

# ATTENUATION AND PENETRATION OF VISIBLE 632.8nm AND INVISIBLE INFRA-RED 904nm LIGHT IN SOFT TISSUES

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**We studied the depth of penetration and the magnitude of attenuation of 632.8nm and 904nm light in skin, muscle, tendon, and cartilagenous tissues of live anaesthetized rabbits. Tissue specimens were dissected, prepared, and their thicknesses measured. Then, each wavelength of light was applied. Simultaneously, a power meter was used to detect and measure the amount of light transmitted through each tissue. All measurements were made in the dark to minimize interference from extraneous light sources. To determine the influence of pulse rate on beam attenuation, the 632.8nm light was used at two predetermined settings of the machine; continuous mode and 100 pulses per second (pps), at an on:off ratio of 1:1. Similarly, the 904nm infra-red light was applied using two predetermined machine settings: 292 pps and 2,336 pps. Multiple regression analysis of the data obtained showed significant positive correlations between tissue thickness and light attenuation ( $p < .001$ ). Student's t-tests revealed that beam attenuation was significantly affected by wavelength. Collectively, our findings warrant the conclusions that (1) The calf muscles of the New Zealand white rabbit attenuates light in direct proportion to its thickness. In this tissue, light attenuation is not significantly affected by the overlying skin, a finding which may be applicable to other muscles. (2) The depth of penetration of a 632.8nm and 904nm light is not related to the average power of the light source. The depth of penetration is the same notwithstanding the average power of the light source. (3) Compared to the 904nm wavelength, 632.8nm light is attenuated more by muscle tissue, suggesting that it is absorbed more readily than the 904nm wavelength or conversely that the 904nm wavelength penetrates more. Thus, wavelength plays a critical role in the depth of penetration of light.**

*key words: Laser Therapy, Light Attenuation, Light Asorption.*

## Introduction

As early as 1968, Mester and his associates [1-3] demonstrated that, at low power, red light promotes tissue repair. In the intervening period since then, the therapeutic value of phototherapy has been argued and debated by many, with several studies supporting the original hypothesis that they promote tissue repair processes in experimental animals [4-32] and human wounds and ulcers [8, 33-38], and others [39-46], suggesting the contrary.

A review of well controlled *in vitro* and *in vivo* laboratory experiments reveals a trend that suggests that low intensity lasers enhance wound healing by promoting cell proliferation [8, 28-30, 41-44], accelerating the formation of granulation tissue, promoting collagen synthesis [3-13, 47-61], fostering the formation of type I and type III procollagen specific pools of mRNA [62], increasing ATP synthesis within the mitochondria, activating lymphocytes, and increasing their abilities to bind pathogens [10, 52]. The trend is not as clear when clinical reports on tissue repair are examined. Rather, sufficient differences of opin-

ion seem evident between studies showing beneficial effects and those reporting no effects whatsoever [10, 33-46].

Given the multitude of treatment parameters used in these studies, i.e., wavelength, pulsed versus continuous wave light, energy fluence, power density, exposure time, frequency and total duration of treatment, it is not surprising that results differ from one study to the next. A good understanding of the nature of light-tissue interaction could simplify and ease the choice of treatment parameters. In this regard, Welch et al [63], have provided a mathematical model that offers a fairly simple method of determining light distribution in tissues. However, their model seems significantly limited, as it applies to CO<sub>2</sub>, Excimer and Er:YAG lasers, situations involving high scattering of light and tissue depths that are less than 40 mm. Beer's Law which indicates that the incident light is exponentially attenuated as it passes through tissue, and Kubelka-Munk theory, a two-flux model used for uniformly diffused incident light, can be used to compute quantitative laser

absorption in blood and skin. However, neither Beer's Law nor Kubelka-Munk theory accounts for the scattering of light in tissues. Other models, e.g., the "Diffusion Approximation Model", assumes that light is scattered almost uniformly after encountering numerous scattering events, but this is not always the case.

