

Laser treatment of onychomycosis: an in vitro pilot study

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Summary

Background: Laser treatment of onychomycosis is the object of considerable interest. Laser therapy could be a safe and cost-effective treatment modality without the disadvantages of drugs. Some studies have described the inhibitory effects of lasers on the growth of fungal colonies. We therefore examined the effects of various laser wavelengths, which have previously shown inhibitory potential, on the fungal isolate *Trichophyton rubrum*.

Patients and Methods: Isolates of fungal colonies were placed clockwise on culture plates. Each culture plate was irradiated on one half with one of the following treatment regimens: 1064 nm-Q-switched Nd:YAG laser at 4 J/cm² and 8 J/cm²; 532 nm-Q-switched Nd:YAG laser at 8 J/cm²; 1064 nm-long-pulsed Nd:YAG laser at 45 J/cm² or 100 J/cm². The other half remained untreated. Standardized photographs were taken and areas of treated and untreated colonies were compared for growth inhibition.

Results: There was no inhibition of fungal growth in any of the treated plates. Differences in size between treated and untreated colonies were not significant ($p > 0.10$).

Conclusions: In this in vitro study Nd:YAG laser treatment of *Trichophyton rubrum* colonies failed to inhibit fungal growth. Nevertheless there might be an effectiveness in vivo which has to be clarified by clinical studies.

Introduction

In more than 99 % of patients, onychomycosis is caused by a dermatophyte infection. The most common causative pathogen is *Trichophyton rubrum* and the second most common is *Trichophyton mentagrophytes* [1, 2]. Only rarely are molds and candidal species the cause [1, 3]. Onychomycosis is the most widespread nail disorder occurring in adults [4, 5]. The reported prevalence ranges between 2 and 13 %. The risk of infection increases significantly with increasing age. About 30 % of patients between the ages of 60 and 70 years of age have infection and among 70-year-olds about 50 % [2, 6]. The incidence appears to be rising in all age groups [5]. The treatment of onychomycosis remains challenging. Both topical and systemic

antifungal agents are associated with treatment failures, need for long-term therapy, high rates of recurrence, and significant costs [7–11]. The commonly used ciclopirox or amorolfine (Loceryl®, Galderma) nail lacquer take a long time to eradicate the infection and rarely completely cures severe onychomycosis [9]. Also, most patients have concomitant fungal infection of the foot which goes untreated. Systemic treatment is usually with terbinafine, itraconazole, or fluconazole. The list of adverse effects and possible drug interactions is long [12, 13]. At present there is no cost-effective, safe, effective, and easy-to-use alternative. Along with photodynamic treatment [14], in recent years [15] there have been increasing reports on the successful use of laser treatment for onychomycosis.

Still, very few data are available. So far, only three clinical studies have examined the positive effect of treatment with long-pulsed Nd:YAG laser (1064 nm) [16, 17] and diode laser with wavelengths of 870/930 nm [18].

There are also a few publications on in vitro results of laser therapy. Kozarev and colleagues treated *Trichophyton rubrum* in vitro once with long-pulsed Nd:YAG laser (wavelength: 1064 nm, fluence: 40 J/cm², spot size: 4 mm, pulse duration: 35 ms) and reported a significant, visible regression of the fungus after three days [16].

Vural and colleagues reported significant inhibition of growth after treatment with q-switched Nd:YAG laser (wavelength: 1064 nm, fluence: 4 and 8 J/cm², as well as wavelength: 532 nm, fluence: 8 J/cm²,

spot size: 2 mm). Photometric analysis showed that after three and six days there was significantly slower growth of treated colonies compared with those that were untreated [19].

Positive effects have also been reported after treatment with Ti: sapphire laser (800 nm) which delivers energy in the femtosecond range [20].

The biological and physical effects of laser treatment on dermatophytes are still uncertain and have been variously discussed in published studies. The advantages of laser treatment of onychomycosis are self-evident. In the present study, we conducted our own tests using the protocols for treatment of *Trichophyton rubrum* in vitro which have been reportedly successful.

Materials and methods

Five selective agar plates were inoculated with six nail specimens each. The specimens were collected from different patients with onychomycosis. The inclusion criteria were as follows: positive fungal culture of the toenails, presence of *Trichophyton rubrum*, and no prior local or systemic treatment. The agar plates were inoculated circularly at 1, 3, 5, 7, 9, and 11 o'clock. The culture media were also marked so that the right and left sides could be distinguished to allow for a comparison of sides (Figure 1). Afterward, the cultures were incubated for ten days at 30° C.

