? *American Journal of Clinical Dermatology*, 4(1), 1-12, https://doi.org/10.2165/00128071-200304010-00001.

[29] Ankersen, J., Birkbeck, A.E., Thomson, R.D. and Vanezis, P., 1999. Puncture resistance and tensile strength of skin simulants. *Proceedings of the Institution of Mechanical Engineers. Part H, Journal of Engineering in Medicine*, 213(6), 493-501, https://doi.org/10.1243/0954411991535103.

[30] Alster, T.S. and Khoury, R.R., 2009. Treatment of laser complications. *Facial Plastic Surgery*, 25(5), 316-323, https://doi.org/10.1055/s-0029-1243080.

[31] Al-Timimi, Z.A., 2021. The performance of the cold laser therapy (GaAlAs-980nm). *Pakistan Journal of Medical and Health Sciences*, 15(10), 3096-3102, https://doi.org/10.53350/pjmhs2115103096.

[32] Ross, E.V., Naseef, G.S., McKinlay, J.R., Barnette, D.J., Skrobal, M., Grevelink, J. and Anderson, R.R., 2000. Comparison of carbon dioxide laser, erbium: YAG laser, dermabrasion, and dermatome: a study of thermal damage, wound contraction, and wound healing in a live pig model: implications for skin resurfacing. *Journal of the American Academy of Dermatology*, 42(1 Pt 1), 92-105, https://doi.org/10.1016/s0190-9622(00)90016-1.