We have used the transected rabbit Achilles tendon and rat skin lesions as models for determining the biomechanical, ultrastructural and biochemical effects of light on tissue repair. Because none of the models described above can be used to account for light-tissue interaction in our experimental model, we determined the depth of penetration and the extent of attenuation of light by the variety of tissues associated with our tendon repair model. Our specific aims were: (1) to determine the depth of penetration of 632.8nm (Ne:He) and 904nm (Ga:As) lasers through specific tissue types and thicknesses, (2) to compare the attenuating effects of skin, muscle, tendon, and cartilage on low energy light, (3) to determine the effects of tissue type and thickness on beam attenuation. Attenuation is operationally defined as the amount of light that is prevented from passing through tissue, primarily due to absorption, reflection and refraction of the light beam.

## Methods

### Animals

Four 4-6 month-old New Zealand white rabbits, housed one per standard 30.5 x 71 x 51 cm rabbit cage in an environment maintained at 22 + 1°C and fed rabbit chow and water ad libitum, were used for this study.

### Animal Preparation

Each rabbit was weighed, then anesthetized with a mixture of 180 mg of xylazene, 900 mg of ketamine and 30 mg of acepromazine by intramuscular injection into the quadriceps muscle of 1 m/1.5 kg body weight. Following satisfactory anesthesia, the animal was prepared for surgery and a longitudinal incision was made, approximately 1 cm lateral to the visible outline of the calcaneal tendon. Blunt dissection was used to separate the tendon and the triceps surae muscle group from adjoining tissue. Thereafter, the tendon was severed sharply and transversely just proximal to its calcaneal attachment, but left connected to its muscle.

### Light Measurement.

A He:Ne laser unit (Dynatronics Inc., Salt Lake City, UT) of 632.8 nm wavelength and 11 mW maximum power, and a Ga:As laser unit (Respond Systems Inc., Madison, CT) of 940 nm wavelength and 7 mW maximum power, were used throughout the study. A customized power meter

(United Detector Technology, model S351L, Hawthorne, CA), calibrated to measure light intensity (power) within the range of 450nm to 1000 nm, was used to measure the amount of light passing through each selected tissue. The photoreceptor of the power meter was secured in a stand as shown in figure 1. The laser applicator was then placed perpendicular to the photoreceptor of the power meter with the target tissue sandwiched between the photoreceptor and the applicator (Figure 2). With the target tissue in place, the power of each laser unit was varied by altering the pulse rate. Pulse rates of 2,336 PPS and 292 PPS were used for the 904nm light, yielding average power outputs of 6.5 mW and 0.8 mW respectively. Zero PPS (i.e. continuous beam) and 100 PPS were used for the He:Ne laser. The target tissues irradiated included the ear, the calcaneal tendon, the triceps surae muscle group and the skin overlying the calf muscles.

### Data Analysis.

Multiple regression analysis was used to determine the effect of tissue thickness on the amount of light attenuated by the tissue under the experimental conditions. Student's t-test was then used to determine the effect of these variables on light attenuation.

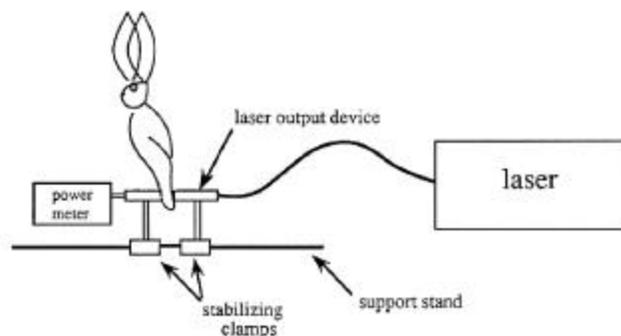


Figure 1. Illustration of our experimental setup.

