UNESP- Universidade Estadual Paulista
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"AVALIAÇÃO DA INFLUÊNCIA DA TERAPIA FOTODINÂMICA ANTIMICROBIANA NA ESTABILIDADE DE COR DA ESTRUTURA DENTÁRIA E DE UMA RESINA COMPOSTA"

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Odontológicas - Área de Dentística Restauradora, da Faculdade de Odontologia de Araraquara, da Universidade Estadual Paulista, para obtenção do titulo de Mestre em Ciências Odontológicas.

Orientadora: Profª Drª Alessandra Nara de Souza Rastelli

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Martin Luther King

Lorenzetti CC. Avaliação da influência da terapia fotodinâmica antimicrobiana na alteração de cor da estrutura dentária e de uma resina composta [Dissertação de Mestrado]. Araraquara: Faculdade de Odontologia da UNESP; 2015.

RESUMO

O objetivo deste trabalho, dividido em dois estudos, foi avaliar: (1) a influência da terapia fotodinâmica antimicrobiana na alteração de cor da dentina utilizando azul de metileno e curcumina como fotossensibilizadores nas concentrações de 20, 40 e 60 μM e nos tempos de pré-irradiação de 2 e 5 minutos, irradiados com fonte de luz LED; e (2) a influência do mesmo tratamento antimicrobiano com os mesmos fotossensibilizadores, concentrações e tempos de pré-irradiação utilizados no estudo anterior, porém sobre alteração de cor em uma resina composta microhíbrida. No primeiro estudo foram confeccionados 130 espécimes a partir de dentes humanos extraídos, os quais foram seccionados em máquina de cortes Isomet. Após a imersão nos fotossensibilizadores, avaliou-se as alterações de cor através de um espectrofotômetro de colorimetria em quatro momentos: antes do início da imersão nas soluções dos fotossensibilizadores (L0), após imersão nos fotossensibilizadores (L1), após irradiação (L2), após 10 dias (L3), após 30 dias (L4) e após 60 dias (L5). Observou-se uma variação alta das médias de ΔE nos grupos com azul de metileno independentemente da concentração e do tempo de exposição, diminuindo a partir da leitura de 10 dias. Com os grupos com curcumina, houve uma variação inicial sem diferença significativa do controle, mantendo-se próxima a ele nas leituras seguintes. Ambos fotossensibilizadores apresentaram alterações significantes clinicamente em dentina segundo a classificação da "National Bureau of Standards" (NBS), sendo a alteração com a curcumina menor que do azul de metileno. No segundo estudo foram confeccionados 130 espécimes de resina composta microhíbrida (Filtek Z250XT) utilizando uma matriz de aço (10x2mm). Após a confecção dos espécimes, estes foram imersos nas soluções dos fotossensibilizadores e posteriormente avaliados com Espectrofotômetro de Colorimetria nos mesmos momentos de leitura de cor realizados no estudo anterior (inicial, após imersão, após irradiação da luz, após 10, 30 e 60 dias). Nos grupos de azul de metileno houve diferença estatística significante em relação ao grupo controle em todos os momentos de leitura, e nos grupos com curcumina houve semelhança ao grupo controle a partir da irradiação da luz. Clinicamente as alterações encontradas segundo a classificação "NBS" no azul de metileno foram significantes e na curcumina foram leves. Com base nos resultados encontrados neste trabalho, pode-se concluir que em ambos os

estudos realizados os fotossensibilizadores levaram à alteração de cor significativa clinicamente, sendo as maiores alterações observadas no azul de metileno.

PALAVRAS-CHAVE: Fotoquimioterapia, Fármacos fotossensibilizantes, Azul de metileno, Curcumina, Resinas compostas, Cor.

Lorenzetti CC. Influence of antimicrobial photodynamic therapy in the color change of the tooth structure and a resin composite [Dissertação de Mestrado]. Araraquara: Faculdade de Odontologia da UNESP; 2015.

ABSTRACT

This study, divided in two studies, was to evaluate: (1) the influence of antimicrobial photodynamic therapy in color change dentin using methylene blue and curcumin as photosensitizers in concentrations of 20, 40 and 60 uM and pre irradiation times 2 to 5 minutes, irradiated with a LED light source; and (2) the effect of the same antimicrobial treatment with the same photosensitizers, concentrations and pre-irradiation times used in the previous study, but on color change in a microhybrid composite. In the first study were produced 130 specimens from extracted human teeth, which were cut in Isomet cuts machine. After immersion in the photosensitizers, the color changes were evaluated using a spectrophotometer colorimeter in four stages: before the immersion in solutions of photosensitizers (L0) after immersion in photosensitizers (L1) after irradiation (L2), after 10 days (L3) after 30 days (L4) and 60 days (L5). There was a high variation of ΔE averages of the methylene blue groups independently of the concentration and exposure time, decreasing from a reading of 10 days. With curcumin groups, there was no significant difference initial variation control, remaining next to it in the subsequent readings. Both photosensitizers showed clinically significant changes in dentin according to the classification of "National Bureau of Standards" (NBS), with a lowest change to curcumin that of methylene blue. In the second study were made 130 specimens microhybrid composite resin (Filtek Z250XT) using a steel matrix (10x2mm). After preparation of the specimens, they were immersed in solutions of photosensitizers and subsequently evaluated with a spectrophotometer Colorimetry in the same color reading moments performed in the previous study (initial, after immersion, after light irradiation, after 10, 30 and 60 days). In methylene blue groups was no statistically significant difference from the control group at all times of reading, and in groups with curcumin were similar to the control group after the irradiation of light. Clinically the changes found in the classification "NBS" in methylene blue were significant and curcumin were mild. Based on the findings of this study, it can be concluded that in both the photosensitizing

studies led to clinically significant color change, with highest changes observed in methylene blue.

KEYWORDS: Photochemotherapy, Photosensitizing Agents, Methylene Blue, Curcumin, Composite Resins, Color.

SUMÁRIO

1 INTRODUÇÃO

A cavidade oral é colonizada por um complexo de microrganismos, incluindo bactérias aeróbias e anaeróbias gram-positiva e gram-negativa, fungos, micoplasmas, protozoários e vírus. Entre as doenças bacterianas mais comuns presentes nos seres humanos estão a cárie dentária e as doenças periodontais, sendo ambas resultantes de acúmulo de biofilme sobre os dentes e tecidos moles orais[23].

As bactérias organizadas na forma de biofilme sobre a superfície dentária apresentam maior resistência a antibióticos, estresses ambientais, e a mecanismos de defesa do sistema imunológico. A remoção mecânica da placa bacteriana, uma boa higiene oral, e o uso de agentes antimicrobianos fazem parte de tratamentos convencionais[28,21,39], porém pela dificuldade de manter agentes químicos convencionais em concentração terapêutica na cavidade oral, eles podem ser ineficazes devido à resistência bacteriana[41,11]. Um dos principais fatores de prevenção e controle das doenças periodontais e da cárie é a redução ou controle de microrganismos patogênicos presentes na superfície dental, tendo assim cada vez mais a utilização de agentes antimicrobianos[41]. Entre os agentes antimicrobianos mais utilizados tem-se a clorexidina, que é altamente eficaz tanto sobre bactérias gram-positiva quanto gram-negativa, assim como em alguns fungos e vírus. Porém, seu uso por longos períodos pode levar a efeitos colaterais, tais como: alteração no paladar, sensação de queimação, manchamento dos dentes e materiais restauradores[5, 14].

Frente a este contexto, a busca por métodos antimicrobianos coadjuvantes têm aumentado significativamente, podendo-se destacar entre eles a terapia fotodinâmica antimicrobiana (aTFD), a qual tem se mostrado eficaz e vantajosa em diversos estudos[41,42,3,11]. Nesta técnica, a resistência bacteriana é improvável devido à ação citotóxica do oxigênio singleto e dos radicais livres presentes na reação, atuando como vantagem também por evitar o uso de agentes antimicrobianos que são utilizados no tratamento de infecções sistêmicas, diminuindo assim o risco de resistência bacteriana nestes tratamentos.

Entre outras vantagens desta técnica estão também a de evitar danos aos tecidos do hospedeiro e a rapidez na morte da célula bacteriana, não necessitando manter o agente químico em altas concentrações e por longos períodos de tempo sobre as lesões, como ocorre no uso de antibióticos[41, 42, 11].

Tratamentos clínicos utilizando-se a terapia fotodinâmica antimicrobiana têm sido aprovados em várias regiões do mundo, como nos Estados Unidos, Canadá, Japão, União

Européia, Brasil, Rússia[1,8,9]. A terapia fotodinâmica tem aplicação principalmente no tratamento de diferentes tipos de câncer[17,8,9,1,2], seja na área médica ou na Odontologia[30]. Porém, muitos estudos tem mostrado que esta terapia também apresenta potencial antimicrobiano, representando uma alternativa como técnica antibacteriana em casos de periodontite, periimplantite, cárie dental e infecções intra-canal, antifúngica (candidíase), e antiviral (herpes labial) para erradicação ou controle dos microrganismos causadores[37,24,20,43,30,12].

