Theoretical and Experimental Study of the Water-Based Drug Delivery under the Nail Plate by Er-Laser Radiation

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Abstract. Double-stage active laser drug delivery (DSLADD) of an aqueous solution of methylene blue using Er:YLF laser radiation experimentally was investigated. Model for describe of the delivery of local drugs under a microporated nail plate taking into account the peculiarities of laser radiation exposure, capillary effects in microholes and diffusion in the nail plate was developed. The phenomenon of spreading of the drug consisting in the fact that after laser action drug is distributed in the nail bed not only in the area of the nail bed located directly under the microhole, but also along the border of the nail plate and the nail bed around the microhole was observed for the first time. Calculations performed within the framework of the developed model showed that, taking into account spreading phenomenon, the density of filling the nail area with microholes can be significantly reduced, which can proportionally increase the productivity of laser delivery in general. © 2022 Journal of Biomedical Photonics & Engineering.

Keywords: dual-stage method for active laser drug delivery; drug delivery model; laser microporation; nail; nail permeability coefficient.

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1 Introduction

Diseases of the nails, especially fungal infections, are difficult to treat [1]. Peroral drug delivery remains the most popular delivery method due to its ease of implementation, but this delivery method can negatively affect the gastrointestinal tract and liver before the drug enters the systemic circulation. Local drug delivery largely avoids these problems, but has very low efficacy, primarily due to the extremely low permeability of drugs through the nail plate. In addition, drugs for local delivery, as a rule, quickly lose their molecular-diffusible form after application due to the rapid evaporation of the solvent, which prevents them from effectively penetrating the nail [2].

High density and hardness of the nail plate provides excellent protection for sensitive fingertips. Unfortunately, these properties make local drug delivery extremely difficult. The penetration of the drug into the nail plate occurs due to passive diffusion and is limited by the strong cross-link's characteristic of the plate structure. On the other hand, the physicochemical properties of drugs also determine the speed and depth of their penetration into the nail. The permeability of the nail plate is mainly influenced by the active molecular weight of the drug [3]. Currently, in the local treatment of fungal diseases of the nail, antifungal agents of the 2nd generation (imidazoles) and antimycotics of the 3rd generation (triazoles and allylamines) [4, 5], methylene blue (MB) [6] and chlorin-containing photodynamic drugs [7] are used.

To increase the permeability and efficiency of delivery of local drugs, chemical (acids, alcohols, glycols, etc.), physical (microporation, ionophoresis, sonophoresis, electroporation, ultrasonic and laser drug delivery) and mechanical methods (injection, removal, grinding or mechanical perforation biological tissue) can be used [8–11]. Effective laser microporation of the nail is realized using CO_2 and erbium lasers [12, 13]. The radiation of erbium lasers is effectively absorbed by water containing in nail plate and α-keratin which is the main component of the nail [14]. As a result of the transformation of absorbed laser energy to heat, a biological tissue is heated and ablated [13]. Compared to other methods of enhancing the drug penetration, including microneedling and microdermabrasion, laser drug delivery (LADD) through microporation of the nail

demonstrates greater safety, convenience, and effectiveness [15].

The delivery of drugs to the microporated nail plate can be passive or active, that is, it can occur without or as a result of any external influence. With passive delivery, water-based drugs do not penetrate through the microporated nail plate to the nail bed due to the high coefficient of surface tension [16]. With active delivery, external influences, for example, laser-induced hydrodynamic processes, can stimulate delivery and increase the rate of drug penetration [17]. The choice of a laser source for delivery is essential. It is important to exclude the possibility of changing the properties of the drug under the action of laser radiation. In this regard optimal is the impact not on the drug itself, but on the substance in which it is dissolved. It is known that the Er:YLF laser has a wavelength of 2.81 μm and is very effectively absorbed by water [18]. The high value of Er:YLF laser radiation absorption in water provides effective heating and transformation of the laser energy into mechanical energy, which is expressed in the generation of acoustic waves leading to the delivery of drug dissolved in water. In this case, the laser radiation acts on water and not on the drug, excluding the transformation of the latter as a result of the direct action of light. Active laser delivery methods are constantly being improved, for example, in articles [19–21], the capabilities of a new double-stage active laser drug delivery (DSLADD) method are discussed and demonstrated *in vitro* for the first time. DSLADD includes nail microporation using Er:YLF laser radiation through the layer of drug applied to the nail surface and subsequent exposure of this layer to the radiation of the same laser to deliver the drug under the already microporated nail plate. It is obvious that *in vivo* the effectiveness of DSLADD may differ from that *in vitro* due to the anatomically tighter connection of the nail to the underlying tissues *in vivo*. Thus, testing DSLADDs *in vivo* is an actual task.

The peculiarity of the current level of development of technologies for the drug delivery through a microporated nail is that its rate is difficult to predict. This is a serious limitation for the widespread use of the microporation method in clinical practice. An important characteristic that determines the drug delivery after nail microporation is the coefficient of drug penetration through such a nail plate. Obviously, the coefficient of drug penetration when using microporation to improve local drug delivery depends on both the properties of the nail plate and the filling density and the dimensions of the microholes (micropores).

