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Antimicrobial Photodynamic Therapy as a Technique for Decontamination of Acrylic Resin Devices Provided by Different Dental Laboratories

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Abstract

Introduction: Dentures, occlusal splints, surgical guides and orthodontic appliances are examples of acrylic resin devices made in dental laboratories, which must be disinfected and even sterilized before insertion into the oral cavity. This study evaluated the antimicrobial effect of photodynamic therapy (PDT) applied to acrylic resin specimens received from different laboratories.

Methods: Three hundred standardized specimens were ordered from six randomly selected laboratories registered in the Council of Dentistry of Ceará (n=50). The PDT consisted in the association of 22 μM erythrosine, as a photosensitizer (P), and a 520-nm LED at 38 J/cm² (L). The specimens of each laboratory were randomly distributed into five groups: positive control, sterilized with ethylene oxide; negative control, untreated (P-L-); erythrosine control, only stained (P+L-); LED control, only irradiated (P-L+); PDT (P+L+). Then, the specimens were individually sonicated in saline solution; the suspension was diluted, plated on culture mediums (blood agar, sabouraud dextrose agar and a non-selective chromogenic agar), and incubated for 48 hours at 37°C. Colony-forming-unit (CFU) counts were done and statistical tests of Kruskal-Wallis/Dunn were carried out.

Results: The specimens from all laboratories were contaminated with bacteria and yeasts. *Staphylococcus aureus, Staphylococcus saprophyticus, Escherichia coli, Enterococcus* spp., *Klebsiella and Pseudomonas* spp. were identified. The PDT significantly reduced CFU counts ($P<0.0001$), compared to P-L-.

Conclusion: PDT was able to effectively decontaminate the acrylic resin specimens provided from dental laboratories.

Keywords: Acrylic resins; Photochemotherapy; Contamination; Disinfection; Sterilization.

Introduction

Photodynamic therapy (PDT) has been promising in the inactivation of pathogenic microorganisms.[1-3] It is applied in dentistry in the areas of cariology, periodontics, endodontics, and oral pathology, being quite effective.[4-7] In the dental prosthesis area, this technique has been reported in the control of *Candida albicans* biofilm.[1,3] However, there is also interest in the control of other microorganisms in order to avoid cross-infection between the dental office and the dental laboratory, and the need to sterilize immediate dentures and surgical guides for implantology, which should be used in an aseptic environment.[8]

PDT involves the application and retention of a photosensitizing agent in the microbial cells, under irradiation with a light source of compatible wavelength with its absorption band. The irradiated photosensitizer undergoes a transition from a low power state to a state of higher energy or triplet. At this stage, the photosensitizer reacts with biomolecules, producing free radicals and radical ions, and it also reacts with molecular oxygen to produce singlet oxygen ($^1O_2$), which is highly cytotoxic, since it causes oxidation of cellular constituents, like the plasma membrane and DNA, resulting in the death of stained microbial cells.[9,10]

The removable dentures or surgical guides are primarily composed of acrylic resins. This material can be sterilized by ethylene oxide gas, gamma ray, hydrogen peroxide

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plasma or microwave irradiation. The first three methods are used in hospitals at high cost, and the fourth method, while promising, has not provided high effectiveness in microbial elimination; besides, there are controversies regarding their effect on the dimensional stability and hardness of the involved materials.[11-13] For disinfection of acrylic resins it is common to use substances such as 1% sodium hypochlorite, peracetic acid, chlorhexidine digluconate and 70% alcohol, which, although reducing the amount of microorganisms, have disadvantages, such as changes in surface roughness and color of acrylic resins, corrosion and irritant effects on skin.[12]