Based on a standardized protocol, all plates were individually photographed with a digital SLR camera (Canon Eos 350D, EFS 60 mm lens 1:2,8, Canon Inc. Tokyo Japan). To obtain comparable images, the photos were taken under identical lighting conditions, the same shutter speed, and by the same person. The photos were taken immediately after treatment of the colonies with various types of laser. Based on the studies published until now, we chose the wavelengths and treatment conditions as follows:

- If the parameters had already been used successfully in other studies to treat *Trichophyton rubrum*.
- If the treatment would have been possible with actual patients under the same conditions in vivo.

Each fungal culture was treated with a different regimen (Table 1). In order to compare sides, in each culture only the three colonies on the right-hand side at

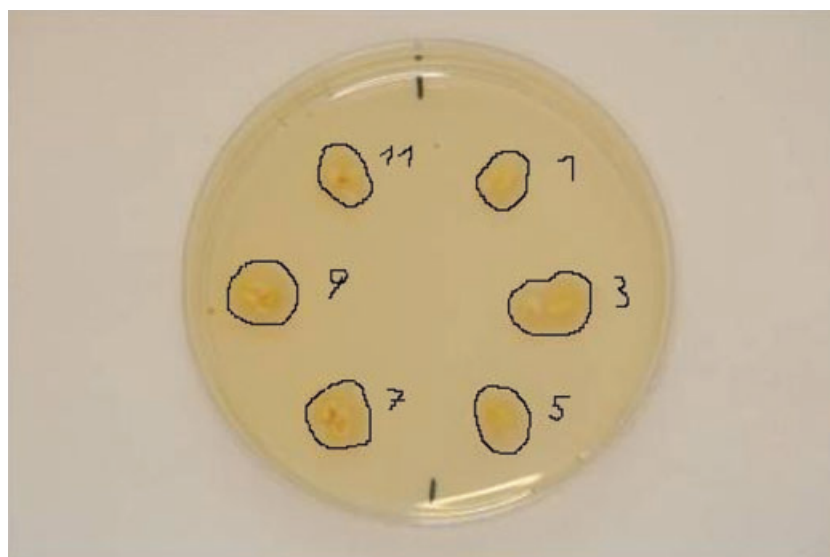


Figure 1: Culture plate with six colonies of *Trichophyton rubrum*. For photometric measurement we defined the limits of the fungal colonies and labelled the plate clockwise.

Table 1: Overview on lasers and parameters used to treat the five culture plates in the study.

	Laser	Wavelength	Fluence	Spot size	Pulse duration
1	Nd:YAG, q-switched	1064 nm	4 J/cm ²	2 mm	6 ns
2	Nd:YAG, q-switched	1064 nm	8 J/cm ²	2 mm	6 ns
3	Nd:YAG, q-switched	532 nm	8 J/cm ²	2 mm	6 ns
4	Nd:YAG, long-pulsed	1064 nm	45 J/cm ²	10 mm	40 ms
5	Nd:YAG, long-pulsed	1064 nm	100 J/cm ²	3 mm	40 ms

1, 3, and 5 o'clock were treated; those at 7, 9, and 11 o'clock were not. Laser treatment was done without covering the fungal cultures and without cooling. The distances were determined by the hand pieces: 3 cm for the long-pulsed Nd:YAG laser, and 2 cm for the q-switched Nd:YAG laser. For protection, the investigator wore gloves and a face mask over the nose and mouth, and the laser plume was suctioned. After treatment, the cultures were incubated at 30° C; the temperature remained constant throughout the study.

Three fungal cultures were treated (once) with q-switched KTP-Nd:YAG laser, (wavelength: 1064 nm, fluence: 4 and 8 J/cm², spot size: 2 mm) and with

q-switched Nd:YAG laser (wavelength: 532 nm, fluence: 8 J/cm², spot size: 2 mm; each "Affinity QS", Cynosure Inc., Westford MA 01886, USA). Two others were treated with long-pulsed Nd:YAG laser (wavelength: 1064 nm, fluence: 45 J/cm², pulse duration: 40 ms, spot size: 10 mm; and with the fluence: 100 J/cm², pulse duration: 40 ms, spot size: 3 mm; "Elite", Cynosure Inc., Westford, MA, 01886, USA) (Table 1).