There is a known mathematical model that makes it possible to estimate the rate of drug delivery through microporated skin [22]. This model leads to a relatively simple equation of skin resistance and demonstrates that the approach based on the calculation of the flow proportional to the total perimeter of the microhole predicts significantly higher flows than the approach based on the calculation of the flow proportional to the area of a microhole. The results of calculations based on this model were in good agreement with the experimental data on drug delivery to the skin presented in the literature [23–25]. However, the model assumes that the drug instantly penetrates into the microhole, which is not entirely true for microholes with small dimensions (radius) where capillary effects are present. In addition, this model assumes that the stratum corneum of the skin is completely impervious to topical drugs and thus does not take into account the diffusion of the drug in the skin. Thus, for an adequate description and optimization of the process of local drugs delivery under the nail plate, it is relevant to create a mathematical model to assess the rate of drug penetration through a microporated nail plate, which takes into account the contribution of capillary effects and drug diffusion in the nail plate.

The aim of this study was (1) to investigate of DSLADD of an aqueous solution of methylene blue using Er:YLF laser radiation experimentally, (2) to develop a model for the delivery of local drugs under a microporated nail plate taking into account laser radiation influence on the process of the drug delivery through the micropores in the nail plate created by the laser radiation, capillary effects in microholes and diffusion in the nail plate, (3) to estimate the coefficient permeability of a microporated nail plate for an aqueous solution of methylene blue using this model, and (4) to compare the simulation and experimental results.

2 Materials and Methods

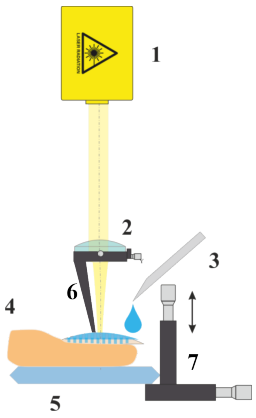
2.1 Experimental Setup for Investigate of DSLADD of a Methylene Blue Aqueous Solution Using Er:YLF Laser Radiation

In an *in vivo* experiment, the distribution of methylene blue (MB) under the nail plate after DSLADD was investigated. For active delivery of MB, an Er:YLF laser radiation ($\lambda = 2.81$ μm) was used. The study used a healthy nail of finger of one volunteer, which was mechanically cleaned of dirt and washed with distilled water before the experiments. The average thickness of the nail plate measured along the free edge of the nail was 365 ± 20 μm. The scheme of experimental setup and the appearance of the experiment are shown in Fig. 1.

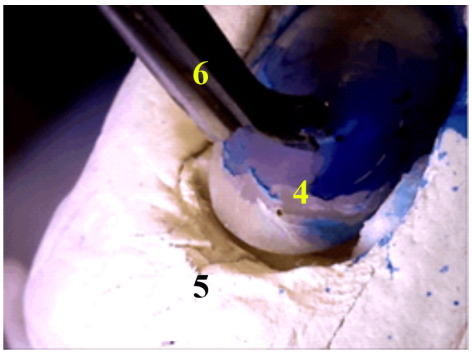
First, according to recommendation of Ref. [19] a microhole was created in the nail plate as a result of *in vivo* exposure the volunteer's nail to ten Er:YLF laser pulses through a layer of aqueous MB solution ($C = 0.001\%$) with a thickness of $h = 100 \pm 10$ μm applied to the nail surface. Laser pulse energy was $E = 4 \pm 0.1$ mJ, and the pulse repetition rate was $f = 30$ Hz. Then, active laser delivery of MB was carried out as a result of exposure the same MB layer to four Er:YLF laser pulses with the same radiation parameters as for microporation. Thus, 14 laser pulses were used for DSLADD through a single microhole. In total, in the experiment, ten microholes located at a distance of about 1 mm from each other were created and analyzed.

After DSLAAD, the appearance of the nail with the results of active laser delivery was recorded using a

microscope “Axio Scope A1” (“Carl Zeiss”, Germany) with a camera “Axiocam ERc 5s” (“Carl Zeiss”, Germany).



(a)



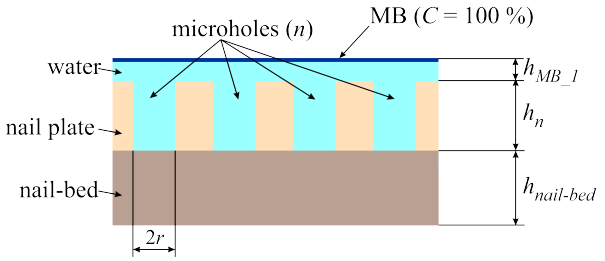
(b)

Fig. 1 Scheme of experimental setup (a) and appearance of the *in vivo* experiment (b) on DSLADD of MB solution using Er:YLF radiation: 1 – Er:YLF laser; 2 – collecting lens (F = 50 mm), 3 – injection needle with MB solution; 4 – volunteer’s finger; 5 – finger fixing device, 6 – focal plane fixator, 7 – XYZ-translator.

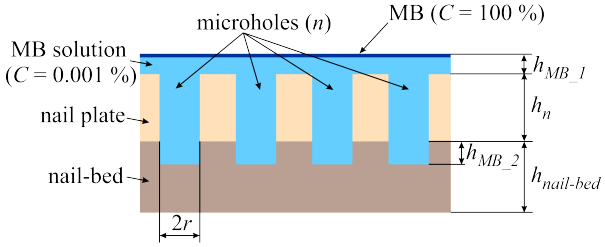
In the course of the study, it was found that with *in vivo* active Er:YLF laser delivery of MB, the effect of laser radiation on the drug leads to its spreading along the surface of the nail bed, i.e. to the fact that it is distributed in the nail bed not only in the area of the nail bed located under the microhole, but also along the border of the nail plate and the nail bed around the microhole. To assess this phenomenon from photographs, the diameter d_{MB} of the area of the nail bed containing MB and the diameter d of the microhole in the nail plate were measured in the “CorelDRAW Graphics Suite” (“Corel”, Canada) software.

