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Skin Pigmentation Characterized by Visible Reflectance Measurements

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Abstract. The epidermal melanin content affects most dermatologic treatments involving light, and can limit the therapeutic success significantly. Therefore, knowledge of the optical properties of skin is required. This study investigates how the concentration of melanin influences visible reflectance spectra of skin and the relationship to threshold radiant energy fluence for melanosomal or melanocyte destruction. Reflectance spectra were measured at 28 pigmented human skin sites in vivo. For Asian and Caucasian subjects, measured reflectance values varied over the same range, while significantly lower values were recorded for African individuals. Epidermal melanin absorption coefficients measured at 694 nm were about 2500 m^{-1} for African, and $300\text{--}1200\text{ m}^{-1}$ for Caucasian and Asian skin. Twenty-five skin sites were exposed to ruby laser pulses (694 nm), where the pulse duration was long enough to allow heat diffusion between melanosomes. Hypopigmentation occurred, on average, at 12 and 26 J cm^{-2} for sun-exposed and sun-protected white skin, respectively, while slightly lower threshold values resulted from the measured spectra. As visible reflectance spectra reveal information regarding skin pigmentation and individual threshold doses for melanosomal damage, a use as a diagnostic tool in various dermatological laser treatments is apparent.

INTRODUCTION

Human skin colour varies significantly between individuals, dependent on race, sun exposure and age. A proper analysis of the reflectance spectrum of human skin in the visible wavelength region (380–780 nm) might, therefore, reveal important diagnostic information. Furthermore, additional information exists in the near infra-red spectrum ranging from 780 to about 1500 nm wavelength. The optical penetration depth in the ultra-violet (u.v.) part of the spectrum is much less than the epidermal thickness. Therefore, little information can be extracted from reflectance spectra in this region.

Normal skin colour originates in the presence of specific chromophores such as melanin, haemoglobin, bilirubin and carotene. However, scattering due to the inhomogeneous dis-

tribution of lipids, water and proteins within each cell, as well as the random distribution of cells, also has a very important impact on the visual appearance of skin. The characteristic redness often observed in Caucasian skin is due to the joint action of light absorption in dermal blood vessels and scattering in the epidermis and upper dermis. Skin colour might vary from pink to almost bluish depending on the degree of blood oxygenation. This phenomenon occurs because oxygen-rich blood has less absorption of red light and higher absorption of blue light than deoxygenated blood. The brownish or sometimes almost black colouration of African skin is due to a higher concentration of epidermal melanin, which has a broad absorption spectrum. Bilirubin is an orange-yellow pigment and has a characteristic absorption spectrum, with an absorption peak around 450 nm that decreases to zero

around 550 nm (1). Bilirubin and carotene (a yellow pigment) are normally present in small concentrations in the blood (2).

Knowledge of light propagation in tissue and light-tissue interactions has improved significantly over recent years, and has resulted in a wide variety of biomedical photonic applications. Several lasers in the visible or near infra-red part of the spectrum are used in dermatology. Melanin located in the epidermis must be considered in all photonic therapies due to its high absorption coefficient for these wavelengths. This results in a decreased optical penetration depth that protects deeper skin layers against unfortunate radiation. However, the light absorption causes localized heating in the epidermis, and for high melanin concentration and sufficiently high radiant energy fluence rates, irreversible thermal damage or even necrosis may occur. Knowledge of these threshold values for when melanosomal damage occurs is, therefore, required to achieve successful laser treatment.

One example where light absorption by melanin may result in epidermal damage is tattoo removal. Lasers for tattoo removal utilize a wavelength that is well absorbed in the pigment particles, together with a short pulse width. These pulses are demonstrated to cause large thermal transients and shock wave generation that may result in mechanical damage confined to the particles (3, 4). The Q-switched ruby laser (694 nm), the Q-switched Nd-YAG laser (532 nm or 1064 nm) and the alexandrite laser (755 nm) are used to selectively remove the pigment granules of a tattoo. The pulse duration selected for these therapies varies from 10 to 100 ns. Lasers in this regime have been used in previous studies to investigate the threshold radiant energy fluence causing melanosomal damage in normal skin when exposed to short (40 ns) Q-switched ruby laser pulses (5, 6). The results showed melanosomal damage dependent on skin pigmentation and incident radiant energy fluence. These threshold values for human sun-protected white skin, sun-exposed white skin and brown lentigo (corresponding to highly pigmented skin) were, on average, 3.1, 2.0 and 1.4 J cm⁻² (5). Histological examination of laser-irradiated skin showed that damage was confined to the melanosomes and immediate neighbourhood, when the radiant energy fluence was limited to the value for where immediate whitening occurred. Above this threshold, keratinocyte nuclei were also disrupted (5).

Removal of port-wine stain (PWS) birthmarks is another example of laser treatment in which high melanin concentrations limit therapeutic success. The currently used flash-lamp pumped dye laser (FLPDL at 585 nm wavelength and 0.45 ms pulse duration) relies on the principle of laser-induced selective photothermolysis. Selectivity is obtained by using an optical wavelength that is well absorbed in the blood vessels comprising the birthmark, and low absorption in the surrounding tissue. Furthermore, the pulse duration should be equal to or shorter than the time required for heat to diffuse across the target blood vessels (3). This time is called the thermal relaxation time. A successful treatment is, however, only obtained if the temperature in the overlying epidermis is kept below the threshold for damage. Epidermal damage induced by the FLPDL is known to increase with increasing melanin content, ie skin of darker colour. The damage is found to vary from elongation of basal keratinocytes and subepidermal microvesiculation, to subepidermal blistering at higher radiant energy fluences (7).

