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Injury thresholds for topical-cream-coated skin of hairless guinea pigs (*cavia porcellus*) in the near infrared region

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ABSTRACT

The reflectance and absorption of the skin plays a vital role in determining how much radiation will be absorbed by human tissue. Any substance covering the skin would change the way radiation is reflected and absorbed and thus the extent of thermal injury. Hairless guinea pigs (*cavia porcellus*) *in vivo* were used to evaluate how the minimum visible lesion threshold for single-pulse laser exposure is changed with a topical agent applied to the skin. The ED_{50} for visible lesions due to an Er: glass laser at 1540-nm with a pulse width of 50-ns was determined, and the results were compared with model predictions using a skin thermal model. The ED_{50} is compared with the damage threshold of skin coated with a highly absorbing topical cream at 1540 nm to determine its effect on damage pathology and threshold. The ED_{50} for the guinea pig was then compared to similar studies using Yucatan minipigs and Yorkshire pigs at 1540-nm and nanosecond pulse duration. [1, 2] The damage threshold at 24-hours of a Yorkshire pig for a 2.5-3.5-mm diameter beam for 100 ns was 3.2 Jcm^{-2}; very similar to our ED_{50} of 3.00 Jcm^{-2} for the hairless guinea pigs.

Keywords: laser, laser safety, skin, Takata skin model, MVL, ED_{50}, guinea pigs, laser induced breakdown

1. INTRODUCTION

As the skin is the largest organ of the body, the probability of tissue exposure from optical radiation is far more likely for the skin than that for the eye. Injury to large areas of skin is a significant incident since these injuries may lead to serious loss of bodily fluids, toxemia, and systemic infections. Yet there is limited research for a protection factor against laser exposure to the skin when compared to laser eye protection. Laser radiation injury to the skin is comparable to that of the eye except in the retinal hazard region (400-1400-nm). The most damaging wavelengths for skin have been lasers operating in the near-infrared and infrared range which penetrate the skin into the subcutaneous tissue causing deep thermal injury.[3] Many of these lasers are used in military settings and are capable of producing high peak-power with short pulses.[2] This type of exposure has proved to cause more extensive damage than continuous wave lasers. The reflectance of the skin plays a vital role in determining how much radiation will be absorbed. Any substance covering the skin would change the way radiation is reflected and absorbed and thus the extent of thermal injury. In this study, the effective dose required to produce an observable response 50% of the time (also known as the ED_{50}) was determined for hairless guinea pigs (*in vivo*) at 1540-nm using 42-65-ns pulses. In addition, these results are then used to evaluate how the minimum visible lesion threshold for single-pulse nanosecond

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laser exposure is changed with the addition of a covering agent on the surface of the skin. Similar studies have been done using modeling to demonstrate contact thermal burns and temperature profile in skin cover for competitive estimation of heat protection properties of materials.[4] Utilization of many regions of the electromagnetic spectrum may warrant a model of possible protection factors against some wavelengths.

The hairless guinea pig has skin which is physiologically similar to humans and has the added advantage that depilation is not required prior to every procedure.[5] The guinea pigs with covering agent applied did not show any kind of visible damage 1-hour and 24-hours later after exposures as a result of plasma shielding. The ED_{50} is compared to a similar study done using Yucatan minipigs.[2] The Yorkshire and Yucatan mini-pig are commonly used as *in vivo* skin models for damage threshold determination for national laser safety standards used in the ANSI Z.136.1-2000.[6] Of the two, the Yucatan mini-pig has been deemed the more applicable animal model for laser-induced skin injury investigations.[7] A comparison of skin thickness between the Yucatan mini-pig and the arms, neck, and face of human skin are statistically identical.[8] The hairless guinea pig epidermis is of similar thickness to that of human skin with distinct strata, serrated/non serrated basil keratinocytes and shallow dermal papillae.[5]

2. MATERIALS AND METHODS

2.1 Subjects

A total of three male hairless guinea pigs were used for ED_{50} exposures and one was used to test the covering agent. The hairless breed was chosen because of its similarity to human skin and because depilation is not required. The guinea pigs were procured from Charles River Laboratories, Wilmington, MA. All procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals under a protocol approved by the Brooks City-Base, TX Institutional Animal Care and Use Committee (IACUC).[9-10] Each guinea pig weighed from 550 to 720 grams and was between three and six months of age. The guinea pigs were fed commercially available diets and had unlimited access to water. Twelve hours prior to procedure, solid food was withheld. The animals involved in this study were procured, maintained, and used in accordance with the Federal Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources --National Research Council.[9] Brooks City-Base, TX has been fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) since 1967.

