Assessment of the Suitability of Excimer Lasers in Treating Onychomycosis

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Abstract

Since it is known that UV-C radiation kills fungus, we wanted to verify the hypothesis that the use of excimer laser could be an alternative method for treating onychomycosis - nail fungus. The aim of the first stage of this work was to determine the transmission, reflection and absorption of nails. In the following stage we focused on irradiation of fungi. Our final task is to assess whether it is possible to determine the parameters of radiation (a total dose, a dose per pulse frequency, a repetition rate, a number of pulses) for which the elimination of fungi would be the most effective but without damaging the nail and soft tissue underneath it. The results so far have showed that UV-C radiation does not pass through a fingernail to such an extent that it could damage the soft tissue beneath it. Fungi are destroyed by the application of only small doses of radiation using the excimer laser. Additional measurements will be required to determine the modulation parameters of the excimer laser radiation for the treatment of onychomycosis.

Keywords: Excimer laser, UV-C radiation, nails, onychomycosis
Introduction

Onychomycosis is caused by various kinds of parasitic fungi, mold or yeast. Our research is focused on the fungus Trichophyton rubrum. This type of fungus was cultured from samples from patients treated for onychomycosis. Such cultivation is time consuming and is carried out in Petri dishes or in glass tubes on glucose agar. Trichophyton rubrum is the anamorphic fungus from the genus of fungi in the family Gymnoascaceae (Teleomorfa Arthroderma). T. rubrum has minor antigenic properties and the human immune system responds to it only mildly. Patients, who are free from any difficulties caused by T. Rubrum, serve as a long-term source of infection. Trichophyton rubrum induces pathological changes in the keratin structures - in skin, hair and nails. The most common cutaneous mycosis caused by T. Rubrum is Tinea Pedis, most often localized on the skin between toes. Onychomycosis is a medical condition where nails are infected by fungus that feeds on keratin (a tissue forming the nail). It gradually grows through and decomposes it. It is said that this disease affects 8% of population. The main causes of the disease include: wearing closed and airtight shoes made of inappropriate materials, use of public swimming pools, trauma affecting legs during longer exercise, certain diseases - especially diabetes and vascular diseases, but also obesity, increased sweating of feet - especially in the summer, wider use of antibiotics and corticosteroids.

Fig.1 - Nail damaged by onychomycosis

Treatment is topical, in more serious cases systemic (oral), or combined, but always long and problematic. Its choice depends on the seriousness of each clinical condition. Local therapy using antifungal (AF) solutions have a chance only if there is a maximum of 30% nail plate damage. AF varnish can be applied in conditions with up to 50% of nail plate damage. When changes affect 50-90% of a nail plate, the topical treatment usually fails. If changes are visible all the way to the rear ball of a nail, it means that the fungal infection (which in fact extends 3-5 mm further beyond the visible changes) has hit the nail matrix. This is a clear indication that the use of an oral, or combined, treatment is needed. The local treatment does not significantly affect the patient’s body; however, it puts high demand on patient's cooperation. Before the administration of medication, it is important to remove all visibly damaged parts of the nail (by cutting out, sanding or chemical ablation) and then the area has to be thoroughly treated until its full clinical recovery. The treatment can take many months. Throughout all this time, the patient must apply AF solution twice a day or AF varnish twice a week. AF creams are not suitable for nail
treatment, as cream can be quickly wiped off from the nail surface, so that the active
substance does not manage to permeate the keratin. AF solution, on contrary, seeps into
crevices of the damaged nail, the nail plate and the nail balls. In addition, AF varnish creates
after drying an occlusion (enclosed film) that further supports the diffusion of the active
ingredient. In the case of a systemic (overall) treatment, the AF ingredient permeates after oral
administration from the blood to the nail bed. If the AF level is sufficient, the originator of the
problem is unable to multiply and destroy the other parts of the nail. A new healthy nail starts
to grow and continuously displaces the affected parts. The overall AF cure must be taken daily
or in monthly cycles. However, systemic therapy has a number of side effects as well as
contraindications.