A técnica da terapia fotodinâmica envolve três diferentes componentes: uma luz com comprimento de onda específico, um fotossensibilizador não tóxico e sensível à luz, e oxigênio molecular presente nos tecidos. Após a aplicação do fotossensibilizador, este absorve os fótons da luz irradiada e os elétrons passam de seu estado fundamental a um estado mais excitado[21]. Com a presença do oxigênio encontrado nas células, o fotossensibilizador ativado pode reagir com moléculas vizinhas por transferência de elétrons ou hidrogênio, formando radicais livres, que em contato com o oxigênio produz espécies altamente reativas, como por exemplo, o peróxido de hidrogênio e radicais hidroxila (reação do tipo I). O fotossensibilizador ativado pode reagir também transferindo energia ao oxigênio, levando à formação de um estado altamente reativo de oxigênio, conhecido como oxigênio singleto (reação do tipo II). Ambos os tipos de processos (I e II) podem levar à morte celular e à destruição da célula[21, 29].

A busca por agentes fotossensibilizadores e seus potenciais para aplicação na terapia fotodinâmica antimicrobiana tem sido investigada por vários estudos na Odontologia[35,23,31]. Para que um corante possa ser considerado e utilizado como fotossensibilizador é necessário que alguns requisitos sejam observados: um fotossensibilizador após a sua fixação à parede bacteriana deve atrair a luz irradiada, apresentando picos de absorção óptica próximos ao comprimento de onda da luz utilizada[40]; permanecer em estado excitado por tempo suficiente a fim de se interagir com as moléculas vizinhas e formar as espécies altamente reativas capazes de promover a morte microbiana, além de não apresentar características tóxicas para as células do hospedeiro[22].

Diversos são os corantes encontrados na literatura como fotossensibilizadores na terapia fotodinâmica com finalidade antimicrobiana, podendo citar: o azul de metileno e azul de toluidina como os mais comumente utilizados em Odontologia, dentre outros como rosa de bengala, derivados de hematoporfirina e mais recentemente a curcumina. São conhecidas mais de 400 substâncias como fotossensibilizadores, incluindo corantes, drogas, cosméticos e compostos naturais. O azul de metileno tem demonstrado em diversos estudos um alto grau de

seletividade para causar danos tanto a bactérias gram-positivas como gram-negativa, além de sua toxicidade ser relativamente baixa em humanos[40, 41, 35].

Os diversos tipos de fotossensibilizadores podem se associar ao laser, porém cada um com sua aplicação que dependerá da absorção de luz em comprimento de onda específico. Entre os fotossensibilizadores mais usados e seus comprimentos de onda estão: derivados da hematoporfirina (620-650nm); fenotiazínicos como, por exemplo, o azul de metileno e azul de toluidina (620-700nm), agentes fitoterápicos (550-700nm); ftalocianinas (660-700nm)[24]. Os fotossensibilizadores devem apresentar picos de absorção próximos ao comprimento de onda da luz utilizada para que haja o efeito antimicrobiano[40].

Mais recentemente, verificou-se o potencial da curcumina a ser utilizada como substância eficaz utilizada para esta finalidade, apresentando como grande vantagem o fato de ser uma substância natural e inofensiva para tecidos orais[4,16,27]. Além disso, a penetração da luz necessária para fotoativar a curcumina a torna uma excelente substância para a terapia fotodinâmica antimicrobiana[38].

Diversas são as concentrações utilizadas para os diferentes fotossensibilizadores para se obter o efeito antimicrobiano desejado. Em 2000, Ivanov et al.[18] relataram que os corantes fenotiazínicos não deveriam ultrapassar a concentração de 0,1% pois acima disto podem tornar-se tóxicos e provocarem o manchamento da dentina, o que não seria interessante em Odontologia pela questão estética. Usacheva et al.[36] (2003) afirmaram também que o azul de metileno em determinadas circunstâncias, como no aumento de sua concentração, podem levar a mudanças das interações entre as moléculas do corante, causando assim agregação do corante, o que poderia comprometer sobremaneira a sua ação fotodinâmica.

No que diz respeito às fontes de luz, as mais utilizadas em aTFD com seus respectivos comprimentos de onda são: LASERs de He-Ne (632,8 nm); de Diodo AsGaAl (620-690 nm); de Diodo InGaAIP (635-685 nm) e de Argônio (488-514 nm)[21]. O LASER apresenta características como emissão de fótons com o mesmo comprimento de onda (monocromaticidade), além da capacidade da luz de se propagar em uma única direção[32], características estas que fazem com que esta fonte de luz seja extremamente eficiente. Outra opção de fonte de luz na Odontologia são os LEDs (Light Emitting Diode), que também apresentam monocromaticidade se diferindo dos LASERs por apresentarem feixe de luz divergente, sendo uma alternativa simples e barata[33] utilizada eficientemente na ação antimicrobiana da terapia fotodinâmica[1, 27].

Na literatura atual encontra-se uma variedade de estudos que avaliam o efeito antimicrobiano da terapia fotodinâmica utilizando diversos tipos de fotossensibilizadores com

diferentes concentrações, bem como doses e fontes de luz[4, 27, 31, 43]. Contudo, ainda há a necessidade de trabalhos que investiguem os efeitos dessa terapia sobre a estrutura dental e materiais restauradores em termos de alteração de cor ocasionada pelos agentes fotossensibilizantes, pois dependendo da aplicação clínica em Odontologia, os agentes fotossensibilizadores entrarão em contato com tecidos, estruturas dentais e materiais restauradores, tendo a resina composta como o material mais utilizado. Caso haja uma alteração de cor significativa após este contato, esta técnica pode se tornar inviável clinicamente com o uso de determinado fotossensibilizador em determinada concentração e tempo de contato com a superfície dentária. Frente a isto, fica destacada a importância de investigar esta possível alteração de cor pela aplicação da terapia fotodinâmica sobre dentina e uma resina composta.

2 PROPOSIÇÃO

2.1 Objetivo Geral

O presente estudo tem como objetivo avaliar a estabilidade de cor da dentina humana e de uma composta após aplicação da terapia fotodinâmica antimicrobiana (aTFD), em função de dois fotossensibilizadores em diferentes concentrações e tempos de contato.

2.2 Objetivos Específicos

1) Avaliar o efeito da terapia fotodinâmica antimicrobiana na estabilidade de cor na dentina de dente humano utilizando azul de metileno e curcumina como fotossensibilizadores.

Artigo 1- Effect of antimicrobial photodynamic therapy on the dentin color stability

2) Avaliar o efeito da terapia fotodinâmica antimicrobiana na estabilidade de cor de uma resina composta microhíbrida utilizando os fotossensibilizadores azul de metileno e curcumina.

Artigo 2- Influence of antimicrobial photodynamic therapy in the color change of a composite resin submitted to two photosensitizers

3 CAPÍTULOS

3.1 Capítulo 1

Effect of antimicrobial photodynamic therapy on the dentin color stability*

*Artigo nas normas da revista "Laser Physics"

Effect of antimicrobial photodynamic therapy on the dentin color stability

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Abstract

Studies have shown that the antimicrobial photodynamic therapy is an effective alternative treatment to antimicrobial agents in oral infections, which uses photosensitizers such as methylene blue and curcumin. This study evaluated the effect on color change in human dentin specimens of these two photosensitizers at concentrations of 20, 40 and 60 μM and pre-irradiation times of 2 and 5 minutes, irradiated with a LED light source. The color readings were obtained from a colorimetric spectrophotometer at four different times: before the immersion in solutions of photosensitizers (L0) after immersion in photosensitizers (L1) after irradiation (L2) after 10 days (L3) after 30 days (L4) and 60 days (L5). It was observed that until L2 with methylene blue there was a high variation of ΔE, regardless of the concentrations and exposure time, which decreased in the L3 reading. With the curcumin, initial variation did not differ significantly from control, which was maintained in all subsequent readings. Both sensitizers had clinically detectable changes in dentin, and the changes with the curcumin was lower than with methylene blue.

Keywords: photodynamic therapy, methylene blue, curcumin, dentin.

1. Introduction

Several microorganisms that colonize the oral cavity can cause some diseases, the most commons are dental caries and periodontal diseases, both resulting from the accumulated biofilm on the oral hard and soft tissues. The microorganisms present in the oral biofilm present greater resistance to antimicrobial agents and to the defense of the host immune system [1]. The oral biofilm environment, where the bacterial cells have close proximity and form a stable structure, is favorable for horizontal gene transfer, which can lead to the spread of antibiotic resistance genes from other cells present in the biofilm [2].

Various antimicrobial agents are used for the control or reduction of pathogenic microorganisms present in the dental surface [3], among the most used is the chlorhexidine due its high effectiveness on both, gram-positive and gram-negative, bacteria. However, the use of chlorhexidine for long periods causes staining on teeth and restorative materials, altered taste and burning sensation [4, 5].