The reflectance spectrum, as well as the colour, of normal human skin is strongly influenced by the melanin concentration. The logarithm of the skin reflectance increases approximately linearly from 620 to 730 nm; increased melanin content, rather than changes in the blood or scattering properties, causes the slope to steepen. The reason is an almost flat blood absorption coefficient and linear scattering and melanin absorption coefficients (8, 9) in this wavelength range. Kollias and Baqer (10) have tried to establish a quantitative relation between reflectance measurements of skin and absorbance measurements of DOPA-melanin (dihydroxyphenylalanine) in solution. Calculations were based on the logarithmic slopes of the spectra in the wavelength region 620–700 nm. A correlation was found between spectral slopes and concentration of melanin pigment in skin, and the total melanin mass in human skin could be estimated non-invasively to a first approximation. Similar comparisons were done by Hajizadeh-Saffar et al (11), who calculated a melanin index for human skin based on the logarithmic slopes of the spectra from 650 to 700 nm. The index is adjusted due to the effects of oxygen saturation and haemoglobin concentration by empirical formulas. The index is compared to those obtained from reflectance measurements of a synthetic melanin compound dissolved in

sodium hydroxide solution. Such simplified and linear models can give reasonable quantitative values for the melanin content. However, the method is not applicable if the tissue parameters deviate much from normal values, eg for extreme changes in blood content. Furthermore, the scattering properties of melanin present in human skin can differ from that of extracted melanin or melanin in solution (10, 12). Therefore, more complex analysis is necessary to determine the optical properties, especially when needed for dosimetry purposes.

There are few simple and adequate methods for *in vivo* determination of the melanin content, and for the purpose of laser dosimetry. Treatment and threshold doses are usually determined from the subjective clinical experience of the attending physician. As human skin is a complex structure, and optical properties can change after each successive laser treatment, development of a non-invasive diagnostic tool that can assist and guide the physician would be advantageous.

This study investigates how melanin influences light-skin interactions and thereby visible reflectance spectra of human skin. It further presents spectra measured from skin with different pigmentation. Optical properties, such as the average epidermal absorption coefficient, are predicted from mathematical simulations using optical diffusion approximations. Furthermore, this information is applied to extract and estimate threshold radiant energy fluences for epidermal damage at two wavelengths and at 0.5 ms pulse width. Actual threshold values are found when exposing the skin to 0.5 ms ruby laser pulses (694 nm), ie at a wavelength with low absorption in blood. Threshold values resulting from Q-switched ruby laser exposure are also presented and compared with reported results.

LIGHT-TISSUE INTERACTION

A brief overview of the functional structure of the epidermis is given, relevant to the light interaction with skin. A simple model describing light propagation in skin is presented.

Epidermis: functional structure

The epidermis is about 50–150 μm thick, except in the soles of the feet and the palms of the hand where it is thicker (13). It consists of four

Fig. 1. Diagrammatic cross-section through the epidermis. One melanocyte is shown with its dendrites and corresponding keratinocytes.

main layers: basal, squamous, granular and horny (Fig. 1). Two types of cells are present in the basal layer, keratinocytes and dendritic melanocytes, where melanin-containing organelles called melanosomes are produced by the melanocytes. Melanosomes can be either oval-shaped eumelanosomes containing brown/black pigment, or round pheomelanosomes containing a red pigment (pheomelanin). The melanosomes undergo four growth stages before being fully melanized. Black colour is caused by the predominance of eumelanin, while freckles and red hair are due to small amounts of eumelanin and greater proportions of pheomelanin. Blond hair, in contrast, is produced by a small number of Stage III and less Stage IV melanosomes (14). Melanosomes are transferred from the melanocytes into one of the surrounding keratinocytes by pinching off the tip of the dendrite. The keratinocyte migrates towards the stratum corneum, and during the migration, melanosomes are degraded, leaving only a melanin polymer, called melanin dust. Melanosomes within the keratinocytes of dark-skinned people are numerous, large (0.8–1.2 μm in diameter), heavily melanized, distributed as solitary units and degrade slowly. Larger melanosomes can be found intact in the stratum corneum, and the melanocytes are more highly dendritic. The opposite is found in fair skin with smaller melanosomes (<0.8 μm in diameter) grouped together in clusters of three or four. For Caucasians, the melanosomal complexes may be degraded even in the basal layer (14). When white skin is exposed to ultra-violet light, however, melanocytes become larger and more dendritic, and the production of melanosomes increases and accelerates. The increased melanin content in the keratinocytes causes the brown coloured 'sun tan' (15).

Fig. 2. Diagrammatic cross-section of human skin. The dashed line indicates the separation between the 'epidermal' and the 'dermal' layer used in the simulation model.

The number density of melanocytes in the human body is thought to be similar in people of all races, but the number of active epidermal melanin units varies in the different regions of the body (16). Furthermore, sunlight acutely increases the melanocyte population, while cold or severe thermal burns decrease the number of pigment cells in affected skin, presumably due to the inflammatory/immune process. The melanocyte density also decreases with age, beginning in the fifth decade of life, but the melanocytes themselves seem to become larger and more dendritic (14). The total epidermal renewal time is 59–75 days (17).

