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Potential of curcumin-mediated photodynamic inactivation to reduce oral colonization

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ABSTRACT

Objective: The present study assessed the susceptibility of salivary pathogens to photodynamic inactivation (PDI), mediated by a water-soluble mixture of curcuminoids (CRM) and LED light.
Methods: A 10 mL sample of unstimulated saliva was collected from volunteers. The inoculum was prepared using 9 mL of saline and 1 mL of saliva. Inoculum suspensions were divided into 14 groups and treated according to the description below. Groups that received the PDI treatment (light for 1 min or 5 min and 1.5 g/L or 3.0 g/L of CRM concentration) were called $C_{1.5}L_{1.8}$, $C_{1.5}L_{9.0}$, $C_{3.0}L_{1.8}$, $C_{3.0}L_{9.0}$. To evaluate the CRM decontamination alone, the $C_{1.5/1}$,$C_{1.5/5}$,$C_{3.0/1}$ and $C_{3.0/5}$ groups were assessed. Likewise, light alone was evaluated through the $L_{1.8}$ and $L_{9.0}$ groups. Chlorhexidine at 0.12% (CLX) for 1 or 5 min was used for the positive control groups (CLX_1 and CLX_5, respectively); saline was used for 1 or 5 min (CTR_1, CTR_5, respectively) for the negative control groups. After the tests, serial dilutions were performed, and the resulting samples were plated on blood agar in microaerophilic conditions. The number of colony forming units (CFU/mL) was determined and $\log_{10}$-transformed. Data were analyzed using a One-way Analysis of Variance with Welch correction, followed by the Games Howell's test ($\alpha = 0.05$). Log reduction (LR) measure for antimicrobial efficacy was also calculated using data from the CTR_5 as untreated samples.
Results: The CHX_5 showed the best antimicrobial result, followed by the CLX_1. The antimicrobial effect of CRM was more pronounced when associated with light (PDI), but significantly lower than the CLX_5 effect. The $C_{3.0}L_{9.0}$ protocol showed similar results to the CLX_1.
Conclusion: The results show that PDI with CRM at the studied concentrations is as effective for oral decontamination in clinical dental care conditions as the CLX at 0.12% for 1 min.

1. Introduction

The human mouth can host more than 700 species of pathogenic and non-pathogenic microorganisms [1]. Thus, the bacterial aerosols generated from a patient's mouth during dental procedures expose the dental staff to a chronic occupational hazard. The bioaerosol can spread by a radius of 60 cm from the oral cavity, contaminating everything in this perimeter [2]. Consequently, several infectious diseases, including those caused by viruses, can be transmitted by the aerosol representing significant health risks for the dental staff, such as chickenpox, hepatitis B, hepatitis A, herpetic conjunctivitis, herpes simplex, herpes zoster, infectious mononucleosis, measles, rubella, mumps and influenza. Additionally, many other diseases may be caused by bacteria and fungi [3]. The bioaerosol can also put healthy patients at risk because of the possibility of cross-contamination at the dental office [1–5].

The biosafety manual recommends the routine use of personal protective equipment (PPE) as standard for the treatment of any patient [5]. However, there are reports showing that the aerosol formed by dental handpieces remains suspended in the environment for at least 10 min to a few hours after treatment [4,5]. In that case, the use of PPE will not guarantee the protection of the dental team. Therefore, measures that can effectively reduce the amount of microorganisms present in the oral aerosol should be incorporated as a biosafety routine associated with the use of PPE to ensure more effective protection of the dental staff and patients. For this

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purpose, some practitioners routinely ask the patient to perform oral decontamination using topical mouthwashes [6].

Currently, 0.12% chlorhexidine digluconate (CLX) is the most widely used antiseptic for mouth disinfection [7]. According to Davies [8], CLX is a broad spectrum antimicrobial agent that exhibits activity against gram-negative and gram positive bacteria and yeasts. However, there is no positive evidence of their effectiveness against spores [9] and the routine use of CLX as an antiseptic may cause alteration in taste sensation and staining of the teeth and tongue [8,10]. Because of its drawbacks, investigations have focused on the search for alternative methods for oral decontamination [11,12]. Therapy based on physical interaction of light and photosensitizing drugs, called photodynamic therapy (PDT), have been developed as an alternative or complementary technique to solve many microorganism-related health problems [12–16]. Photodynamic inactivation of microorganisms, also known as Photodynamic Antimicrobial Chemotherapy (PACT) [16] or Photodynamic Inactivation (PDI) [17], is based on the combination of a photosensitizer (PS) and light of the appropriate wavelength to excite the PS molecule [18]. The killing is a result of the formation of radicals and reactive oxygen species that can react with multiple targets at a cellular level. Thus, advantages of PDI include the few undesired side effects and the improbable chances of promoting resistance by microorganisms [14–19].