Animals were anesthetized with a single injection of xylazine (5 mg/kg of body weight) intramuscular (IM) and ketamine (40 mg/kg) IM. After sedation, the guinea pig's skin was cleansed to remove any debris on the skin surface. The cleansed skin was inspected and photographed to make sure scratches or any other irritations that existed prior to the procedure were noted. Pulse rate was monitored using a reflectance pulse oximeter on the foot. The animal's internal temperature was monitored using a rectal digital thermometer and maintained using a heated water blanket throughout all of the procedure.

2.2 Laser

An Er:glass laser (Megawatt Lasers, 75 joules/pulse) was modified to produce nanosecond pulses by installing an opto-mechanical switch (Taboada Research Instruments, Inc., San Antonio, TX).[11] The modified laser was used to deliver various pulse energies in the range of 0.28-1.62 J/cm² per pulse for a pulse duration range of 42-65-ns. Pulse durations were measured by a model ET-3000 InGaAs Electro-Optics Technology, Inc. photodiode connected to at Tektronix model TDS 220 oscilloscope. Energy measurements were made at the location of the beam splitter using an Ophir Laserstar energy meter with Ophir model number: 30(150)-A-HE energy probes. A HeNe laser was used as a sighting beam to locate the exposure point. The Er:glass laser was aligned with an articulating arm so that exposures were made perpendicular to the subject with a consistent distance from the focusing lens and the flank skin every time to produce a consistent spot size. The setup is shown in Figure 1.

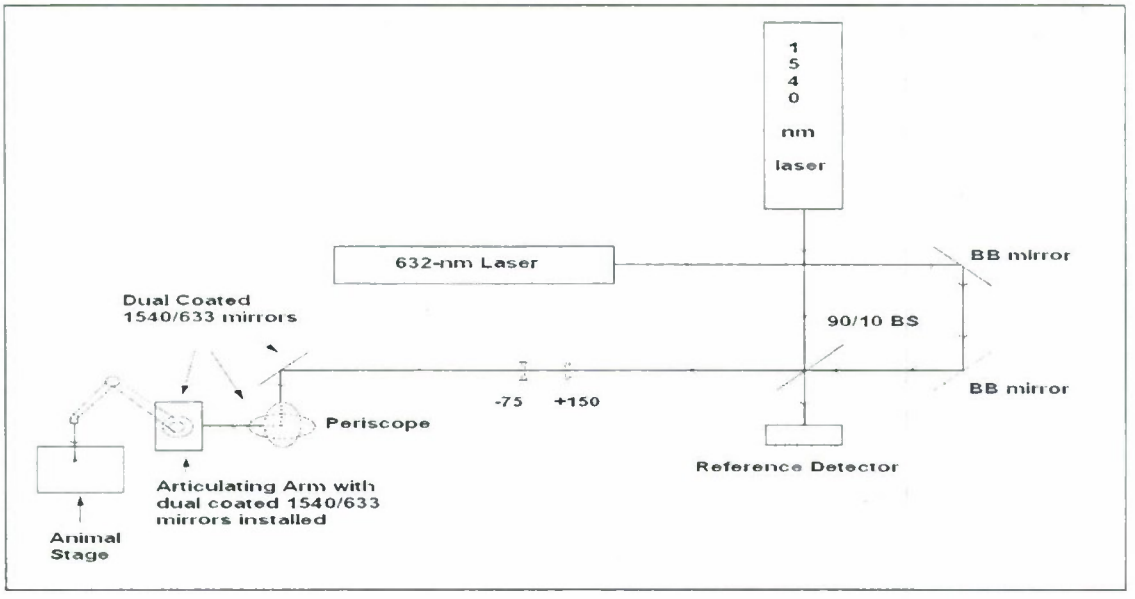

Figure 1: Schematic of the experiment setup. The 632-nm HeNe laser is the sighting beam for the 1540-nm Er:glass laser and was used to help guide 1540-nm pulse delivery. BS: Beam Splitter. BB: Broad Band.

