Effect of Nail Thickness on Visible Radiation Transmittance: Implications for New Photodynamic Therapy Technologies in Onychomycosis

Enrique Navarrete- de Gálvez, José Aguilera, María Victoria de Gálvez, Pablo Fonda-Pascual, Enrique Herrera-Ceballos, Alfonso Gago-Calderón

First published: 13 June 2020

https://doi.org/10.1111/php.13296

Abstract

Photodynamic therapy is taking importance as a nonintrusive treatment for nail onychomycosis. Knowledge of true transmittance values across nails could lead to qualitative and quantitative improvements in light-based treatments. We have characterized the spectral transmittance of healthy and fungally infected human fingernails and toenails according to nail thickness, and we propose a surface transmittance model for the small-scale optimization of light-based treatments. Transmittance of fingernails and toenails was analyzed by means of spectroradiometric measurements under solar-simulated visible light radiation (400 nm to 750 nm). The nail thickness was measured by means of microscope measurement. Transmittance was highest at longer wavelengths and decreased gradually as the wavelengths became shorter but with a significant nail transmittance of around 20% in the blue region of the spectrum. In the case of nails affected by onychomycosis, transmittance fell to under 10% because of the thickness of the nails, with no changes in spectral characteristics of transmitted light. Nail thickness is the main variable controlling exponentially light transmission in the visible spectrum and not only red radiation is effective for nail onychomycosis PDT. Blue light, the spectral band more effective for PPIX absorption is also effectively transmitted.

Introduction

Nails are exposed to potentially harmful environmental influences, such as bacteria and fungi, which, alongside other factors such as injury, unsuitable footwear and genetic susceptibility, can cause nail lesions. Onychomycosis, which is a fungal infection of the nail, is the most common cause of nail lesions (1, 2). It

affects 2.18% of the population, and its incidence is growing due to the aging of the population, the rising incidence of immune disorders and use of immunosuppressive therapy, and the growing practice of sport and use of nonbreathable footwear(1). Onychomycosis can have a significant impact on quality of life, causing pain, impaired tactile function and anxiety due to noticeable cosmetic defects (2). Conventional treatment for onychomycosis includes proper hygiene and a combination of topical and systemic treatments (3). Although systemic treatment clears the infection in 70% to 95% of cases, it is associated with adverse effects, such as nausea, toxic hepatitis and gastrointestinal problems. In addition, it requires long treatment periods (3–6 months) and a high degree of self-discipline and can also interact with other drugs (3).

Photodynamic therapy (PDT), a newer treatment option for onychomycosis, involves the administration of a topical photosensitizer that is absorbed by target cells and converted to a light-sensitive substance. When activated by light in the presence of oxygen, this substance undergoes a photochemical reaction that leads to oxidative stress and ultimately cell death. The level of activation varies according to the photosensitizer and wavelength used. Although several studies have shown that PDT is effective in onychomycosis (4-6), none of them have explored in-depth parameters that are critical for the correct application of PDT, such as nail thickness and, more importantly, transmission of light through the nail.

It has been reported a very high variation of doses applied to PDT, in the range of 18–108 J cm−2. This has been used based on experience of different dermatological units treating nail onychomycosis and dermatophytes with TFD. Moreover, selected wavelength in nail treatments is based on the presence of the type of light source normally used for skin PDT in medical services. Most of works use *in vivo* red led light exposure (630 nm maximum wavelength) due to the presence of red lamps for normal PDT (4, 5). Thus, nail PDT does not take into account nail thickness and structure, as well also nail curvature in finger for a real light transmittance of light. In addition, the doses used to treat fungal infections of the nails are similar to those used to treat skin conditions, with no consideration given to the potential loss of irradiance that occurs as the light crosses the keratinized nail plate. In addition, this loss could be even greater in nails thickened by fungal infection.

The aim of this study was to perform a quantitative and qualitative analysis of light transmitted through healthy and infected nails at different wavelengths to determine the most effective parameters for delivering optimal PDT outcomes in conditions affecting the nails.