The concern over the issue of cross-infection in dental offices and dental laboratories has been increasing in recent years, especially after studies showing that the contamination of technicians in a prosthetic laboratory is mainly caused by contaminated impressions or mishandling of items from the clinic after arrival at the laboratory. Research reported that more than 60% of the prostheses delivered to the laboratories from dental clinics were contaminated with pathogenic microorganisms from the oral cavity of patients.[13-16] One study reported that 39.5% of the technicians in a prosthetic laboratory never wore gloves when working.[17] Furthermore, the lathes used for polishing and finishing acrylic prostheses have been described as a major source of contamination.[18] Kugel et al[8] concluded that no prosthetic laboratory is immune to the presence of potentially pathogenic microorganisms.[8,19]

Given this, the search for an alternative method of simple and effective disinfection or even sterilization of these acrylic devices is of great clinical interest.[8,20,21] Photodynamic therapies for the inactivation of microorganisms can be a solution because of its proven effectiveness in other areas, combined with the simplicity of the technique and the possibility of using low-cost materials. The aim of this study was to evaluate the effect of PDT technique on combating microorganisms present on the surface of acrylic resin specimens made in different dental laboratories from the city of Fortaleza (CE – Brazil), using 22 μM erythrosine associated with a 520-nm LED with energy density of 38 J/cm^2. Therefore, the null hypothesis tested in this study was that the combination of a photosensitizer and LED could have no antimicrobial effect on acrylic resin substrates.[21,22]

Material and Methods

Preparation of the Samples

A self-curing acrylic resin (Classico, Articles Dental Classic Ltda. São Paulo, SP, Brazil) was selected for the preparation of the specimens. A total of three hundred specimens were ordered from six selected dental laboratories in Fortaleza (CE - Brazil), according to the manufacturer's specifications, following the model of matrices provided by the main investigator (n = 50).

The matrices provided to the laboratories were fabricated in vinyl polysiloxane (Adsil, Vigodent, São Paulo, SP, Brazil) measuring 1 cm in diameter and 2 mm in thickness, packed in surgical paper and sterilized by autoclaving. In dental prosthetic laboratories, they were included in flasks, so they could be reproduced with the indicated pressing of acrylic resin. The finishing and polishing of the specimens were also conducted by the laboratories, indicating the use of sandpapers numbers 220, 400 and 600, followed by polishing around with a felt cone and canvas polishing discs with the pumice stone and Spanish white powder.

Selection of Laboratories

This selection was randomly performed by a raffle, via a computer program (Microsoft Office Excel 2007), from a list provided by the Regional Council of Dentistry of Ceará (CRO-CE).

The dental prosthetic laboratories drawn were called L1, L2, L3, L4, L5 and L6 and received no information about the survey but the order of service, the requirement on the trademark of resin, the finishing system and the matrices for the preparation of the specimens. The operator of the experiments also had no knowledge about which laboratories were being tested, making this a blinded study.

The prepared specimens were transported in a sterile metal box from the dental laboratory to the microbiology laboratory at the Faculty of Pharmacy, Dentistry and Nursing in Federal University of Ceará (FFOE -UFC). The shipping box was opened inside a laminar flow for the removal of specimens and beginning of the experimental phase.

Selection of Photosensitizer and Light Source

Erythrosine (Vetec, Rio de Janeiro, RJ, Brazil) was used for the sensitization at the concentration of 0.22 μM.[9] Powdered erythrosine was dissolved in phosphate-buffered saline (PBS, pH 7.4). The solution was filtered through 0.22 μm pore diameter membranes (Merck Millipore, Billerica, Ma, USA) and stored in the dark. The absorption spectrum (400-800 nm) of the photosensitizer was confirmed in a spectrophotometer (Cary 50 Bio, Varian Inc., Palo Alto, Ca, USA).

A light-emitting diode (LED) (DMC, São Carlos, SP, Brazil) was used, with a tip diameter of 8.3 mm, a wavelength of 520 nm, an average output power of 90 mW, an energy of 30 J, a fluence of 38 J/cm^2, and an irradiation time of 5 minutes and 33 seconds.