The first trials in treating fungal nail disease using laser radiation appeared in 1980. Initially,
however, it was more of a thinning or ablation of the afflicted nail, or digging holes in the nail
using high power lasers with the subsequent application of ointment. It was not until 2009,
when the use of lasers that cause no destruction of the nail began. This type of lasers should
act upon the originator of the mold.

For the treatment of onychomycosis, there are currently used so called Footlasers. As of 15
October 2010, the Pinpointe FootLaser (Nd: YAG pulse laser operating near 1064 nm) has
been granted FDA (Food and Drug Administration) clearance for use on onychomycosis.
Nd: YAG laser (1064 nm) is a fixed-phase device with active environment consisting of
yttrium aluminum garnet. The laser is driven by a burning krypton discharge tube and has a
power output of 100-200 W. Some modern workplaces use a Q-switched Nd: YAG laser,
which uses very short pulses with high energy.

The CoolBreeze laser uses 1320nm mid-infrared wavelength.

The next type of such a laser is the Noveon laser that uses 870nm and 930nm concurrently.
Results have been published in the Journal of the American Podiatric Medical Association. In
2010 physicians in 12 podiatric offices conducted a study of 199 toenail patients with a total
of 687 infected toes. Patients were evaluated at 90 day intervals. Following treatment, 85% of
the treated toenails showed clear linear nail improvement at day 180 and 35% of these showed
further improvement at day 270.

A problem is that there are no studies that would explain how these lasers affect
onychomycosis from the physical point of view. Considering their wavelength and high
energy, it is assumed that it is the heat effect that acts on the mold. Infrared radiation,
especially in the area of IR-A (760-1400 nm), penetrates into the tissue most deeply. It can
penetrate skin to a depth of several millimeters.

Similarly, there is a lack of extensive clinical studies that would in the longer term evaluate
and compare this method with pharmacological treatments.

No study provides information about the long term safety of this method, since there is a risk
of damage to the nail bed and, therefore, temporary or even permanent loss of a nail.
Published experience indicates great effectiveness of this treatment, but these are files of only
several dozen patients, and the evaluation criterion is only a photograph and evidence of nail
growth without any signs of onychomycosis.

Last year an article about laser systems for the new treatment area of onychomycosis was
published in the Journal of the American Podiatric Medical Association. As of January 2012,
the US FDA has approved four laser systems for the “temporary increase of clear nail in
onychomycosis.” The FDA has approved these devices on the basis of “substantial
equivalence” to predicate devices with similar technical specifications and applications. Laser
therapy appears to be a promising alternative to traditional pharmacotherapy, but these
systems have been tested in only limited clinical trials.
Radiation with a wavelength less than 300 nm is extremely effective in destroying microorganisms. Most sterilizing is the UVC light at a wavelength of 253.7 nm. This area is called the germicidal. UVC has a very small penetration capability and does not penetrate beyond the dead skin cells. Germicidal UV radiation does not kill cells directly. It damages their DNA, and the cells then become sterile.

We assume therefore that the use of a laser emitting a laser beam in the UVC area should damage the DNA mold even by lower density of the energy radiation, and should not result in the transmission of radiation into the soft tissue beneath the nail.

Excimer laser active area consists of a specific type of molecules called dimers. Most excimer lasers radiate in the UV spectrum. They have minimal absorption depth in the tissue, allowing the removal of microscopic layers of tissue with minimal damage to the surrounding area. In our experiment, we used an excimer laser with a wavelength of 248 nm (with krypton-fluorine content).

Excimer is an unstable molecule that is produced only on a temporary basis as a result of the interaction of excited atoms (or excited molecules) with the atom (or molecule) in the basic state, and that cannot be created if both atoms (molecules) are in the basic state. Excimer lasers use electrical discharge excitation and electron beams. Excitation by electron beam is utilized in the construction of large lasers because these exhibit greater efficiency (about 10%).

Lasers excited by an electrical discharge are of a simpler structure than lasers excited by an electron beam. The mixture produces ions and excited atoms of rare earth elements. Ions, then, in a triple collision with atoms of the same type create molecular ions. These are then, in a subsequent process of recombination, converted into excimers.

