

## **Development and comparison of two devices for treatment of onychomycosis by photodynamic therapy**

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# Development and comparison of two devices for treatment of onychomycosis by photodynamic therapy

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**Abstract.** Onychomycosis is the most common nail disorder. The treatment for this type of infection is one of the main difficult ones in clinical practice, due to the fact that the nails are nonvascularized structures, which compromise the penetration of drugs delivered systemically and favor slow nail growth. We present two devices based on light-emitting diode arrays as light sources for the treatment of onychomycosis by photodynamic therapy (PDT). PDT is an emerging technique that uses a photosensitizer (PS) activated by light in the presence of oxygen. The PS absorbs energy from light and transfers it to oxygen, producing reactive oxygen species such as hydroxyl radicals, superoxide, and singlet oxygen which inactivate fungi and bacteria. Our proposal is the use of a portable and secure light source device in patients with onychomycosis. Additional advantages are the low cost involved, the possibility of topical treatment rather than systemic and the simplicity of operation. These advantages are important to ensure the implementation of this technology for the treatment of an impacting health problem. © The Authors. Published by SPIE under a Creative Commons Attribution 3.0 Unported License. Distribution or reproduction of this work in whole or in part requires full attribution of the original publication, including its DOI. [DOI: [10.1117/1.JBO.20.6.061109](https://doi.org/10.1117/1.JBO.20.6.061109)]

Keywords: onychomycosis treatment; light-emitting diode; photodynamic therapy; photodynamic inactivation; antimicrobial photodynamic therapy.

Paper 140635SSRR received Oct. 3, 2014; accepted for publication Apr. 9, 2015; published online May 8, 2015.

## 1 Introduction

The correct illumination of a lesion is a requirement for a successful treatment by photodynamic therapy (PDT). In this sense, an illumination device that properly delivers such illumination becomes essential. With that focus in mind, this article describes two devices produced specifically to work as light sources for the treatment of fungus nail disease by PDT.

Onychomycosis is the most common nail disease and may be caused by dermatophytes, yeast, and nondermatophytes fungi. The conventional treatment consists of the administration of topical and systemic antibiotics and antifungals for long periods and may be the cause for the increased microbial strains resistant to the currently available drugs.<sup>1-3</sup> The treatment for this type of infection is one of the main difficult ones in clinical practice, due to the fact that the nails are nonvascularized structures which compromise the penetration of drugs delivered systemically and favor slow nail growth.<sup>4</sup> This, associated with a high incidence of this type of infection, shows the importance of developing new technologies and treatment options.<sup>5,6</sup>

Therapies for onychomycosis in initial clinical studies using lasers, PDT, and iontophoresis have been shown to be promising.<sup>7</sup> This new style of treatment approach can be advantageous because they are conducted within a clinic and only require patient compliance.<sup>5-7</sup> Those techniques involve noninvasive procedures. Laser treatment of onychomycosis infections using the principle of absorption of light energy by the fungi results in

the conversion of mechanical energy into heat or energy.<sup>8,9</sup> Fungi are sensitive to heat above 55°C, which results in fungicidal effects.<sup>10,11</sup> However, heating the dermal tissue to temperatures above 40°C results in pain and necrosis. Therefore, the energy delivery with a laser source must be performed either by pulses, to enable heat dissipation by the tissue—which has improved heat conduction compared to nails—or using a moderate energy delivery rate to prevent tissue thermal damage.<sup>12</sup>

Iontophoresis is a technique that uses an electrical current to increase drug transport through semipermeable barriers. This treatment in association with terbinafine topical treatment has been tested because it has the highest antifungal effect on dermatophytes.<sup>13</sup> The disadvantage of this technique, however, is that it still requires the application of antifungals.

PDT uses light to activate a photosensitizing agent applied topically, which generates reactive oxygen species (ROS) that initiate the destruction of cells by necrosis or apoptosis. The photosensitizers (PSs) for PDT can also be absorbed by fungi.<sup>14,15</sup> Therefore, PDT may also be an alternative for patients susceptible to onychomycosis infection due to a comorbidity, since these therapies do not interact with other drugs.<sup>16,17</sup> We believe that this therapeutic area has the potential to continue expanding and that broader clinical investigations shall result in new options for professionals.