Materials and methods

We analyzed a total of 18 nails from human fingernails and toenails. The toenails were all from the first toe and included healthy nails and nails affected by onychomycosis. The fingernails were all healthy and were from all five fingers. They had been removed from recently deceased individuals in the anatomy department. The infected toenails were coming from normal surgical procedures in which the patient has received total removal of the nail, and in normal hospital procedure, this material is destroyed. We informed to the patients that before destruction of the nail it was going to be analyzed for our work, and we received written consent that is enough for this type of procedure following the laws in Spain. No ethical approval from Ethical board of our institution was necessary. Secondly, the nails proceeding from Anatomy Department of the University of Málaga are from human donation to the University education and/or research finality that have followed all the legal donation regulation. After light transmission analysis, nails were returned to the Anatomy Department. We followed ethical declaration of Helsinki for this kind of study.

To be included in the study, the nails had to have a uniform circular area that was large enough (at least 1 cm2) to cover the optical sensor. This assessment was made macroscopically. Nails that met these criteria were cleaned by soaking them in soap and water for 3 min at 25°C. They were then scrubbed with a nail brush, cleaned with ethanol, rinsed and dried to ensure that all traces of dirt and cosmetic products had been removed. Before each measurement, the nails were placed in distilled water for 3 min and dried with filter paper to reproduce as closely as possible the conditions of natural hydration and transparency.

Spectral transmittance was measured at wavelengths between 400 nm and 750 nm to cover most of the visible light portion of the electromagnetic spectrum (7). Transmittance was measured using a 300-W Oriel solar simulator (Newport Corporation, Irvine). The spectral irradiance emitted by the solar simulator and transmitted by the different nails analyzed were measured using a Ulbricht UV-visible integrating sphere connected by fiber optic to a double monochromator spectroradiometer (Macam SR-2274, Irradian Co, Scotland). The wavelength and irradiance of the spectroradiometer are calibrated annually at the Spanish National Metrology Center using a certified UV-visible calibration lamp.

For the measurements, the integrating sphere was placed 5 cm from the solar simulator and perpendicular to the light beam. The spectral transmittance of each nail was measured by placing the outer part of the nail facing the light beam, just above the aperture of the sphere (Fig. 1).

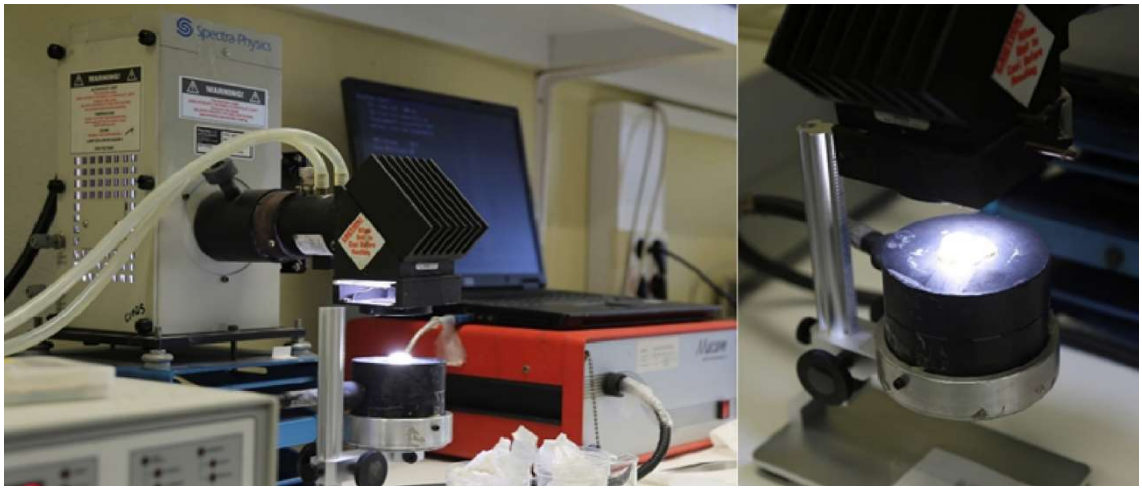

Figure 1

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Image of the illumination system (solar UV-visible simulator) as well as the transmitted light acquisition system with the nail directly situated on the light entrance of the integrating sphere connected to the double monochromator spectroradiometer

Prior to measurements, we have verified that no significant effect of the nail curvature in the light transmission was observed. Nail was disposed on a plane by help of two laboratory tweezers, and light transmission was compared with measurements in the same nail following the normal curvature. No significant differences were observed, and no differences were observed in light transmission across the nail positioned to the light beam in the normal external side or to the internal side. By using an integrating sphere as light sensor, the effect of dispersion of light due to nail structure is minimized.