Three and six days after laser treatment we performed follow-up with photo documentation based on the above-mentioned criteria. The photos of the fungal cultures were adjusted to scale (consistent diameter of agar plates). Using the ImageJ program (Rasband,

W.S., ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, <http://imagej.nih.gov/ij/>, 1997-2011) for image processing, the surfaces of the fungal colonies were digitally surrounded by a border by manually drawing a polygon around them and their surface was quantified in square millimeters (Figure 1). In every fungal culture, the average increase in the size of the treated and untreated colonies were compared using one-tailed T tests. The significance level α was 0.05; p values that were the same or less than α led to rejection of the null hypothesis.

Results

At three and six days after laser treatment, none of fungal colonies in the five culture dishes showed signs of regression. Nor were there any significant differences between the various laser systems used (or parameters) or between treated and untreated colonies on the same plate ($p \geq 0.13$) (Figures 2, 3). There were also no differences in growth between the different plates.

Discussion

Laser therapy is considered by some authors to be a promising new method for treatment of onychomycosis. We thus conducted a study on the effects of laser treatment on in vitro growth of *Trichophyton rubrum*.

The antimicrobial efficacy of various laser systems in vitro has been reported by various studies. Meral and colleagues reported the fungicidal effects of Nd:YAG laser on *Candida albicans* [21]. In another study, the diode laser "Noveon" (870/930 nm) made by Nomir Medical was found, in a manufacturer's in vitro study, to have a fungicidal and bactericidal effect on *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Trichophyton rubrum* [22].

A working group led by Kozarev treated *Trichophyton rubrum* cultures in vitro with Nd:YAG laser (wavelength: 1064 nm, fluence: 40 J/cm², spot size: 4 mm, pulse duration: 35 ms). After only three days, there was a visible significant regression of the fungi. We were unable to reproduce these results using the identical parameters. A difference in our treatment scheme was the larger spot size of 10 mm. Yet this would

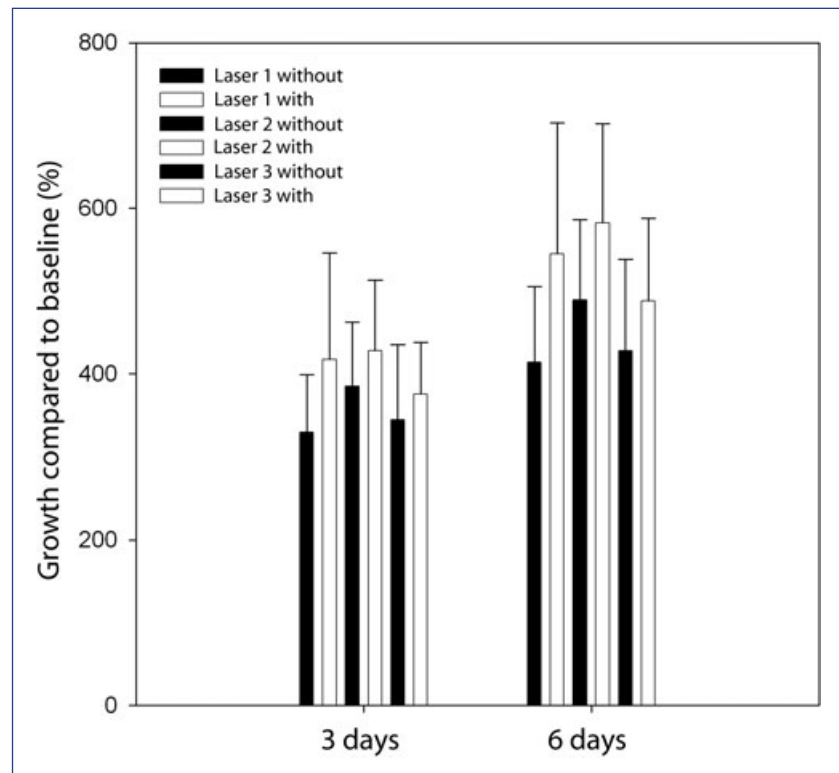


Figure 2: The quantified areas of the fungal colonies 1–3 on days 3 and 6 after laser treatment with q-switched lasers. Laser 1: 1064 nm (4 J/cm²), Laser 2: 1064 nm (8 J/cm²), Laser 3: 532 nm (8 J/cm²), each from left to right and without (control, black bars) and with (white bars) laser treatment. The picture shows the increase in area in relation to baseline, differences are not statistically significant, p value > 0.10.

