Attenuation coefficient of the light in skin of BALB/c and C57BL/6 mice

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ABSTRACT

Optical properties of the biological tissue play an important role to a correct use of optical techniques for therapy and diagnosis. The mice skin presents morphological differences due to characteristics such as gender, body mass and age. Murine models are frequently used in pre-clinical trials in optical therapy and diagnosis. Therefore, the assessment of the skin tissue in animal models is needed for a proper understanding of how light interacts with skin. Noninvasive techniques such as optical coherence tomography (OCT) have been used to obtain optical information of the tissue, as the attenuation coefficient, with the advantage of obtaining sectional images in real time. In this study, eight female BALB/c albino mice (twenty-four weeks old) and eight male C57BL/6 black mice (eight weeks old) were used to measure the attenuation coefficient of the light in the skin, utilizing the OCT technique, aiming to check for influence of the aging process. Two moments were assessed twenty-two weeks apart from each other. Our data show that the aging process significantly affects the light attenuation coefficient in mice skin. Twenty-two weeks after, statistical significant differences were observed between groups within a same strain. We conclude that light attenuation coefficient of mice skin may be influenced by factors such as disorganization of the dermis. Morphological aspects of skin should be taken into account in studies that involve optical strategies in murine models.

Keywords: BALB/c mice, C57BL/6 mice, murine model, skin tissue and Optical Coherence Tomography (OCT).

1. INTRODUCTION

Optical therapy and diagnosis have been used to a broad range of clinical trials[1]. These strategies have been widely applied in some skin diseases such as laser therapy for wound healing, wrinkles, acne scars, skin resurfacing, skin cancer and inflammatory process[2, 3]. To investigate the effects of optical techniques in the biological tissue, murine model is widely utilized since it presents low cost and easy manipulation. However, epidermis and dermis morphology can have different aspects that are dependent on the age, body mass and gender[4]. In this context, to determine more appropriately the optical skin characteristics plays a pivotal role to a correct use of light-based practices.

The classical way to study skin is by histological techniques that assess morphological differences in the tissue. However, these tools are invasive whereas need coloring to visualize structure, thickness and modification. Optical coherence tomography (OCT) is a non-invasive image technique relying on low coherence interferometry Michelson, cross-sectional visualization that has been used to evaluate structures without modifying the sample[5]. OCT may be applied in vivo, showing real time images reaching up to 3 mm depth into biological tissue using a near infrared light source. OCT can give information about backscattered light that is important to determine the light attenuation coefficient (α) [6].

In this study, we used BALB/C albino female and C57BL/6 black male mice in different biological moments of life to evaluate skin tissue characteristics to understand if the aging process influences the light attenuation in the mouse skin.

2. MATERIALS AND METHODS

All the experimental procedures were submitted and approved by the Ethical Committee in animal research at IPEN (Project n° 047/09 CEPA-IPEN/SP).

Animals - The animals were female BALB/c mice (n=8) and male C57BL/6 mice (n=8). The animals received food and water *ad libitum* and were kept in a controlled environment in the vivarium of the IPEN- CNEN/SP. The study was realized in two moments: firstly (moment I), the animals were 24 (BALB/c) and 8 (C57BL/6) weeks old, respectively.

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On the second moment (II), the animals were 46 (BALB/c) and 30 (C57BL/6) weeks old. For the experiment, the animals were anesthetized intraperitoneally with ketamine hydrochloride association (0.32 mL / kg) and xylazine (0.2 mL / kg) and shaved in the abdominal region of the lower quadrant.

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Optical Coherence Thomography (OCT) - The OCT equipment (OCP 930RS THORLABS- INC) has a central wavelenght of 930 nm, 2 mW power, lateral and axial spatial resolution of 6 μm and a half height width of 100 nm. The animals were placed on a standardized platform with the abdominal region of the lower quadrant facing the OCT camera. A stream (sequence of images of the same region) of the abdominal region of each animal was performed.