The effect of nail thickness was also analyzed. Two cross-sectional strips of 1 mm in width were cut from each nail: one from the lunula (the white crescent at the proximal end) and one from the free edge of the

nail. Both strips were placed between the two arms of a pointed-tip forceps and compressed until they were completely flat. The forceps were placed on a graphic scale and an image captured of both the nail and the scale using a digital microscope with a magnification range of 10–200X and a resolution of 640 × 480 (Dino-Lite AM3113T). All images were processed in AutoCAD version 2019. The thickness of each nail strip was measured at three points (the two ends and the center) (Fig. 2). No differences in measurements were observed from the plane of the nail in vertical position with respect to the graphic scale situated on bottom or at the same plane of the nail measurements.

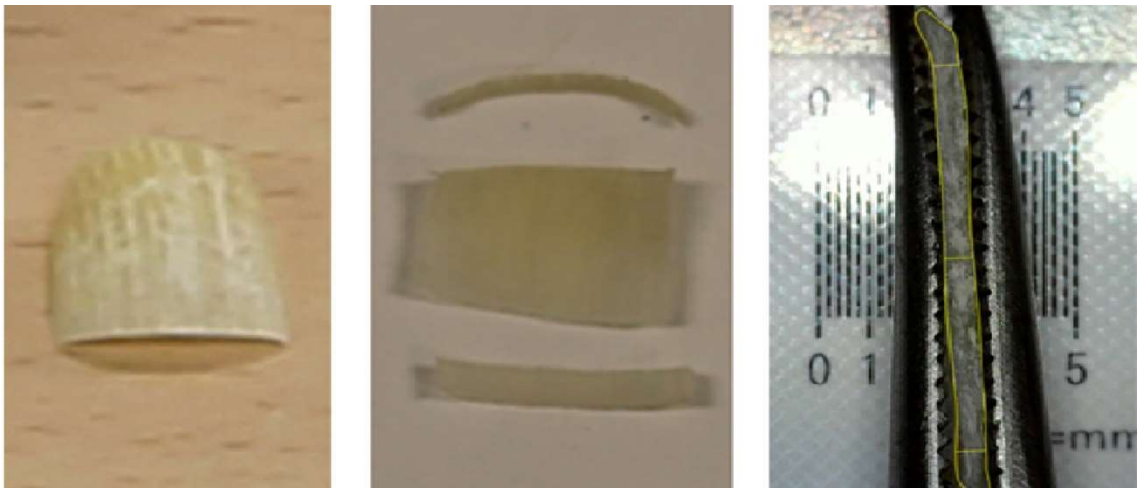

Figure 2

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Nail measurements. Left, full nail sample; Centre, strips cut from the lunula and free edge of nail. Right, microscopic image of free edge strip showing the three sections where thickness is measured.

Since nails become thicker from the base to the free edge, measurements of the nail thickness were taken from mean values of lunula piece and of from the free edge of the nail and we consider the mean value from those side measurements (8).

Statistics

We performed a regression analysis for relationship between light transmittance *vs* nail thickness for wavelengths of 400–409–450–460–500–509–540–550–600–630–634–650 and 700 nm. From this first individual wavelength analysis, we could perform a second regression analysis for new values of the constants “a” and “b” (Formula 1) with respect to each wavelength.

Results

Our results showed a decrease in irradiance transmitted through the nail plate across the visible spectrum. Reduction was greatest at wavelengths with higher energy.

The transmission spectra for the different nails shows how the nail plates behaved differently depending on the wavelength used, with greater transparency seen at lower-energy (longer) wavelengths. A similar tendency was observed for the reduction in irradiance, with lower transmittance values observed at short wavelengths and higher values observed at long wavelengths. The differences in transmittance were attributable to varying nail thickness (Fig. 3).