In this context, we are presenting and comparing two devices based on light-emitting diode (LED) arrays for use in PDT. These devices have a low thermal component and a relatively narrow emission band around a wavelength. The time required for the absorption of the PS between its administration and illumination (the drug-light interval) is important because this

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interval is the parameter that allows one to estimate whether the drug has reached the intended location, which is central to treatment.<sup>18</sup> One advantage of the technique is the low probability of selection of resistant microorganisms, since the resistance to ROS is virtually impossible.<sup>18</sup>

The microbial photodestruction is most commonly achieved with fluence rates of hundreds of milliwatts per square centimeters. In addition, the light absorption effects obtained by this therapy do not include high temperatures; instead, it induces photochemical reactions between PS, light, and the substrate.<sup>19</sup>

The PDT requires the presence of three factors that interact simultaneously: a PS, a source of light emitting an appropriate wavelength, and the availability of oxygen.<sup>20</sup> The PDT mechanism of action occurs based on two types of physical-chemical reactions: type I and type II reactions.<sup>21,22</sup> Type I reaction occurs through the generation of highly reactive free radicals,<sup>23</sup> resulting in a complex mixture of ROS which can oxidize a variety of biomolecules.<sup>23,24</sup> Type II reactions, however, are based on generation of singlet oxygen ( $^1O_2$ ), a highly reactive species of oxygen, which is produced by an excited-state reaction between an excited PS molecule and a vital oxygen molecule.<sup>24,25</sup>

Another advantage of PDT is that the PS is preferentially absorbed by the target cells, and the illumination is designed to be applied only on the region to be treated.<sup>26–28</sup>

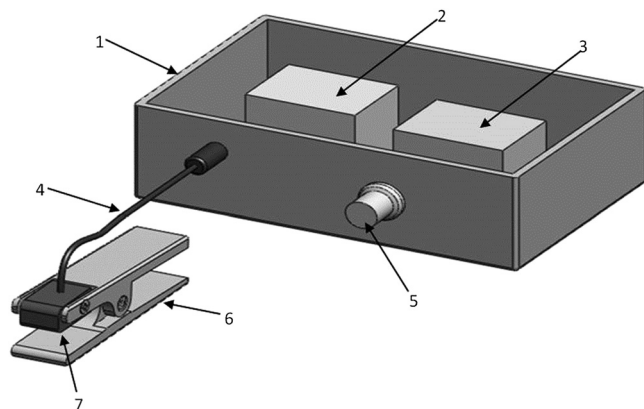
The use of PDT for onychomycosis provides fast results without recurrence.<sup>5,6</sup> In addition, aspects such as the low cost of the instrumentation involved, the possibility of local treatment rather than systemic, and simplicity of operation are important to ensure the implementation of this technology for the treatment of an impacting health problem.

The purpose of this article is the presentation and comparison of new devices to be used as light sources for PDT in the treatment of onychomycosis as an effective and safe technique with a lower cost in comparison with the conventional treatment.

## 2 Materials and Methods

### 2.1 Devices' Setup

Figure 1 shows a schematic drawing of the equipment and its main parts. Those parts were idealized considering the following aspects:



**Fig. 1** Portable equipment used for treatment of infections (onychomycosis) of the toes and hands of humans, consisting of: (1) a power source with module, (2) current and (3) voltage, (4) a connection cable, (5) on/off bottom with or without adjustment control voltage, (6) clamp of contact (7) with articulated head.

1. the fingers and the nail plate are one solid structure composed of different layers in contact;
2. the LED displays the incident spot pattern, i.e., with an intensity and energy dose that do not vary;<sup>30</sup>
3. the light radiation penetrates the nail, considering that it is very thin;
4. the injuries were not considered as a single region, because the patient rarely has a single lesion; and<sup>5,6</sup>
5. the light source is chosen in accordance with the PS to be used for patients.