The spot size could be varied by changing the distance between the focusing lens at the end of the arm to the exposure site and was adjusted until the $1/e^2$ diameter was 6-mm. A metal "aiming ring" was attached to the end of the articulating arm. Exposures were made on each lateral side in a grid pattern consisting of four to six rows and away from the folds of skin near the legs. The number of exposure sites was dependent on the size of the subject on the respective lateral side. Each row consisted of five to six individual exposure locations denoted by 1 cm by 1 cm boxes using black ink marker. Surgical markers were found to smear and interfere with evaluation. Energy delivered was systematically varied for each exposure and randomly delivered at each exposure site. Each subject received a range of 48-56 total exposures for each procedure.

2.3 Evaluations

Three independent evaluations were performed for each exposure site for the presence of laser-induced skin lesions and subjects were photographed at 1 and 24-hours. Before a site of exposure was counted as a lesion, at least two out of three evaluators had to agree a lesion existed. Biopsy specimens were not collected for histological examination.

The cream was diluted to a 1-part cream and 4-parts mineral oil solution for measurement, and demonstrated an absorbance greater than 78 cm^{-1} at 1540-nm when the diffuse reflectance and total transmittance were measured using a single integrating sphere. The undiluted cream was added to evaluate how the minimum visible lesion threshold for a single-pulse laser exposure is changed with the topical agent on the skin. The amount of cream was carefully measured using a needleless syringe, and 0.03 cc were added to each space in the 4 X 7 grid. The topical cream was then carefully smeared with a flat edged tool, and the energy was delivered with randomly-varying levels to each square of one grid. A picture of the cream on skin pre exposure is shown in Figure 2.

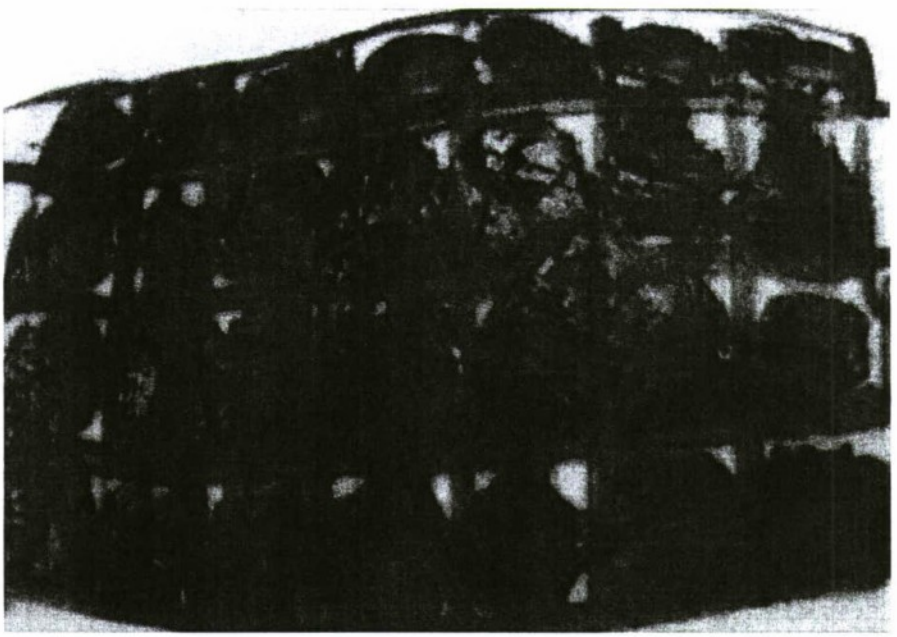

Figure 2: 0.03 cc of cream added to each square on the skin and then spread over each square area before exposures.

Probit[12] analysis was the statistical method used to determine the estimated dose for a 50% probability of producing a lesion, also known as the ED_{50}. Data from each exposure evaluation was input into Probit analysis to calculate the ED_{50} along with their fidcucial limits at the 95% confidence level and slopes.