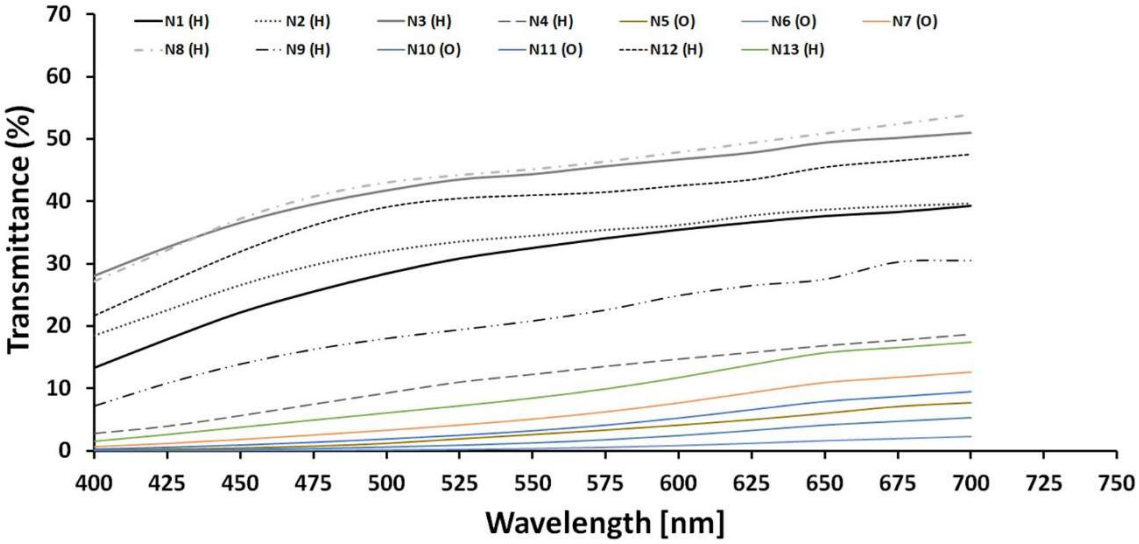

Figure 3

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Spectral transmittance at 400 nm to 750 nm for thirteen nails, with air as reference. H= healthy, O= Onychomycosis.

It is difficult to provide a representative transmittance value for a given wavelength, as this value is an exponential function of nail thickness and therefore highly sensitive to change. Transmittance must therefore be measured separately in each case. Nevertheless, in order to show how such calculations could be used to optimize PDT, we will conservatively use the mean transmittance values calculated for our sample at three main wavelengths: 14% for violet (409 nm), 25% for green (509 nm) and 31% for red (634 nm).

The association between nail thickness and transmittance at the three wavelengths is shown in Fig. 4. Violet, green and red were chosen as these wavelengths coincide with the maximum absorption peaks for protoporphyrin IX. The distribution of the data was fitted to a negative exponential regression model, and the correlation coefficients were above 0.9 in all three cases (Fig. 4A–C).