Diffuse reflectance and light distribution in human skin

Several mathematical models have been applied to analyse light propagation in human skin. The complex structure of skin necessitates use of simplified models, such as the diffusion approximation to the equation of radiative transfer or Monte Carlo simulations. The latter is, however, very time consuming. This paper will use an analytical model based on optical diffusion theory developed by Svaasand et al (8). Normal human skin is described by a planar model (Fig. 2), where an upper 100 μm layer includes epidermis and papillae, limited by air on one side and dermis on the other. Melanin is assumed to be distributed uniformly in the 'epidermal' layer, and the papillae are included to ensure all melanin is contained in this layer. The dermal blood volume fraction, B_d , is based on blood being distributed uniformly, and a small epidermal fraction ($B_e=0.2\%$) is included due to the papillae. Furthermore, the blood absorption spectrum is approximated by an analytical expression (8). Oxygen saturation and haema-

tocrit are held constant at, respectively, 80% and 0.41. Increased haematocrit to 0.5 would only give slightly lower reflectance around the oxyhaemoglobin absorption peaks (around 540 and 580 nm). Oxygen saturation certainly affects the absorption characteristics of whole blood, but the blood volume fraction in normal skin is low and, therefore, has less impact on the reflectance spectra.

The true scattering coefficients are reported to decrease approximately proportional to the inverse wavelength in the visible spectrum (8, 18–20), and several studies have reported the coefficients at 577 nm wavelength (18–20). True scattering coefficients for, respectively, dermis and epidermis, $\mu_{s,d}$ and $\mu_{s,e}$, will, therefore, be evaluated by values at this wavelength. Reported coefficients vary from about 12 to 50 mm^{-1} (18–24). The melanin absorption coefficient is reported to decrease approximately proportional to the fourth power of the inverse wavelength (25), and is evaluated by the value at 694 nm, $\mu_{a,m}$, ie the ruby laser wavelength. Absorption coefficients are reported in the range 970–3200 m^{-1} , depending on skin colour (18, 19, 26). The simulation model uses distributed sources, and the diffusion approximation can, therefore, be applied close to the skin surface as well as deeper inside the skin. The optical diffusion approximation fails for wavelengths below 450 nm due to the high blood absorption in the Soret band, and the wavelength range is limited to 450–800 nm. The full mathematical description of the model is presented in Svaasand et al (8) and Norvang et al (9).

Using this analytical model, diffuse reflectance from normal skin with different pigmentation can be simulated (Fig. 3). All optical properties other than the melanin absorption coefficient are held constant. Skin with low melanin content, as in fair skin (upper curve), is represented by a melanin absorption coefficient of $\mu_{a,m}=300 \text{ m}^{-1}$. Influence of blood absorption is seen from the reflectance minima at 540 and 580 nm. The reflectance increases rapidly from 580 to 620 nm, before flattening at 800 nm. In skin with increased melanin content, $\mu_{a,m}=800 \text{ m}^{-1}$, corresponding to tanned white skin (middle curve), the characteristic blood spectrum is visible but suppressed. The slope of the reflectance spectrum between 620 and 800 nm has steepened compared to the curve corresponding to low melanin content. The overall reflectance has also decreased. The spectrum is clearly influenced by the

Fig. 3. Simulated diffuse reflectance from human skin with different pigmentation given by $\mu_{a,m}$ at 694 nm. $\mu_{s,e} = \mu_{s,d} = 30 \text{ mm}^{-1}$ at 577 nm, and $B_d = 1\%$.

characteristic wavelength-dependent melanin absorption. A high melanin content, as in African skin, can be simulated with $\mu_{a,m} = 2500 \text{ m}^{-1}$ (lower curve). The blood absorption peaks are masked and reflectance values have decreased significantly.

MATERIALS AND METHODS

Eleven Caucasian males (one Persian and 10 North European), four Asian males and two African females, aged 20–60 years, volunteered for this study. Visible reflectance spectra were measured at the upper arm or forearm, and average melanin absorption coefficients were determined. Furthermore, threshold radiant energy fluences necessary for melanosomal damage were calculated at 694 nm wavelength. Actual threshold values were found by exposing the skin sites in all male volunteers to short (<100 ns) and long (≈ 0.5 ms) ruby laser pulses (694 nm wavelength). All skin sites were shaved to exclude light absorption by melanin in the hair shaft. Melanin in hair follicles was, however, not removed. The test sites (maximum $4 \times 10 \text{ cm}^2$) were located where the skin appeared uniform.

Reflectance measurements

Diffuse reflectance spectra were measured in two or three locations within the test area. A small difference was found, presumably due to variation in pigmentation and blood content normally occurring in the human body, and spectra were therefore averaged. The skin sites

were held against the instrument aperture at constant pressure, and with the volunteers standing in a comfortable position to avoid extreme changes in blood flow or pressure during the measurement. The room temperature did not vary and is assumed to be constant throughout the experiment.

The spectra were measured using a diode-array spectrophotometer with an integrating sphere (HP-8452A Diode-Array Spectrophotometer[™] with a Labsphere RSA-HP-84 Accessory[™]). The sphere aperture was 20 mm in diameter, and the integration time was 10 s. Although the instrument recorded data over the wavelength range 380–820 nm, only reflectance from 450 to 800 nm was analysed (8). The spectra were corrected using a diffuse reflecting standard with known reflectance. Thus, the correction procedure compensated for electronic noise and spectral non-uniformities in the lamp emission and detector response.