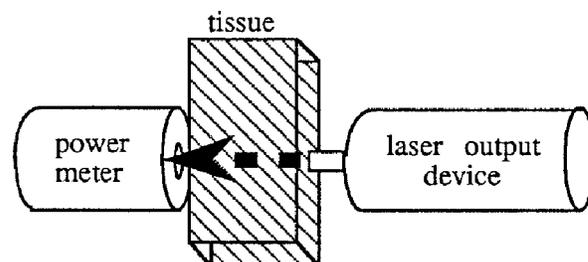


Figure 2. Illustration showing a close up view of laser application, tissue specimen, and beam measurement with the power meter.

## Results

In each of the tissues tested, there was a significant posi-

tive correlation between tissue thickness and beam attenuation (Tables 1 & 2). At both wavelengths beam attenuation per millimeter of tissue was greater for muscle and cartilage compared to all other tissues, i.e., skin and muscle combined, tendon, and skin and tendon combined (Figs. 3 & 4), suggesting that these wavelengths, i.e., 632.8 nm and 904 nm are absorbed more by muscle and cartilage than any of the other tissues tested. Furthermore, attenuation per millimeter of tissue was significantly greater for cartilage(the ear) than tendon, two similarly dense connective tissues; (cartilage = 2.44 and 2.27 mW/mm and tendon = 1.22 and 1.48 mW/mm, for 904 nm and 632.8 nm wavelengths respectively). The low amount of beam attenuation by tendon relative to its analogue, cartilage, and the similarity in the magnitude of beam attenuation of both wavelengths by two morphologically dissimilar tissues, muscle and cartilage, suggest that tissue density is not a primary determinant of beam attenuation.

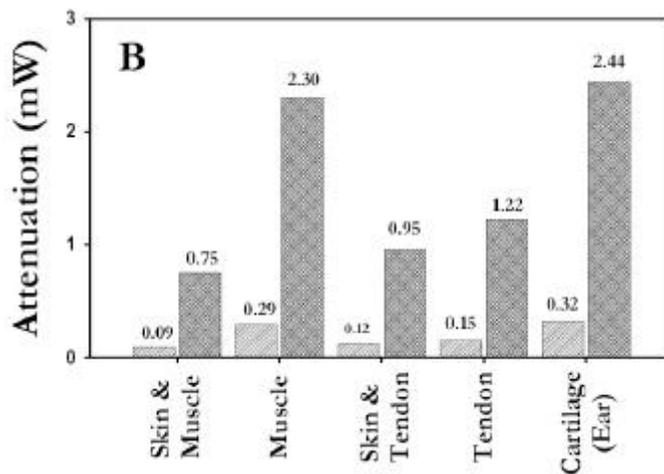
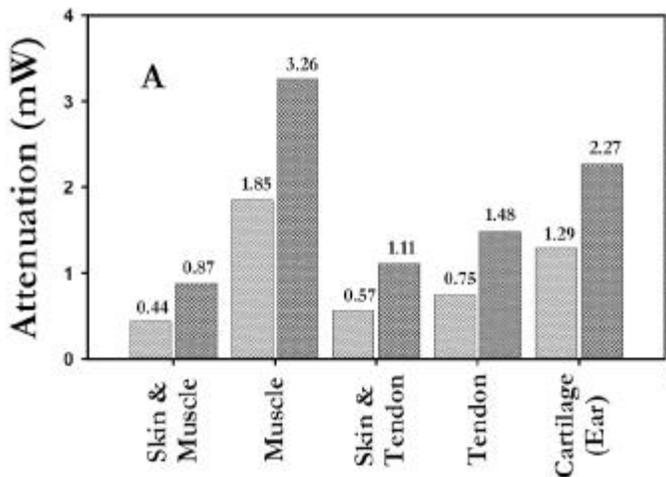


Fig 3: Attenuation of light by various tissues, (A) Attenuation of 632.8 nm red light per millimeter of tissue. Lighter bar=pulsed beam; Dark bar=continuous beam. (B) Attenuation of invisible infra-red 904 nm light per millimeter of tissue (Light bar=292 pps; Dark bar=2,336 pps).