Difficulties in complete elimination of microorganisms by antimicrobials and the consequences that their use can lead, led the need to create alternative treatments for biofilm control and treatment of bacterial diseases, standing out among them in several studies for its effectiveness and advantages, the antimicrobial photodynamic therapy (PDT) [3, 6, 7]. Among the advantages of this technique is the improbability of bacterial resistance due to high cytotoxicity of reactive oxygen species and free radicals that are formed, the speed of bacterial killing and the atraumatic character to the host [3, 8]. Many studies have investigated the effect of this technique on dental caries [9, 10, 11] which is still a highly prevalent disease in humans nowadays.

The dentin caries has an outer layer of highly infected by bacteria, softened, impossible remineralization and must be completely removed during the excavation process, but also other inner layer, this being contaminated with low numbers of microorganisms and susceptible to remineralization [12]. However, clinicals can find difficults to have the exact distinction between these two layers [8] and this may lead to excessive removal of dentin and even the pulp exposure [13].

In this context, the antimicrobial photodynamic therapy has appeared as an effective and conservative approach. The mechanism of action of this technique is based on the use of the light sensitive dyes (photosensitizers), which are specific for a certain application area, a luminous energy with a wavelength equivalent to the absorption spectrum of the photosensitive agent and oxygen present in the tissues. With the interaction of these three

factors (photosensitizer, light and oxygen) there is an electronic excitation causing two types of reaction (Type I and Type II). In Type I reaction, there is the interaction of the excited photosensitizer molecules with neighbor molecules, transferring hydrogen ions or electrons, giving rise to free radicals such as hydrogen peroxide, superoxide and hydroxyl reactive. The type II reaction arises from the interaction of the excited photosensitizer with the molecular oxygen, resulting in the singlet oxygen, which is a highly reactive state, leading to cell death, as well as in type I reaction [1, 14].

There are several chemical compounds used as photosensitizers present in studies in the literature, but for a compound be used as a photosensitizer it should attract the radiated light after its attachment to the bacterial wall, presenting absorption peaks near the wavelength of the emitted light [15], have their excited molecules for a sufficient time to form the reactive species, and should not be toxic to the host cells [16]. Among the photosensitizers used in dentistry, studies have shown good antimicrobial results with the use of methylene blue [3, 17] and curcumin [18, 19], which were the compounds used in this study.

Since the beginning of photodynamic therapy, various light sources have been tested, being the laser emission those who were more employed by presenting characteristics such as monochromaticity (emission of photons with the same wavelength) and emission of large amounts of energy to injury target [20]. However, with the development of other light sources, such as light emitting diodes (LEDs), the lasers have been replaced by the use of this alternative technology for antimicrobial action of photodynamic therapy. The LEDs besides having features like emission of diverging and not coherent beams, which favor the complementarity with the photosensitizer [21], has a lower cost of acquisition and maintenance of the appliance, and is more easily present in dental office [22].

For use in clinical practice of antimicrobial photodynamic therapy is necessary to investigate if the photosensitizing agents used can lead to staining of the tooth structure, which could compromise its use. The aim of this in vitro study was to evaluate the effect of this antimicrobial treatment modality in color stability in human tooth dentin using methylene blue and curcumin as photosensitizers.

2. **Materials and Methods** (Apêndice A1)

This research was approved by the Research Ethics Committee of the Faculty of Dentistry of Araraquara, São Paulo State University (CAAE: 30682114.0.0000.5416)

(Anexo). The human teeth used were obtained from the Teeth Bank of the same Institution and stored in a 1% thymol solution for 24 hours for decontamination thereof.

2.1. Intra-examiner Calibration

To evaluate the reliability of the measurements, intra-examiner calibration was conducted to test the reproducibility, where the researcher 2 examined 10 specimens in duplicate for color stability, with one-hour interval between assessments. The intra-examiner agreement was estimated using the intraclass correlation coefficient (ρ) [23]. The degree of agreement between the data was classified according to the proposal of Fermanian *et al* [23], and obtained an agreement level intra-examiner classified as "excellent" (> 0.91).

2.2. Confection Dentin Specimens

The selected teeth were cleaned and stored in distilled water with exchanges held weekly, and later sectioned in the middle third using cutting machine under refrigeration (Isomet; Buehler Ltd, Lake Bluff, IL, USA), obtaining the dentin fragments in dimensions 6 x 6 x 2 mm. After this procedure, the fragments were placed in a circular array to the resin inclusion Siquiplás CrisLight (Siquiplás) to enable correct positioning of the specimens in the spectrophotometer for color readings.

From this, each specimen was immersed in artificial saliva with exchange performed weekly and stored in an oven (EBC1-Odontobras- Trade Eq. Medical-Dental LTDA, Ribeirão Preto, SP, Brazil) at a temperature of 37 ° C ± 1 ° C and were only removed to the immersion in solutions with photosensitizers and readings in the spectrophotometer.

2.3. Photosensitizers

The specimens were exposed to the photosensitizer methylene blue and curcumin (Sigma Aldrich, St. Louis, MO) at concentrations of 20, 40 and 60 μM, for times of 2 and 5 minutes (pre-irradiation time). Due to very long intervals of time may hamper the clinical practice, it opted to these times, which besides being feasible in clinical practice, have satisfactory results in the literature in ttgerms of antimicrobial efficiency [24, 25, 26].

2.4. Light Source

It was used LED lighting system (BioTable, LAT, USP, IFSC, San Carlos, SP) in the wavelength of about 440nm (FS curcumin base) or 630nm (methylene blue FS) resonant with the absorption peak of the selected photosensitizer at a dose of approximately 15 J / cm^2.

2.5. Evaluation change color

The color measurements were obtained through the Colorimetric Spectrophotometer (Color guide 45/0, PCB 6807 BYK-Gardner GmbH, Gerestsried, Germany) with a wavelength varying from 400 to 700 nm through direct transmission with standard lighting D65 on white background [27]. After calibrate the spectrophotometer, four readings were taken for each specimen: before the immersion in solutions of photosensitizers (L0), after immersion in the photosensitizers (L1), after irradiation (L2), after 10 days (L3), after 30 days (L4), and after 60 days (L5).

The color values were recorded by the spectrophotometer according to the CIE- Lab System recommended by CIE (Commission Internationale de l'Eclairage) [28, 29].

In the coordinate CIE L*a*b *system, L * refers to the lightness coordinate (gray scale) with values of zero (black) to 100 (white). The values of a * and b * are the chromaticity coordinates of the red-green and yellow-blue axis, respectively. Values of positive a* indicate deviation of chromaticity towards the red hue and negative values shift towards green hue. Similarly, positive b* values indicate a shift towards yellow hue and positive values indicating a shift to blue hue [28, 30].

For the evaluation of possible color changes of the specimens composed and dentin resin, a certain classification was adopted by the "National Bureau of Standards" (NBS), where: ΔE values between 0.0 to 0.5: extremely slight change; 0.5 to 1.5: slight change; 1.5 to 3.0: perceptible change; 3.0 to 6.0: significant change; 6.0 to 12.0: extremely significant change; 12.0 or more, change to another color.

2.6. Data analysis

Statistical evaluation of the color change in human dentin specimens caused by two photosensitizers in three different concentrations, with two contact times, was performed by analysis of variance for repeated measures. These analyzes were complemented by multiple comparisons of means by Tukey test. It was adopted the 5% significance level for decision making.

3. Results

In the Table 1 shows the averages and standard of deviations ΔE color deviation from the initial color dentin samples according to the photosensitizer (A = methylene blue and curcumin = C), concentration (20, 40 and 60 μM) exposure time (2 and 5 minutes), and

timing of reading color (L1 to L5 after an initial reading L0). It is observed that until the L2 reading there was a high variation of the mean ΔE for methylene blue, regardless of concentration and exposure time, which decreased in L3 reading, maintaining the same level until the end. However, in the groups treated with curcumin, the initial variation did not differ significantly from control, keeping in all subsequent readings.

In Figure 1(a) e (b), the average ΔE are shown in the pre-irradiation times of 2 minutes (Figura 1a) and 5 minutes (Figura 1b), along with the standard errors, which provide an estimate of the accuracy of them. It facilities the display of results in the Table 1, although without statistically significant, it showed an influence of higher concentration and exposure time increased the variation in color to reading 3. Curcumin clearly, as indicated by statistical analysis, remains throughout the trial period almost the same level of control.

Table 1 - Mean (standard deviation) of ΔE color deviation from the initial color (L0) in dentin samples, according to the treatment (concentration - time of exposure), and A = methylene blue; C = curcumin; concentration in μM and exposure time in minutes, and the color reading time (L1 to L5).

