# Light attenuation in rat skin following low level laser therapy on burn healing process

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## ABSTRACT

Low-level laser therapy (LLLT) is commonly used to accelerate wound healing. Besides, the technique of imaging the light distribution inside biological tissues permits us to understand several effects about light-tissue interaction. The purpose of this study was to determine the relative attenuation coefficient of the light intensity in healthy and burned skin rats during cutaneous repair following LLLT or not. Two burns about 6mm in diameter were cryogenerated using liquid $N_2$ on the back of 15 rats. Lesion L was irradiated by a He-Ne laser ($\lambda$= 632.8nm) and fluence 1.0J/cm$^2$; Lesion C was control and received sham irradiation. A healthy skin area (H) was also analyzed. The lesions were irradiated at days 3, 7, 10 and 14 post-burning. The animals were euthanized at days 3, 10 and 31 and skin samples were carefully removed and placed between two microscope slides, spaced by z= 1mm. A laser beam irradiated the sandwiched tissue from epidermis to dermis. A CCD camera was placed orthogonal to the beam path and it photographed the distribution of the scattered light. The light decay occurred according to the Beer's Law. Significance was accepted at $p < 0.01$ by using t-Student test. Our results show that the light decay along any direction was close to an exponential. Burned skin samples presented decay significantly faster than healthy skin samples. Besides, attenuation coefficient changed during burning healing comparing treated and control lesions. These findings suggest that the relative attenuation coefficient is a suitable parameter to optimize LLLT during wound healing.

**Key words:** absorption; attenuation coefficient; laser therapy; polarized light; red laser; scattering; skin repair

## 1. INTRODUCTION

Low power lasers were introduced as therapeutic modality in 60's due to the required low energy densities and to the high penetration of red and infrared lasers in biological tissue. Since then, many studies on cells have been reported in the literature[1-4]. However, neither the correlation between exposure and response nor the basic mechanism responsible for the observed effects on therapeutic fluences is completely understood.

The positive clinical results of laser therapy are attributed to a sequence of cellular and molecular events. The determinant factors in photochemical, photophysical or photobiological response are the wavelength, energy density, power density, chromophores concentration and the optical properties of the treated tissue (reflection, transmission, absorption, scattering, anisotropy and birefringence), as well as its physiologic state. In the case of low level laser therapy (LLLT), respiratory chain's components are the first absorbers of light quantum, signaling a cascade of events, which leads to the final effect, for example, to wound healing[5].

Although several studies have been published, frequently discrepancies are found and only a few of them have presented some scientific argument[1-4]. In addition, the optical properties of pathological tissue have been more extensively studied by the scientific community [6-8].

This study was carried out to determine the relative attenuation coefficient of the light intensity in normal and burned skin rat after treatment with LLLT. The technique of imaging the light distribution allows us to obtain a relative attenuation coefficient for the light intensity that, once known, allows the understanding of several effects of light tissue interaction and consequently, the possibility of optimization of light parameters for LLLT.

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## 2. MATERIALS AND METHODS

Two round burns of about 6mm in diameter were cryogenerated at the end of the spinal column of male adults Wistar rats using a cylindrical brass rod cooled at 77K. The brass rod was kept for five seconds on rats, twice a day, with an interval of five minutes between them, during three days.

The source of laser light was a He-Ne laser at $\lambda$= 632.8nm, with 10mW of output power (Uniphase, USA) arranged in a convenient setup. A lens system and an optical filter were used to ensure a uniform exposure at the wound position, obtaining an expanded beam with 6mW at 1 $cm^2$. A Glan-Thompson prism was inserted in the beam path to obtain a linearly polarized beam. The polarizer was held on a rotator disk, which managed the parallel or antiparallel alignment of the linear polarization of the incident laser beam to the spinal column of the rats.

### 2.1. Treatment

After the last cooled application (third day), lesion "C" was not irradiated (control), and lesion "L" was irradiated using laser polarization aligned in parallel with the rat's spinal direction. During the experiment, the rats were singly housed in solid-bottomed cage in a 12h light/12 h darkness schedule at 22ºC, with unlimited access to food and water. National and international principles of laboratory animal care were followed. A healthy skin area labeled "H" was also analyzed. The energy density was 1J/$cm^2$ per irradiation, corresponding to an exposure time of approximately 3 minutes. The animals were irradiated on the 3rd, 7th, 10th and 14th day and killed on the 3rd, 10th and 31st day post-wounding.