2.2 Theoretical Model for the Delivery of Local Drugs under a Microporated Nail Plate

When creating a model describing the process of drug (MB) penetration under a micropore nail, it was assumed that after laser microporation, cylindrical microholes (with a radius of r) are formed in the nail plate, which reach the nail bed. The structural model of a microporated nail illustrating its state before and after drug (MB) penetration into the nail bed is shown in Fig. 2. It is assumed that the microholes are located at equal distances from each other and form a certain density of filling the area of the nail with microholes n_p (number of microholes per cm^2). The concentration of the applied drug (MB) is kept constant and equal to 100 % at a distance of h_{MB_1} = 100 μm above the nail plate. The penetration of the drug to a depth of h_{MB_2} = 100 μm is determined by the diffusion of the drug into the nail bed (nail epidermis). After diffusion, the concentration of drug (MB) in the aqueous solution becomes 0.001%.



(a)



(b)

Fig. 2 Structural model of a microporated nail to describe passive drug (MB) delivery through the nail plate – before (a) and after (b) delivery of drug (MB) through microholes.

We assume that the flow of the drug through the microporated nail plate will be equal to the sum of the flow through the microholes and the flow through the intact nail plate. In addition, the model takes into account the spreading mentioned above (see Section 2.1) of the MB under the nail plate caused by hydrodynamic processes during DSLADD. In this regard, we believe that with such spreading of the drug, the complete filling of the area under the nail plate with the drug, which is obviously necessary for the effective treatment of fungal disease, can be achieved with a smaller number of microholes in the nail plate than without spreading. However, this assumption has not been previously investigated.

According to Ref. [22], for an intact nail plate, the coefficient of drug penetration through the nail bed is defined as:

$$K_{p\_o} = \frac{1}{R_0} = 0, \quad (1)$$

where R_0 is the diffusion resistance to the drug penetration through the intact nail plate and the nail bed.

In the absence of a nail plate, the permeability coefficient of the drug through the nail bed is determined as [22]:

$$k_{p\_no\_nail} = \frac{1}{R_{no\_nail}} = \left(\frac{h_{MB\_1}}{D_{drug}} + \frac{h_{MB\_2}}{K_s \times D_{nail-bed}} \right)^{-1}, \quad (2)$$

where D_{MB} is a coefficient of drug (MB) diffusion in a layer of water with a thickness of 100 μm located on the surface of the nail, $D_{nail\text{-}bed}$ is a coefficient of drug diffusion in the nail bed, K_s is distribution coefficient characterizing the change in the concentration of the drug at the border between the nail bed and water, R_{no_nail} is diffusion resistance to the penetration of the drug through the nail bed, h_{MB_1} is the thickness of the applied drug (methylene blue) in the form of an aqueous solution over the nail plate, h_{MB_2} is the depth of the drug penetration into the nail bed.

Considering the model developed for the skin and presented in Ref. [22], under the assumption that the delivered drug (MB) instantly penetrates into the microhole, and the stratum corneum is completely impervious to local drugs, and replacing the skin model with a nail model (see Fig. 2), we find that the coefficient of drug permeability through the microporated nail and nail bed can be determined as:

$$k_{p\_no\_tr} = \frac{1}{R_{no\_tr}} = 4n_p \times r \times \times \left((1 + \frac{4h_n}{\pi r}) \times \frac{1}{D_{MB}} + \frac{1}{K_s \times D_{nail-bed}} \right)^{-1}, \quad (3)$$

where D_{drug} is a coefficient of drug (MB) diffusion in a layer of water with a thickness of 100 μm located on the surface of the nail and in a microhole initially filled with water, $D_{nail\text{-}bed}$ is a coefficient of drug diffusion in the nail bed, K_s is distribution coefficient characterizing the change in the concentration of the drug at the border between the nail bed and water in microhole, R_{no_tr} is diffusion resistance to the penetration of the drug through the microporated nail and the nail bed, on the assumption that the aqueous solution of drug (MB) instantly penetrates into the microhole and the nail is completely impervious to local drugs, h_n is the thickness of the nail plate, r is the microhole radius, n_p is the density of filling the nail area with microholes.

Considering the model presented in Ref. [22], in the case of the skin, it is assumed that the water into which the drug diffuses instantly penetrates the micropore, and also that the stratum corneum is completely impervious to topical drugs. Therefore, in case of the nail, diffusion of the drugs in the nail plate also is not taken into account. At the same time, the coefficient of permeability of the drug through the nail plate k_{p_nail} depends on the molecular weight MW of the drug as follows [4]:

$$k_{p\_nail} = 10^{(-3.11-1.75\times\log(MW))}. \quad (4)$$

In case of MB molecular weight MW = 319 Da [26] and $k_{p\_nail} = 3.224\times10^{-8}$ cm/s.

In addition, in Ref. [22], it is assumed that initially the microholes are filled with water and not air, as is usually the case after microporation. Consequently, capillary effects arising at the interface between the solid (nail plate), liquid (water) and gaseous (air in the microholes in the nail plate) phases are not taken into account. At the same time, the movement of liquid in micro-holes with a small radius depends on the surface tension force. With a high coefficient of surface tension, water-based drugs do not penetrate into microholes due to the resistance caused by surface tension forces Δp_σ, which can be estimated by the formula [27]:

$$\Delta p_\sigma = \frac{4\sigma}{d}, \quad (5)$$

where σ is the coefficient of surface tension, $d = 2r$ is the diameter of microhole.