Methods

Measuring transmission, reflection and absorption of nails.

A fiber spectrophotometer from Ocean Optics, model DH2000, was used as a source of UV light. This device emits electromagnetic radiation at wavelengths of 220-1050 nm. It consists of two sources. Deuterium, radiating at wavelengths between 220-400 nm, and halogen, radiating at wavelengths of 300-1050 nm. It was used to determine transmission, absorption and reflection properties of nail tissue. For the actual measuring of nail parameters, we used four samples. The first two were taken from a big toe, the other two from a thumb. Each of them had different thicknesses, which was necessary to take into account when analyzing the results. The nails were cleaned before taking the measurements mechanically as well as in an ultrasound bath. After assembling the apparatus for measuring transmission (see Figure 2), we performed reference measurements without the sample.
For observed reflections we used the same equipment as in the previous experiment. The only difference was in the optical cable, which was adapted to collect the reflected radiation (see Figure 3). The nail transmissions were measured for an area of 248 nm using high-performance KrF laser in pulse mode.

Monitoring the thermal response

For the actual monitoring of the thermal response we used the KrF (krypton - fluorine) excimer laser COMPEX F 205 Pro, Lambda Physik, radiating at a wavelength of 248 nm. Heat distribution was scanned by a Fluke Ti 50 thermal imaging camera from Ahlborn. With the help of double sided tape we fixed the sample nail onto a piece of cardboard. The laser beam was narrowed down to an aperture diameter of 4 mm. Laser power was set to 188 mJ. The laser pulse length is fixed - 25 ns. Given that the thermal camera does not capture images in a synchronized way with the laser, the scanning interval was set at a frequency of 20 Hz. The laser was operated manually at the highest frequency. In at least one experiment we hoped to approach as closely as possible to the state of a nail after the laser beam reaches it. This solution is not ideal, however, for determining whether the nail was warmed up, this experiment sufficed.
Laser energy (E): 188 mJ. Effective surface beam (S): 0.126 cm². The energy density is given by: \( \omega = \frac{E}{S} \), \( \omega = 188 / 0.126 \) which corresponds to \( \omega = 1492 \) mJ/cm².

**Mold irradiation**

T. Rubrum mold we irradiated using the excimer laser Lambda Physik, Compex Pro 248nm. To measure the energy applied in a pulse, we used a COHERENT FieldMax II device (Fig. 3). This value was particularly important to determine the energy density. To document the irradiated samples, we used a Sony A200 DSLR camera with a Tamron 90/2.8 macro lens, and an Olympus microscope. Irradiated samples were placed on the optical table using a stand (Fig. 4). A cover lid of the Petri dish with the mold Trichophyton rubrum was uncovered just before the start of irradiation. The experimental setup was standard, used for similar experiments. We used a circular diaphragm of diameter 0.5 cm, i.e. 0.196 cm². The irradiated sample mold was placed behind the diaphragm as closely as possible (a contact position is not possible because of the sample parameters) so as to prevent irradiation from scattered radiation. For the full assembly please see Figure 4.

The energy in the pulse was varied in the range from 144 mJ to 304 mJ (higher values caused undesired heating of tissue). The corresponding energy density varies in an interval from 0.74 J/cm² to 1.55 J/cm². Repeating frequency was set to 10Hz and the number of pulses was changed for every value of energy - to 200, 500 and 1000. After irradiating a circular footprint, we observed it with our eyes and with a help of microscope for one month for bad or unwanted changes (growth of fungus).

**Search for ablation thresholds on a sample of a human tissue**

On some dissected and freshly frozen human skin we sought to determine ablation thresholds. Before and after irradiation, we took detailed photographs of the tissue. Parameters were the same throughout the whole experiment - a circular footprint of a 2.6 mm in diameter, i.e. 5.31 mm²; and a repetition rate of 10 Hz.
Ablation threshold is the highest laser output, which does not interfere with the structure of a tissue, is for a toenail 128.37 mJ/cm². For therapeutic purposes, this extreme value must not be exceeded.