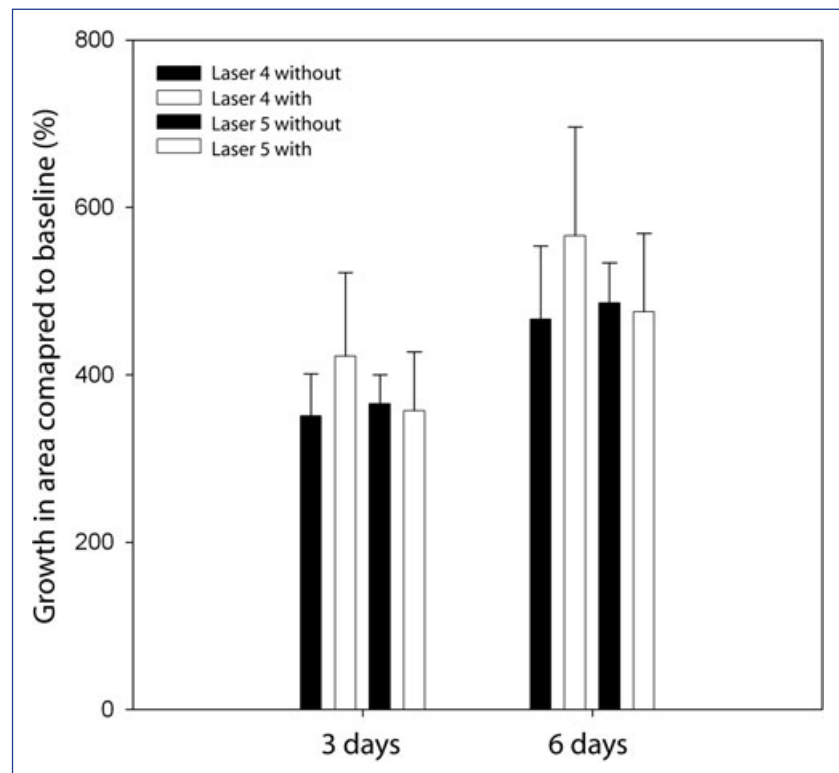


Figure 3: The quantified areas of the fungal colonies 4 and 5 on days 3 and 6 after laser treatment with long-pulsed lasers. Laser 4: 1064 nm (45 J/cm²), Laser 5: 1064 nm (100 J/cm²), each from left to right and without (control, black bars) and with (white bars) laser treatment. The figure shows the increase in area in relation to baseline, differences are not statistically significant, p value > 0.10.

not explain the lacking efficacy; the total energy delivered to a 10 mm spot size is more than twice that delivered to 4 mm. We also treated a fungal culture of a similar size of 3 mm and higher energy density of 100 J/cm². This more intense treatment also failed to produce significant results. It would be erroneous to think that regression might have occurred after the observation period of six days. Fungal cultures typically grow strongly in the first days if there is sufficient nutrient agar available. After about two weeks, growth stops due to lacking nourishment. Any regression due primarily to laser treatment would thus occur in the early phase of growth. For reasons related to infection prevention, we did not cool the cultures during laser application as would be done to the fungus in vivo. Yet this should not have any significant influence on the study results. The purpose of cooling in vivo is to make the procedure less painful for the patient and also to counteract overheating of the nail plate. If, as Kozarev and colleagues suggest, thermal effects are responsible for the results, then cooling would be counterproductive to obtaining a good treatment outcome. In personal correspondence with Kozarev, there was some discrepancy with the published results. During our conversation, the pulse duration was 25 ms rather than 35 ms as reported. In addition, the authors selected strongly pigmented colonies. Unpublished photos of a treated fungal culture show a mold species and not the *Trichophyton* colony as reported. In addition, the study was not peer-reviewed and thus only partly meets scientific quality standards.