Taking to account the Eq. (5), speed of steady liquid flow $\Delta v = \sqrt{\frac{4\sigma}{\rho r}}$ [28] and nail plate thickness h_n, the resistance R_{CE}, caused by only the forces of the surface tension can be defined as

$$R_{CE} = h_n \times \Delta v = h_n \times \sqrt{\frac{2\Delta p_\sigma}{\rho}} = h_n \times \sqrt{\frac{4\sigma}{\rho r}}. \quad (6)$$

Since the studied MB aqueous solution has a low concentration ($C = 0.001\%$), in calculations it can be assumed that the surface tension of the MB solution at the interface with air in microholes is equal to the surface tension of water at the interface with air in microholes: $\sigma = 72.86\times10^{-3}$ N/m (at temperature T = 20 °C) [29], and the density of aqueous solution of MB is assumed to be equal to density of water $\rho = 997$ kg/m^3.

The coefficient of drug permeability through a microporated nail plate and nail bed, taking into account R_{no_tr} (3) and R_{CE} (6) is determined as:

$$k_{p\_with\_CE} = \frac{1}{R_{with\_CE}} = 4n_p r \times \times \left((1 + \frac{4h_n}{\pi r}) \times \frac{1}{D_{MB}} + \frac{1}{K_S \times D_{nail-bed}} + \frac{1}{h_n \sqrt{\frac{4\sigma}{\rho r}}} \right)^{-1}, \quad (7)$$

where r is the radius of the microhole, R_{with_CE} is the diffusion resistance to the penetration of the drug through the microporated nail and the nail bed considering capillary effects.

The permeability coefficient of the drug through the microporated nail plate and the nail bed considering capillary effects and the coefficient nail plate permeability is determined as:

$$k_p = \frac{1}{R} = 4n_p r \times \left((1+\frac{4h_n}{\pi r}) \times \frac{1}{D_{MB}} + \frac{1}{K_S \times D_{nail-bed}} + \frac{1}{h_n \sqrt{\frac{4\sigma}{\rho r}}} \right)^{-1} + \left(1 - n_p \pi r^2\right) \times k_{p\_nail}. \quad (8)$$

The coefficient $1 - n_p \pi r^2$ allows to consider the diffusion only through the part of the nail plate, which does not have micropores. In the absence of micropores ($n_p = 0$), this coefficient is equal to 1, and the permeability coefficient $k_p = k_{p\_nail}$. With the maximum possible density of filling the surface of the nail plate with micropores without overlapping, $n_p = n_{p\ max} = 1/(2r)^2$, and k_p reaches the maximum value in this case.

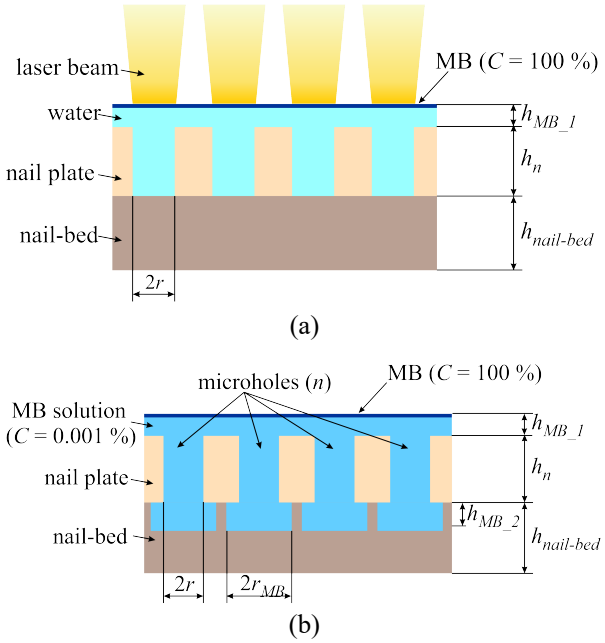


Fig. 3 Structural model of a microporated nail to describe active drug delivery (MB) through the nail plate – before (a) and after (b) delivery of drug (MB) through microholes.

To overcome the resistance caused by the forces of surface tension, laser radiation can be used. It stimulates the acoustic waves and pressure, which can be much greater than the forces of hydraulic resistance. As noted above (see Section 2.1), during *in vivo* active Er:YLF laser delivery of MB, the action of laser radiation on the drug leads to spreading of the drug, i.e. it is distributed in the nail bed not only in the area of the nail bed located directly under the microhole, but also along the border of the nail plate and the nail bed around the microhole (see Fig. 3 (a, b)). To take into account the contribution of this phenomenon to the area from which diffusion into the nail bed occurs, we used the drug distribution coefficient k_d, which is defined as the ratio of the diameter $d_{MB} = 2r_{MB}$ (where r_{MB} is the radius of the nail bed area under the nail plate containing the delivered drug) to the diameter of microhole in the nail plate $d = 2r$ (see Fig. 3b).

The spreading effect of is associated with the action of laser radiation only on the drug in the microholes and therefore does not affect the drug diffusion through the part of the nail plate, which does not have micropores. In this regard, the contribution of the drug distribution coefficient k_d should be taken into account only in the first term of Eq. (8) associated with $k_{p\_with\_CE}$.