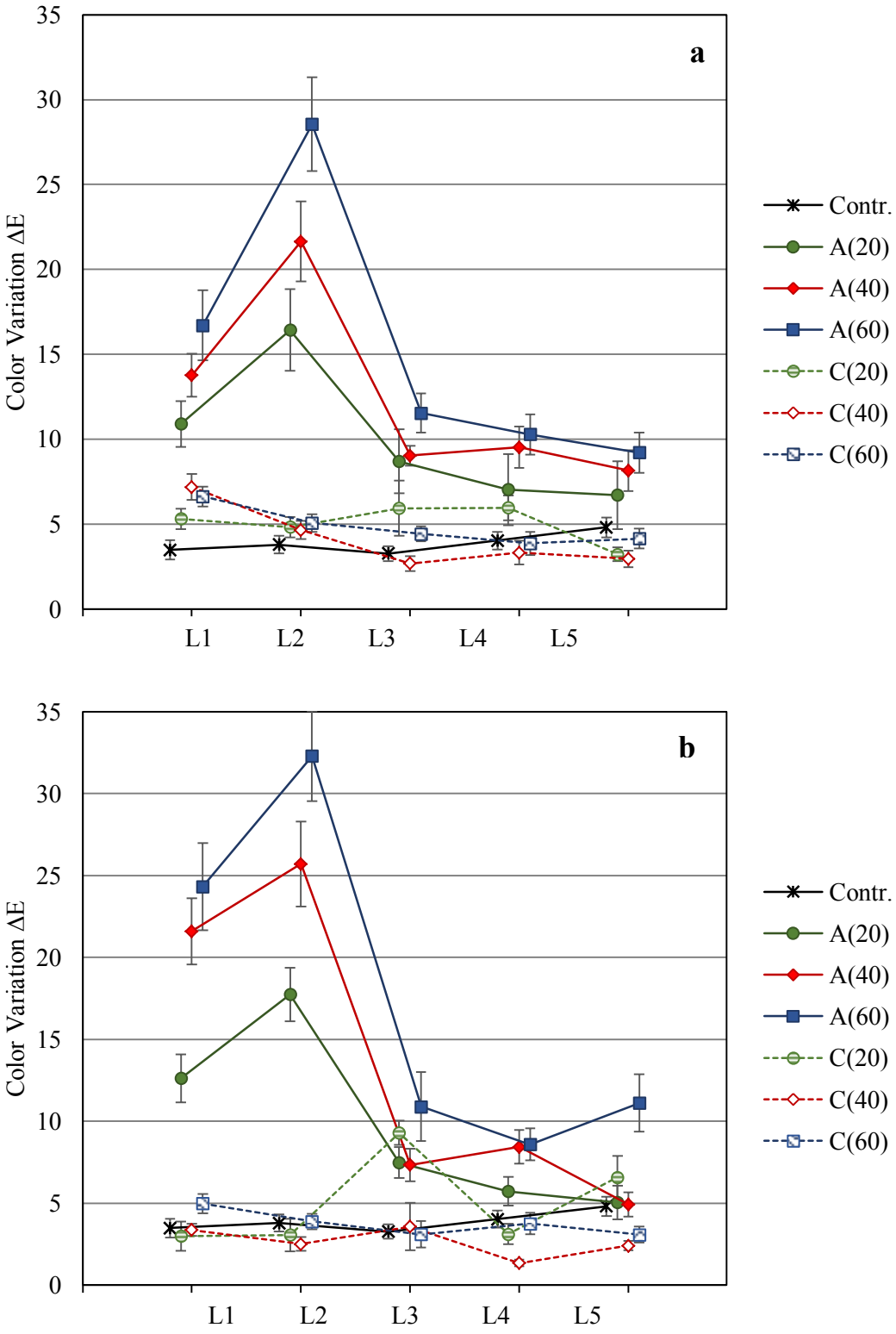
Groups	Reading Occasions				
	L1	L2	L3	L4	L5
Control	3,48 (1,79) a	3,79 (1,67) a	3,27 (1,39) a	4,03 (1,62) a	4,81 (1,85) a
A(20-2)	10,90 (4,25) aBCDE	16,44 (7,58) bB+	8,70 (5,95) aAB	7,04 (6,63) aAB	6,71 (6,31) aAB
A(20-5)	12,62 (4,66) bCDE+	17,74 (5,13) bB+	7,48 (2,99) aAB	5,73 (2,75) aAB	5,05 (3,25) aAB
A(40-2)	13,77 (4,04) aDE+	21,65 (7,46) bBC+	9,04 (1,83) aAB	9,53 (3,85) aB	8,17 (3,80) aAB
A(40-5)	21,60 (6,37) bFG+	25,70 (8,19) bCD+	7,34 (3,10) aAB	8,44 (3,26) aAB	4,93 (2,32) aAB
A(60-2)	16,71 (6,53) bEF+	28,55 (8,76) cCD+	11,55 (3,69) abB+	10,28 (3,78) aB	9,21 (3,75) aAB
A(60-5)	24,33 (8,40) bG+	32,28 (8,67) cD+	10,90 (6,68) aB+	8,58 (3,09) aAB	11,12 (5,52) aB
C(20-2)	5,31 (1,91) aABC	4,83 (1,92) aA	5,94 (5,11) aAB	5,96 (2,30) aAB	3,22 (1,31) aA
C(20-5)	3,00 (2,83) aA	3,07 (3,14) aA	9,30 (2,36) bAB	3,12 (1,95) aAB	6,59 (4,15) abAB
C(40-2)	7,19 (2,41) aABCD	4,65 (1,72) aA	2,68 (1,37) aA	3,32 (2,23) aAB	2,96 (1,54) aAB
C(40-5)	3,37 (1,20) aA	2,51 (1,33) aA	3,57 (4,58) aA	1,35 (0,63) aA	2,41 (0,90) aA
C(60-2)	6,63 (1,86) aABCD	5,07 (1,62) aA	4,42 (1,38) aAB	3,86 (2,17) aAB	4,15 (1,84) aAB
C(60-5)	4,98 (1,89) aAB	3,88 (1,45) aA	3,10 (2,58) aA	3,76 (2,11) aAB	3,09 (1,52) aA

Averages followed by the same letters (lowercase or uppercase on the line in the column) are not significantly different ($p > 0.05$)
+ Indicates that the mean is significantly different from the control mean

Legend:

Column Groups □ photosensitizer (concentration - exposure time)

Figure 1 - Mean (symbols) and standard errors (vertical bars) of ΔE color variation in relation to the initial color (L0) in dentin samples, according to the concentration in the time of 2 minutes **(a)** and 5 minutes **(b)**, with A = methylene blue; C = curcumin; µM concentration and exposure time in minutes, and the color reading time (L1 to L5).



4. Discussion

Photodynamic therapy has been considered as an effective alternative antimicrobial treatment for diseases of the oral cavity such as dental caries, and as one of the essential factors for antimicrobial activity have the use of photosensitizing agents [1]. There are several chemical compounds that are used as photosensitizers, which are dyes, fact that can lead to the staining of dental structures. In this study, after evaluating the influence of methylene blue and curcumin in color change in specimens of human dentin, it was found that with the curcumin no statistically significant difference in relation to the control group was found. However with the methylene blue the statistically significant differences to the control group were observed to L2 (after irradiation of light), decreasing after the L3 (reading after 10 days) onwards. However, analyzing clinically, according to the classification determined by the "NBS", already in the control group was observed a significant color change, observing also similar changes to curcumin, however there are extremely significant changes in the groups that used methylene blue.

These significant changes obtained in general in all groups of dentin specimens can be justified by the difference in dentin composition of each specimen, since it comes from different teeth, which leads to different behaviors even in the face to the same interventions. Dentin is a semi-permeable membrane with tubular channels which move fluids, and a process of complex permeability dependent of physical and chemical conditions, and the influence of the molecular size, geometric shape and chemical affinity of the fluid to be penetrated [31, 32]. The tubular structure has a higher density and dentin tubule diameter towards the pulp tissue. This morphological change, which also causes the permeability, vary in different regions of dentin [33]. In preparation of the specimens, a 2 mm thick dentin of the middle third was used for standardize the dimensions. For variations in the sizes of dental crowns, this region may vary. In teeth with greater crowns this region would be located more superior than in small crowns, so there is difference in the number and diameter of the tubules of these regions between the various teeth, even though the third molars. This may influence the amount of dye penetration in areas with a greater quantity and higher tubular diameter. Thus, it is possible to suggest that the optical characteristics vary dentin to dentin, also considering, in addition to the above-mentioned factors, a specimen with higher chromaticity dentin may have a different susceptibility to the impregnation of a pigment specimen with clearest dentin.

The staining observed to the control group, with only the presence of artificial saliva, even with the change of the liquid weekly, can be attributed to the composition of saliva. In study Omata *et al* [34], who assessed the possibility of hybrid composite's staining immersed in some solutions, found that the artificial saliva alone resulted in a staining of specimens, assuming that this coloration was caused by the presence of the mucin in saliva's composition, which has a yellowish color. This statement was based on the study of Meyer-Lueckel *et al* [35], who also suggested that saliva led to an extrinsic staining of the composite. Although the present study had a different saliva composition, containing potassium chloride, sodium chloride, magnesium chloride, potassium phosphate dibasic, calcium chloride, carboxymethylcellulose (CMC 3000), sorbitol 70% nipagin and distilled water, and one or more of these compounds may have influenced the saliva staining.