To determine the range of possible thicknesses of each fastener, averages of measurements taken using calipers were used,<sup>5,6</sup> but the variation in the thicknesses and widths of fingers was considered. Figure 2 shows how this device can be used in the fingernail and toenail at the same time.<sup>5</sup>

Due to excellent clinical results with two distinct classes of PS excited in different wavelengths, two devices emitting at different wavelengths were developed: one emitting at 470 nm, for curcumin activation [Figs. 2(a) and 2(b)], and one emitting at 630 nm, for porphyrin activation [Figs. 2(c) and 2(d)]. Both were developed at the São Carlos Institute of Physics (Laboratory of Technology Support, São Carlos, SP, Brazil) with fastening loops coupled to LED arrays, anatomically designed for the toenails and hands as shown with more detail in Fig. 3.

### 2.2 Optical Characteristics

Table 1 presents the optical characteristics for both wavelengths provided by the company LUXEON Rebel Color Portfolio with Test Current Thermal at 25°C.

### 2.3 Photosensitizers

Two different PSs were used for each wavelength: a hematoporphyrin-derivative (Photogem®, Limited Liability Company Photogem, Moscow, Russia) for excitation at 630 nm, and a mix of curcumins and curcuminoids (PDT Pharma, São Paulo, Brazil) for excitation at 470 nm.

### 2.4 Photodynamic Therapy Treatment

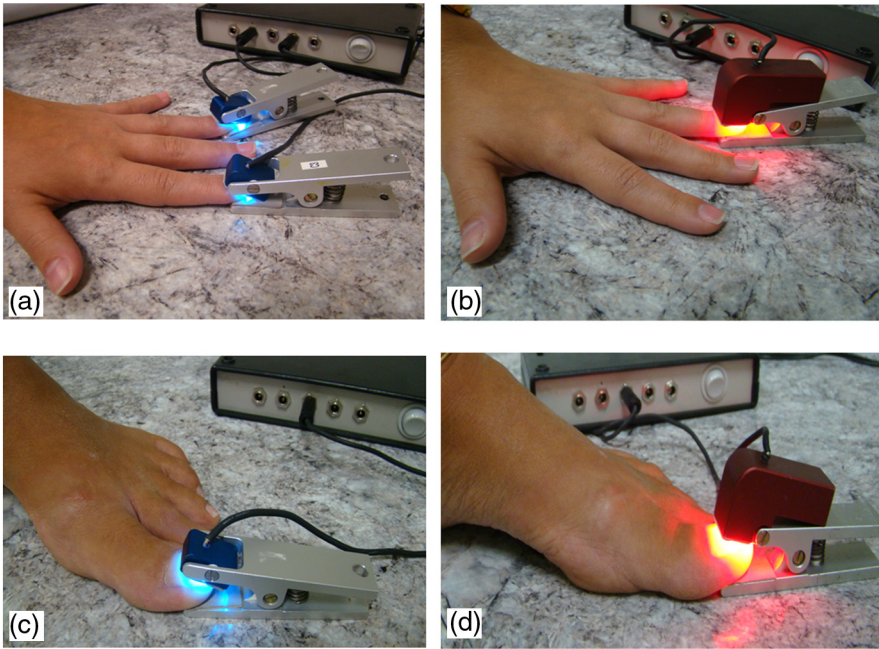
To calculate the amount of energy delivered by PDT, one must use Eq. (1):

$$D = I \cdot T. \quad (1)$$

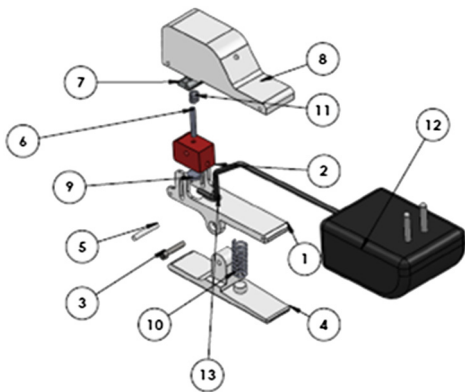
In Eq. (1),  $D$  is the total dose or fluence of energy (in  $J/cm^2$ ),  $I$  is the fluence rate of the light emitted by the equipment ( $0.1 W/cm^2$ ), and  $T$  is the total time of illumination (in s). Thus, since  $D$  is a treatment parameter and  $I$  depends on the device,  $T$  can be obtained by Eq. (1), with known  $D$  and  $I$ .