Based on the foregoing, the coefficient of the drug permeability through the laser-microporated nail plate into the nail bed, taking into account capillary effects, the permeability coefficient of the nail plate k_{p_nail} and the drug distribution coefficient k_d, is determined as:

$$k_{p\_\Sigma} = \frac{1}{R_\Sigma} = 4n_p r k_d \times \left((1+\frac{4h_n}{\pi r}) \times \frac{1}{D_{MB}} + \frac{1}{K_S \times D_{nail-bed}} + \frac{1}{h_n \sqrt{\frac{4\sigma}{\rho r}}} \right)^{-1} + \left(1 - n_p \pi r^2\right) \times k_{p\_nail}, \quad (9)$$

where R_Σ is the diffusion resistance to the penetration of the drug through the microporated nail and the nail bed, taking into account capillary effects, the coefficient of permeability of the nail plate and the coefficient of distribution of the drug.

For the subsequent modeling, the following coefficients were taken: $D_{MB} = 0.38 \cdot 10^{-5}$ cm^2/s [41], diffusion coefficient of the drug (MB) in the nail bed $D_{nail\text{-}bed} = 1.98 \cdot 10^{-6}$ cm^2/s [30], coefficient $K_s = 5.58$ [31].

3 Results and Discussion

3.1 Experimental Study of DSLADD of an Aqueous Solution of Methylene Blue Using Er:YLF Laser Radiation. Estimation of the Coefficient of Drug Distribution under the Nail Plate k_d.

In our study, methylene blue was used as the topical drug to be delivered under the nail plate. Methylene blue is a well-known antiseptic, has disinfecting and analgesic effect. The choice of this drug is also often due to the fact that it has a deep blue color and is well visualized. Usually in clinical practice, an aqueous or alcoholic solution of MB with a concentration of $C = 1$–3 % is used [32]. In some cases, including during photodynamic

therapy (PDT), the concentration of MB can be reduced [33]. Methylene blue is a widely used photosensitizer for PDT of fungal diseases [34]. It has a high redox potential and has an absorption maximum in the spectral range 550–700 nm [33]. In photodynamic therapy using MB, radiation is used that corresponds to its maximum absorption. It is known that the concentration of MB in water from the range $C = 0.001 \pm 0.01\%$ is most effective for the generation of singlet oxygen $_1O^2$ during antimicrobial photodynamic therapy with a diode laser with a wavelength of 660 nm [33]. At $C = 0.001\%$ in water, methylene blue is in the monomeric form [35]. It is known that the combined effect of an aqueous solution of MB with $C = 0.001\%$ and laser radiation with a wavelength of 662 nm on lactobacilli, Candida Albicans fungus, and Staphylococcus Aureus bacteria significantly reduces their colonies [36]. In our study, the concentration of MB in an aqueous solution is $C = 0.001\%$.

A typical appearance of the dorsal surface of the nail after an *in vivo* DSLAAD of methylene blue through a single microhole as a result of exposure to 14 pulses of Er:YLF laser radiation with an energy of 4 ± 0.1 mJ is shown in Fig. 4.

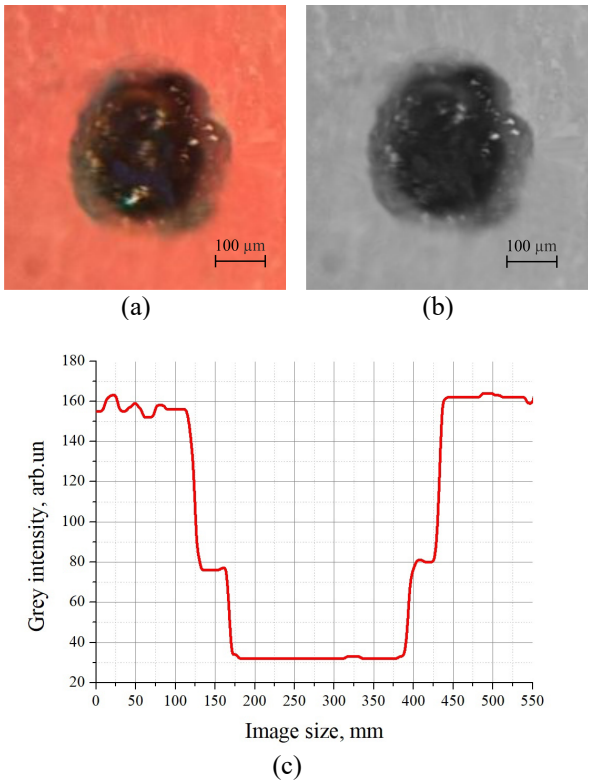


Fig. 4 (a) Typical color photo of a nail plate after an *in vivo* DSLAAD, (b) converted Gray image of the color photo shown in Fig. 4aб and (c) the Gray intensity distribution along the diameter of the microhole shown in Fig. 4b.

During image processing, a color photograph of the nail plate (Fig. 4a) was converted into a Gray image (Fig. 4b). To estimate the diameter of the MB-containing area under the nail plate in this image, the Gray intensity (*GI*) distribution along the diameter of the microhole was analyzed (Fig. 4c). Gray intensity analysis was carried out using the "Mathcad 14" (PTC, Inc., Canada) software package, where intensity "0" is black and "255" is white. Gray intensity was measured in two mutually orthogonal directions and averaged over 10 photographs of the nail plate obtained after DSLAAD.