Experimental Groups

For each of the six selected laboratories, the specimens were randomly distributed into 5 groups (n = 10):

- Positive control (EO) - sterilized with ethylene oxide gas;
- Negative control (P-L) - no treatment;

- Control of the photosensitizer (P + L-) - stained with erythrosine and unirradiated;
- Control of the green LED (P-L +) - irradiated with a green LED and unstained;
- Experimental (P + L +) - stained with erythrosine and irradiated with a green LED.

The EO group was the positive control because after the arrival from the lab, their specimens were individually wrapped in surgical paper and subjected to ethylene oxide sterilization, considered the gold standard. The negative control group received no treatment in order for the present researchers to permit the verification of actual contamination of the specimens after arrival from the laboratories.

The group (P + L-) aimed to search a possible antimicrobial effect of the erythrosine alone at 22 μM. The specimens of this group, after the arrival from the laboratory, were immersed in 2 mL of the dye solution, ensuring contact in all their surfaces, for 5 minutes in glass containers.

The group (P-L +) aimed to check the possible antimicrobial effect of irradiation with a 520-nm LED alone. The active tip of the LED device was fixed in a universal stem and positioned perpendicularly to the surface to be irradiated from a distance as close as possible (0.8 cm), so the entire specimen would be covered with light. Irradiation was performed at 38 J/cm^2 on all surfaces of each specimen as soon as they came from the laboratory.

The experimental group (P + L +), which consisted of the combination of the photosensitizer and the LED, tested the effect of the PDT on microbial reducing in acrylic resin specimens made in dental prosthetic laboratories from the city of Fortaleza- CE – Brazil (Figure 1). The procedures of staining and irradiation were performed as described above. The interval between the application of dye and LED irradiation was 5 minutes.

Microbiological Procedures

After treatments, the specimens were placed in individual tubes containing 1 mL of 0.9% sterile saline solution. Each tube was shaken by an agitator (MPL – Materiais para Laboratório, MPL QL-901, Piracicaba, SP, Brazil) for 1 minute.

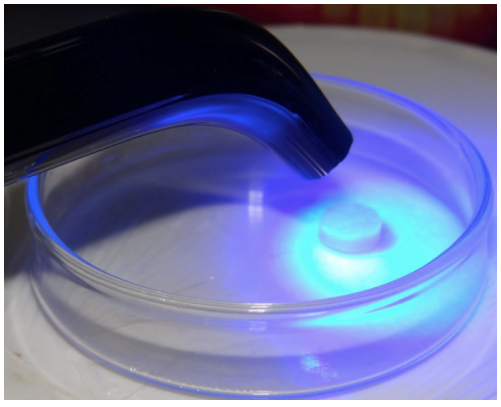

Figure 1. PDT being performed on a sample (p + L +)

The suspension went through a process of dilution 1:10, 1:100, 1:1000 and the neat and diluted forms were plated (10 μL) in petri plates containing the culture media Blood Agar, Sabouraud Dextrose (Eximlab LTDA, São Paulo, SP, Brazil), and CHROMagar Orientation® (Probac, São Paulo, SP, Brazil). The plates were incubated for 48 hours at 37°C for subsequent counting of colony-forming units (CFUs).

The Blood Agar culture medium allows the growth of viable bacteria, whereas Sabouraud Dextrose Agar allows the growth of viable yeasts and fungi. CHROMagar Orientation® is a non-selective chromogenic medium that allows identification of specific colonies through differences in their shades.

Statistical Analysis

The CFUs were counted for each group from each laboratory. Adherence to the assumptions of normality was verified using the Cochran tests ($\alpha = 0.05$). The data did not conform to the requirements of a normal distribution, so Kruskal-Wallis and Dunn's multiple comparison tests were performed at 95% confidence intervals ($\alpha = 0.05$).

Results

The results of CFU counts of viable bacteria in different groups for different laboratories are shown in Figures 2b to 2g, while the results of CFU counts of viable yeasts in different groups for the analyzed laboratories are shown in Figures 3h to 3m.

The antimicrobial activity of PDT against viable bacteria was statistically significant according to the results from (P + L +) groups compared to (P-L-), (P-L +) and (P + L-) groups ($P < 0.0001$).