Before starting the procedure, preparation was carried out by disinfecting the nail with alcohol 70%, then nail scraping was done, followed by the application of the PS (Fig. 4).

After application of the PS, the lesion was occluded with aluminum foil for protection against light [Fig. 5(a)] and, after a period of 1 h, the nail plate was illuminated with a light source equivalent to the chosen PS [Fig. 5(b)]. Following treatment, the collection of images for later analysis was performed [Fig. 5(c)]



**Fig. 2** Light-emitting diode (LED) coupled loops anatomically designed for the toenails and fingernails as shown. (a) and (b) LED at 470 nm for curcumins activation and (c) and (d) LED at 630 nm for porphyrins activation.



**Fig. 3** Schematic drawing of each the pieces of one of the devices with LEDs emitting at 630 nm: 1—top piece in white PVC; 2—aluminum heatsink; 3—Allen M screw, 24 × 20 mm; 4—base in lower white PVC; 5—axis in white PVC; 6—aluminum threaded axle; 7—aluminum top piece; 8—PVC jacket; 9—plate with LED 630-nm label; 10—spring coil 5 × 0.5 × 25; 11—spring coil 5 × 0.3 × 10; 12—a power source 220/110 V; and 13—power cable.

2.5 Analysis of Photosensitivity Nail

Since we cannot remove the nail to verify the sensitization of the fungus part of the nail, we have used fluorescence images excited by 532 or 408 nm to observe the evidences that the actual part containing the fungus is, in fact, sensitized. In both cases, we have verified this fact. The use of urea to produce permeation of the nail material is fundamental for making sure that some of the sensitizers definitely reach the local site for treatment. This was done by a careful analysis by confocal microscopy.

We observe through the confocal images the penetration of the nail PSs: in the sample without PS, in the sample with curcumin, and sample with Photogem. After that we performed the same tests on samples with both PSs; however, these were treated with urea 1 h before the PS.