Results

Reflection, absorption and transmission of a nail sample are shown in the following graphs: (Figures 5 and 6)

Fig.5 - Reflection, Absorption and Transmission of a nail sample
(Sample nail– thickness 0.43 mm)
As the graph above shows, reflection emitted by the source across the spectrum is low - around 1%. As we measured both - transmission and reflection, we can, using the following formula $A = 100 - T - R$ [%] (where $A$ is absorption, $T$ is transmission and $R$ is reflectivity), calculate the value of absorption. All figures are shown in percentages. The measured results of transmission indicate that nails are impervious to radiation of wavelengths lower than approximately 400 nm. Results of this experiment clearly indicate an important fact - during nail laser irradiation in the UV spectrum (specifically UV-C) we do not need worry about the UV rays passing deeper into the skin, where it could cause undesirable thermal, biochemical and photo-physical effects. Reflection emitted by the source across the whole spectrum is low - around 1%.

Measurements of nail transmission for 248 nm using a high-performance KrF laser in pulse mode:

| Sample number | 1  | 2  | 3  |
|---------------|----|----|----|
| Thickness (mm)| 1,1| 0,7| 0,45|
| Energy before the impact (mJ) | 37 | 50 | 37 |
| Energy from the impact (mJ) | 9  | 11 | 7  |
| Transmission (%) | 24 | 22 | 19 |

Table 1 - Measurements of nail transmission
Verification of absorption of UVC radiation by thermal imaging camera.

The result of the heat area experiment after the use of the laser beam is that with values of energy needed for fungus elimination we do not reach temperatures in nail or underneath the nail tissue that would have unwanted effects. As inappropriate, we considered lasting heat exceeding 40°C, although we can exceed this temperature for a short time without the destruction of tissue. Here is a typical image of the heat impacted area, which we made using a thermo-camera.

Evaluation of the mold irradiation

Fungi are successfully destroyed even when using minimal values – energy in pulse 144 mJ and density of energy 0.74 J/cm², and they are not growing again. We have been observing the reaction of Trichophyton rubrum in relation to the number of pulses. For higher numbers of
pulses (500 and 1000) the destruction can be observed by the naked eye, for a lower number of pulses (200) we were able to observe the damage caused just through a microscope and we have only verified that there was no more growth of fungi. This value we consider as a limit. During our experiments all the fungi were destroyed even with these values. Because of the small size of the footprint compared to the size of a nail in medical use it will be necessary to make the footprint bigger, or (what is in our opinion better) move the footprint in two directions and in that case it would be better to use a lower number of pulses for each radiation and divide the footprint into layers. Part of the experiment, we focused on the influence of the frequency of laser radiation to destroy fungi. We used a frequency of 10-40 Hz. From monitoring the samples, it is obvious that the change in frequency with the same number of pulses has no significant impact on the disposal of mold. The crucial factor is rather the actual number of pulses.

Fig.9 - Case photographs with samples of mold irradiated by higher value. The result is immediate mold destruction with no re-growth even after 4 weeks. (Parameters: f 20Hz, E 13mJ, T 5s, Ec = 1300mJ)

Fig.10 - Microscopic image of mold after laser irradiation
Evaluation of the thermal response of the irradiated tissue.

In other experiments performed in our department, frequency has proved to be an important factor. Even a small increase in frequency resulted in increased temperature and thus increased risk of tissue damage. For the treatment of onychomycosis, it is absolutely crucial to know the limit value adjustment of the laser, with which it is safe to use the laser for treating patients. Ablation threshold - the highest laser power, which does not interfere with the structure of tissue, we set out for the nail to 128.37 mJ/cm². This limit, therefore, cannot be exceeded during the therapy. The time of irradiation (irradiation product - power density in W cm⁻² and radiation exposure time) is a fundamental parameter that determines whether the effects of radiation will be thermal (long exposure), acoustic, or other (very short pulses). In determining the ablation threshold of human skin the following parameters for excimer laser were used: a circular footprint of 2.6 mm in diameter, i.e. 5.31 mm²; repetition rate of 10 Hz. Results are in Table 2 below.