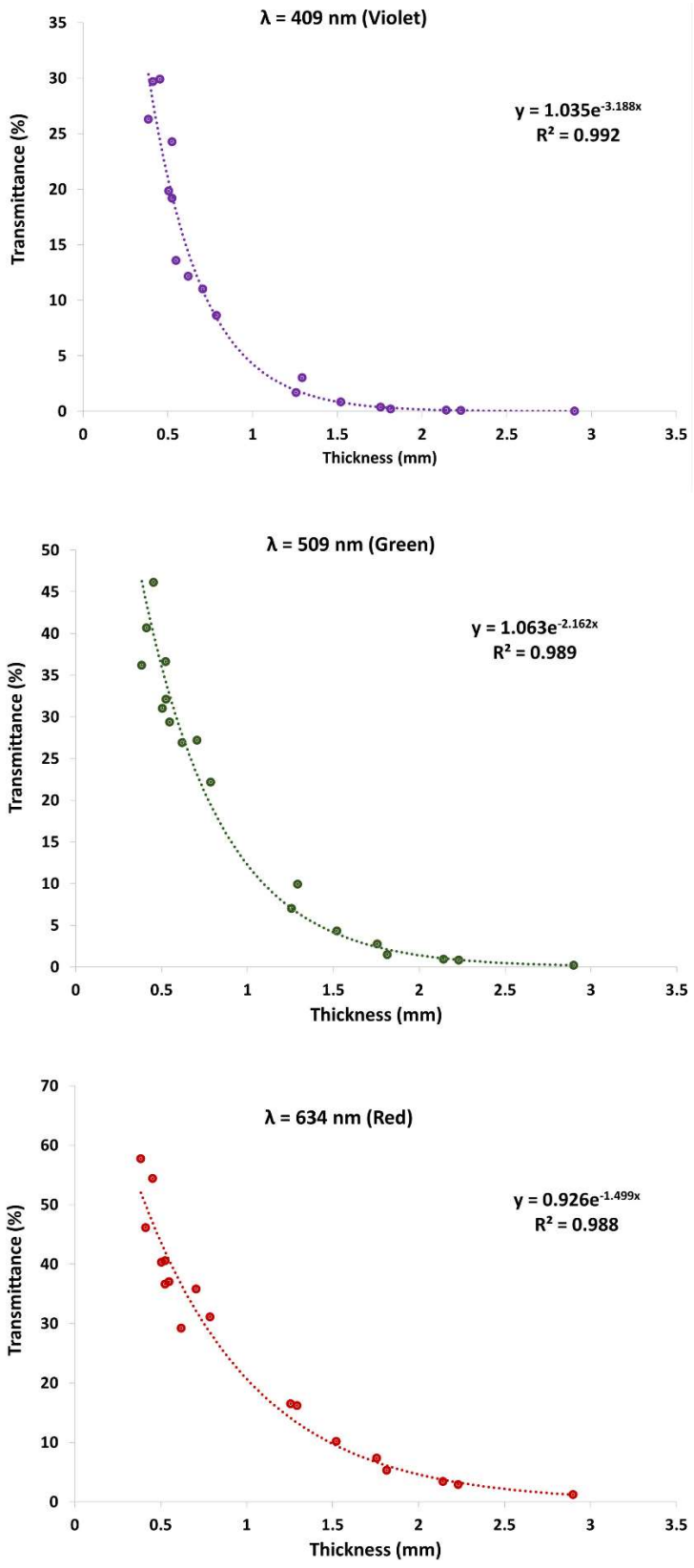

Figure 4

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Transmittance of nails at wavelength maxima peaks for protoporphyrin IX absorbance with respect to nail thickness. (A) 409 nm (violet), (B) 509 nm (green) and (C) 63 nm (red)

The following exponential regression functions were calculated for transmittance relative to nail thickness: = 1.03516e−3,18839x at 409 nm, = 1.06325e−2,16164 x at 509 nm and = 0.92603 e−1,49881x at 634 nm.

The spectral transmittance of the nail with respect to the thickness and for each wavelength was obtained from this multiple superficial regression model:

T=a·eb·x(Formula 1)

a=c1·λ4+c2·λ4+c3·λ3+c4·λ2+c5·λ+c6

b=c7·λ3+c8·λ2+c9·λ+c10

where as follows: T: Transmittance; x: Thickness of nail in mm; λ: Wavelength in nm; C_1: 2212E-12; C_2: −6727E-09; C_3: 0,000008119; C_4: −0,004859; C_5: 1,43928; C_6: −167,326; C_7: −4267E-09; C_8: −6621E-06; C_9: 0,01808; C_{10}: −9,1856.

Figure 5 features a 3D diagram showing how transmittance varies according to nail thickness and wavelength. Note how loss of transmittance increases with thickness at the shorter (blue-purple) wavelengths. The slope of the curve is also steeper at these wavelengths, indicating that PDT protocols that use these wavelengths are more amenable to optimization.