For viable yeasts, it was observed that PDT was also effective, but the irradiation with the light source alone showed a significant reduction in CFU counts in almost all laboratories tested, except for L6. Erythrosine alone also played a role in reducing viable yeasts for L3 and L4.

The comparison between laboratories as the initial contamination of specimens by viable bacteria and viable yeasts are shown in Figures 4n and 4o respectively. The microorganisms *Staphylococcus aureus, Staphylococcus saprophyticus, Escherichia coli, Enterococcus* spp., *Klebsiella and Pseudomonas* spp. were detected on the specimens from all laboratories analyzed.

Discussion

The risks of cross infection in dental offices are established and well known. The same cannot be said of the dental prosthetic laboratories because they generally do not have

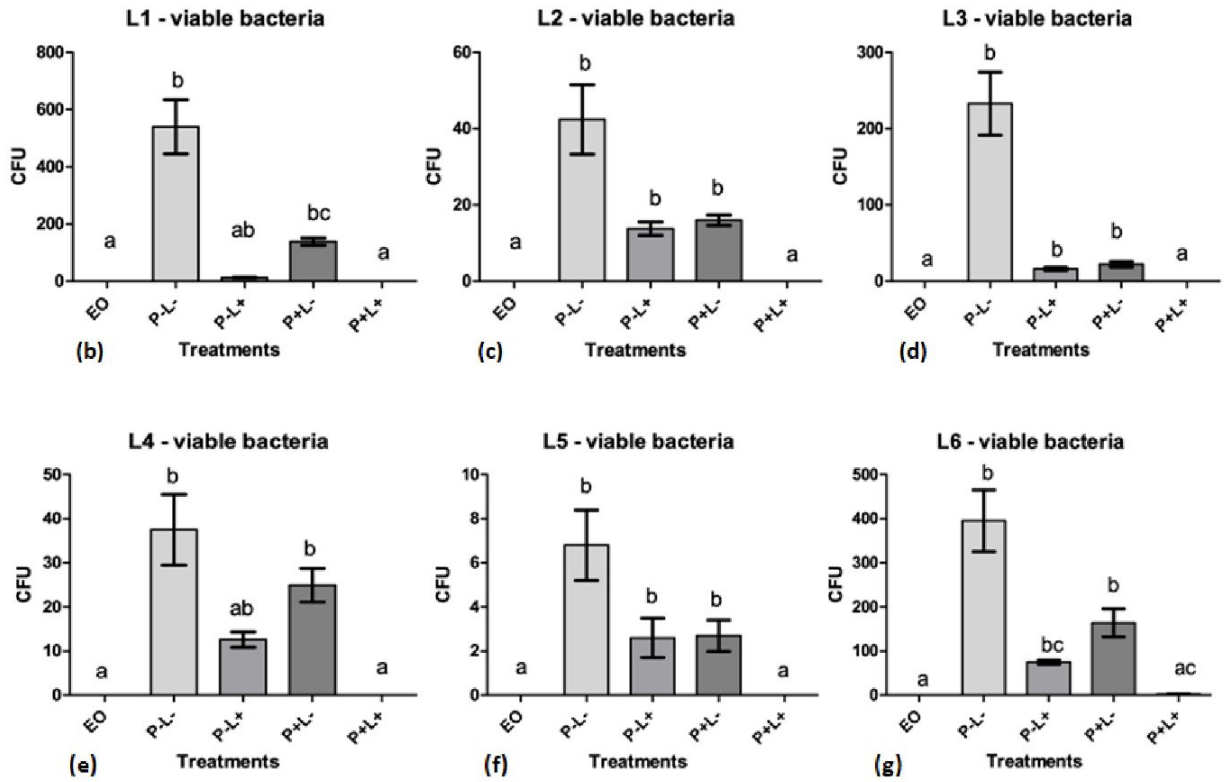

Figure 2. (**b**) The results of CFU counts of viable bacteria in different groups for Laboratory1 (L1). (**c**) The results of CFU counts of viable bacteria in different groups for Laboratory 2 (L2). (**d**). The results of CFU counts of viable bacteria in different groups for Laboratory 3 (L3). (**e**) The results of CFU counts of viable bacteria in different groups for Laboratory 4 (L4). (**f**) The results of CFU counts of viable bacteria in different groups for Laboratory 5 (L5). (**g**) The results of CFU counts of viable bacteria in different groups for Laboratory 6 (L6). Different lowercase letters indicate $P<0.0001$