3. EXPERIMENT RESULTS

Erythema was defined as the minimum damage at 1 and 24-hour inspections. The majority of the lesions were of this sort and appeared anywhere from immediately after exposure up to 24-hours later. Lesions close to the threshold that were either visible or undetectable at 1-hour became the opposite at 24-hours. At the highest energies and pulses ranging from 42-56-ns, immediate whitening of the exposed area surrounded by pink inflammation occurred. The lesions from the higher exposure energies formed scabs on the skin that were present weeks later. The damage can be seen in Figure 3. The ED_{50} at 1 and 24-hours for persistent erythema were found to be 2.99 J/cm² and 3.04 J/cm² respectively. A total of 160 exposures were statististically processed for the ED_{50} at 1 and 24 hour postexposure and are shown in Tables 1 and 2. The Chi-Square distribution ranged from 0.97 to 1.00 at the 1-hour readings and dropped significantly after 24-hour readings because of insufficient distribution for the Probit program. There was consistent damage above a specific exposure level after the 24-hour period. The fiducial limits calculated for all ED_{50} thresholds at both the 1-hour and 24-hour times were within ± 22 percent of the ED_{50} value.

▪ Experimental Setup ▪ Number of exposures ▪ Animal Model	MVL-ED_{50} (Jcm^{-2}) 1-Hour Reading	MVL-ED_{50} (Jcm^{-2}) 24-Hour Reading	Probit Curve Slope = $\delta p/\delta d$ 24-Hours
6.0-mm diameter spot 3 guinea pigs, 6 flanks, 160 exposures	2.99 (2.7 – 3.4)	3.04 (2.8-3.4)	7.4

Table 1. MVL-ED_{50} data for a Q-switched pulse duration of 30-ns at 1054-nm. The Fiducial limits are shown in parenthesis.

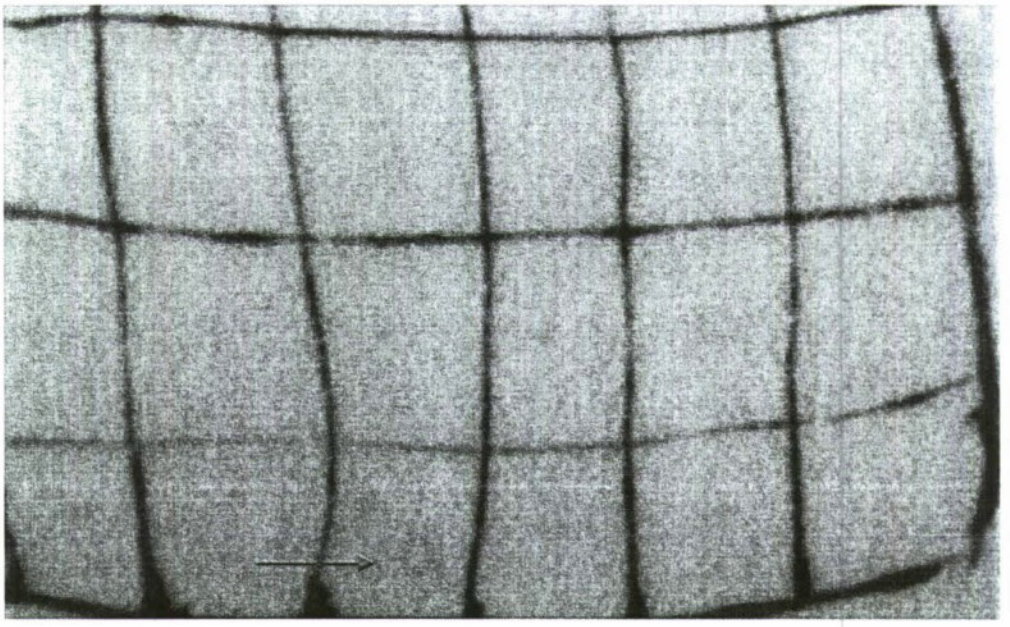

Figure 3: 1540-nm laser exposures after one hour showing erythema at some sites. The arrow points to a lesion.

When laser exposures were made to the skin with the covering agent on it, a very loud popping sound and a bright flash of light occurred at the exposed surface. Only 28 sites were exposed of the 56 originally planned because it was believed extreme thermal damage may have been occurring on the skin surface. The cream was gently removed using baby wipes, and independent evaluations were made of the skin surface for any damage lesions. All three observers agreed that no lesions existed at 1 and 24-hour observations. The cream was applied to a chamois to reproduce the loud pops and flashes observed in the experiment. A photo was taken and can be seen in Figure 4 below.