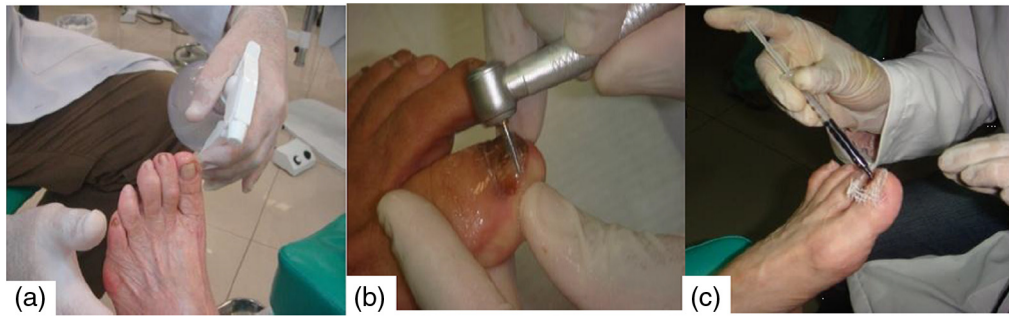
3 Results

3.1 Devices' Setup

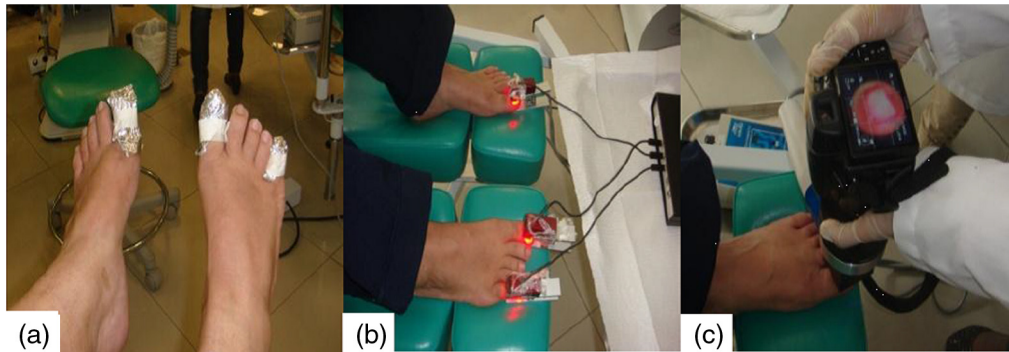
The prototype was designed for patients with onychomycosis. Temperatures considered tolerable by the patient were determined

**Table 1** The optical characteristics for comparison wavelengths: blue 470 nm and red 630 nm.

|       |          | Dominant Wavelength $\lambda_D$ or Peak Wavelength $\lambda_P$ |          |                     | Typical Spectral Half-width (nm) | Typical Temperature coefficient of Dominant Wavelength (nm/°C) | Typical Total included Angle (degrees) | Typical Viewing Viewing (degrees) |
|-------|----------|----------------------------------------------------------------|----------|---------------------|----------------------------------|----------------------------------------------------------------|----------------------------------------|-----------------------------------|
| Color | Min      | Typ.                                                           | Max.     | $\Delta\lambda$ 1/2 |                                  | $\Delta\lambda_D/\Delta\lambda_J$                              | $\theta$ 0.90 V                        | $2\theta$ 1/2                     |
| Blue  | 460.0 nm | 470.0 nm                                                       | 490.0 nm | 20                  |                                  | 0.05                                                           | 160                                    | 125                               |
| Red   | 620.0 nm | 627.0 nm                                                       | 645.0 nm | 20                  |                                  | 0.05                                                           | 160                                    | 125                               |



**Fig. 4** (a) Asepsis with alcohol 70%; (b) nail scraping; and (c) application of photosensitizer (PS).



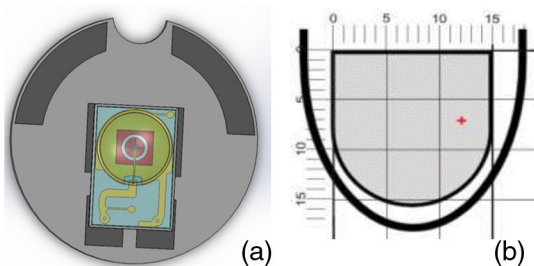
**Fig. 5** (a) Occlusion site; (b) photodynamic therapy; and (c) collection of nail lamina images.

in all points in the treated field, according to the size of the nail plate, and still allowing to evaluate the different substances in medicines, both of which differ in chemical structure and in the absorption spectrum.<sup>29</sup> The medication was kept in direct contact with the lesion for just an hour, and was subsequently illuminated for 20 min, resulting in an energy dose of  $120 \text{ J/cm}^2$ .<sup>30</sup>

Both the prototype device and the technique were patented (MU 9102265-7 U2 05/12/2011). The medications used are commercial and already approved for experimental clinical studies: one from Russia (Photogem®) and other from a Brazilian pharmaceutical company (PDT Pharma, São Paulo, Brazil).