The application of antimicrobial photodynamic therapy involves the use of photosensitizers, which are found in the literature as several compounds and with various applications and use protocols. There are studies that detected sensitivity of cariogenic microorganisms in human dentin treated with photodynamic therapy using ortho toluidine blue associated with LED light [9, 36]. However, other studies have shown high efficacy of this treatment in cariogenic bacteria using other photosensitizers, varying its concentration. In study by Araujo *et al* [18], after evaluating the use of curcumin at five different concentrations (0.75, 1.5, 3.0, 4.0 and 5.0 g / L) associated with the LED on and biofilm carious dentin lesions observed a significant microbial reduction when used the higher concentration, and that microorganisms of dentin had lower light penetration and dispersion of the dye in the biofilm. Another photosensitizer also used in photodynamic antimicrobial therapy is methylene blue. Guglielmi *et al* [37] used in their study of methylene blue 0.01% irradiated with laser InGaAIP on dentin, samples of permanent molars with deep caries lesions, demonstrating a clinical potential of the technique for caries treatment and thus avoid the risk of pulp exposure.

Evaluating the influence of the photosensitizer methylene blue and curcumin at concentrations of 20, 40 and 60 μM and pre-irradiation time of 2 and 5 minutes in a color change in dentin specimens, it was observed that both photosensitizers showed statistically significant changes after pre-irradiation time, showing no statistically significant influence on the concentration and exposure time highest increase in variation. Curcumin showed color changes, already in the first reading, closest of the control of the methylene blue, while maintaining a decrease in the value after the application of light, with no significant difference of the values found in the control group in the last reading. This decrease can be

explained by photobleaching process, which is the dye degradation front a strong optical excitation, that due to oxidizing chemical species formed, cause the destruction of neighboring molecules [38]. Dovigo *et al* [39] due to the high photobleaching rate of curcumin observed, suggested that in longer periods of exposure of light formed low levels of reactive oxygen species, because would have lesser extent in the solution of optically active curcumin molecules in the visible spectrum. Study of Chignell *et al* [40] also confirmed that curcumin has undergone photobleaching, observing a reduction of the dye after light absorption. The authors affirmed that this degradation of curcumin after the light appears to be an independent process of its nature. Although methylene blue, as well as other compounds, also presents degradation after the light, the photosensitizer´s color change reduction was observed only at the time of immersion in artificial saliva, since 10 days. With the results obtained, it can be suggested that curcumin deserves attention to be used. So that it can remain in sufficient concentration to interact with the light source and produce reactive species for the desired antimicrobial effect even being more sensitive to degradation in contact with light.

Studies have evaluated the optical characteristics of dentin and found that the transdentinal light transmission has a direct relation to the shape and arrangement of the dentinal tubules [41, 42] and may have a direct relationship between dentinal permeability and light transmission through the dentin [43]. However, in Turrioni *et al* [43] study, that assessed the influence of the permeability of human dentin in the transdentinal transmission with LED light, showed that the transmission of light through the dentin did not vary significantly, regardless of the greater or lesser permeability of dentin. In study Vaarkamp *et al* [41], evaluated the transmission of light at different sides dentin blocks radiating laser light at different angles, concluding that dentinal tubules directly affect the dispersion of light in the dentin. They found that when the light was applied parallel to the direction of the tubules, the light transmission was more intense. In another study, which used light perpendicular to the axis of the tubules, a greater loss of light intensity was found, which shows the anisotropy characteristic of the dentin [42]. This information may suggest that in certain regions of dentin, it may contain photosensitizers that suffered less degradation by light (photobleaching), due to different ways of transmission of light by dentin, and can therefore remain regions with high concentrations of the dye. The photodegradation of curcumin leads to accumulation of photoproducts that can not be detected in the light spectrum range used [39], which can thus also suggest that remains a quantity of pigment absorbed into the dentine. The reactions leading to the formation of photoproducts depend on internal factors

(such as the level of oxygen and the pH) and also external factors (such as the wavelength and fluence of the light source used) [44]. Most photobleaching processes studied are oxidative and seem to involve singlet oxygen [45].

5. Conclusion

Curcumin and methylene blue sensitizers are already widely used for antimicrobial purposes in photodynamic therapy, however, both lead to clinically perceptible color changes in dentin, with a lowest change of the curcumin than methylene blue. Based on the results obtained in this study, it was concluded the need for future studies for evaluation of the color change by photosensitizers in antimicrobial photodynamic therapy to reduce the limitations and thus to be a treatment for clinical application. It might be suggested future studies with different parameters that what were used in this study (for example, use a lower concentration of photosensitizer and lower pre-irradiation time) to reduce the influence on color changes of these compounds, but taking into consideration the antimicrobial effect of the new parameters.

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3.2 Capítulo 2

Influence of antimicrobial photodynamic therapy in the color change of a resin composite submitted to two photosensitizers*

*Artigo nas normas da revista –Photodiagnosis and Photodynamic Therapy”

Influence of antimicrobial photodynamic therapy in the color change of a resin composite submitted to two photosensitizers

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ABSTRACT

Background: Due to limitations of antimicrobial agents on oral microorganisms, photodynamic therapy has been highlighted as an effective treatment using photosensitizing agents and light source. This study evaluated the influence of two photosensitizers used in this antimicrobial technique (methylene blue and curcumin) in the color change of a microhybrid composite (Filtek Z250XT).

Methods: One hundred and thirty specimens were prepared from the resin using steel die (10x2mm), and subsequently immersed in the photosensitizers in three differents concentrations (20, 40 and 60 μM) and two pre-irradiation time (2 to 5 minutes) with a LED light. The color readings obtained on a spectrophotometer colorimetry were performed before immersion in photosensitizers (L0) after immersion (L1) after irradiation (L2) after 10 days (L3) after 30 days (L4) and after 60 days (L5).

Results: In both photosensitizers was observed that there was no significant influence of different concentrations, pre-irradiation times and reading times. Methylene blue showed a statistically significant difference from the control group at all readings, and curcumin appeared di d not change since L2.

Conclusion: This study may suggest that the clinical use of curcumin leads to less color changes compared to methylene blue.

Keywords

Photodynamic therapy; Methylene blue; Curcumin, Resin composite

INTRODUCTION

A variety of species of microorganisms colonize the tooth surfaces and soft tissues[28] in the oral cavity, hosting more than 700 different species of bacteria, fungi, viruses, due to the presence of secretions, nutrients and epithelial debris[44]. Among the most commons bacterial diseases in humans are dental caries and periodontal diseases, both resulting from biofilm accumulation in the oral tissues[28].

A concern of dentists is the risk of infections during and after surgery due to aseptic conditions of the oral cavity, considering the large number of microorganisms present[47]. Due to this, the common recommendation for dentists is the use of systemic antibiotics to reduce the risk of postoperative infections and can thus lead to side effects such as bacterial resistance[12]. In order to decrease bacterias in the oral environment, antimicrobial agents are widely used, and the one that is most used and has high efficiency, is the chlorhexidine. Chlorhexidine solution has wide spectrum of action, which means it has the ability to cause an immediate reduction in the number of bacteria in the oral cavity, but it has side effects with prolonged use as change in taste and staining of teeth and restorative materials[2, 19].

With the limitations of several antimicrobial agentes, alternative treatments were developed, having highlighted the antimicrobial photodynamic therapy, which has high efficacy against oral pathogens (TFDA)[9, 27, 55]. Photodynamic therapy is a treatment already known in medicine for treating certain benign and malignant lesions in different regions of the body[48], but there are already too many studies showing its effectiveness in reducing microbial cases in dentistry intra-canal infections, dental caries, periodontitis, peri-implantitis, and other applications[20, 27, 31, 36, 57]. This technique has the advantages of causing bacterial kill quickly as well as to be improbable the bacterial resistence due the cytotoxicity of reactive species which are formed during the process as well as being a technique atraumatic to host[7, 55].

The mechanism of action of photodynamic therapy consists of interaction between three elements of fundamental importance: photosensitive chemical compound

(photosensitizer), light source and molecular oxygen. After penetration of the photosensitizer in the bacterial wall, it will attract photons emitted from the light source at a wavelength equivalent to the absorption spectrum of the photosensitizer, and there will be an electronic excitation by transferring hydrogen ions or electrons from neighboring molecules that give rise to free radicals hydrogen peroxide and hydroxyl radicals (type I reaction). Also the energy is transfered to oxygen by the photosensitizer, resulting in a highly reactive condition called singlet oxygen[28, 49].

The photosensitizers used in photodynamic antimicrobial therapy should provide absorption peaks near the wavelength of the light source used[54], to be non-toxic to the host and form the reactive species to lead to cell death[32]. There are several classes of chemical compounds that has proven to be effective in this technique, having the phenothiazine dyes, such as methylene blue, which has a high degree of selectivity, and ortho-toluidine blue, in wide use in oral infectious diseases[51, 54]. A natural substance of origin of the Curcuma Longa root also has excelled results in studies by the powerful antimicrobial effects that it presents, with its broad absorption peak (300 - 500nm)[1,14,22,42].

In relation to the light source to be used in photodynamic therapy, various types have been tested, and the lasers were, until recently, the most widely used, with good features like monochromaticity (same wavelength) and large amount of energy given to target lesion[56]. However, the cost-benefit of the emergence of LED devices, these have been widely used, having characteristics of divergent and not coherent beams, which are favorable to the complementarity of the photosensitizer[20], and is a device already present in the dental office and at a lower cost[37].