| Trial number | Energy in pulse [mJ] | Energy density [J/cm²] | Number of pulses | Manifestation | Voltage/Tension (approximate value) [kV] |
|--------------|----------------------|------------------------|------------------|--------------|----------------------------------------|
| 1            | 4,5                  | 0,85                   | 200              | Without damage | 18                                     |
| 2            | 7,5                  | 1,42                   | 200              | Very mild smoke in later phase and very weak footprint | 22                                     |
| 3            | 9,7                  | 1,83                   | 200              | Smoke all the time and visible but weak footprint | 26                                     |
| 4            | 7,5                  | 1,42                   | 1000             | Occassional mild smoke and weak footprint | 22                                     |
| 5            | 6,4                  | 1,2                    | 1000             | Indistinct footprint (more „shiny“ at places) | 20                                     |

Table 2 – Evaluation of the thermal response of irradiated tissue

The footprints were evaluated using the human eye and magnifying glass. "Smoke" mentioned in table 2 is probably caused by the ablation threshold of the surface layers, whether or not it is caused by the thermal effect, it needs to be verified in the future.

Discussion

Nails affected by mold vary considerably in their structure and thickness. In order to obtain a sufficient amount of information, it will be necessary to examine as many samples of different thicknesses as possible as well as nails in varying degrees of onychomycosis. In further tests it will be necessary to determine under what parameters damage to the skin during radiation might occur.

The results so far indicate that only a small dose of radiation is needed for eradication of nail fungus, which is favourable due to the fact that we must not damage the soft tissue under the nail. The objective of each single experiment was to determine the limits of the use of laser
radiation in destroying fungi. As found and verified in previous experiments, fungi responds to wavelengths radiation in the UV spectrum positively and even relatively low power radiation is reliably destroying them.

However, for medical applications, it is necessary to know the minimal values required for total eradication of fungi, which is not needed to be known in laboratory conditions for experiments aimed at mere destruction of the mold.

In the search for the limiting values, it is necessary to take into account, firstly, that the transmitted radiation is substantially absorbed by the nail (relevant parameters of nails were examined in the previous experiments), and secondly, that the radiation is also absorbed by the tissue under the nail, not only by fungi, and therefore heat generation occurs, but temperature over 40 °C is undesirable.

In our experiment, we focused on determining such a value of the transmitted radiation that would ensure destruction of mold without an excessive increase in the tissue temperature.

Conclusion

The results of transmission measurements indicate that nails are impervious to radiation of wavelength less than about 400 nm. Reflection is low across the whole spectrum emitted by the source - at around 1%.

Measurements of nail transmission at wavelengths of 248 nm, using a high performance KrF laser in pulse mode, showed that the percentage of transmission depends on thickness of the nail, so for thicker nails the value is lower. Energy passing through the sample is very small and with usual nail thickness there will not be any undesired heat effects.

When verifying the absorption of UVC radiation by a thermal imaging camera, we found that the temperature of the irradiated nail sample does not exceed 40 °C, and thus will not damage the soft tissue under the nail, and is not perceived as painful by the patient.

We have found out and subsequently verified that fungi are destroyed even when using energy values in pulse of 144 mJ and energy density 0.74 J/cm² and that they did not regrow. With those values we can use 200 pulses at a repeating frequency of 10 Hz.

Modulating the frequency of the laser radiation, according to our measurements, does not affect the destruction of mold.

The evaluation of monitoring the ablation threshold of human skin shows that this value does not exceed the ablation threshold of the nail.

Among factors that most influence the degree of tissue damage and thus are relevant to the determination of the parameters for the possible excimer laser treatment of onychomycosis include, according to our results, absorption, reflection and transmission of irradiated tissue, its size, laser wavelength, intensity of the laser beam and time of irradiation.

Determination of the precise parameters for excimer laser treatment requires additional tests but the results of our measurements show that this method could be one of the possible ways of treating onychomycosis, either on its own, or in combination with pharmacologic therapy.

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