### 3.2 Optical Characteristics

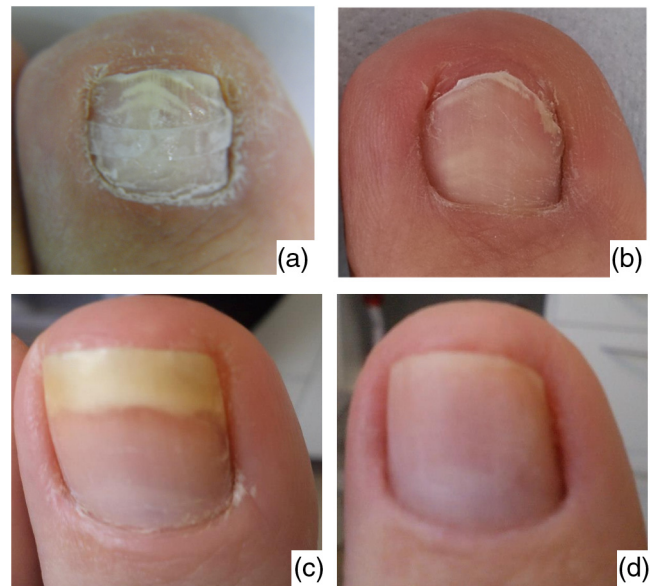
The illumination tests were conducted during a total time of 20 min, with a fluence rate of up to  $100 \text{ W/m}^2$  and varying wavelengths (630 and 470 nm). It was shown that the light penetration across multiple layers of the nail was possible without causing any irreversible thermal damage to tissues around the nail plate (Fig. 6).



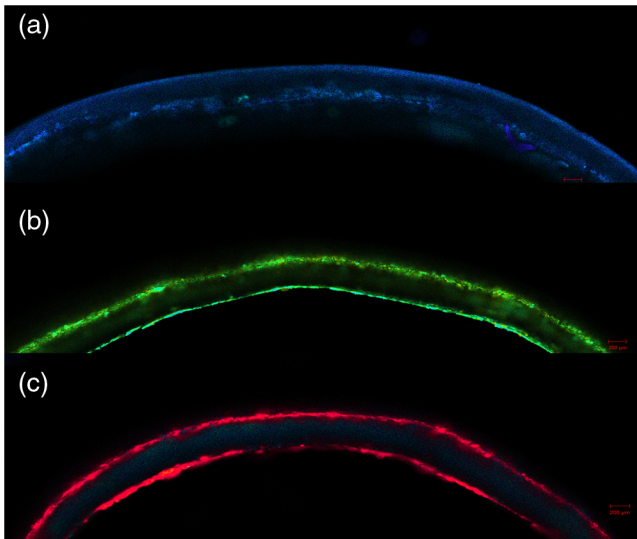
**Fig. 6** Schematic representations of (a) an LED with diameter of 1 cm and (b) average-sized Hallux with scale in millimeters.

### 3.3 Photodynamic Therapy Treatment

The first version of the prototype with LEDs emitting at 630 nm (red light) was designed by considering the tissue penetration of this wavelength. However, despite the fact that the blue light at a wavelength of 470 nm has less penetration than red light in

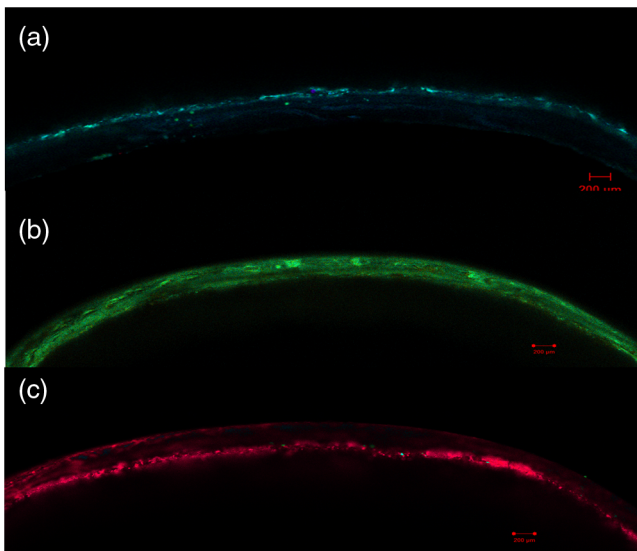


**Fig. 7** (a) Toenail left hallux with onychomycosis of the female patient 55-years-old with lesion more than 5 years. (b) Six months of treatment with Photogem® and the device with LEDs at 630 nm. (c) Toenail left hallux with onychomycosis of the female patient 46-years-old with lesion more than 10 years. (d) Clinical result 2 months after treatment with curcumin and curcuminoids excited with the device emitting at 470 nm.