As a result of processing the entire array of photographs after DSLAAD, it was found that the image of a microhole in the nail plate corresponds to Gray intensity $GI = 32 \pm 11$. Thus, the average microhole diameter is 220 ± 10 μm, which coincides with the values presented earlier in Ref. [19]. The image of the intact nail plate has $GI = 160 \pm 10$, and the image of the area around the microhole containing the MB has $GI = 76 \pm 16$. Thus, the average diameter of the area of the nail bed under the nail plate containing the delivered drug (MB) is 312 ± 32 μm. Thus, in *in vivo* DSLAAD with Er:YLF laser radiation, the drug distribution coefficient is $k_d = 1.42 \pm 0.22$.

3.2 Theoretical Study of the Delivery of Local Drugs under Microporated Nail Plate

The results of modeling the coefficient of MB permeability through a laser-microporated nail plate into the nail bed depending on the area of microholes formed in the nail plate, obtained at various approximations described above for microhole diameter 220 μm and $k_d = 1.42$ are shown in Fig. 5.

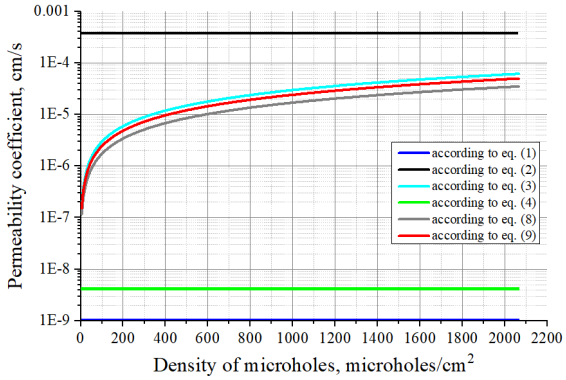


Fig. 5 Dependence of the permeability coefficient k_p on the density of filling the nail area with microholes n_p.

It can be seen that an intact nail plate has a minimum permeability coefficient, according to Eq. (4) (green line) $k_{p\_nail} = 3.224 \times 10^{-8}$ cm/s. In the absence of a nail plate, the permeability coefficient, according to Eq. (2) (black line), is maximum and corresponds to $k_{p\_no\_nail} = 28.61 \times 10^{-5}$ cm/s.

With a microhole diameter of 220 μm, the maximum possible density of filling the nail area with microholes without overlapping of microholes corresponds to 2066 microholes/cm^2. It can be seen that, without taking into account capillary effects and the coefficient of permeability of the nail plate, the coefficient of permeability of the drug through the microporated nail plate and the nail bed, according to Eq. (3) (blue curve)

$k_{p\_no\_tr} = 6.15\times10^{-5}$ cm/s. The results of calculation the permeability coefficient of the drug taking into account capillary effects, according to Eq. (7), very close to results of calculation taking into account capillary effects and the coefficient nail plate permeability according to Eq. (8) (gray line). The value of the drug permeability coefficient, when taking into account capillary effects and the permeability coefficient of the nail plate, obtained according to Eq. (8) (gray curve) is $k_p = 3.506\times10^{-5}$ cm/s, which is 1.75 times less than the value of permeability coefficient obtained according to the Eq. (3). Obviously, this result is related to the resistance to the penetration of liquid into the microhole in the nail plate caused by the forces of surface tension and diffusion resistance to the penetration of the drug through the nail plate. In this case, the contribution of diffusion resistance to the penetration of the drug through the nail plate is insignificant in comparison with the contribution of the resistance to penetration of liquid into the microhole in the nail plate caused by surface tension forces.

The model, which takes into account the coefficient of distribution of the drug k_d in addition to capillary effects and the coefficient of permeability of the nail plate k_{p_nail}, increases the coefficient of permeability of the drug through the microporated nail plate and the nail bed by increasing the area on which the MB solution diffuses into the nail bed. In this case, with a filling density of 2066 microholes/cm^2 and $k_d = 1.42$, according to Eq. (9) (red curve), the permeability coefficient of the drug through the microporated nail plate and the nail bed increases by 1.42 times relative to the model that takes into account only capillary effects and the coefficient of permeability of the nail plates, and reaches $k_{p\_\Sigma} = 4.978\times10^{-5}$ cm/s. This can be explained by the fact that since in this case the second term $(1 - n_p\pi r^2)\cdot k_{p\_nail}$ in Eq. (9) is negligible, then the change in k_{p_Σ} is mainly determined by the drug distribution coefficient k_d. That is, due to the effect of drug spreading along the surface of the nail bed associated with the action of laser radiation during DSLAAD the density of filling the nail area with microholes can be significantly reduced (in this case, 1.42 times) without change in k_{p_Σ}, which can proportionally increase the productivity of laser delivery in general.

It should be noted that the values $k_{p\_nail} = 3.224\times10^{-8}$ cm/s and $k_{p\_\Sigma} = 4.978\times10^{-5}$ cm/s obtained in this work allow to conclude that the effect of Er:YLF laser radiation increases the permeability of the drug (MB) under the nail proportion to the ratio of these coefficients, i.e. 1544 times. It is known [37] that an effective photodynamic therapy of onychomycosis when applying the MB to the surface of a nail plate, which does not contain micropores, takes 8 sessions, separated by interval in 2 weeks (the duration of each session is 480 s including 180 s for drug application and 300 s for photodynamic irradiation). As a result, a significant reduction in the Onychomycosis Severity Index (OSI) was observed, and 70% of patients reached complete cure 12 weeks after the end of therapy. Thus, the laser microporation and delivery of MB under the nail plate by radiation of Er:YLF laser, taking into account the spreading effect, may allow to achieve a similar treatment effect, significantly reducing the duration of drug application to 0.12 s.