Furthermore, attenuation of the invisible infra-red 904 nm wavelength was significantly higher in all the tissues tested and in all modes of application, i.e., pulsed or continuous, when compared with the 632.8 nm beam, except in muscle tissue where the 632.8 nm wavelength was more attenuated (Fig. 4;  $p < .05$  in each case). Although the absolute amount of beam attenuation was higher when both wavelengths were applied at each machine's optimal intensity compared to their respective pulsed intensities, as shown in figure 3B, the relative amount of beam attenuation was essentially the same for each tissue in both modes of application. Thus, the attenuation of either wavelength of light was not affected by the two modes of application, pulsed or continuous.

**Table 1: 632.8nm Wavelength**

Tissue	r	R <sup>2</sup>	p	N
Muscle	.591	.350	<.0001	36
Skin & Muscle	.887	.786	<.0001	9
Muscle	.725	.526	<.0001	36
Tendon	.690	.476	<.0001	9

Table 1. Correlation of 632.8 nm beam attenuation and tissue thickness for the various tissues tested. (r = Pearson's correlation coefficient; R<sup>2</sup> = coefficient of determination, i.e., the amount of variance in attenuation predicted by knowing the average power of the light source, and N = number of specimens tested).

**Table 2: 904nm Wavelength**

Tissue	r	R <sup>2</sup>	p	N
Muscle	.801	.641	<.0001	36
Skin & Muscle	.904	.818	<.0001	9
Muscle	.876	.767	<.0001	24
Tendon	.976	.953	<.0001	9

Table 2. Correlation of 904 nm beam attenuation and tissue thickness for the various tissues tested. (r = Pearson's correlation coefficient; R<sup>2</sup> = coefficient of determination, i.e., the amount of variance in attenuation predicted by knowing the average power of the light source, and N = number of specimens tested).

The gastrocsoleus muscle was the only tissue that we were able to vary the thickness appreciably enough to derive predictive equations for beam attenuation at various thicknesses. For this tissue, it was possible to predict the attenuation of 632.8 nm and 904 nm light at increasing thicknesses as indicated in the following equations:

$$y = 4.38 \times 10^{-2} x + 6.59 \text{ (for 904nm light pulsed at 2,336 pps)}$$

$$y = 6.17 \times 10^{-3} x + 8.23 \times 10^{-1} \text{ (for 904nm pulsed at 292 pps)}$$

$$y = 3.17 \times 10^{-2} x + 7.67 \text{ (for 632.8 nm continuous wave light)}$$

$$y = 2.06 \times 10^{-2} x + 3.91 \text{ (for 632.8 nm light pulsed at 100 pps)}$$

(Where  $y$  = the amount of attenuation and  $x$  = the average power of the laser device).