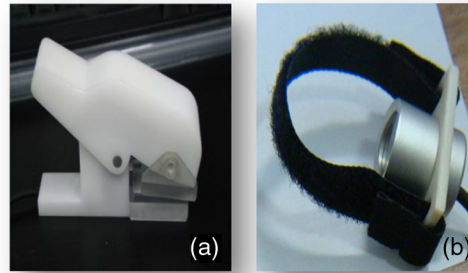


**Fig. 8** Observation through the confocal images of the penetration of the nail PSs: (a) the sample without PS; (b) the sample with curcumin; and (c) Photogem sample.

biological tissue in general, a decision was made to develop this second version of the device with the aim to use it to activate a natural PS, the curcumin. The clinical protocol was followed according to previously published studies.<sup>6</sup> In Fig. 7, the results of two cases of patients treated with PDT using these new devices are shown. Figure 7(a) shows the left hallux toenail of a 55-year-old female patient with an onychomycosis lesion for more than 5 years. Figure 7(b) shows the clinical result 6 months after PDT sessions with Photogem<sup>®</sup> and the 630-nm LED device. The second case is shown in Figs. 7(c) and 7(d), which show a left hallux toenail of a 46-year-old female patient with an onychomycosis lesion for more than 10 years [Fig. 7(c)]. The clinical result with curcumin and curcuminoids 2 months after PDT session which was activated by LED device (470 nm) is shown in Fig. 7(d).



**Fig. 9** The images of the same samples with PSs performed; however, these were treated with urea 1 h before the PS.



**Fig. 10** Two different versions of the equipment both emitting at 450 nm, with 100 mW/cm<sup>2</sup>: (a) equipment in the form of loop; (b) newer device with LED connected with velcro.

### 3.4 Analysis of Photosensitivity Nail

In Fig. 8, we observe through the confocal images the penetration of the nail photosensitizers. Figure 8(a) is the sample without PS, Fig. 8(b) is the sample with curcumin, and Fig. 8(c) is the Photogem sample.

In Fig. 9, the images of the same samples with photosensitizers were performed; however, these were treated with urea 1 h before the PS.

## 4 Discussion

Although techniques such as laser and iontophoresis have significant clinical results, PDT stands out because of its low cost, no side effects, and because it is a light source based on LED technology. This article concerns the characterization of two devices for the treatment of onychomycosis by PDT. The technique has solved fungal nail problems with excellent results in previous studies, showing that 87 of 90 patients had a satisfactory clinical response.<sup>5,6</sup> Providing hyperkeratotic nail penetration, reaching the underlying areas, is sufficient for the success of the treatment.<sup>31</sup> However, we aim to improve the method and the geometric pattern to provide the application of a greater amount of light in order to decrease the illumination time. This new approach for the treatment of onychomycosis can save treatment time and should show excellent acceptance by patients.

## 5 Conclusions

This article showed the importance of developing this device as a light source for the treatment of onychomycosis by PDT. The results in clinical research<sup>5,6</sup> led to a modification in the prototype [Fig. 8(a)] to include anatomical improvements, such as a larger contact area due to the curvature (modifying from a flat area to a concave one), the external start button, the introduction of a timer, the device width—which was reconsidered for use in all the fingers at once, and an autoclave protection to prevent cross-contamination among patients and among fingers. These improvements were made to provide more comfort for the patient and the operator.

Another project has been designed with only one LED connected with velcro [Fig. 8(b)] for better comfort of the patients regardless of the size of the feet, which was a limitation of the last version [Fig. 8(a)].

Since the success of any application of photodynamic technique needs the correct illumination for reaching the desired success, we have described and tested two illumination devices

for special application to nail onychomycosis (Fig. 10). The devices aim to follow the anatomy of the site to be treated for better reproducibility of the procedure as well as give correct information about illumination devices for this specific application for those who want to use the procedure.

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