

The effects of laser irradiation on *Trichophyton rubrum* growth

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Abstract The effects of various laser wavelengths and fluences on the fungal isolate, *Trichophyton rubrum*, were examined in vitro. Standard-size isolates of *T. rubrum* were irradiated by using various laser systems. Colony areas were compared for growth inhibition on days 1, 3, and 6 after laser irradiation. Statistically significant growth inhibition of *T. rubrum* was detected in colonies treated with the 1,064-nm Q-switched Nd:YAG laser at 4 and 8 J/cm² and 532-nm Q-switched Nd:YAG laser at 8 J/cm². Q-switched Nd:YAG laser at 532- and 1,064-nm wavelengths produced significant inhibitory effect upon the fungal isolate *T. rubrum*

in this in vitro study. However, more in vitro and in vivo studies are necessary to investigate if lasers would have a potential use in the treatment of fungal infections of skin and its adnexa.

Keywords Laser · Dermatophyte · Fungus

Introduction

Trichophyton rubrum—a keratinophilic filamentous fungus that infects skin, nails, and hair follicles—is the most common causative agent of dermatophytosis worldwide [1–6]. Current management of *T. rubrum* includes topical and systemic antifungal pharmacologic treatments. However, these approaches may not be successful in every case due to such factors as difficulty with long-term application of topical medications, side effects of certain medications in systemic use, and failure to deliver the medication to the target area in sufficient concentration [7, 8]. It is not uncommon that a fungal infection persists despite appropriate topical and/or systemic chemical treatment(s), and this may be one of the most important factors responsible for the high cost of treatment in dermatophyte infections [9–11]. Obviously, there is a need for safe, efficient, easily performed, and cost-effective treatment modalities without the abovementioned disadvantages of topical and systemic antifungals in the management of dermatophyte infections.

Photodynamic therapy has been recently proposed to treat *T. rubrum* infections, and promising results were obtained from these in vitro studies using certain photosensitizers and light sources [12–14]. However, the direct effect of laser light on fungal isolates has not been rigorously examined for their possible inhibitory potential.

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In this *in vitro* study, we report the effects of various laser systems on *T. rubrum* colonies.

Materials and methods

An isolate of *T. rubrum* obtained from a toenail scraping was serially passed on Difco™ Sabouraud Dextrose Agar (BD diagnostics, Sparks, MD). After pure culture was obtained, this fungal strain was used for the entire experiment to provide standardization. Four-millimeter punch biopsy samples (total of 18) of the primary colonies were transplanted to three new fungal plates (six colonies per plate). Standardized photographs were obtained utilizing a Nikon CoolPix 5400 digital camera (Nikon, Torrance CA) at 4× optical magnification with 10-cm distance from the fungal plates. Twenty-four hours after obtaining standardized photographs, the colonies were exposed to various wavelengths and fluences of laser light (Table 1). Fluences for each laser system were chosen based on tolerable fluences that are commonly used for treating unwanted hair, tattoo, or port-wine stains in clinical settings. Each colony was treated with a sufficient number of pulses to cover the entire area of the colony with minimal overlapping. One colony in each plate was left untreated as the control colony and marked accordingly. The colonies were rephotographed under identical photography parameters as described above on the first, third, and sixth posttreatment days. Assessment of growth was made by converting the standardized digital images into bitmap format and calculating the size of each fungal colony in pixels utilizing Microsoft® Paint program (Microsoft, Seattle, WA).

Colonies irradiated with IPL, FPD, Er:YAG, or KTP lasers did not show any inhibitory effect, and thus were not further studied (data not shown). A second phase focusing on 532- and 1,064-nm wavelengths of Q-switched Nd:YAG laser system was conducted, as these were the only two wavelengths showing inhibitory effect on fungal isolates. For this purpose, Q-switched Nd:YAG laser system was used at 532-nm wavelength with 1, 2, 4, 6, 8, and 10 J/cm² and at 1,064 nm wavelength with 2, 4, 6, 8, and 10 J/cm².

We used a 2-mm-diameter beam and a pulse frequency of 10 Hz for both wavelengths. In this series, each laser fluence was used to treat five colonies in an agar plate containing six colonies. One colony was left untreated for control. Areas of treated colonies and control colonies were calculated in pixels from standardized photographs obtained before treatment, and 1, 3, and 6 days after treatment as described above.

The effect of laser treatment on fungal growth was analyzed in two ways. First, the general effect of laser treatment on colony size was assayed, using a paired sample *t* test. Second, the effect of specific laser treatments on fungal growth rate was determined, using the slope of a simple regression of colony size against colony age. Before analysis, all the colony-size data were log transformed to ensure homogeneity of variance, normality of error, and to linearize the relationship between colony size and age. All analyses were conducted on JMP 5.0 (SAS Institute).