A comparison measurement was employed, maintaining a constant Q-value of the sphere. The skin was positioned at the reference port when measuring the reflectance standard for the correction procedure, while switching positions during the skin measurements. With this method, no error is introduced due to the different reflectance levels between skin (~ 10 –70%) and the reflectance standard ($\sim 100\%$).

Ruby laser exposure

A Laseaway[™] Q-switched ruby laser emitting at 694 nm wavelength was used in the free-running and the Q-switched mode. Pulse duration in the free-running mode was 0.5 ms full width half maximum (FWHM), and slightly less for radiant energy fluences below 5 J cm^{-2} . In the Q-switched mode, the pulse length varied from 30 to 100 ns (FWHM) for decreasing fluence values, and increased to 150–200 ns for radiant energy fluences less than 1 J cm^{-2} . Pulse duration shorter than 100 ns should ideally be sufficient to confine the heat to single melanosomes, since this is much shorter than the thermal relaxation time for these organelles (27). For 0.5 ms pulses, however, heat diffusing from neighbouring melanosomes also contributes (27). The radiant energy fluence was increased until an immediate whitening could be seen in the irradiated site, similar to what is reported in earlier studies (5, 6), or other immediate changes in the skin texture. The radiant

Fig. 4. Set-up for ruby laser exposure. The distance, x , from the laser hand piece to the aperture and thereby the spot size, d , was varied.

energy fluence was calculated from measurements of spot size and energy for each laser setting, and varied from, respectively, 2.8–50 and 0.25–4.0 J cm⁻² for the free-running and the Q-switched mode. The laser beam had a non-Gaussian distribution, ie it was multimode and the beam profile appeared almost flat-top. The pulse-to-pulse variation in radiant energy fluence was less than 5%.

Skin sites were positioned against the aperture (Fig. 4) out of the focus of the laser beam at a distance x from the laser hand piece. The spot diameter, d , was varied from 5 to 2 mm to achieve the highest radiant energy fluences in the free-running mode. Immediate changes were, however, not observed within the maximum radiant energy fluence in one skin site.

Doses for minimal sensation, immediate erythema (5–10 min after irradiation), delayed oedema and immediate whitening or other texture changes were noted for each test site. The volunteers were observed for at least 3 months after laser irradiation for possible hyper- or hypopigmentation, scarring and/or necrosis.

RESULTS

Reflectance spectra were measured at 28 different skin sites, in which 25 were exposed to the ruby laser. The skin of the African females was not irradiated by the laser. Measurements were performed at both volar and dorsal sites of the arm of, respectively, 10 males (upper arm) and one female (forearm), while measurements for the remaining individuals were performed only at the dorsal or volar sides. Fourteen sites were of skin type III, seven sites were of type IV, and four sites were of type V. The two Africans had approximately the same, moderately dark brown skin colour (skin type VI). Dark hair was removed from a Persian and a North European subject.

The skin sites are grouped according to sun tan rather than skin type, since degree of sun

tan is an average of all melanin present in epidermis and upper dermis (14). Furthermore, the skin type may be misleading if the skin has not been exposed to the sun, which was the case for six skin sites. A sun tan coding is selected from 1 to 6, where 6 denotes moderately dark African skin (three sites). White skin is coded with 5 for darkly tanned (one site), 4 for tanned (five sites), 3 for moderately tanned (eight sites), 2 for lightly tanned (five sites), and 1 for sun-protected skin (six sites). White skin sites are also combined in larger groups of, respectively, sun-exposed sites (tanning code, TC=3, 4 and 5) and sun-protected sites (code 1 and 2). All 11 sun-protected sites were located on the upper arm; four on the dorsal and seven at the volar side. Eleven sun-exposed sites were located on the dorsal side of the arm, four on the forearm and seven on the upper arm. The last three sun-exposed sites were located at the volar side of the upper arm.

Reflectance measurements

Diffuse reflectance spectra were measured twice at two or three different locations within each test area. The variation in reflectance among these locations was less than 5% at 550 nm and less than 3% at 650 nm. Measured spectra for three different pigmented skin sites are shown in Fig. 5(a). Highest reflectance was achieved in the Caucasian with fairest skin, measured at the volar side of the upper arm (upper curve). The middle spectrum was measured at the dorsal side of the upper arm of a moderately tanned Scandinavian, while the lowest reflectance spectrum was measured at the dorsal side of an African subject.

Measured spectra for all skin sites enrolled in the study are presented according to race in Fig. 6. Three additional spectra are presented in the Caucasian-Persian figure (CP1 volar, CP2 volar and CP3 volar), where CP1 volar is from the same volunteer as CP1 dorsal. The skin site, CP2 volar, was slightly more tanned than the other in this group. The spectra of North Europeans are presented in two figures to avoid overlap. The reflectance values measured at 585 and 694 nm for all test sites are presented as a function of tanning code [Fig. 7(a)]. The diffuse reflectance at both wavelengths decreases with increasing melanin content.