Among the applications of antimicrobial photodynamic therapy already mentioned, there is the general decontamination of the mouth, which is an important application when considering the preoperative disinfection and as prevention of infection in the postoperative treatment, treatment in immunocompromised patients (condition this by side effect any disease or even some treatment), and in patients in intensive care units, which have several limitations. The general decontamination of the oral cavity is an alternative that in the near future will be more widespread, with advantages such as the control of resistant fungi in denture wearers, and because it lead to a reduction in loads of pathogens in place, which prevents the use of drugs with side effects[40].

To date, there are no studies in the literature that show side effects of the technique of antimicrobial photodynamic therapy. Staining of tooth structure and restorative materials is one of the side effects of chlorhexidine. However, the fact of using photosensitive dyes so that there is the desired effect, is of great importance to investigate whether they could lead to staining of restorative materials such as composite resin, which could compromise its use, especially in areas that would compromise the aesthetics. Facing this context, the aim of this study was to evaluate the effect of antimicrobial photodynamic therapy on color stability of a composite resin using methylene blue and curcumin as photosensitizers.

MATERIALS AND METHODS (Apêndice A2)

Intra-examiner calibration

The reproducibility study was conducted to evaluate the reliability of measurements obtained intra-examiner, where the researcher 2 examined in duplicate 10 specimens for color stability, with one-hour interval between assessments. The intra-examiner agreement between the data was estimated by the intraclass correlation coefficient (ρ), was classified as proposed by Fermanian[18] (1984), as "excellent" (>0.91).

Preparation of specimens in composite resin

For this research was used the composite resin whose characteristics are shown in Table 1.

The composite resin specimens were fabricated inside a steel die with 10 mm diameter holes and a thickness of 2 mm. Then, in order to obtain a smooth surface, a polyester strip was placed on the surface of the composite, a glass plate, and a weight of 1 kgf for 30 seconds to allow the flow of excess material and to leave a smooth surface and standardized[3]. After this period the weight and the glass plate were removed and light-curing polyester strip by the time of 40 seconds by Radii Plus® (SDI, Australia) with irradiance of about 1,500 mW / cm2. After curing, the polyester strip was removed and both the metal matrix hemisecções were separated allowing the removal of the clamp clinical specimens.

From this, the specimens were immersed in artificial saliva with exchange performed weekly, and stored in an oven (EBC1-Odontobras- Trade Eq. Medical-Dental LTDA, Ribeirão Preto, SP, Brazil) kept the temperature of 37 ° C ± 1 ° C and until the color readings.

Photosensitizers

The photosensitizers used for immersion of the specimens were methylene blue and curcumin (Sigma Aldrich, St. Louis, MO) at concentrations of 20, 40 and 60 μM, for times of 2 to 5 minutes (pre-irradiation time). The use of these times has antimicrobial satisfactory results in literature[25, 35, 59] and very long time intervals could hinder the clinical practice in the art.

Light Source

It was used LED lighting system (BioTable, LAT, USP, IFSC, San Carlos, SP) in the wavelength of about 440nm (FS curcumin base) or 630nm (methylene blue FS) resonant with the absorption peak of the selected photosensitizer at a dose of approximately 15 J / cm2.

Evaluation change color

The color values were obtained through the Colorimetric Spectrophotometer (Color guide 45/0, PCB 6807 BYK-Gardner GmbH, Gerestsried, Germany) with a wavelength varying from 400 to 700 nm through direct transmission with standard lighting D65 on white background[15]. After the calibration of the spectrophotometer, four readings were taken for each specimen: before the immersion in solutions of photosensitizers (L0), after immersion in the photosensitizers (L1), after irradiation (L2), after 10 days (L3), after 30 days (L4), and after 60 days (L5). The readings were performed everyday following the same time.

The color values were recorded by the spectrophotometer according to the CIE-Lab System recommended by CIE (Commission Internationale de l'Eclairage)[24, 53]. In this system, L * refers to the lightness coordinate (gray scale) with values of zero (black) to 100 (white). The values of a * and b * are the chromaticity coordinates of the red-green and yellow-blue axis, respectively. Values of positive a* indicate deviation of chromaticity towards the red hue and negative values shift towards green hue. Similarly,

positive b* values indicate a shift towards yellow hue and negative values indicating a shift to blue[21, 24].

For the evaluation of possible color changes of the specimens composed and dentin resin, a certain classification was adopted by the "National Bureau of Standards" (NBS), where: ΔE values between 0.0 to 0.5: extremely slight change; 0.5 to 1.5: slight change; 1.5 to 3.0: perceptible change; 3.0 to 6.0: significant change; 6.0 to 12.0: extremely significant change; 12.0 or more, change to another color.

Analysis Data

Statistical analysis of the color change in the resin composite samples subjected to two photosensitizers in three different concentrations and in two contact times, was performed by analysis of variance for repeated measures. For multiple comparisons of means, additional analyzes were performed with the Tukey test, adopting a significance level of 5%.

RESULTS

Table 2 shows the averages and standard deviations ΔE color variation relative to the initial color composite samples in accordance with the photosensitizer (A = methylene blue and curcumin = C), concentration (20, 40, and 60 μM) exposure time (2 and 5 minutes), and timing of reading color (L1 to L5 after an initial reading L0).

It is observed that both, in the methylene blue as in the curcumin, there was no significant difference between the different groups, ie, there was no significant influence of different concentrations, time of pre-irradiation and reading times, with only statistically significant difference between the L1 (after immersion reading) and the other readings of occasions. Comparing the mean of photosensitizers groups relative to the control group, it is observed that all groups with methylene blue showed statistically significant differences at all times of reading. However in curcumin, only the groups in the first reading (L1) showed a significant difference compared to control, reducing this change and keeping similar to the control group from L2.

In Figure 1(a) e (b), the average ΔE are shown in the pre-irradiation times of 2 minutes (Figura 1a) and 5 minutes (Figura 1b), along with the standard errors, which provide an estimate of the accuracy of them. This figure makes it easy to see the results in Table 2, with a little more pronounced influence of the highest concentration in the 5

minutes exposure time, even without significant difference. Curcumin as indicated by statistical analysis, remains throughout the trial period almost the same level of control.

DISCUSSION

Due to the limitations of antimicrobial agents in removing microorganisms in the oral cavity antimicrobial photodynamic therapy has been featured, which is an alternative treatment using a photosensitizer. The photosensitizers are activated by a light source and interacts with tissue oxygen[28]. Considering the need to assess if the use of the photosensitizer will lead to staining of restorative materials present in dental structures, this study evaluated the influence of methylene blue and curcumin on the color change of a microhybrid composite, noting that the methylene blue showed statistically significant difference from the control group even in the final reading (after 60 days), that clinically according to the classification determined by the "NBS" represents changes between extremely significant and meaningful changes. In the curcumin, there were no statistical significant differences from L2, and considering clinical evaluation the changes were mild.

The fact of the color changes seen more pronounced in the methylene blue even over time may suggest a lower degradation of the dye compared to curcumin, which after application of light showed similar results to the control group. The photobleaching occurs by optical excitation of the photosensitizer molecules[34]. Studies have shown a high curcumin degradation during periods of exposure light in a process called photobleaching, decreasing the absorption of the dye after lighting and producing low levels of reactive oxygen species[10, 14], which would suggest be advantageous in the case of color change the structures present in the oral environment, but as the work Mang et al.[34] (1987), this would reduce the efficiency of the treatment and its antimicrobial results. The photobleaching processes that occur with many photosensitizers are oxidative and suggest the involvement of singlet oxygen[6]. In the control group there was a color change over time of the readings, but with no significant difference, suggesting a change by the composition of the artificial saliva. In the study of Lee and Powers[30] (2006), stated that there enzymes in human saliva, such as salivary esterases, which can lead to internal or external color change of the composites due to the fact that they can change their surface properties.

There are several literature studies assessing the susceptibility of composite resins to staining after immersion in pigments solutions such as juices, coffee, tea and other

solutions[26, 39, 41, 52]. When it comes to PDT, there are no studies evaluating color changes of photosensitizers on resin. Microhybrids composites, as used in this study, are widely used because of its physical, chemical properties associated with a good polishing of restorations[16]. However, studies have shown that nanocomposites exhibit properties such as wear resistance, equal or greater than microparticulate and microhybrid composite resins[50, 58]. The composition and characteristics of the particles of the composite resin has a direct influence on the surface smoothness and extrinsic pigmentation[45, 46]. The resin matrix[52] and polymerization of the composite[26] are intrinsic factors with an influence on the color stability of the material. The microhybrid resins have low degree of water absorption due to the presence of hydrophobic monomers in the matrix, having the degree of water sorption a direct relationship with the color stability of the material[16]. Finishing and polishing is another factor that influences the surface of the composite and it is associated initial discoloration of the material [43]. But there are studies that found no difference in color stability in specimens submitted to the procedures for finishing and polishing of a nanoparticulate resin, which was suggested by the fact that the polyester strip used during the production of the specimens already produce a surface smoothness, which decreases surface roughness and consequently the possibility of staining[4, 38, 41]. In the study performed by Nasim et al.[39] (2010) the effect of drinks in color stability of different composites were evaluated, concluding that all beverages led to color changes, but with clinically acceptable variations. It also observed that the microhybrids composites had greater color stability than the nanocomposites and microparticulated, stating that the color stability is related to both the type of drink while the composition of the composite resin.