To detect whether laser treatment affects fungal growth, a paired sample *t* test was used to compare the mean size of the laser-treated and untreated colonies on a plate, across all plates, for days 1, 3, and 6. Growth rate of fungal colonies receiving different laser treatments was subsequently compared using a general linear model (GLM). The statistical model was: $\log(\text{colony size}) = \text{day} \times \text{treatment} + \text{error}$, where day is a continuous variable and treatment is a categorical variable. Differences in fungal growth rate between treatments were detectable as a significant interaction between the factors “day” and “treatment” (day × treatment). The analysis subsequently tested whether the interaction term for each treatment was significantly different from zero using a *t* test; that is, whether a particular laser treatment resulted in slower or more rapid growth of the fungal colonies compared to average growth across all laser treatments.

Results

With the exception of Q-switched Nd:YAG laser with 532- and 1,064-nm wavelengths, none of the lasers tested in the first phase of this study revealed significant growth

Table 1 Laser systems, wavelengths, and fluences used during the initial phase of the study

Laser system	Wavelength (nm)	Fluence (J/cm ²)
Intense pulsed light (Lumenis, Vasculight, Santa Clara, CA)	695 to 1,000	38, 45, 57
Intense pulsed light (Lumenis, Vasculight, Santa Clara, CA)	755 to 1,000	38, 45, 57
Pulsed dye laser (Candela, ScleroPlus, Wayland, MA)	585	8, 11, 14
Q-switched Nd:YAG laser (Surgical Laser Technology, CMLD, Malvern, PA)	532	8, 10
Q-switched Nd:YAG laser (Surgical Laser Technology, CMLD, Malvern, PA)	1,064	6, 8, 10, 12
Erbium YAG laser (Sciton Model Contour, Palo Alto, CA)	2,940	25
KTP laser (Laserscope, Aura, San Jose, CA)	532	2, 4, 6, 8

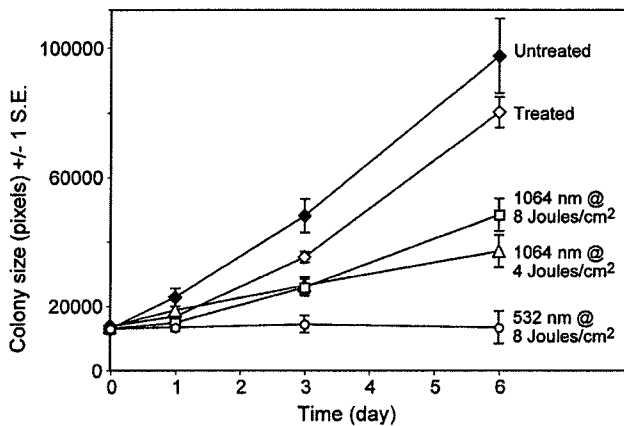


Fig. 1 Growth of fungal colonies receiving different laser treatments. Chart shows average size of untreated and laser-treated colonies as well as size of colonies receiving specific laser treatments. From day 1 to 6, laser-treated colonies were significantly smaller than untreated colonies on the same plate (paired sample *t* test, $p < 0.05$ for all). Treatment with 1,064 nm at 4 and 8 J/cm² and 532 nm at 8 J/cm² significantly reduced fungal growth rate compared to the average growth of all laser-treated colonies (GLM, $p < 0.0005$ for all)

inhibition on *T. rubrum* colonies (Table 1, data not shown). Figure 1 shows the average size of the control colonies and laser-treated colonies on days 0 to 6 after treatment. For days 1, 3, and 6, the mean size of colonies that had been laser treated was significantly smaller than the size of colonies that had been untreated on the same plate, across all plates (paired sample *t* test, $t_{\text{day } 1} = 4.28$, $t_{\text{day } 3} = 3.67$, $t_{\text{day } 6} = 2.744$; $df = 21$, $p < 0.05$ for all). Different laser-treatments had significantly different effects on the growth rate of the fungal colonies (GLM, $F_{\text{day} \times \text{treatment}, 10, 198} = 22.75$, $p < 0.0001$). Specifically, treatment with 1,064-nm Q-switched Nd:YAG laser at 4 and 8 J/cm² and 532-nm Q-switched Nd:YAG laser at 8 J/cm² resulted in a much lower growth rate than the average for all laser-treated fungal colonies ($t > 3.54$, $p < 0.0005$ for all; Figs. 1 and 2).

Discussion

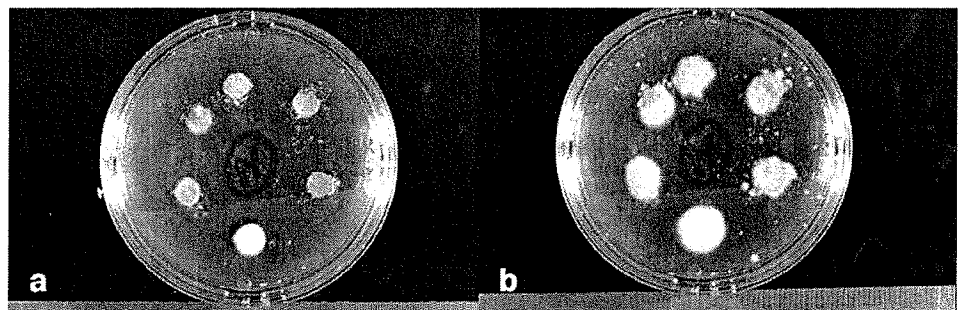
We have investigated the effects of various laser systems on the growth of a common dermatophyte *T. rubrum* in this in