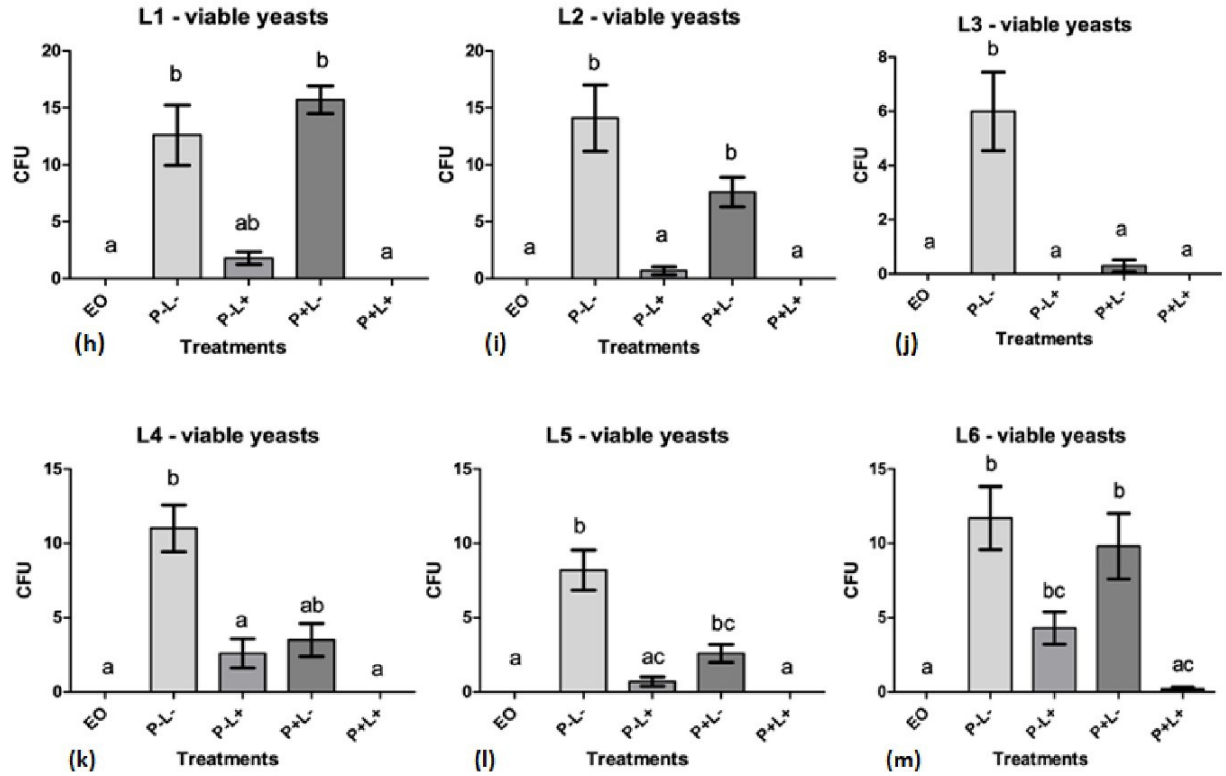

Figure 3. (**h**) The results of CFU counts of viable yeasts in different groups for Laboratory 1 (L1). (**i**) The results of CFU counts of viable yeasts in different groups for Laboratory 2 (L2). (**j**) The results of CFU counts of viable yeasts in different groups for Laboratory 3 (L3). (**k**) The results of CFU counts of viable yeasts in different groups for Laboratory 4 (L4). (**l**) The results of CFU counts of viable yeasts in different groups for Laboratory 5 (L5). (**m**) The results of CFU counts of viable yeasts in different groups for Laboratory 6 (L6). Different lowercase letters indicate $P<0.0001$