Due to the large number of microorganisms present in the oral cavity, it is common to need treatment with antibiotics, which has led to the worldwide increase in resistance of gram-positive bacteria and negative[33], and of treatment with antimicrobial agents, such as chlorhexidine, for oral disinfection procedures as the pre and post-surgery, patients in intensive care units, patients with immunosuppression, and even in cases of recurrent infections by bacterial and fungal resistance. The use of oral antimicrobial decontamination with photodynamic therapy would be an excellent use in these cases because of its advantages. Diseases such as mucocutaneous oropharyngeal candidiasis is a common manifestation of infection by the human immunodeficiency virus (HIV)[11]. Studies have shown the susceptibility of *Candida albicans* to death by antimicrobial photodynamic therapy using various photosensitizers[5,8,29].The disinfectant

solution most commonly used is chlorhexidine, which although lead to immediate reduction in the number of oral bacteria[19], its use causes effects already reported as altered taste, burning sensation, and staining of teeth and restorative materials[2]. The study by Araujo et al.[1](2012), evaluated the use of curcumin associated with lighting as an oral antimicrobial mouthrinses in some volunteers, and noted that there was no burning sensation reports, or pain, or ulcers. The reduction of bacteria is compared with the use of traditional oral solutions containing chlorhexidine and alcohol in its compositions[23].

Although there are no studies evaluating the photosensitizing staining in composite resins, Donnelly et al.[13] (2007), stated that it is undesirable to use photosensitizers for mouthwash due to their highly colored nature and potential for staining the teeth, oral mucosa and lips, suggesting only use photosensitizer directly at the infection site. However, in the study by Araujo et al.[1] (2012), argued that in general, the decontamination with antimicrobial photodynamic therapy is a simple procedure and allows advantages in the precaution of complications in oral surgical procedures.

CONCLUSION

Photodynamic therapy technique is an interesting alternative to antibiotics and traditional antimicrobial mouthwash to reduce oral microorganisms, but there are factors that can influence their clinical use as staining of restorative materials. In clinical situations, due to the many effects that can influence the color change of photosensitizers in composite resin, as their concentration, resin composite type, finishing and polishing procedures, saliva, tooth brushing and other factors, there is the necessity of researchs still to be carried out in this field to determine its clinical use without compromising the color of dental restorative materials and structures. The present study may suggest that the use of curcumin lead to lower color changes compared to methylene blue.

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Table 1 - Identification and characteristics of the resin composite used in the study.

Material	Fabricante	Tipo	Composição da matriz	Tamanho médio (carga)	Cor
Filtek Z250 XT	3M ESPE, St. Paul, MN, EUA	Microhybrid	Bis-GMA Bis-EMA UDMA	Particle 0,01µm to 3,50 µm, with average of 0,6µm	A_2

Table 2 - Mean (standard deviation) of ΔE color variation from the initial color (L0) of resin samples in accordance with treatment (concentration - time of exposure), and A = methylene blue; C = curcumin; μM concentration and exposure time in min, and the color reading time (L1 to L5).

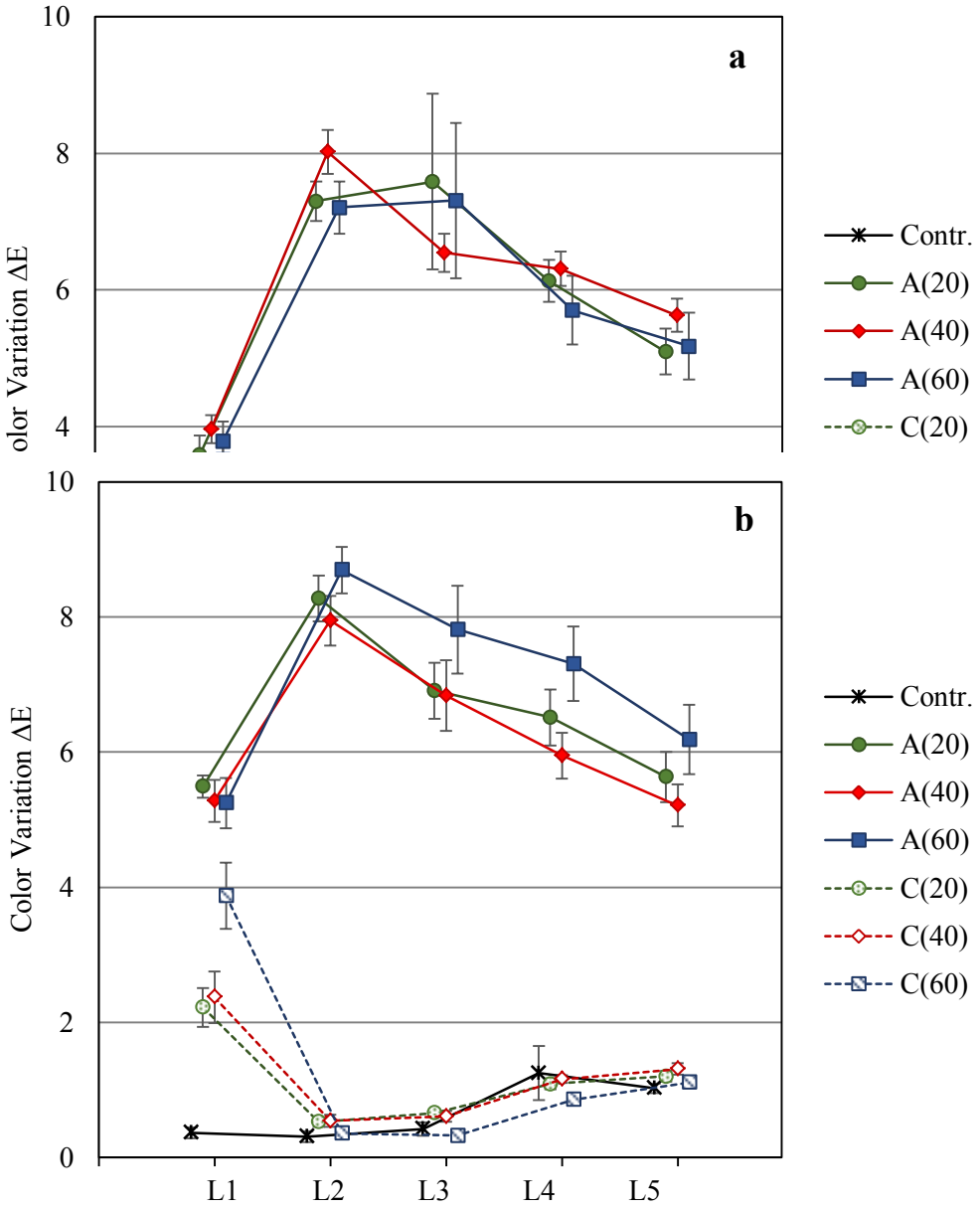
Groups	Reading Occasions				
	L1	L2	L3	L4	L5
Control	0,36 (0,24) a	0,31 (0,25) a	0,42 (0,31) a	1,25 (1,27) a	1,03 (0,22) a
A(20-2)	3,59 (0,91) aABC+	7,30 (0,91) cB+	7,58 (4,06) cB+	6,13 (0,98) bcB+	5,10 (1,06) abB+
A(20-5)	5,49 (0,53) aC+	8,28 (1,07) bB+	6,91 (1,31) abB+	6,51 (1,30) abB+	5,63 (1,17) aB+
A(40-2)	3,96 (0,64) aABC+	8,02 (1,01) cB+	6,54 (0,88) bcB+	6,31 (0,79) bcB+	5,63 (0,76) abB+
A(40-5)	5,28 (0,99) aBC+	7,95 (1,17) bB+	6,83 (1,65) abB+	5,95 (1,08) aB+	5,21 (0,98) aB+
A(60-2)	3,79 (0,93) aABC+	7,21 (1,21) cB+	7,31 (3,60) cB+	5,70 (1,59) bcB+	5,18 (1,55) abB+
A(60-5)	5,25 (1,17) aBC+	8,70 (1,09) cB+	7,81 (2,06) bcB+	7,31 (1,75) bcB+	6,18 (1,62) abB+
C(20-2)	2,27 (1,14) bA	0,48 (0,28) aA	0,41 (0,30) aA	0,87 (0,23) abA	0,91 (0,24) abA
C(20-5)	2,22 (0,91) aA	0,53 (0,22) aA	0,66 (0,19) aA	1,09 (0,25) aA	1,20 (0,25) aA
C(40-2)	3,14 (1,38) bAB+	0,36 (0,09) aA	0,42 (0,16) aA	0,89 (0,22) aA	1,16 (0,20) aA
C(40-5)	2,37 (1,20) bA	0,54 (0,27) aA	0,61 (0,26) aA	1,16 (0,18) abA	1,31 (0,26) abA
C(60-2)	3,51 (1,15) bABC+	1,17 (2,61) aA	0,42 (0,18) aA	0,93 (0,16) aA	1,08 (0,12) aA
C(60-5)	3,88 (1,55) bABC+	0,36 (0,16) aA	0,32 (0,14) aA	0,86 (0,21) aA	1,11 (0,17) aA

Averages followed by the same letters (lowercase or uppercase on the line in the column) are not significantly different ($p > 0.05$)
+ Indicates that the medium in a column are significantly different from the control mean

Legend:

Column Groups □ photosensitizer (concentration - exposure time)

Figure 1 - Mean (symbols) and standard errors (vertical bars) of ΔE color variation from the initial color (L0) of resin samples in accordance with treatment (concentration - time of exposure), and blue A = methylene; C = curcumin; µM concentration and exposure time in 2 minutes **(a)** and 5 minutes **(b)**, and the color reading time (L1 to L5).