4 Conclusion

For the first time, the possibility of a two-stage active laser delivery of a photodynamic drug (MB) under the nail plate by Er:YLF laser radiation has been investigated and demonstrated *in vivo*. The distribution coefficient of the drug k_d was experimentally estimated, which is defined as the ratio of the diameter of the nail bed area under the nail plate containing the delivered drug to the diameter of the microhole in the nail plate. An original, relatively simple analytical model was developed to assess the permeability of a drug through a laser-microporated nail plate. The model considers the contribution of the drug spreading effect under the nail plate caused by effect of laser radiation on the drug during DSLADD and the resistance caused by surface tension forces. As a result of calculations within the framework of the developed model, it was found that for DSLAAD of an aqueous solution of MB with Er:YLF laser radiation with a laser pulse energy of 4 ± 0.1 mJ, the distribution coefficient is $k_d = 1.42 \pm 0.22$, and the density of filling the nail area with microholes is $n_p = 2066$ microholes/cm^2, and the permeability coefficient MB through the microporated nail plate and the nail bed reaches $k_{p\_\Sigma} = 4.978\times10^{5}$ cm/s.

Disclosures

All authors declare that there is no conflict of interests in this paper.

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References

1. C. C. Lai, C.-K.Tan. Y.-T. Huang, P.-L. Shao, and P.-R. Hsueh, "Current challenges in the management of invasive fungal infections," Journal of Infection and Chemotherapy 14(2), 77–85 (2008).
2. J. C. T. Wang, Y. Sun, "Human nail and its topical treatment: brief review of current research and development of topical antifungal drug delivery for onychomycosis treatment," Journal of Cosmetic Science 50(1), 71–76 (1999).

3. S. Murdan, "Drug delivery to the nail following topical application," International Journal of Pharmaceutics 236(1–2), 1–26 (2002).
4. S. M. Baswan, S. K. Li, and G. B. Kasting, "Diffusion of uncharged solutes through human nail plate," Pharmaceutical development and technology 21(2), 255–260 (2016).
5. A. K. Gupta, F. C. Simpson, "Investigational drugs for onychomycosis," Expert Opinion on Investigational Drugs 23(1), 97–106 (2014).
6. L. W. Figueiredo Souza, S. V. T. Souza, and A. C. C. Botelho, "Randomized controlled trial comparing photodynamic therapy based on methylene blue dye and fluconazole for toenail onychomycosis," Dermatologic Therapy 27(1), 43–47 (2014).
7. A. V. Belikov, Yu. V. Semyashkina, S. N. Smirnov, and A. D. Tavalinskaya, "Investigation of Changes in the Absorption Spectrum of Modern Chlorine-Containing Medicines for Photodynamic Therapy and Methylene Blue as a Result of Exposure to LED Emissions with a wavelength of 656±10 nm," Optics and Spectroscopy 128(7), 980–988 (2020).
8. V. Mathur, Y. Satrawala, and M. S. Rajput, "Physical and chemical penetration enhancers in transdermal drug delivery system," Asian Journal of Pharmaceutics 4(3), 173–183 (2010).
9. S. Mitragotri, J. Kost, "Low-frequency sonophoresis: a review," Advanced Drug Delivery Reviews 56(5), 589–601 (2004).
10. N. A. Monteiro-Riviere, A. O. Inman, and J. E. Riviere, "Identification of the Pathway of Iontophoretic Drug Delivery: Light and Ultrastructural Studies Using Mercuric Chloride in Pigs," Pharmaceutical Research 11(2), 251–256 (1994).
11. D. I. J. Morrow, P. A. McCarron, A. D. Woolfson, and R. F. Donnelly, "Innovative strategies for enhancing topical and transdermal drug delivery," The Open Drug Delivery Journal 1(1), 35–59 (2007).
12. M. T. Tsai, T.-Y. Tsai, S. C. Shen, C. Y. Ng, Y.-J. Lee, J.-D. Lee, and C.-H. Yang, "Evaluation of Laser-Assisted Trans-Nail Drug Delivery with Optical Coherence Tomography," Sensors 16(12), 2111 (2016).
13. J. Neev, J. S. Nelson, M. Critelli, J. L. McCullough, E. Cheung, W. A. Carrasco, A. M. Rubenchik, L. B. Da Silva, M. D. Perry, and B. C. Stuart, "Ablation of human nail by pulsed lasers," Lasers in Surgery and Medicine: The Official Journal of the American Society for Laser Medicine and Surgery 21(2), 186–192 (1997).
14. E. G. Bendit, "Infrared absorption spectrum of keratin. I. Spectra of α-, β-, and supercontracted keratin," Biopolymers: Original Research on Biomolecules 4(5), 539–559 (1966).
15. X. Chen, D. Shah, G. Kositratna, D. Manstein, R. R. Anderson, and M. X. Wu, "Facilitation of transcutaneous drug delivery and vaccine immunization by a safe laser technology," Journal of Controlled Release 159(1), 43–51 (2012).
16. A. V. Belikov, A. V. Skrypnik, A. N. Sergeev, S. N. Smirnov, and A. D. Tavalinskaya, "Er:YLF-laser microperforation of the nail plate for drug delivery," Proceedings of SPIE 10716, 107160X (2018).
17. D. F. Lyons, V. Le, G. L. Bidwell III, W. H. Kramer, E. A. Lewis, D. Raucher, and J. J. Correia, "Structural and hydrodynamic analysis of a novel drug delivery vector: ELP [$V_5G_3A_2$-150]," Biophysical Journal 104(9), 2009–2021 (2013).
18. G. Hale, M. Querry, "Optical constants of water in the 200nm to 200micron wavelength region," Applied Optics 12(3), 555 (1973).
19. A. V. Belikov, A. D. Tavalinskaya, and S. N. Smirnov, "Investigation of the Dual-Stage Method of Active Er:YLF Laser Drug Delivery Through the Nail and Laser-Induced Transformations of the Drug Extinction Spectrum," Lasers in Surgery and Medicine 53(8), 1122–1131 (2021).
20. A. V. Belikov, A. D. Tavalinskaya, S. N. Smirnov, and A. N. Sergeev, "Active Er-laser drug delivery using drug-impregnated gel for treatment of nail diseases," Biomedical Optics Express 10(7), 3232–3240 (2019).
21. A. V. Belikov, S. N. Smirnov, and A. D. Tavalinskaya, "Laser delivery and spectral study of a chlorin-containing drug for the treatment of onychomycosis with sequential laser (λ = 2819 nm) and photodynamic (λ = 656 ± 10 nm) exposure," Optics and Spectroscopy 129(6), 698–706 (2021).
22. A. S. Rzhevskiy, R. H. Guy, and Y. G. Anissimov, "Modelling drug flux through microporated skin," Journal of Controlled Release 241, 194–199 (2016).
23. G. Yan, K. S.Warner, J. Zhang, S. Sharma, and B. K. Gale, "Evaluation needle length and density of microneedle arrays in the pretreatment of skin for transdermal drug delivery," International Journal of Pharmaceutics 391(1–2), 7–12 (2010).
24. S. Zhang, Y. Qiu, and Y. Gao, "Enhanced delivery of hydrophilic peptides in vitro by transdermal microneedle pretreatment," Acta Pharmaceutica Sinica B 4(1), 100–104 (2014).
25. Y. Bachhav, S. Summer, A. Heinrich, T. Bragagna, C. Böhler, and Y. N. Kali, "Effect of controlled laser microporation on drug transport kinetics into and across the skin," Journal of Controlled Release 146(1), 31 – 36(2010).
26. A. K. Saikia, S. Aggarwal, and U. K. Mandal, "Electrically induced swelling and methylene blue release behaviour of poly (N-isopropylacrylamide-co-acrylamido-2-methylpropyl sulphonic acid) hydrogels," Colloid and Polymer Science 293(12), 3533–3544 (2015).