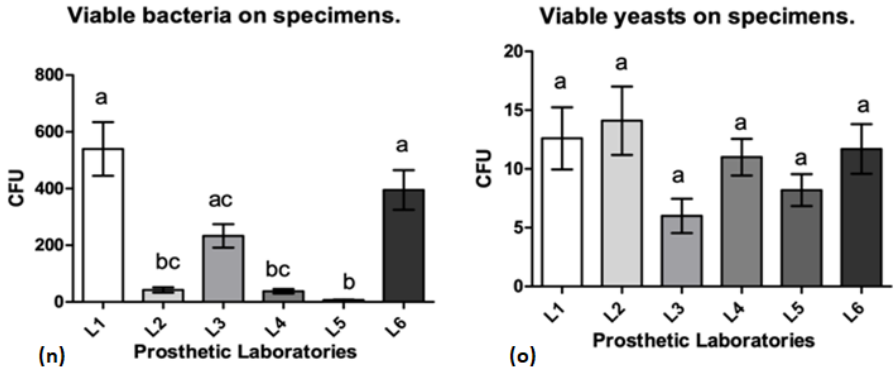

Figure 4. (n) The laboratories contamination of specimens by viable bacteria (CFU/mL). Different lowercase letters indicate $P<0.0001$. (o) The laboratories contamination of specimens by viable yeasts (CFU/mL) ($P=0.1241$)

direct contact with the patient, and thus it is believed that they are not exposed to biological material.[22]

Prosthesis, molds, models or other objects having contact with the patient's saliva or blood can serve as an indirect transmission of microorganisms to the personnel involved in the handling of the prosthesis in the laboratory, including laboratory technicians, via direct contact or by aerosols produced during abrasion procedures and polishing. Due to the high degree of contamination of the prosthesis, if appropriate disinfection procedures are not implemented in laboratories, microorganisms may be transferred from the laboratory to the patient, triggering a cross infection.[23]

In addition to concerns related to molds, studies have shown that the compartment that holds the pumice stone and felt cones for polishing can spread diseases.[15,24] In this study, it was found that although there was no contact with the oral cavity of patients, specimens were contaminated with different types of microorganisms, including many species of bacteria, as well as yeasts.

Witt and Hart[15] reported that all samples of felt disks with the pumice stone and water, in three dental prosthetic laboratories, were contaminated with microorganisms like aerobic positive Gram-bacilli, including *B. cereus*, *B. brevis*, *B. licheniformis* and members of the group *coli*. In this study, such microorganisms as *Enterococcus* spp., *Pseudomonas* spp., *S. aureus*, *Klebsiella*, *E. coli* and *S. saprophyticus* were identified.

The use of PDT in this study consisted of an alternative for disinfection or sterilization of acrylic resin-based materials, which proved to be quite effective, showing statistically significant difference between the group that received therapy and the negative control group without any form of treatment, resembling the gold standard, sterilized in ethylene oxide. In all laboratories, there were different levels of contamination, but in all, the therapy has proven to be a safe and efficient alternative for the control of infections caused by pathogenic microorganisms.[24,25]

In addition to the work of Demidova and Hamblin,[22] Dovigo[3] and Wood et al[9] when associating a photosensitizer with a compatible light emitting device, it was observed that there was a significant decrease in the quantity of microorganisms evaluated. Similar to this work, the sterilization of some specimens occurred when subjected to PDT in question, which means a reduction that reached zero CFU.

