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Effect of different pre-irradiation times on curcumin-mediated photodynamic therapy against planktonic cultures and biofilms of *Candida* spp

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ABSTRACT

Objectives: The aim of this study was to evaluate the effects of pre-irradiation time (PIT) on curcumin (Cur)-mediated photodynamic therapy (PDT) against planktonic and biofilm cultures of reference strains of *Candida albicans*, *Candida glabrata* and *Candida dubliniensis*.
Materials and methods: Suspensions and biofilms of *Candida* species were maintained in contact with different concentrations of Cur for time intervals of 1, 5, 10 and 20 min before irradiation and LED (light emitting diode) activation. Additional samples were treated only with Cur, without illumination, or only with light, without Cur. Control samples received neither light nor Cur. After PDT, suspensions were plated on Sabouraud Dextrose Agar, while biofilm results were obtained using the XTT-salt reduction method. Confocal Laser Scanning Microscopy (CLSM) observations were performed to supply a better understanding of Cur penetration through the biofilms after 5 and 20 min of contact with the cultures.
Results: Different PITs showed no statistical differences in Cur-mediated PDT of *Candida* spp. cell suspensions. There was complete inactivation of the three *Candida* species with the association of 20.0 μM Cur after 5, 10 and 20 min of PIT. Biofilm cultures showed significant reduction in cell viability after PDT. In general, the three *Candida* species evaluated in this study suffered higher reductions in cell viability with the association of 40.0 μM Cur and 20 min of PIT. Additionally, CLSM observations showed different intensities of fluorescence emissions after 5 and 20 min of incubation.
Conclusion: Photoinactivation of planktonic cultures was not PIT-dependent. PIT-dependence of the biofilm cultures differed among the species evaluated. Also, CLSM observations confirmed the need of higher time intervals for the Cur to penetrate biofilm structures.

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1. Introduction

Species of the genus *Candida* are considered commensal yeasts frequently isolated from the oral cavity of healthy patients.[1–3] However, these microorganisms can act as opportunistic pathogens under certain circumstances, such as impairment of salivary glands, long-term use of immunosuppressive drugs and antibiotics, denture wear, and malignancies.[4,5] *Candida albicans* is the most commonly isolated species, being present in around 20–50% of the cases of oral infections.[6] Recently, infections with species other than *C. albicans*, notably *Candida glabrata and Candida dubliniensis* have been increasingly described.[7–9] *C. glabrata* has become the second most frequently isolated commensal yeast from the oral cavity,[2,7,8] and it is responsible for 15% of mucosal lesions.[2] *C. dubliniensis* is a recently described species of the genus *Candida*[10] primarily associated with oral candidiasis[11] in acquired immunodeficiency syndrome (AIDS) patients.

Denture stomatitis is a common superficial infection of the palate oral mucosa that affects more than 65% of denture wearers.[12] This condition develops under the influence of denture plaque, which consists of a structured community of microorganisms surrounded by a self-produced polymeric matrix and adherent to an inert or living surface.[13] Studies[14,15] have demonstrated that different species of yeast and bacteria are associated with denture biofilm, including *Candida* spp., *Staphylococcus* spp., *Streptococcus* spp., *Lactobacillus* spp., *Pseudomonas* spp., *Enterobacter* spp. and *Actinomyces* spp. Clinically, denture stomatitis is characterised by erythematous points on the denture-bearing tissues and diffuse erythema.[16] The most susceptible hosts are the elderly, who concomitantly wear dentures and use immunosuppressive medications or prophylactic antifungal agents, which can promote substantial switching of the oral ecology,[17] and further facilitate the installation[6] and dissemination of opportunistic infections.[22]

Oral candidiasis may be treated with either topical[19,20] or systemic[21] antifungal therapy, according to the severity of the infection. The therapy of choice for immunocompromised patients is usually a course of systemic antifungal agents such as fluconazole or amphotericin B.[6] However, some conventional antifungal drugs, such as azoles, present fungistatic activity rather than fungicidal, resulting in an inadequate treatment outcome for immunocompromised patients.[9,22,23] Therefore, recurrent candidiasis is common, and retreatments are often needed. In this context, *C. glabrata* and *C. dubliniensis* are of special importance because of the innate resistance to antifungal agents of the former,[2,9] and the ability of the latter to develop rapid *in vitro* stable fluconazole resistance.[23–25]

With the increase of microbial resistance, many researchers have focused on finding non-conventional therapies to treat oral infections. Photodynamic therapy (PDT) is a promising therapeutic method[26–30] originally developed for the treatment of tumours.[28] Recently, PDT has been investigated to treat other pathologies such as viral, fungal and bacterial infections.[28,30] Although PDT does not replace conventional systemic antimicrobial therapy, improvements may be obtained using the photodynamic approach in the clinical treatment of local infection.[30] PDT involves the application of a photoactive drug denominated photosensitiser (PS) and its exposure to a light source with appropriate wavelength to activate the PS. After the absorption of photons, and in the presence of oxygen, an excited state of the PS can be generated.[28] These events result in a cytotoxic photodynamic reaction, involving the production of reactive oxygen species and sequential oxidative reactions, which lead to cell death.[31] It seems that PDT acts primarily against the cell membrane, and after increasing its permeability, the PS moves into the interior of the cell, and damages the intracellular organelles.[32,33] Therefore, differently from conventional antifungal drugs, whose mode of action is limited to a single target,[23] PDT acts against several targets, thus it is unlikely that resistance will emerge.[34] Among other factors, successful PDT depends on the pre-irradiation time (PIT),[35] which is the time required by the PS to remain in contact with the target cells before irradiation. This period will enable the PS to bind to the cytoplasmic membrane and/or penetrate into the cells.[33,34] The following exposure to light will allow the PSs to exert their function in promoting cell death.

Many researchers have focused their attention on effective PSs for the photoinactivation of microorganisms.[26,27,29,32–45] Curcumin (Cur) is a yellow-orange dye extracted from the rhizomes of the plant *Curcuma longa*.[46] It is commonly used as a spice in traditional Asian cookery, and has been shown to exhibit a variety of pharmacological properties such as antitumor, anticancer, anti-inflammatory, antioxidants, and antimicrobial activities,[18,46,47] some of which can be enhanced by light application.[44,48] Cur has been used as a PS in antimicrobial PDT, mainly on photoinactivation of *Candida* species, with positive results.[41] However, some studies have stated that in contrast to that which occurs with several PSs, Cur does not bind to cells, or binds to them weakly, leaving about 90% in an extracellular bulk phase.[37] The removal of the non-associated Cur promotes a substantial reduction in its phototoxic effects.[36,41]

The aim of this study was to evaluate the effects of PIT on curcumin-mediated PDT in the inactivation of planktonic and biofilm cultures of three *Candida* species: *C. albicans*, *C. glabrata*, and *C. dubliniensis*.

2. Materials and methods

2.1. Microorganisms

Two *Candida* strains obtained from American Type Culture Collection (ATCC) and one from the Centraal bureau voon Schimmelcultures (CBS) were evaluated in this study: *C. albicans* (ATCC 90028), *C. glabrata* (ATCC 2001), and *C. dubliniensis* (CBS 7987). All three *Candida* strains were maintained in a freezer at −70 °C until the assay.

2.2. Photosensitiser and light source

Curcumin (Sigma–Aldrich, Saint Louis, Missouri, USA) was prepared with 10% of Dimethyl Sulfoxide (DMSO) to originate a stock solution, from which other solutions were prepared at final concentrations of 5.0, 10.0, 20.0, 30.0 and 40.0 μM. A light emitting diode (LED) was used to activate the PS. The LED