Fig. 5. (a) Measured and (b) simulated diffuse reflectance spectra for: African skin ($\cdots$) with simulation parameters $B_d=1\%$, $\mu_{s,e}=\mu_{s,d}=50 \text{ mm}^{-1}$ at 577 nm, $\mu_{a,m}=2500 \text{ m}^{-1}$ at 694 nm; moderately tanned Scandinavian skin ($---$), $B_d=3\%$, $\mu_{s,e}=30 \text{ mm}^{-1}$, $\mu_{s,d}=35 \text{ mm}^{-1}$ at 577 nm, $\mu_{a,m}=800 \text{ m}^{-1}$ at 694 nm; fair Caucasian skin ($—$) $B_d=0.7\%$, $\mu_{s,e}=30 \text{ mm}^{-1}$, $\mu_{s,d}=35 \text{ mm}^{-1}$ at 577 nm, $\mu_{a,m}=300 \text{ m}^{-1}$ at 694 nm. For all sites: $B_e=0.2\%$.

The average melanin absorption coefficients were found from fitting simulated to measured spectra in an iterative procedure. All measurements were performed from healthy volunteers, and the blood oxygenation can be assumed to be equal to 80%. Thus, the resulting blood absorption spectrum is flat in the wavelength region 620–800 nm. Changes in the spectrum curvature in this wavelength region is, therefore, primarily determined from changes in melanin absorption, and a spectrum was first fitted according to the slope in this region. Next, the blood volume fraction was adjusted until the difference in reflectance between 585 and 800 nm was approximately the same for the simulated and measured spectra. The reflectance level is strongly influenced by the true scattering coefficient, and this can, therefore, be used to scale the simulated spectrum. The scattering coefficient is slightly wavelength dependent, but small changes do not affect the slope. The melanin absorption and true scattering coefficients were varied in steps of, respectively, 50 m^{-1} and 5 mm^{-1} . The blood volume fraction was first varied in steps of 0.5%, followed by 0.1% steps. This method gives a good approximation when the reflectance values are high (8, 9). The melanin absorption coefficient derived from a specific spectrum was then relatively insensitive to variations in the scattering and blood absorption properties. The accuracy in the determined coefficients are, therefore, relatively high, $\pm 50 \text{ m}^{-1}$. For low reflectance values, eg the reflectance from African skin and for wavelengths below approximately 580 nm, changes in the melanin absorption coefficient causes

small changes in the reflectance. The accuracy in the determined coefficients is, therefore, lower, and the procedure gives a rough estimate only. Simulated spectra giving the best fit for three measured spectra in Fig. 5(a) are shown in Fig. 5(b). The achieved average melanin absorption coefficients are presented as a function of tanning code for all skin sites [Fig. 7(b)], in which the $\pm 50 \text{ m}^{-1}$ accuracy caused overlapping data points. The absorption coefficient are demonstrated to increase with increasing tanning codes. Mean values at 694 nm were 770 m^{-1} (550–1200) for sun-exposed skin (TC=3, 4, 5) and 360 m^{-1} (300–450) for sun-protected skin (TC=1, 2), respectively. For African skin, the corresponding mean was 2500 m^{-1} .

Scattering coefficient and blood volume fraction for African skin were, respectively, around 50 mm^{-1} and 1%. For Asian skin, the corresponding values ranged from 20 to 55 mm^{-1} and 0.5 to 1.5%. Scattering coefficients varied from 20 to 45 mm^{-1} for Caucasian skin (20–25 for Persian, 25–35 for Scandinavian, 20–35 for North European above 30 years old and 25–45 for those below 30 years of age). Blood volume fraction varied from 0.5 to 3% (0.5 for Persian, 0.5–3 for Scandinavian, 0.5–2.5 for North European above 30 years old and 0.5–1 for those below 30 years of age).

The average melanin absorption coefficient at 585 nm is approximately 50% higher than at 694 nm (1). Average absorption coefficients at 585 nm were, therefore, about 1540 and 720 m^{-1} for, respectively, sun-exposed and sun-protected skin, and 5000 m^{-1} for African skin. Using these absorption coefficients and

Fig. 6. Measured diffuse reflectance spectra for different pigmented sites (a–f). The spectra are grouped according to race. For the Caucasian-Persian (CP) spectra (c), only the CP1 dorsal skin site was exposed to ruby laser.

Equation A1 (see Appendix), a threshold radiant energy fluence was found for melanosomal damage. The resulting threshold values for sun-exposed skin were, respectively, 8.4 and 10.9 J cm⁻² at 585 and 694 nm. The corresponding values for sun-protected skin were 14.3 J cm⁻² at 585 nm and 20.4 J cm⁻² at 694 nm. The reflectance values used for sun-exposed skin sites were, respectively, 0.26 and 0.48 at 585 and 694 nm, and for sun-protected skin, 0.36 and 0.55. The calculated threshold radiant energy fluence for African skin was 3.5 J cm⁻² at 585 nm and 5.0 J cm⁻² at 694 nm, where the corresponding reflectance values were 0.15

(585 nm) and 0.28 (694 nm). Threshold values calculated from the absorption coefficients at 694 nm, together with those obtained from ruby laser exposure, are presented in Fig. 8.

Ruby laser exposure

Minimum radiant energy fluences required for specific clinical changes were averaged for each tanning code (Tables 1 and 2). Minimal sensation corresponded to the minimum radiant energy fluence at which the volunteer sensed the laser pulse. Erythema was a red,

Fig. 7. (a) Measured diffuse reflectance for the skin sites at 694 nm (+) and 585 nm (◇), and (b) simulated average melanin absorption coefficient (m^{-1}) at 694 nm vs tanning code.