### 2.2. Digital Imaging

After sacrifice, the areas from healthy and burned skin were carefully collected and placed between two microscope slide mounts, spaced by 1mm. A thin anti-reflector film was previously deposited in the microscope slides. A He-Ne laser beam at $\lambda$= 632.8nm with 10mW of output power (Uniphase, USA) and 0.1mm of spot size irradiated the sandwiched tissue, from the epidermis to dermis (figure 1).

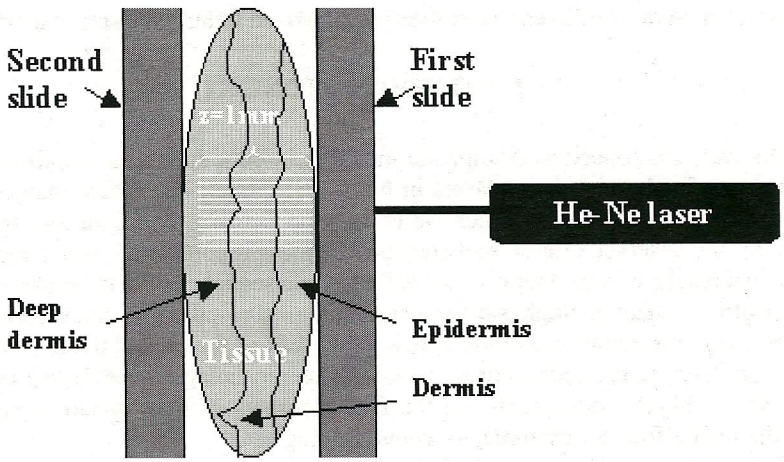


**Figure 1: Measurement scheme of the attenuation coefficient.**

A CCD camera (MVC-FD73 Digital Mavica, Sony, Japan) was placed orthogonal to the beam path and photographed the intensity distribution of the scattered light. Two lenses were placed between the objective and the microscope slides to enhance the camera's magnification, resulting 10x increase (figure 2). The images were recorded as bitmap files with 8-bit resolution, yielding a 256 gray levels image. The camera captures the scattered light, which is proportional to the local light intensity, and the photograph corresponds to a two-dimensional light intensity distribution model. Along the laser propagation direction, it was possible to extract the intensity variation.

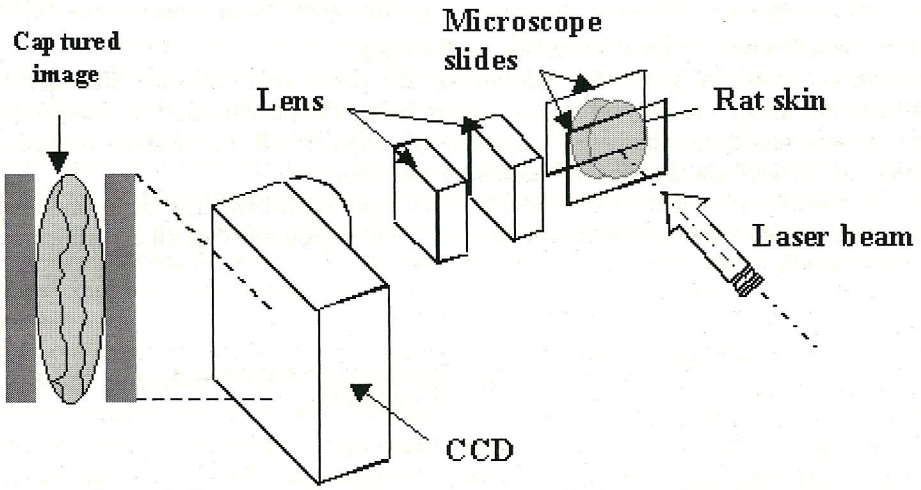

Figure 2: Experimental setup to capture scattered light from the skin sample.