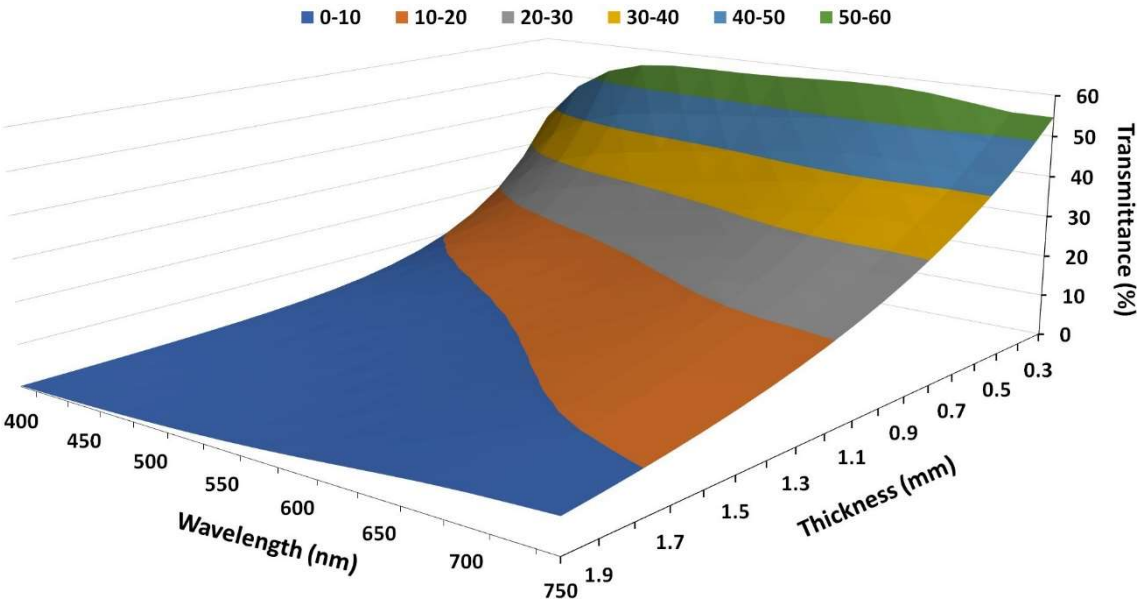

Figure 5

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Regression surface showing transmittance as a function of wavelength and nail thickness

Discussion

The advent of PDT led to significant improvements in the treatment of numerous skin disorders, many of which represent a major public health problem worldwide. Thanks to modern spectrum analyzers, we now have a better understanding of the different dynamics that occur during PDT, but several aspects need to be further investigated whether we are to optimize this treatment.

We have demonstrated that transmittance is affected by nail thickness. In particular, we have shown that the amount of light capable of passing through the nail plate decreases exponentially with increasing thickness

and that the constants vary according to the wavelength of light employed. Using the results of the different experiments conducted, we have performed a new reference equation, based in application of Lambert–Beer´s law model, for estimating transmittance losses in nails treated with PDT according to the wavelength of light applied and the thickness of the nail. We put deep attention in the special characteristics of infected nails, with differences in nail structure and change of color to darker nails in most of them. However, no changes in light quality transmission were observed for infected nails. In case of change of color, we must detect a change in transmission depending on wavelengths absorbed by pigmentation, but gray color did not lead to transmittance measurements any change in quality of transmission. The change in structure of the infected nail could confer a differential behavior due to differences in light dispersion. However, the very high increase in nail thickness of infected nails compared to the healthy ones makes that the decrease of light transmission is so high in the whole spectrum that possible changes due to the other variables (color, structure) were no significant. When we included infected nails in the models of light transmission due to thickness, those one fitted with a very high correlation to the model.

This formula can be used to determine optimal light delivery (intensity, frequency and/or duration) tailored to individual treatment sites, regardless of their characteristics, thereby preventing over- and underexposure and ultimately ensuring that the photosensitizer (drug) is used to maximum effect. Such corrections will enable both qualitative and quantitative improvements in PDT applied in different settings.

Beyond the fact that the proposed empirical model presents a precise adjustment to the reality of the phenomenon, it is considered interesting for future studies to analyze the adjustment of new mathematical models developed for heterogeneous media with strong light absorption (9).