Fig. 8. Average threshold radiant energy fluence at 694 nm for melanosomal damage vs tanning code; determined from immediate whitening (stippled bars), hypopigmentation (open bars), and calculated from measured reflectance spectra (hatched bars). Minimum and maximum values are represented by error bars, and number of skin sites contributing to each average is given inside each column.

inflammatory colour observed within 5 min following exposure. Delayed oedema corresponded to a slight swelling of the skin observed 5–10 min following laser exposure, while hyperpigmentation corresponded to a light brown colour observed one to several weeks after exposure. When the laser light destroyed epidermal melanin, the skin appeared white a few weeks following laser exposure, a condition denoted as hypopigmentation. Immediate whitening was the white crust formation observed at the irradiated site when exposed to nanosecond pulses, and similar to what was observed in earlier studies (5,

6). This white ash-like crust most likely resulted from acoustic shock wave generation and explosive vaporization, and at a temperature corresponding to a reported threshold of 110°C (6). Crust formation was absent for most sites exposed to longer laser pulses. Instead, blanching of the irradiated skin was observed, and in a few cases, the epidermis may have separated from the dermis and a small scar was observed. For the Q-switched mode, the results were compared with those of Hruza et al (5) (Table 2), where 12 white males were examined. The threshold doses observed in the two studies are approximately equivalent.

Table 1. Minimal radiant energy fluence introducing clinical changes

Clinical change	Sun-protected skin (J cm^{-2})	Sun-exposed skin (J cm^{-2})
Minimal sensation	13 (8–31)	8 (3–15)
Erythema	18 (7–31)	10 (5–20)
Delayed oedema	35 (35–35)	18 (12–30)
Hyperpigmentation	15 (8–23)	12 (6–26)
Hypopigmentation	26 (23–27)	12 (8–26)
Immediate whitening	41 (31–50)	21 (12–31)

Long, 0.5 ms, ruby laser pulses. Range of fluence values in parentheses.

One volunteer in the present study, with very fair skin, considered the pain intolerable when irradiated to high Q-switched energy fluences. The radiant energy fluence was, therefore, not increased above 2.3 J cm^{-2} , and immediate whitening was not observed. Hypopigmentation was, however, observed 3 weeks later. At four additional fair-pigmented sites, the radiant energy fluence in the Q-switched mode was not increased to a maximum (10 J cm^{-2}) due to occurrence of strong erythema or oedema reactions.

A mathematical description for melanin heating is presented in Svaasand et al (27) and Norvang et al (28). Single melanosomal and average melanin absorption coefficients can be calculated from the clinical response to short and long ruby laser pulses, where the mathematical expressions are presented in Equations A1 and A2 (see Appendix). As the melanosomes are assumed to be of one size and distributed uniformly in the entire $100 \mu\text{m}$ epidermal layer, the model is only approximate. Figure 9 shows the calculated average absorption coefficients for sun-protected and sun-exposed skin, based on the clinical response to the ruby laser pulses and from the measured reflectance spectra. Immediate whitening and hypopigmentation were used as criteria for melanosomal damage.

DISCUSSION

Measurements of optical properties of human skin in vivo require use of simplified models, such as the layered simulation method (8). Still, this model is demonstrated to account for individual differences in scattering and absorption properties. The true scattering

coefficients measured in this study fitted the range of reported values (18–24) well, while the average melanin absorption coefficients covered a wider range than the few reported values (18, 19, 26). The observed overlap between tanning codes and differences within codes [Fig. 7(a)] indicate the existence of a wide variety of optical properties among individuals, eg determined by body location, blood pressure, age, sun tan or race.

No significant differences were observed among the Caucasian groups or among these groups and the Asian group (Fig. 6). Quite different optical properties were, however, found within each group or race. The Caucasian-Persian spectra demonstrated slightly lower reflectance values than the other Caucasian spectra. This might be a result of few tested skin sites. All Caucasian and Asian spectra demonstrated lower melanin absorption coefficients than the African spectra, and showed very low reflectance and with curvature dominated by melanin absorption. Additional measurements are needed to detect possible differences in optical properties among Africans.

The reflected signal was averaged over the spectrophotometer aperture ($\approx 20 \text{ mm}$ in diameter) and in depth down to the optical penetration depth, about 1–2 mm, in the red to near infra-red part of the spectrum. This justifies the use of a planar model, in which average optical properties can be found, eg the average melanin absorption coefficient. The measured spectra do not contain information about localized absorbers or scatterers, since visible and near infra-red light is effectively scattered in skin. Exact localization of chromophores is, however, not critical when the pulse duration is long enough for heat to diffuse between them, as for 0.5 ms pulses.

The measured scattering and blood absorption properties are not significantly changed if the epidermal thickness differs from the chosen $100 \mu\text{m}$. This is not the case for the melanin absorption coefficient. The reflected signal 'sees' a total absorption rather than an absorption coefficient, and the melanin absorption coefficient is, therefore, only valid for the $100 \mu\text{m}$ thickness. Coefficients for thinner epidermal layers, in particular, are higher than the calculated ones, resulting in lower threshold radiant energy fluences. Separate measurements of the epidermal thickness can be performed, eg using low coherence methods or by normal and/or confocal microscopy.