Vural and colleagues reported that after one week there was significantly slower growth of *Trichophyton rubrum* cultures which were treated in vitro with q-switched Nd:YAG laser [19]. We also used these treatment parameters but could not reproduce their reported success. A possible reason is the different preparation of the cultures. Vural and colleagues first prepared a "preliminary culture" and extracted a uniformly large piece of the colony to inoculate a new plate. Thus, the plate that was treated did not contain a nail specimen. Our agar plates did, and this may be what helped promote the growth of the fungus.

Manevitch and colleagues reported that after a single application of laser to fungal nail infection in vitro with a femtosecond laser (Ti:sapphire laser, wavelength: 800 nm) there was no more growth in the fungal culture that was cultivated afterward [20]. According to the authors, selective photothermolysis successfully treated the fungus while protecting the surrounding tissue. Yet in dermatology, femtosecond lasers are not widely used, except in experimental studies for economic reasons.

In our study, long-pulsed Nd:YAG laser with a wavelength of 1064 nm, q-switched Nd:YAG laser with a wavelength of 1064, and KTP laser with a wavelength of 532 nm were all unable to reproduce the inhibitory effects on fungal growth as described by other working groups. Even at a high fluence of 100 J/cm², which has not yet been otherwise studied, a comparison of treated/untreated sides showed no effect on fungal colonies.

It is unclear why we were unable to reproduce the results. One possibility is "publication bias." Studies reporting positive or significant results are more often published and garner more attention. Also, authors are less inclined to publish studies that have not led to significant results. This is a well-known "file-drawer problem." Finally, conflicts of interest may also have distorted some of the scientific data by influencing the publishing of insignificant findings related to new technologies.

Few clinical studies have been published on the efficacy of laser treatment of onychomycosis. Kozarev and colleagues reported that after four treatments in weekly intervals with long-pulsed 1064 nm Nd:YAG laser that 95 % of patients healed after three months. One must consider, though, that only the fungal infection of the nail was treated. The usually responsible and accompanying tinea pedis is an undisputed reservoir for reinfection of the nail. Combination therapy consisting of laser treatment and an oral antifungal agent (for 2–3 months) would be – assuming the results were reproducible – in our opinion a "conditio sine qua non." The PinPointe™ Foot-Laser™ (NuvoLase™ Inc., Chico, CA, USA) by Cynosure™ (Cynosure™ Inc., Westford, MA, USA) is a 1064 nm Nd:YAG laser (100 µs pulse duration). In Germany, treatment with this laser is

available at several PinPointe™ laser centers. Only a single treatment session is needed; laser therapy must be done in combination with a two-week regimen of terbinafine cream. After a careful search of the literature, we could find no scientific studies confirming the efficacy of the PinPointe™ laser.

A clinical study by Landsman and colleagues reported on the treatment of onychomycosis with a diode laser (Noveon® Laser, Nomir® Medical Technologies Inc., Waltham, MA, USA) using wavelengths of 870 and 930 nm [18]. In 63 % of patients there was healthy re-growth of the nail measuring 3 mm, and the results of histology were negative in 30 % of patients. The study was very small, however, with 26 patients. Yet the fact that the working group cooperated with Nomir® Medical, the manufacturer of Noveon® lasers, is a concern.

Further studies are needed to investigate the effect of laser on dermatophytes, particular in regard to possible use on onychomycosis in vivo. The mechanism of effect of laser light on fungal cultures in vitro and in vivo is controversially discussed and is still uncertain at this point. Kozarev and colleagues have suggested that thermal effects are the primary cause; Bornstein and colleagues described the formation of free radicals as well as an influence of laser on cellular metabolic reactions, while ruling out thermal effects [22]. Vural and colleagues believed the absorption of energy by xanthomelanin and melanin to be primarily responsible for the effectiveness. Both substances are found in abundance in *Trichophyton* species. In our opinion, the effect could be due to unspecific tissue heating with a subsequent increase in circulation due to vasodilatation and stimulation of immunological processes. Before regular use on humans, one must determine the associated risks of laser treatment, especially permanent damage of the nail matrix associated with the use of higher energy densities. For the use of laser therapy for routine treatment of onychomycosis, as has been advertised and propagated by laser manufacturers and franchises, we believe that sufficient evidence is still lacking. Randomized clinical studies are urgently needed. <<<

Conflict of interest

None.

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