27. K. F. Pavlov, P. G. Romanov, and A. A. Noskov, Examples and tasks in the course of processes and apparatuses of chemical technology, Ripol Classic, Moscow (1987) [in Russian].
28. G. V. Taranenko, "Calculation of the hydraulic resistance of failure-type trays in the mode of a moving gas-liquid layer," ScienceRise 6(2), 23 (2016) [in Russian].
29. M. V. Masterov, M. W. Baltussen, and J. A. M. Kuipers, "Influence of the free surface on hydrodynamics in a bubble column," Chemical Engineering Science: X 8,100077 (2020)
30. E. A. Basco, M. V. Klement'eva, A. N. Bashkatov, V. V. Tuchin, and E. A. Genina, "Diffusion of methylene blue into rat skin ex vivo," Problemy opticheskoy fiziki i biofotoniki. SFM-2017 47 (2017) [in Russian]. ISBN 978-5-98116-224-4.
31. S. Mishra, P. Kumar, "Attenuation of methylene blue dye during riverbank filtration through sandy aquifers," Water and Environment Journal 29(4), 507–514 (2015).
32. I. I. Jack Clifton, J. B. Leikin, "Methylene blue," American Journal of Therapeutics 10(4), 289–291 (2003).
33. C. Komine, Y. Tsujimoto, "A small amount of singlet oxygen generated via excited methylene blue by photodynamic therapy induces the sterilization of Enterococcus faecalis," Journal of Endodontics 39(3), 411–414 (2013).
34. L. W. F. Souza, S. V. T. Souza, and A. C. de C. Botelho, "Distal and lateral toenail onychomycosis caused by Trichophyton rubrum: treatment with photodynamic therapy based on methylene blue dye," Anais Brasileiros de Dermatologia 89(1), 184–186 (2014).
35. W. Spencer, J. R. Sutter, "Kinetic study of the monomer-dimer equilibrium of methylene blue in aqueous solution," Journal of Physical Chemistry 83(12), 1573–1576 (1979).
36. A. A. Selifonov, O. G. Shapoval, A. N. Mikerov, and V. V. Tuchin, "Determination of the diffusion coefficient of methylene blue solutions in dentin of a human tooth using reflectance spectroscopy and their antibacterial activity during laser exposure," Optics and Spectroscopy 126(6), 758–768 (2019).
37. E. Alberdi, C. Gomez, "Efficiency of methylene blue-mediated photodynamic therapy vs intense pulsed light in the treatment of onychomycosis in the toenails," Photodermatology, Photoimmunology & Photomedicine 35(2), 69–77 (2019).