Table 2. Minimal radiant energy fluence introducing clinical changes

Clinical change	Sun-protected skin (J cm^{-2})		Sun-exposed skin (J cm^{-2})	
	This study	Hruza et al (9)	This study	Hruza et al (9)
Minimal sensation	1.7 (1.2–2.2)	—	1.1 (0.8–1.6)	0.5 (0.2–0.9)
Erythema	1.7 (0.6–2.7)	1.8	1.1 (0.6–1.7)	1.1 (0.6–2.1)
Delayed oedema	2.1 (1.5–3.2)	—	1.6 (1.1–2.1)	1.4 (0.8–2.5)
Hyperpigmentation	2.6 (1.8–3.7)	1.5–2.5	1.5 (1.1–1.9)	1.5–2.5
Hypopigmentation	2.8 (1.4–4.0)	—	1.5 (1.1–1.9)	1.5–2.5
Immediate whitening	3.2 (2.2–3.6)	3.1 (1.6–4.2)	1.8 (1.3–2.5)	2.0 (1.5–2.8)

Short (<100 ns) ruby laser pulses. Range of fluence values in parentheses.

Fig. 9. Average absorption coefficient (m^{-1}) at 694 nm vs sun exposure based on response to ruby laser exposure in the free-running (FR) mode and from measured reflectance spectra. Solid bars, μ (whitening) (FR); open bars, μ (hypopigmentation); hatched bars, μ (simulated).

Threshold radiant energy fluences calculated from the reflectance spectra corresponded well with observed doses for when hypopigmentation occurred (Fig. 8). As hypopigmentation remained for more than 2 months for all skin sites, not only the melanosomes, but also the melanocytes, may be destroyed. The melanosomal population is more dense near the melanocyte nuclei (15). Light absorption by these melanosomes causes heating that may have brought the melanocyte nucleus temperature above the threshold for damage (28). Hypopigmentation might, therefore, occur at slightly lower temperatures than where the melanosomes themselves are destroyed, eg at temperatures for thermal denaturation, around 65–70°C (3). Furthermore, the critical exposure time for these longer laser pulses may be determined from the integral over the temperature rise rather than from the peak temperature rise (27). Threshold radiant energy fluences for immediate clinical changes, on the

other hand, were higher than the calculated ones for all tanning codes. The blanching observed instead of white crust formation was most likely due to separation of epidermis from the dermis. This is severe damage and probably occurred at high temperatures.

Hyperpigmentation occurred at approximately the same radiant energy fluences for sun-exposed and sun-protected skin (Table 1) when exposed to the ruby laser pulses. Hyperpigmentation limits the radiant energy fluence significantly in future laser treatments and should be avoided. The mechanisms for when it occurs are, however, not fully understood, and further investigations are required.

In port-wine stain therapy using the flash-lamp pumped dye laser, successful coagulation of the ectatic blood vessels usually requires radiant energy fluences in the range 6–10 J cm^{-2} . The regular laser therapy is, therefore, not suitable for treating African skin, where the threshold for melanosomal damage was found to be equal to 3.5 J cm^{-2} . No epidermal damage should occur at regular treatment doses for sun-protected white skin, while in sun-exposed skin, the treatment doses must be carefully chosen. One should remember that the threshold values may be lower, eg when the epidermis is thinner than the assumed 100 μm or the local density of melanosomes is higher. A possible solution to avoid both hypo- and hyperpigmentation, however, is using selective epidermal cooling (27, 29). A short cooling pulse delivered to the skin prior to laser admission protects the melanocytes in epidermis while preserving the temperature in dermis. Approximate absorption coefficients for single melanosomes can also be calculated from threshold radiant energy fluence values (Equations A1 and A3 in Appendix). Thus, the response to

short nanosecond pulses results in coefficients around $3\text{--}5.5 \times 10^3 \text{ m}^{-1}$ for sun-protected to sun-exposed skin, while for 0.5 ms pulses, the coefficients are about 10 times higher. This factor is, however, expected to be 100 times higher if the calculations are only based on thermal heating of the melanosomes (27). Other factors must, therefore, be involved, eg explosive evaporation resulting in expansion of the melanosomal interaction volume.

A reliable model for single melanosomal damage must account for acoustic wave generation and for the non-uniform melanosomal density. The latter may be modelled using a denser melanosomal population close to the melanocyte and keratinocyte nuclei, and with a larger distance between these 'packages'. Furthermore, threshold temperatures should be determined for both short and long pulses. Lastly, more research is needed to determine influence of melanin scattering, due to the different size and form, ie free melanin, inside melanosomes or melanosomes inside melanocytes. Scattering is very difficult to handle mathematically when the particle size is about equal to the wavelength, eg for melanosomes.

Analysis of reflectance spectra seems to be very useful for estimating average optical properties in vivo, such as the average melanin absorption coefficient. In the simulation model used in the present study, all parameters or concentrations of chromophores can easily be changed, and others included to analyse specific conditions or disease. Bilirubin, which has a characteristic absorption peak of 450 nm, is one such pigment that can be added. It has little impact on the analysis in this study, since bilirubin is normally present in small amounts in blood. Furthermore, the analysis did not emphasize this wavelength region. For analysis of spectra measured from patients suffering from hyperbilirubinaemia, however, the simulation method may be very useful.