Path length of the light in the mice skin (z)- the technique of OCT obtains sectional images in real time, which show the optical path of the light. For obtaining z we used the program *ImageJ* considering 1 pixel = 3.088803 μm (that would be the value of the axial distance utilized in OCT) and calculated the ratio z/n, where n is the refractive index of the mice skin.

Attenuation coefficient of light (α) - To calculate α, we utilized the program *V10_desmineralizacao_media_fit_stream*, with n= 1.44 for refractive index of the mice skin[7]. The selection area adopted for all measurements was in the range between 1000 and 2000 μm (Fig. 1A). For the signal adjustment, it was adopted the region of the dermis and subcutaneous tissue of the mice, i.e, the exponential area between the first peak (on the skin) and the second peak (corresponding to muscle tissue) (Fig. 1B).

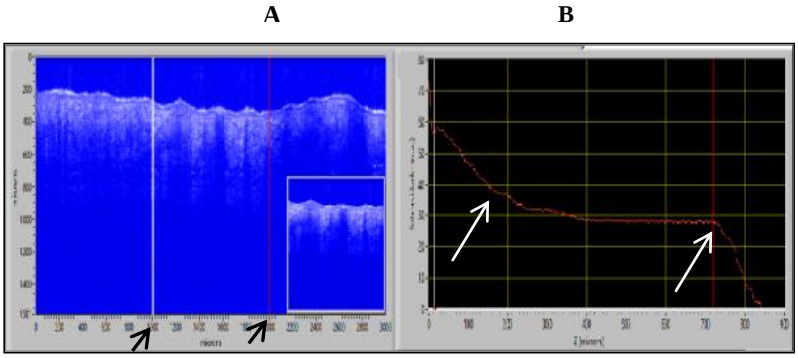

Figure 1. A: Image by OCT of the mice skin . Vertical lines delimit the region of interest analyzed. B: The arrows indicate the exponential curve that was swept by the software used.

Thereafter, the images obtained by the software for each animal were analyzed. For the calculation of α, it was used a simple model of exponential decay in intensity of the detected light (backscattered) according to the equation:

$$I(Z) = I_0 . e^{-2\alpha z} + C \qquad (1)$$

where *I* represents the value of the detected intensity; I_0 is the value of the intensity of the light source; α is the total attenuation coefficient; *Z* is the depth analyzed and C is a constant used in the signal due to background noise[8].

Statistical analysis: Values are presented as means, and error bars are standard errors of the mean (SEM). Data were analyzed with Shapiro-Wilk W test to assess the normality. Homogeneity of variance was verified by Brown-Forsythe test. To determine the effects at each time point, data were analyzed by paired T-test. Data were considered statistically significant when $P<0.05$.

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3. RESULTS

The figure 2 shows the optical depth of the light into mice skin in the two moments as described above. Here we show a sample illustration and caption for a multimedia file:

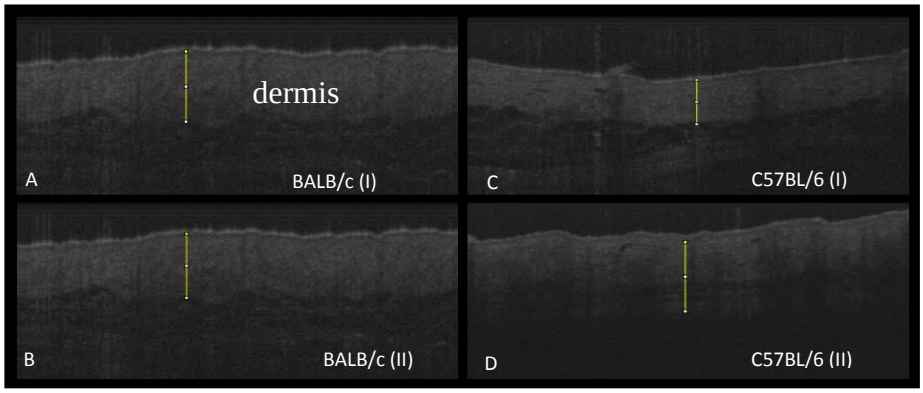

Figure 2 - Optical depth of the light in the dermis of mice Images A/B – OCT image of a BALB/c mouse in the moments I and II, respectively. Images C/D – OCT image of C57BL/6 mice in the moments I and II, respectively.