The light intensity data I(z) was recorded in ASCII format and analyzed by the public domain software called ImageJ® (National Institutes of Health, USA). From this data, it was plotted the logarithm of $I/I_0$ versus the distance z. $I_0$ is the maximum intensity of laser measured when it reached the first pixel of tissue. The exponential decay was fitted to the equation

$$I(z) = I_0 e^{-\mu_r z} \qquad \text{(Eq. 1)}$$

where $\mu_r$ ($mm^{-1}$) is the relative light attenuation coefficient.

The measurements were achieved approximately 90 minutes after the animals' death and the samples were kept immersed in saline solution during this period. The laser propagation direction was chosen for analysis. Ten measurements with z= 1mm were made in each skin sample. Mean values and standard deviations of the relative attenuation coefficient in skin sections were computed and analysed by using the t-Student test. Significance was accepted at $p < 0.01$.

## 3. RESULTS AND DISCUSSION

Biological systems are complex and comprise a wide variety of cellular and tissue fluids, each one with different absorption characteristics. The elements of the tissue that exhibit a high absorption coefficient of a particular wavelength or a region of the spectrum are called chromophores. Besides water, chromophores such as melanin, hemoglobin and proteins exert significant influence on the interaction of radiation and tissue.

One example of such a medium would be skin. Within the medium, the particles either travel uninterrupted or collide with the nuclei that constitute the medium. A collision results in either an absorption of the particle into the nucleus or a scattering to a new direction. The sum of the absorption and the scattering coefficients is designated as the total attenuation coefficient ($\mu_t$) such that[9]:

$$\mu_t = \mu_a + \mu_s \qquad \text{(Eq. 2)}$$

The total attenuation coefficient thus represents the probability of photon interaction per unit of path length ($mm^{-1}$).

In this way, the light entering into the tissue is subject to scattering and absorption phenomena. A collimated laser beam normal to the surface has a small portion of the light reflected at the surface and the remaining light is attenuated into the tissue by absorption and scattering. Unscattered light is attenuated exponentially following Beer's Law (Eq. 1)[10].

When the absorption is the predominant effect in a medium, the absorption coefficient $\mu_a$ can be determined through the curve of $\ln[I(z)/I_0]$ versus optical path of the sample. But when absorption and scattering are

important effects to be considered in the same medium, like for biological tissue, the curve of $\ln[I(z)/I_0]$ versus the optical path of the sample gives the total attenuation coefficient $\mu_t$.

In this context, the effects of scattering, transmission and penetration depth are highlighted in this article using the Beer's Law, but as we are only interested in describing the phenomenon of attenuation compared to healthy skin, skin lesions treated and untreated, they were determined only the relative attenuation coefficients between the samples, not the total attenuation coefficients for each sample.

The figure 3 displays the typical exponential decay of the light inside biological tissue and figure 4 shows the mean values of the relative attenuation coefficient for laser and control groups during all experimental period.

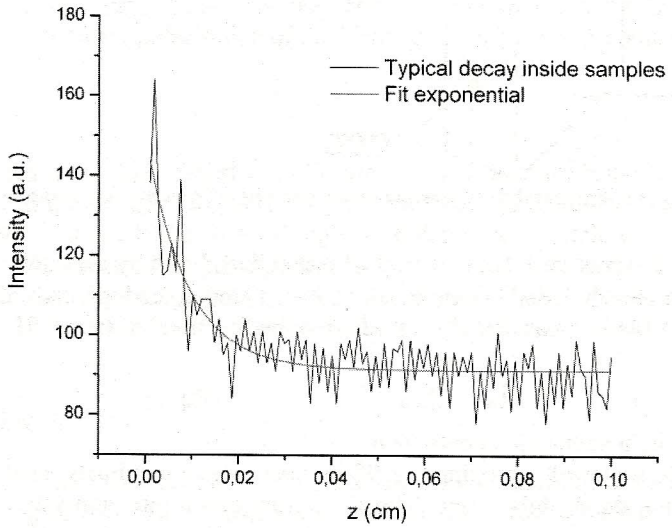


Figure 3: Exponential decay of the light inside biological tissue.