CONCLUSION

The important issue in this study has been to relate threshold for damage to some parameter that can be measured in vivo. Visible reflectance spectra of human skin are demonstrated to reveal important and rational information about the skin pigmentation, despite simplifications and uncertainties in the simulation model. A wide range of optical properties is found within the same race and among races,

and individual analyses are therefore required. Average melanosomal absorption coefficients were used to calculate threshold radiant energy fluences, eg necessary for port-wine stain therapy. The calculated threshold doses were lower than threshold for both hypopigmentation and immediate clinical changes for all tanning codes. Furthermore, threshold values for hyperpigmentation were approximately equal to calculated threshold doses for melanosomal damage. Therefore, use of the lower, calculated threshold values prevents irreversible damage and may only result in minimal pigmentary changes. Reflectance spectra also contain information about other skin pigments, such as bilirubin, and estimating its concentration could be very useful in treating hyperbilirubinaemia.

Important optical properties can be estimated from visible reflectance spectra of human skin. The measurements are simple and quick to perform, and analyses of the spectra can, therefore, be used to determine threshold radiant energy fluences for laser treatments, such as for port-wine stain therapy.

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Key words: Threshold fluence; Melanin absorption; Light propagation; Skin reflectance; Absorption coefficients; Thermal diffusion

APPENDIX

The mathematical model for melanosomal heating and destruction of organelles is presented in Svaasand et al (27), and further investigated in Norvang et al (28). The model assumes spherical melanosomes with the same size, distributed uniformly in the entire 100 μm epidermal layer. The mean radius is based on either a single melanosome for heavily pigmented individuals or a cluster of melanosomes for lightly pigmented people. Furthermore, it is assumed that the laser energy is absorbed uniformly over the melanosomal volume. The melanosomal diameter is here assumed to be equal to 1 μm , with 4 μm distance between them.

Svaasand et al (27) found that the average melanin absorption coefficient could be found from the response to 0.5 ms ruby laser pulses, corresponding to heating a homogeneous medium. A threshold radiant energy fluence, E_{th} , determining melanosomal damage, denotes the dose for when immediate whitening occurs. This is assumed to correspond to a threshold temperature, ΔT_{th} , of approximately 110°C (6). The radiant energy fluence will be transmitted into the skin, subtracted from the specular reflectance, R_{sp} ($\approx 2.8\%$ for $n_{\text{skin}} = 1.4$). The fluence inside the skin is higher than the radiant energy fluence, E_{in} , due to a

high portion of back scattering inside the skin. This is described by the build-up factor of fluence $(1+\gamma/A)$. It is dependent on the diffuse reflectance value of the skin, γ , and the refractive index of skin and air, respectively, n_{skin} and n . The constant A describes the Fresnel reflection at the tissue-air interface from within the medium (30). For a refractive index of the skin of 1.4, $A \approx 1/6$. The average melanosomal absorption coefficient, $\mu_{a,m}$, then becomes:

$$\mu_{a,m} = \frac{\kappa \Delta T_{th}}{\chi} \frac{1}{\left(1 + \frac{\gamma}{A}\right) E_{th} (1 - R_{sp})} - \mu_{a,n} \quad (\text{A1})$$

where $\mu_{a,n}$ denotes a small background absorption in the epidermis [around 25 m^{-1} (8)] due to other absorption effects than melanin. The thermal diffusivity is χ , while κ is the thermal conductivity. Reported values for human skin are, respectively, $\chi = 1.1 \cdot 10^{-7} \text{ m}^2 \text{ s}^{-1}$ and $\kappa = 0.45 \text{ W mK}^{-1}$ (31). The base temperature is assumed to be 30°C , and the temperature rise from this level is ΔT .

The melanosomal absorption coefficient should, in principle, be found using the same method as described in Equation A1. The threshold radiant energy fluence, E_{th} , is obtained from the short nanosecond laser pulses, in which the pulse duration should be less than the thermal relaxation time ($< 2\text{--}3 \mu\text{s}$).

The melanosomal absorption coefficient can also be found using the longer 0.5 ms pulses. The total temperature rise in the single melanosome, with radius m , results from heat produced in the melanosome itself [Equation 12 in Svaasand et al (27)] and heat diffusing from the neighbouring melanosomes [Equation 16 in Svaasand et al (27)]. Damage occurs when the sum of these contributions bring the temperature rise above the threshold of 80°C . The corresponding threshold energy, Q_{th} , is then found for the single melanosomes. This threshold energy is also equal to the absorbed fluence in the single melanosome with radius m :

$$Q_{th} = \frac{4}{3} \pi m^3 \mu_{a,m,\text{single}} \left(1 + \frac{\gamma}{A}\right) E_{th} (1 - R_{sp}) \quad (\text{A2})$$

Combining the two expressions for the threshold energy, Q_{th} , gives the melanosomal absorption coefficient, $\mu_{a,m,\text{single}}$:

$$\mu_{a,m,\text{single}} = \frac{\kappa \tau_p \Delta T_{th}}{\left(\frac{m^2}{2} + \frac{4}{3} \pi m^3 N \chi \tau_p\right)} \frac{1}{\left(1 + \frac{\gamma}{A}\right) E_{th} (1 - R_{sp})} \quad (\text{A3})$$

where the melanosomal density is given by the average distance, d , between the melanosomes, $N = 1/d^3$, and the laser pulse duration is given by τ_p .