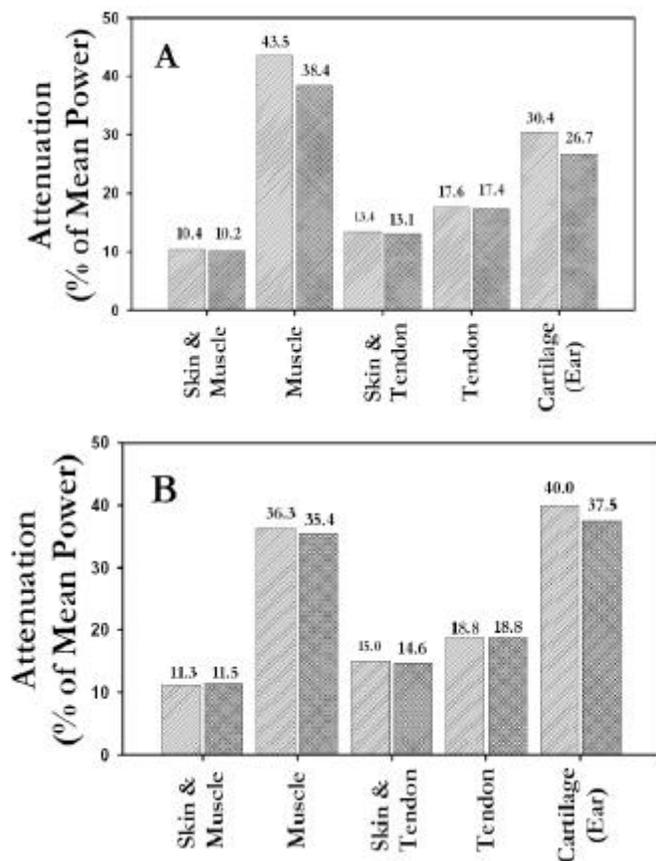


Fig. 4: Attenuation of light by various tissues as a percentage of the average power of the laser source. (A) Percent attenuation of 632.8 nm light; (Light bar=pulsed; dark bar=continuous). (B) Percent attenuation of 904 nm light. (Light bar=292 pps; dark bar=2,336 pps)

## Discussion

Attenuation of light can be accounted for by three major factors, light absorption, light reflection, and light refraction. In soft tissue absorption accounts for a significant amount of beam attenuation, except in situations involving an unusually high amount of beam reflection or when light

is applied to the tissue in the non-contact mode. Our findings indicate that the 632.8nm and the 904nm wavelengths of light are capable of penetrating skin, muscle, tendon, cartilage, and also a combination of skin and muscle, and skin and tendon. And that each tissue type attenuates both wavelengths of light. Given the optical characteristics of these tissues, and the fact that the applicator of each light source was used in direct contact with each tissue, it seems safe to assume that these two wavelengths of light are significantly absorbed by all the tissues tested, in particular, muscle and cartilage, which attenuated more than 35% and 26% of either wavelength of light per millimeter of tissue respectively.

Attenuation of light per millimeter of tissue differed significantly from tissue to tissue. Cartilage and muscle attenuated light more than any of the other tissues tested. And although cartilage and tendons are more akin to one another, both being dense connective tissues, light attenuation by both tissues was significantly different. In contrast, muscle and cartilage, two morphologically dissimilar tissues, attenuated 904 nm light to the same extent, buttressing the notion that factors other than tissue thickness account for light attenuation by soft tissues.

Compared to the infra-red 904 nm wavelength, red 632.8 nm light was attenuated more by muscle than any of the other tissues. Thus, the data clearly indicate that 904 nm light penetrates muscle tissue more than 632.8 nm light. This finding is consistent with that of Al-Watban and Andres (reported in this issue of the journal). However, whether or not the observed differences in absorption and penetration have any effects on tissue healing is beyond the scope of this study.

Skin was found to have no significant effect on light attenuation through muscle (Figure 3). The ability of low intensity light to penetrate skin with minimal beam scattering (reflection and refraction) should permit the use of therapeutic light to effectively treat sub-dermal lesions.

Attenuation, in the context of this paper, represents the amount by which light energy is diminished as it passes through the tissue. Thus, it represents the amount of light absorbed, reflected, or refracted such that the beam could not continue in the direction of application. Given this definition, it will be erroneous to assume that the attenuation figures obtained in this study represents the amount of light absorbed by the tissues. Actual determination of beam scattering and measurement of the exact amount of light absorbed by the tissue are subjects of future studies. However, the relative amounts of light attenuated by the various tissues tested could reflect the relative amounts of light absorbed by the tissues.

## Conclusion

Our findings mandate the following conclusions: (1) The calf muscles of the New Zealand white rabbit attenuates light in direct proportion to its thickness. In this tissue, light attenuation is not significantly affected by the overlying skin, a finding which may be applicable to other muscles. (2) The depth of penetration of a 632.8nm and 904nm light is not related to the average power of the light source. For each of the wavelengths tested, the depth of penetration is the same notwithstanding the average power of the light source. (3) Compared to the 904nm wavelength, 632.8nm light is attenuated more by muscle tissue, suggesting that it is absorbed more readily than the 904nm wavelength or conversely that the 904nm wavelength penetrates more. Thus, wavelength plays a critical role in the depth of penetration of light.

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