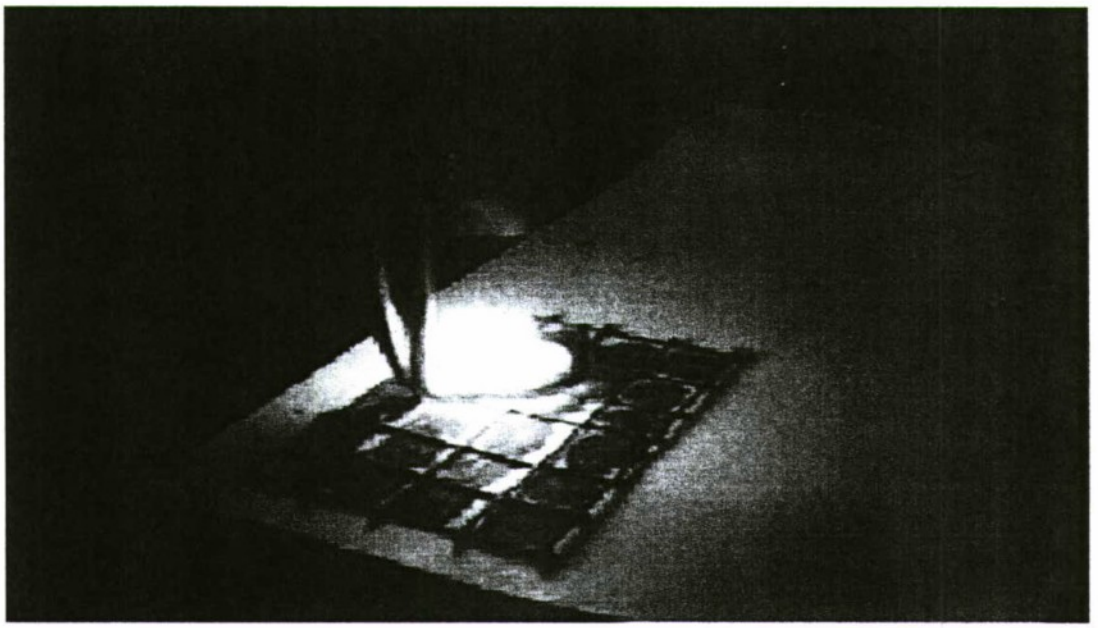

Figure 4: 1540-nm Er:glass laser exposure at 50-ns on cream coated chamois producing a plasma plume. Also seen is the "ring" of the articulating arm for the 1540-nm laser setup.

The ED_{50} for a 50-ns pulse at 1540-nm presented in this paper is discussed along with a comparison to other reported measurements and a mathematical thermal model. Our results compare very closely with that of Lukashev.[1] They used a 1.2-2 month old "Big white" pig (which is believed to be a Yorkshire pig) for exposure of 100-ns at 1540-nm for a 2.5-3.5-mm diameter. The ED_{50} was found to be 3.0 ± 1.1 J/cm² and 3.5 J/cm² for 1 and 24 hour postexposure respectively. They reported no dependency between ED_{50} and laser beam spot size for beam diameters between 2-10 mm.[1] Cain reported an ED_{50} of 6.3 J/cm² and 6.1 J/cm² for a Yucatan minipig at 1-hour and 24- hour postexposure for 31-ns at 1540-nm for a 5-mm spot size.[2] These results are close to the results for the guinea pig. Table 2 shows the comparison.

▪ Experimental Setup ▪ Number of exposures ▪ Animal Model	MVL-ED_{50} (Jcm^{-2}) 1-Hour Reading	MVL-ED_{50} (Jcm^{-2}) 24-Hour Reading
5.0-mm diameter spot 30 ns 216 exposures (Cain) Porcine	6.3	6.1
3.5-mm beam diameter 100 ns 266 exposures (Lukashev) Porcine	3.2	3.1
6.0-mm diameter spot 160 exposures Guinea Pig (*Cavia Porcellus*)	3.0	3.0

Table 2. Comparison of ED_{50} values for Q-switched 1540-nm laser at 42-56-ns pulse durations and 6-mm beam diameter.

The ANSI (Z136.1-2000)[6] allows the maximum permissible exposure (MPE) to 1540-nm for the experiment parameters used in this study to be 1 J/cm². The findings are consistent with the standard and are above the MPE. Other studies for damage evaluation of lasers down to the cellular level using guinea pigs have been done at 355, 532,

694, and 1064-nm.[13-15] The damage evaluation procedures described in the papers are different than the procedure described in this paper, but the responses to laser exposures are similar.