Different types of PS have been investigated in recent years [20–28]. Curcumin (CUR) is a phenolic compound, member of the curcuminoid family, which can be extracted from the rhizomes of *Curcuma Longa*. CUR has been traditionally used as a cooking spice [29], but several *in vitro* and *in vivo* investigations suggested that CUR has great therapeutic potential, including anti-inflammatory [30], antiseptic [31], antifungal [32,33], antioxidant [34,35] and especially, antiviral effect against hepatitis B [36] and H1N1 [37]. Recently, CUR has shown great potential as a PS for PDI because of its ability to absorb light, especially in the blue spectral region [21,23,38,39]. The low penetration depth of the blue wavelength into tissues also points out that CUR would be a suitable PS for oral superficial decontamination. Unlike the phenothiazine dyes, which have been massively investigated for PDI, the staining of teeth, aesthetic tooth restorations and oral mucosa is improbable after using CUR. For those reasons, the efficacy of PDI-mediated by CUR against microorganisms in saliva needs to be evaluated. Most of the microorganisms in saliva are bound to oral epithelial cells [40], so the response to photodynamic action may change from the effect during *in vitro* situations [41,42].

Apart from its efficacy, CUR has poor aqueous solubility and bioavailability and constant investigations have suggested some possible ways to increase its biopharmaceutical properties [43]. One such approach can be the use of a water-soluble mixture of CUR and others curcuminoids (Curcumin, Desmethoxy curcumin and Bis-Desmethoxy curcumin). Thus, in an attempt to minimize the amount of pathogens in human saliva and thus decrease the contamination risk of dental staff and patients, the use of PDI for mouth decontamination is proposed. The study assessed the efficacy of a water-soluble mixture of curcuminoids (CRM), previously validated by Rego-Filho et al. [44] in 2014 in reducing the viability of microorganisms found in human saliva.

2. Material and methods

2.1. Study design

This was a blind, *in vitro,* experimental study (FOAr Research Ethics Committee—Case number: 68/10). The colony forming units per milliliter (CFU/mL) was the response variable. The decontamination treatment was the independent variable which resulted in the 14 study groups (n = 6) that are described in Chart 1.

Single Treatments Groups			
CUR (g/L)	**Light (J/cm²)**	**CLX**	**Group**
1.5 (1 min)	–	–	$C_{1.5/1}$
1.5 (5 min)	–	–	$C_{1.5/5}$
3.0 (1 min)	–	–	$C_{3.0/1}$
3.0 (5 min)	–	–	$C_{3.0/5}$
–	1.8	–	$L_{1.8}$
–	9.0	–	$L_{9.0}$
–	–	0.12% (1 min)	CLX_1
–	–	0.12% (5 min)	CLX_5
–	–	–	CTR_1*
–	–	–	CTR_5*
PDI Groups			
CUR (g/L)	**Light (J/cm²)**	**CLX**	**Group**
1.5	1.8	–	$C_{1.5}L_{1.8}$
1.5	9.0	–	$C_{1.5}L_{9.0}$
3.0	1.8	–	$C_{3.0}L_{1.8}$
3.0	9.0	–	$C_{3.0}L_{9.0}$

Chart 1. Treatment groups.
*CTR_1 and CTR_5 refer to control groups in which samples received only saline solution during 1 and 5 min, respectively.

2.2. Representative oral microbiota

The present study used representative samples of aerobic species commonly found in the human oral cavity. For this purpose, a saliva sample was obtained from pre-selected volunteers from the Araraquara Dental School (UNESP- Univ Estadual Paulista, Brazil). The volunteers were selected following these criteria: no signs and symptoms of disease, non-alcoholic, non-smoker, must be over 18 years of age, not using antibiotics or other mouthwashes, had not used antibiotics in the last month, not using toothpaste with antimicrobial activity and have not been submitted to dental prophylaxis in the last week. A sample of 10 mL of non-stimulate volunteerís saliva was collected in a sterile 50 mL *Falcon* tube to perform the tests.

2.3. Treatments

The photosensitizer chosen was a water-soluble mixture of three curcuminoids (curcumin, desmethoxy curcumin and Bis-desmethoxy curcumin; $C_{33}H_{50}O_{16}$, 730.32 g/mol)(PDT Pharma, Cravinhos, SP, Brazil) which is a topical preparation with acceptable stability and solubility of the curcumin derivatives (Fig. 1). A stock solution of the photosensitizer (3.0 g/L) was used and also diluted in distilled water to obtain the concentration of 1.5 g/L. The CRM was filtered through a sterile membrane with a 0.22 μM pore and was prepared immediately before use.

A light emitting diode (LED) device containing 54 blue LEDs (440 nm ± 10 nm) was used to excite the CRM. This equipment, called “Biotable”, provides uniform emission of light in which the lamps (1W) were arranged to simultaneously irradiate an entire