Table 1 shows the mean values ±SEM of the optical depth of the light in both stains used in this study.

Table 1- Mean values of the optical depth of the light in the dermis of mice OCT.

Groups	z (μM)
BALB/c (I)	175.90±4.29
BALB/c (II)	199.50±2.74
C57BL/6 (I)	153.83±2.76
C57BL/6 (II)	307.70±8.90

The mean values of α for BALB/c groups utilized in the moments of the experiment are demonstrated in the figure 3. Figure 4 displays α for C57BL/6. Statistically significant differences were observed for both strains in the two moments analyzed.

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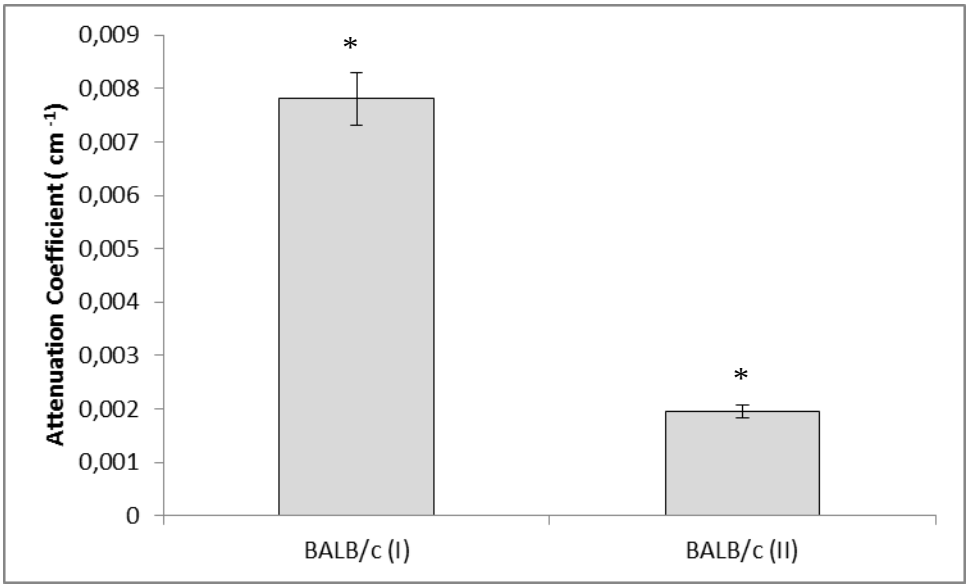

Figure 3 - Mean values of the attenuation coefficient of BALB/c mice in the two experimental moments. The bars represent the SEM (*p= 0.0071*).