Loud "pops" and mini flashes of light, especially at the higher energies, occurred during the ED_{50} experiments. For each exposure, the sounds and light flashes that were observed were noted for each respective exposure parameter. The ED_{50} for 1 and 24 hours did not change much, and the data suggest that some of the lesions close to the threshold that were either visible or undetectable at 1 hour became the opposite at 24 hours. Lesions produced by the highest energies remained for weeks after exposure. It was suspected that some of the damage may have been attributed more to photoacoustic effects than thermal effects because of the "pops" and flashes of light. Part of the discussion will include an explanation of the thermal damage model that was used to help determine the damage mechanism.

4.1Thermal Model

The model employed to estimate temperature effects along with the evaluation of thresholds for tissue damage was a validated legacy model, commonly referred to as the Takata skin model.[16] The Takata model is a time-dependent finite-difference method solution of the two-dimensional (cylindrical symmetry) bio-heat equation. Features of the model include a user-configurable multi-layer tissue model. Thermo-mechanical as well as optical properties of the tissue layers are user inputs. Laser parameters are also user configurable, and sources may include a multitude of single-wavelength sources. The spectrum for a broadband source, that is not specified as an input, can be given as a computed black body distribution corresponding to a known color temperature. Spatial profiles may be flat top, Gaussian, annular, or user defined. Single or multiple pulses or the temporal behavior defined by the user for each point in time may be selected for the temporal profile. The geometric model of beam irradiance is employed along with linear absorption of the tissue to estimate energy deposition rates at various points within the computational grid. Boundary conditions include constant flux surface convection at the tissue-air interface. Thermal effects of variable blood flow with tissue depth are evaluated. Phase change of the water content of the tissue, as well as increased absorption for charred tissues is evaluated through empirical relationships. The model does not incorporate tissue optical scattering effects.

For all runs, the model determines an adaptive time step which captures rapid changes in temperature at high time resolution. The adaptive time step also provides for large time steps in regions for which there is a "steady state" or little change in the temperature distribution. The minimum and maximum coordinates for the grid along with the grid point spacing is defined by the user. The Takata model execution results in a time-temperature history at each point within the computational grid. Each point within the grid is evaluated for potential damage over the duration of the simulation through an Arrhenius damage integral, shown in equation (1), with temperature dependent damage rate coefficients. The damage integral is normalized against experimental data for first-degree through third-degree burns. Henriques setup the rate equation such that a first-degree burn is represented by a damage integral value of 0.1 and second-degree burn is represented by a value of 1.0 (14)

$$\Omega(r,z) = A\int_0^t \exp(-E/RT)dt \qquad (1)$$

where A is the pre-exponential factor (s^{-1}), E is activation energy, R=2.0 cal/(MK) is the universal gas constant, T is the absolute temperature of a given coordinate in time, T(z,r,t), and t is the time at final recovery of temperature after exposure.

The variable A is a normalized constant and E is the activation energy for a reactive process leading to damage. The values for each are respectively given as:

$$A = 3.1 \times 10^{98}\ (1/s) \qquad 317 < T < 323\ K$$
$$E = 628{,}000\ (cal/M).$$

The values are taken from the work of Henriques for controlled temperature exposure on skin.[17] Critical parameters within the thermal model are the absorption-coefficients as a function of wavelength. There are limited data for absorption-coefficients of skin in the infrared range, and the greatest sources of uncertainty are the absorption-

coefficient parameters. The absorption-coefficient values for 1500-1550 range from ~ 1.5 cm^{-1} to 15 cm^{-1} for human and Yucatan mini-pig skin.[18]

4.1.1 Model Results

An absorption-coefficient of 8 cm^{-1} for porcine at 1500-nm provided by Du was used for the model.[19] The model predicted that the ED_{50} pulse power produced no damage and increased the surface temperature to 4.5 $C°$. Lukashev also used a model to predict temperature increases and received a temperature of 8.0 $C°$ at 1540-nm for nanosecond pulses.[1] No damage was achieved in the model until the pulse power was increased to three times larger than the ED_{50} value of power. At this pulse energy, the temperature rise to create a second degree burn was 22.3C°. An absorption coefficient of 1.5 cm^{-1} for human skin measured *in-vitro* was also used, and a similar prediction was given.[18] A second layer was added to emulate the epidermal and dermal layers of skin with respective absorption-coefficients (obtained from Cain) without any success of lowering the pulse power needed to produce damage.[20] The epidermal and dermal layers are considered homogenous and of "infinite thickness", providing a solution for axial boundaries at which little energy is conducted within the simulation time. The coefficient values and predicted temperature rises are shown in Table 3.