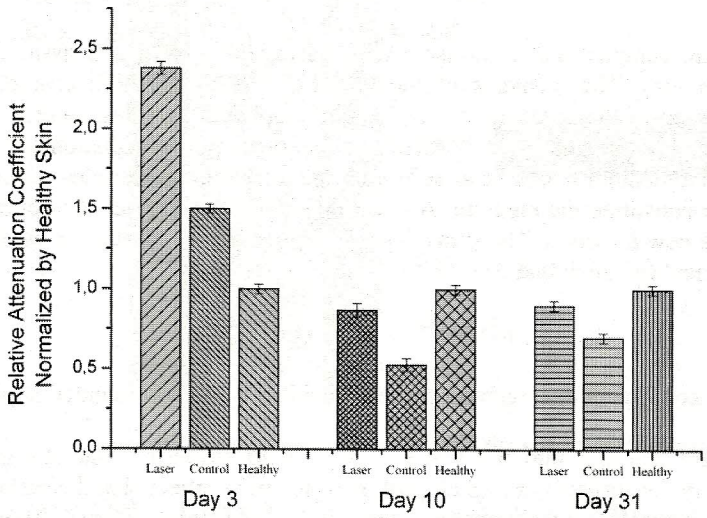


Figure 4: Relative attenuation coefficient during experimental period.

The light attenuation intensity was significantly enhanced in burned skin samples compared to healthy skin on the 3rd day after the wound creation. This result agrees with Papazoglou *et. al.* which showed that there was an

increase in the light attenuation due absorption and scattering inside wounds compared with the nonwounded sites. The changes were correlated with the healing stage of the wound. The data obtained were supported by immunohistochemical analysis of wound tissue[11]. Melo *et al.* also concluded that healthy liver presents about four times higher penetration depth when compared to cirrhotic liver[12]. In fact, the healthy skin penetration depth was two times higher when compared to burned skin in our work. This finding could be explained by the variety of inflammatory cells and cell debris present in the dermis subjacent to the damaged epidermis. Neutrophils are the predominating cell type in the superficial dermis whereas monocytes, macrophages, and giant cells are present in the deep dermis. The vascularization is also affected and many small blood vessels are dilated, most of them filled with blood cells[13]. Indeed, according to Latha *et. al.*, the burn granulation tissue has unique characteristics and changes in very aspects when compared with normal skin[14]. Therefore, it is possible that cells act like light attenuators in damaged specimens.

In addition, our results show that irradiated skin present statistically significant differences when compared to control lesion without irradiation on day 3 and 10 post-wounding. Studies show that laser radiation can accelerate the wound healing process acting in the inflammatory stage, reducing the duration of inflammation and, consequently, precipitating the proliferative repair stage when the granulation tissue is produced[15]. This may be the reason for the higher attenuation coefficient presented by the irradiated group compared to the control group.

When the relative attenuation coefficient was measured on the 31$^{st}$ day, no significant differences were observed between irradiated and healthy skin; however significant differences were observed between lased and control lesions. These results indicate that the burned skin that received laser treatment is probably healed because it is optically similar to healthy skin.

It should be emphasized that in this study, linear polarized light was used to irradiate the tissue during the first 14 days of healing process. The extracellular matrix (ECM) is the scaffold of the skin that supports cells in either unwounded or wounded states. The ECM is dynamic during healing process; it is constantly undergoing remodeling[16]. Thus, one can expect that light propagation into a live tissue during the healing process will change due to the dynamic changes inside the tissue. After the initial inflammatory phase, the early wound matrix is gradually replaced by granulation tissue. Besides, the degree of linear polarization was investigated in healthy and burned skin[17]. The results indicated that linearly polarized light could survive in the superficial layers of skin; the preservation was even higher in burned skin. Therefore, the high cellular content and the increased cellular activity on the granulation tissue could contribute to the optical changes during healing process.

## 4. CONCLUSION

The light intensity in burned skin is significantly more attenuated that in healthy skin. Also, the attenuation coefficient changes during the healing process of burns when treated with linearly polarized He-Ne laser or when no treated. The differences found on optical properties should be taken in account in the optimization of the light parameters used in LLLT.

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