Wood et al[9] applied PDT in *S. mutans* biofilms, using erythrosine 22 μM and 500–650 nm light, 400 W, for 15 minutes, with a reduction of 2.2 ± 0.2 $\log_{10}$ for biofilms of 48 hours and 3.0 ± 0.3 $\log_{10}$ for biofilms of 28 hours. Metcalf et al[23] also studied erythrosine in PDT on *S. mutans,* and they got a bacterial reduction of $2\log_{10}$ after 5 minutes of continuous irradiation and of $3.7\log_{10}$ with fractioned light into time intervals of 10 to 30 seconds. This concentration of erythrosine is considered minimal, making it easy to remove from the acrylic resin sample surface and consequent removal in dental prosthesis, not causing stains on such devices.[9,23]

In their work, Rolim et al[24] evaluated the PDT with different photosensitizers against *S. mutans* planktonic cultures. The $^1O_2$ production of each photosensitizer was analyzed. 163.5 μM methylene blue, toluidine blue ortho and malachite green were activated with a light-emitting diode (636 nm), while eosin, erythrosine and rose bengal were irradiated with a curing light (570 nm). Light sources were operated at 24 J/cm^2. Toluidine blue ortho was the only photosensitizer that effectively reduced 99.9% of this microorganism.

Peloi et al[25] studied the red LED associated with methylene blue, which promoted reductions over 93% for *S. aureus*, *E. coli* and *C. albicans*. Soares et al[26] verified an adhesion reduction of 55% of *C. albicans* on buccal epithelial cells, corresponding to a reduction of $3.41\log_{10}$, using the red LED and toluidine blue. Zanin et al[27] applied PDT with the red LED and toluidine blue on in vitro biofilms and observed a reduction of 95% in the viability of *S. mutans*, *S. sobrinus* and *S. sanguinis*.

As for the action of only LED on the inactivation of microorganisms, a decrease was obtained, though not statistically significant. This result is also identified

in the study of Maclean et al[28] who demonstrated the susceptibility of resistant bacteria to blue light (405 nm) with no photosensitizer. Gram-positive and Gram-negative bacteria were inactivated, including methicillin-resistant *S. aureus,* with reductions near $9\log_{10}$. In the aforementioned study, erythrosine and rose bengal were used, which are xanthenic dyes and have good light absorption, with maximum absorption at approximately 530 nm, a range corresponding to that emitted by blue and green light-emitting diodes, as used in our study.[28]

PDT with erythrosine and the green LED reduced cell counts in planktonic cultures and biofilms of *C. albicans* and *C. dubliniensis* according to Costa et al.[29] Similar results were obtained in this study - there was a significant decrease, reaching zero, in the growth of yeasts in acquired specimens in many cases.

The absence of pre-defined parameters for using PDT makes a reliable comparison of the results among the different papers difficult. Moreover, the concentration of the photosensitizer, photosensitizer type, incubation time, the exposure period of the LED, the type of light source, and the physiological condition of the microorganisms may influence the results of the PDT technique.[25,27]

Certain limitations were identified in this work, as the use of a single type of photosensitizer, on a single concentration and fluency. More research is needed, with different dyes and equipment, to find options more viable and equally effective for PDT.[20,29]

Antimicrobial PDT is promising and deserves researchers' attention because its major advantage is the fact that the development of microbial resistance after multiple treatments is unlikely. The interaction of singlet oxygen and free radicals with various cellular and metabolic structures makes it effective against bacteria resistant to antibiotics.[30] It also does not have toxic, corrosive or allergen effects as certain disinfectants and immersion solutions.

Through this study it was possible to demonstrate the effectiveness of PDT in combating cross-contamination between dental prosthetic laboratories and dental offices, in addition to being a simple, rapid and low-cost alternative for the sterilization of acrylic devices that are used in the surgical field, as surgical guides and immediate removable dentures and without apparent harm to the properties of the acrylic resin.

Conclusion

It was concluded that PDT mediated by erythrosine 22 μM and 520-nm LED promoted antimicrobial activity in acrylic resin specimens provided from different dental laboratories from Fortaleza-CE - Brazil.

Competing Interests

The authors of this article declare that they have no conflicts of interest.

Ethical Approval

Not applicable.

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