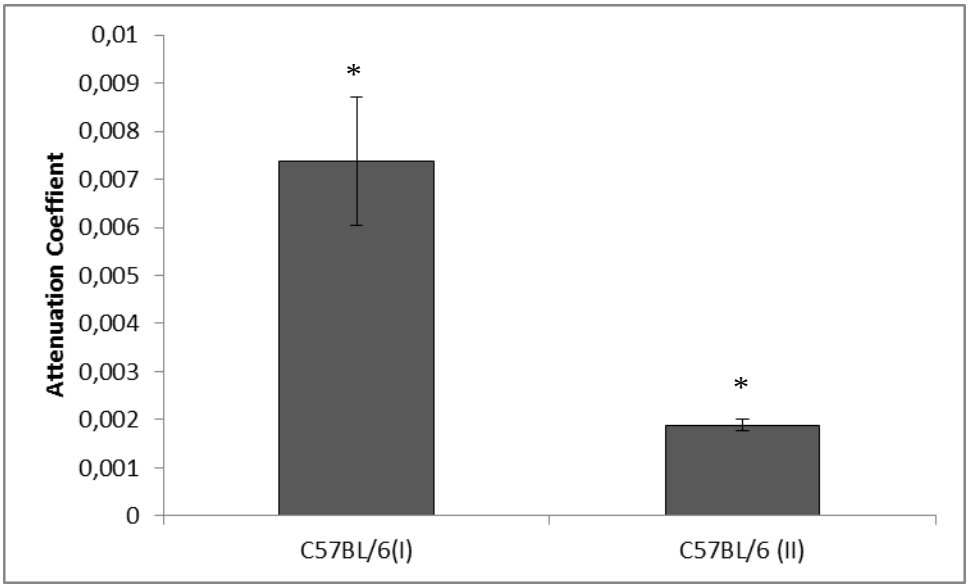

Figure 4 - Mean values of the attenuation coefficient of C57BL/6 mice in the two experimental moments. The bars represent SEM. (*p=0.0216*).

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4. DISCUSSION

Literature is rich about the use of optical therapy for skin diseases[9-12] and murine models are largely used to understand the interaction of the light with biological tissues. Biotissues present a broad spectrum of variance related to optical properties such as absorbance, scattering and transmission of the light[13]. Therefore, studies investigating the light dosimetry in skin need to consider the optical properties due the tissue heterogeneity. The absorbing and scattering centers of the biological tissue can influence the penetration of light. In view of this, is important know the light attenuation coefficient in skin for a correct application of light-based therapies.

In this study, the OCT technique was used for obtaining sectional images in real time to measure the α in the mice skin. According to equation 1, the attenuation coefficient can be estimated from the change in the intensity of light that interacts with the biotissue.. Therefore, through this physical quantity we can assess differences about the aging process andthe dermal matrix tissue organization of these animals.

Our results demonstrated that α obtained in moment I is significantly greater than that obtained in moment II, independent of the strain. This difference could be explained because the aging process of the animal. On the first moment, mice were younger that at moment II. The literature reports that the aging plays an important role in the dermis organization, which is connected to collagen synthesis. Other components like elastic fibers, fibronectin, glycosaminoglycans and proteoglycans also suffer degradation and decrease according to the aging[4]. In consequence, the disorganization and laxity can cause an increase in the dermal extension as can be observed in the image on C57BL/6 mice on the second moment

Definition mathematics for attenuation coefficient is $\boldsymbol{\alpha = \alpha_s + \alpha_a\ [\ cm^{-1}]}$, i.e, the light attenuation is a reduction in the intensity of a light beam as the beam propagates owing to the joint action of the absorption and scattering of light. Our data showed that α decreased as the mice aged. Thus, we can suggest that scattering and absorption decreased or so one remained constant while the other decreased[14].

In biological tissues, absorption is mainly caused by macromoleculas such as proteins and pigments as melanine[15]. The melanization in the epidermis is located so that we hypothesize that the scattering is the major responsible by the light attenuation decrease.

Studies report that the greatest scattering in the dermis is due to collagen fibers. If dermis organization suffers degradation and collagen synthesis decreases, we suggest that the disorganization of the dermis contributes to a decrease of the light scattering[16]. These findings corroborate with another studies realized by R. Rox Anderson[17] and our group[18].

In fact, according to Graaff and coworkers, despite black skin having a higher absorption coefficient than white skin, white and dark skin in humans present similar scattering coefficients of the light, i.e, $\boldsymbol{\alpha_{s\ (\ dark)\ =}}$ 229 cm^{-1} and $\boldsymbol{\alpha_{s\ (\ white)\ =}}$ 237 cm^{-1}. Therefore, different strains not influenced the results of the α[19] . However, more further studies are warranted for identifying other factors that could influence the light attenuation in the biological tissue.

5. CONCLUSION

Our study highlights the importance of the age in the design of a pre-clinical trial. The aging process strongly influences the connective tissue structure that contributes to a decrease of the light attenuation coefficient in skin.

6. ACKNOWLEDGEMENT

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