vitro study as a potential research area for the treatment of dermatophyte infections. Only a few articles have previously examined the effects of laser irradiation on the growth of fungal colonies. One such study examined the effects of argon fluoride gas excimer laser at 193 nm on *Candida albicans* and *Aspergillus niger*. This study indicated eradication of the target organisms as evidenced by the loss of viability of the colonies on subculture [16]. Laser irradiation has been studied experimentally in the agricultural industry to decrease the burden of opportunistic fungi on germinating seedlings, and it has been shown that a substantial diminution of *Fusarium solani* colonization of seedlings can be achieved by using He/Ne gas laser emitting light at 632.8-nm wavelength with a power output of 7.3 mW [17]. However, we did not find data regarding the effects of direct laser light irradiation on dermatopathogens.

The inhibitory effect of Q-switched Nd:YAG laser on fungal colonies seen in this study is most probably due to more than a nonspecific thermal damage. It is known that 532-nm Q-switched Nd:YAG laser is well absorbed by red pigment, which is abundant in *T. rubrum* because of its xanthomegnin content [18, 19]. Xanthomegnin was originally isolated from *T. megninii* and subsequently demonstrated to be the diffusible pigment produced by *T. rubrum* which confers its prominent red pigment seen in culture [19]. The presence of this red chromophore may explain the sensitivity of *T. rubrum* to 532-nm range.

It is known that IPL system used between 695- and 1,000-nm-wavelength range at 38 to 47 J/cm² and 585-nm pulsed dye laser at 8 to 14 J/cm² can induce significant thermal damage [20–23]. Lack of growth inhibition in our study with these settings might also be a supporting evidence regarding pigment-related photothermolysis of *T. rubrum* rather than inhibition due to nonspecific thermal damage. Although the wavelength of Q-switched Nd:YAG laser at 1,064 nm is beyond the absorption spectrum of xanthomegnin, we have observed similar inhibitory effects on the colonies treated with this wavelength. This might be due to another chromophore absorbing at 1,064 nm, such as melanin, as it is known that *Trichophyton* species contain melanin in their cell walls [24, 25].

Fig. 2 Culture plate showing *T. rubrum* colonies 24 h (a) and 72 h (b) after treatment with 532 nm at 8 J/cm² in standardized photographs. Control colony is at 6 o'clock position. Note the inhibition of growth in treated colonies as compared with the control



In addition to its wavelength, another important feature of Q-switched Nd:YAG laser is its relatively short pulse width (i.e., nanoseconds). These short pulses will induce microcavitation and acoustic shock waves that could result in significant inhibition of the fungal colonies [26]. The short pulse times (much shorter than the thermal relaxation time) will also induce thermal shocks in the target chromophore via rapid heating and cooling. These extreme thermal cycles and shock waves do not occur when the laser light is delivered in relative long pulse times, as seen in this study with the use of continuous wave KTP laser with 532-nm wavelength or a pulsed dye laser. Therefore, it can be speculated that effective inhibition of the *T. rubrum* also requires very short pulses of 532-nm wavelength that generates mechanical damage in the irradiated fungal colony.

Considering epidermal colonization of dermatopathogens within the skin, one can speculate that a laser light mainly being absorbed within the epidermis might cause significant fungal inhibition in dermatophyte infections by nonspecific thermal damage. One such laser system that we used in the first phase of this study was Er:YAG laser with a 2,940-nm wavelength. This laser is well known for its epidermal absorption because of the epidermal water content and has been extensively used in skin resurfacing [27]. However, we did not observe any significant inhibition on fungal colonies with Er:YAG laser. This may suggest that the inhibitory effects seen on the colonies not be related to nonspecific thermal damage or water content of *T. rubrum*.

Laser irradiation with the capability of delivering destructive high energy pulses to specific targets with minimized surrounding tissue damage would seem to be well suited for the task of eradicating superficially located organisms in the skin. The current experiment differs significantly from other studies, as we have selected the same strain of a specific organism and serially tested it against a wide array of laser wavelengths and fluences. Although this preliminary in vitro study does show a statistically significant level of growth inhibition at two separate laser wavelengths at certain fluences, the study design makes accurately determining percent viability problematic because of the thickness of the colonies treated. In typical dermatophyte infections, the thickness of the layer of organisms is rarely more than a few organisms. In this study, we examined the effects of laser irradiation on cultured colonies with an estimated thickness of 2–3 mm. It is likely that the more superficial organisms absorbed the majority of radiant energy, thus suffering the burden of damage, leaving the subjacent organisms protected, even at the critical fluences. This may have confounded our growth analysis, leading to an erroneous underestimation of growth inhibition. To address this issue, future in vitro studies are needed to

develop thin agar preparations which may confine fungal growth to a significantly thinner layer and may render interpretation of growth inhibition more accurate. In addition to more in vitro studies, in-vivo studies are necessary to investigate the possible therapeutic effects of various laser systems on various dermatopathogens, as laser–fungus interaction might be different when these microorganisms are embedded within the skin and its adnexa.

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