Skin Type	Wavelength (nm)	Epidermis μ_a (1/cm)	Dermis μ_a (1/cm)	Predicted Temp Rise (C°)
Yucatan Mini Pig	1500	8	--	4.5
Yucatan Mini Pig	1540	6	5.42	3.5
Human (*in-vitro*)	1550	1.5	--	1.1

Table 3: Optical properties of skin at 1540-nm and model predictions for temperature rise on skin surface. The dashes under the dermal column denote one layer used in the model.

It has been determined that a single pulse of the laser energy and duration was within thermal and stress confinement and that the pulse duration was much shorter than relaxation time of the tissue.[21] This suggests that the damage mechanism is not entirely attributed to photothermal interactions as much as thermomechanical interaction. To see if thermomechanical interactions were at play, laser induced breakdown thresholds (LIB) were looked at. The calculated incident irradiance of 9.7 x 10^7 W/cm² was very close to the plasma threshold of 10^8 W/cm² in the presence of local impurities such as dead skin.[21] This suggests that plasma formation was likely and would help to explain the "pops" and flashes of light seen at exposure sites. To find out if the threshold for LIB had been exceeded, the electric field intensity is given by equation 2:

$$E = \left(\frac{2\Phi}{cn\varepsilon_0} \right)^{1/2} \qquad (2)$$

where Φ is the power density, ε_0 is the permittivity of free space, c is velocity of light, and n is the refractive index.[22] The index of refraction for hydrated stratum corneum is 1.41.[23] The calculated electric field intensity was 2.28 x 10^7 V/m and was sufficient to cause LIB.[22]

It is believed that plasma had been generated via an adiabatic process and that it created a shield by absorbing the incident radiant energy and prevented some of the energy from being deposited in the skin. Any damage that occurred

had resulted from acoustic and shock waves from the plasma as well as the high plasma temperature, which can be as greater than 10,000 K.[24]

When the skin was coated with the cream, it was noted that a loud popping noise and an intense flame plume approximately ~ 5-8 cm in height occurred when the laser exposures were delivered. After exposures, the paint was gently wiped off using baby wipes. Photos were taken of the skin after the paint was removed, and exposure sites were inspected by three evaluators for any lesions. All three evaluators agreed no lesions existed at 1 and 24 hour inspections, even at the highest energy of 5.62 J/cm². The conclusion was that because paint had been highly absorbing in the near infrared, it caused ionization and induced an electron avalanche via an adiabatic process. Because the absorption coefficient of the plasma is much greater than the covering agent, nearly all of the incident energy had been absorbed by the plasma and prevented any appreciable penetration into the skin, thus the effect was plasma shielding. The expectation had been that lesions would still exist because of acoustic effects produced by the intense plasma. Since no lesions existed, it was questioned whether thermomechanical effects had helped to generate the lesions on the skin for the ED_{50} determination or if it should be attributed more to the thermal effects of the plasma or other unaccounted phenomena. More studies should be done to help clarify the damage mechanism.

5. CONCLUSION

In this study we experimentally determined the reaction of guinea pig skin (*in vivo*) to 1540-nm radiation of an Er:glass laser for 30-ns pulses at a spot size of 6-mm. The ED_{50} value was found to be 3.0 J/cm² and 3.04 J/cm² for 1 and 24 hours respectively and was above the MPE of 1 J/cm² as given by the ANSI (Z136.1-2000)[6] . A cream added to the skin and exposed at the same energies used to determine the ED_{50} prevented all damage at those energies because of plasma shielding thus increasing the ED_{50} for 1540-nm using 30-40 ns pulses. When we compare our results to similar studies using porcine, the ED_{50} values are close suggesting that guinea pigs may be a suitable model for laser exposure studies. Thermal modeling using the Takata skin model of the experiment parameters at the ED_{50} threshold revealed that the damage induced on the skin for the experiments did not match the predicted damage and temperature rise on the skin surface. The predicted temperatures were too low to cause the observed ED_{50}s and may be attributed to unaccounted for heat or photoacoustic and shock waves from plasma formation on the skin. More research is needed to clarify the damage mechanism at short pulse and peak irradiances of *in vivo* subjects.

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