PDT protocols described in the literature vary in terms of number of sessions, doses (J cm−2), and fluence rates (mW cm−2), type and concentration of photosensitizer, and light source (4, 5, 10, 11). In the case of conditions that directly affect the nail plate or nail bed, such as onychomycosis and psoriasis, we found fluences ranging from 2 J cm−2 to 144 J cm−2 and fluence rates ranging from 10.3 mW cm−2 to 100 mW cm−2 (4, 5, 10). None of the protocols had been adapted to account for nail thickness. According to our formula, a fluence of 37 J cm−2 at a red light wavelength of 636 nm in a PDT protocol for the treatment of skin psoriasis would need to be increased to 84.77 J cm−2 to treat the skin underlying a nail with a thickness of 0.5 mm, as the transmittance for this thickness and wavelength according to formula 5 is 43.65%. Similar calculations could be made to adapt other protocols to varying situations, including nails thickened by onychomycosis. In the case of particularly thick nails, filing down the nail or slicing off part of the plate before applying PDT is recommended for higher transmittance efficiency.

Improvements to light source design can be critical to optimizing the photodynamic reactions that determine the success of PDT (10, 11). One of the main features of LED technology is that it can be used to create light sources (lamps) with very well-defined photometric emission characteristics that can operate at a single output spectrum or at several spectra through the combination of different sources or the use of photon-conversion filters like phosphorus coatings (12, 13). This flexibility permits the creation of PDT devices that can be used to treat a range of disorders, such as onychomycosis, acne and rosacea, superficial and nodular basal cell carcinoma, Paget disease and nail psoriasis (4). When designing a PDT lamp for onychomycosis and other nail disorders, it is important to characterize the frequencies required to ensure that the lamp meets the needs detected during experimental analyses like ours.

Modern LED lamps can be designed to emit radiation in very narrow wavelength bands, meaning that the full power of the lamp can be concentrated at the most effective peaks for the photosensitizer being used. According to data from several studies, the most effective wavelengths for destroying target cells in the absorption spectrum of protoporphyrin IX are 409, 509, 544, 548 and 634 nm (6, 14).

From our nail transmittance results, LED PDT lamps must be provided with lamps that emit in the three wavelengths (409–509–634 nm) to maximize the absorption of the photosensitizer protoporphyrin IX. LED technologies actually provide lamps in those maxima peaks. These wavelengths can be used separately or combined. So, it is possible to reach similar dose of light under all the three wavelengths at the defined target independent of the wavelength transmittance properties.

The results of our experiments imply an optimization of PTD treatment of nail and nail bed disorders. They also update results from previous studies on the transmission of optical radiance through human nails, which showed significantly lower values than those detected in our study using double monochromator spectroradiometer connected to an Ulbricht sphere (15). The values in our study ranged from 10% higher for the 400–600 nm region to 23% higher for the 360 nm region, independently of nail thickness. Overall, the mean difference in transmittance was approximately 18%. We also extended the wavelength range analyzed to the whole visible region from 600 nm to 750 nm.

Finally, by generating a 3D thickness-wavelength-transmittance regression surface using exponential thickness-transmission regression adjustments for different wavelengths, all with correlation coefficients above 0.9, we derived a sufficiently precise mathematical function for calculating nail transmittance at given wavelengths to optimize PDT light delivery according to the thickness of the nail being treated. This thickness will need to be calculated in each case. High correlation coefficients for the regression models allow to us to predict the selection of wavelength and light doses for fingernails and toenails with different characteristics as well as it opens the possibility of development of intelligent light devices for nail irradiation.

Conclusion

It has been possible the determination of constants “a” and “b” of a formula based on Lambert–Beer´s law in order to predict light transmission across the nail depending on the wavelength and the nail thickness. To calculate optimal treatment parameters, it is necessary to know the absorption spectrum of the photosensitizer and the thickness of the nail that is being treated. Nail thickness is the variable that mainly affects the exponential light transmission in the visible spectrum. As well as to red radiation, mainly used in nail PDT treatments, blue light, the spectral band more effective for PPIX absorption, is also effectively transmitted. With this model, by calculating the thickness of the nail or nails being treated, we will be able to calculate transmittance at the working wavelength and adapt the dose accordingly, resulting in both qualitative and quantitative improvements in the use of PDT.

Acknowledgements

Authors would like to thank specially to Dr Pablo Salinas and Dr Santiago Vidal Asensi for providing samples for the present study. The patients in this manuscript have given written informed consent to publication of their case details. This work is part of the research of the Institute of Biomedicine of Málaga (IBIMA) and the Junta de Andalucía working group CTS-162.

Conflict of interests

None declared.

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