4 Considerações Finais

A terapia fotodinâmica tem sido um tratamento de grande importância na odontologia, demonstrando um efeito antibacteriano altamente eficaz, incluindo a ação contra microrganismos presentes na cárie dental [15, 19].

A lesão dentária por cárie possui áreas altamente infectadas com bactérias que devem ser removidas pela impossibilidade de remineralização, e áreas passíveis de remineralização, que devem ser mantidas [26, 44]. Porém, clinicamente é um processo crítico ter a precisão das áreas a serem removidas [10], removendo estrutura dentinária com risco de exposição pulpar [7]. Com a aplicação da terapia fotodinâmica antimicrobiana há a possibilidade de evitar esta exposição pulpar, pois ocasiona uma morte bacteriana eficaz e sem possibilidades de resistência bacteriana devido à formação de espécies altamente reativas no seu mecanismo de ação[10].

Com o propósito de redução de microrganismos presentes no meio oral, há a utilização de diversos agentes antimicrobianos, tendo a clorexidina entre os mais utilizados e com alta eficácia antimicrobiana, porém com efeitos colaterais[14]. Outra aplicação da terapia fotodinâmica com futuro promissor é a descontaminação geral da cavidade oral, que é uma alternativa muito interessante principalmente em procedimentos pós-cirúrgicos e em pacientes imunodrepimidos[25]. Um obstáculo da terapia fotodinâmica para uso na prática clínica é a possibilidade de alteração da cor dos dentes e materiais restauradores devido à cor dos fotossensibilizadores, podendo assim comprometer a estética.

Com a avaliação neste trabalho dos fotossensibilizadores azul de metileno e curcumina, variando-se suas concentrações e tempo de contato com espécimes de dentina e de resina composta, verificou-se que houve alterações de cor significantes clinicamente. As maiores alterações foram observadas nas leituras iniciais, diminuindo após a aplicação da luz, principalmente nos grupos com a curcumina. Fato este pode ser explicado pela fotodegradação do fotossensibilizador, que em contato com a luz diminui a absorção do corante[13]. Porém, mesmo com a leitura após 60 dias em imersão em saliva artificial, foi possível observar alteração de cor nos espécimes com ambos fotossensibilizadores, sendo esta alteração menor com o uso da curcumina e sobre a resina composta.

Analisando ambos os estudos, as alterações de cor encontradas em espécimes de dentina foram mais altas e com maior variedade, que pode ser explicado pelo fato da estrutura de dentina ser muito variável por ter diversos fatores que podem influenciar a absorção de

pigmentos, como, por exemplo, a permeabilidade, difusão da luz sobre a superfície[34], quantidade e disposição dos túbulos dentinários[45]. Entretanto, nos espécimes de resina composta, estas variações encontradas em dentina não ocorrem, pois a composição do material é a mesma em todos os espécimes, além de serem confeccionados de maneira a obter uma lisura superficial. Com a superfície lisa, que pode ser atribuída ao uso da tira de poliéster durante a confecção dos espécimes, eles apresentam menor rugosidade e consequentemente ficam menos susceptíveis a impregnação de pigmentos[6]. Em relação ao fotossensibilizadores utilizados, é possível concluir que a curcumina leva a alterações de cor menores que o azul de metileno, porém há a necessidade ainda de estudos, tanto laboratoriais quanto clínicos, que avaliem estes fotossensibilizadores em concentrações e tempos de pré-irradiações menores, assim como o uso de outros fotossensibilizadores, para que assim seja um tratamento antimicrobiano que alie sua eficácia antimicrobiana ao não comprometimento estético dental.

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APÊNDICE A1 - METODOLOGIA CAPÍTULO 1

1) Fixação do dente em aparato de madeira com godiva

2) Corte no plano axial do dente com máquina de cortes Isomet para obtenção do disco do terço médio da dentina com 2mm de espessura

3) Nova fixação do disco do terço médio da dentina com godiva

4) Marcações no disco dentinário para obtenção de fragmento com dimensão de 6x6mm através da máquina de cortes

5) Fragmentos posicionados em matriz circular para inclusão em resina **poliéster CrisLight para obtenção de espécimes circulares de 10x2mm para correto posicionamento no espectrofômetro**

6) Após confecção dos espécimes, estes foram armazenados em saliva artificial em estufa a 37ºC por 24 horas até a primeira leitura de cor

7) Posicionamento do aparelho Espectrofotômetro de calorimetria sobre um aparato onde se encaixa os espécimes para leitura de cor inicial (L0)

8) Imersão nos fotossensibilizadores azul de metileno e curcumina nas concentrações e tempos de pré-irradiação determinados, e posterior leituras de cor L1 e L2

9) Armazenamento dos espécimes após as imersões novamente em saliva em estufa a 37ºC para posteriores leituras de 10, 30 e 60 dias (L3, L4 e L5)

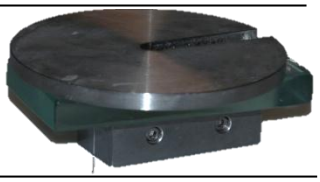
APÊNDICE 2 - METODOLOGIA CAPÍTULO 2

1)Matriz de aço bipartida com orifícios de 10x2mm para confecção dos espécimes em resina composta

2) Resina composta microhíbrida para inserção no interior da matriz em incrementos

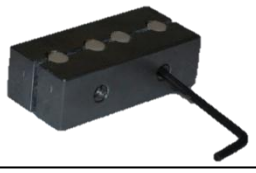
3) Após inserção da resina, foi colocada uma tira de poliéster e uma placa de vidro sobre os espécimes

4) Em seguida aplicou peso de 1kg por 30 segundos para escoar excesso da resina e permitir lisura superficial



5) O peso e a placa de vidro foram removidos e fotoativou-se através da tira de poliéster pelo tempo de 40 segundos

6) Remoção da tira de poliéster após a fotoativação e abertura da matriz bipartida para remoção dos espécimes



7) Após confecção dos espécimes, estes foram armazenados em saliva artificial em estufa a 37ºC por 24 horas até a primeira leitura de cor

8) Posicionamento do Espectrofotômetro de calorimetria sobre um aparato onde se encaixa os espécimes para leitura de cor inicial (L0)

9) Imersão nos fotossensibilizadores nas concentrações e tempos de pré-irradiação determinados, e posterior leituras de cor L1 e L2

10) Armazenamento dos espécimes após as imersões novamente em saliva em estufa a 37ºC para posteriores leituras de 10, 30 e 60 dias (L3, L4 e L5)

ANEXO – Aprovação Comitê de Ética

FACULDADE DE ODONTOLOGIA DE ARARAQUARA - UNESP

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação da influência da terapia fotodinâmica antimicrobiana no manchamento da estrutura dentária e de uma resina composta

Pesquisador: Alessandra Nara de Souza Rastelli

Área Temática:

Versão: 1

CAAE: 30682114.0.0000.5416

Instituição Proponente: Faculdade de Odontologia de Araraquara - UNESP

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 645.911

Data da Relatoria: 12/06/2014

Apresentação do Projeto:

O projeto de pesquisa intitulado "Avaliação da influência da terapia fotodinâmica antimicrobiana no manchamento da estrutura dentária e de uma resina composta" constitui um projeto de dissertação de Mestrado do Programa de Pós-Graduação em Ciências Odontológicas da Foar. Para atingir seus objetivos serão utilizados 120 terceiros molares (obtidos em banco de dentes) e 120 amostras de resina composta Z250. O projeto possui 12 grupos (n=10), para que a cor da dentina e da resina sejam avaliadas em espectrofotômetro antes e após o uso do azul de metileno e da curcumina nas concentrações de 20, 40 e 60 M e tempos de contato iguais a 2 e 5 minutos.

Objetivo da Pesquisa:

O presente estudo terá como objetivo avaliar o manchamento ocasionado por dois fotossensibilizadores (azul de metileno e curumina), em diferentes concentrações (20, 40 e 60 M), utilizados na terapia fotodinâmica antimicrobiana (aTFD) na dentina humana e em uma resina composta por tempos de contato iguais a 2 e 5 minutos.

Avaliação dos Riscos e Benefícios:

Os riscos possíveis com o desenvolvimento desta pesquisa se restringe ao pesquisador durante a confecção e manuselo dos corpos-de-prova, sendo eles minimizados com a utilização correta e

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FACULDADE DE ODONTOLOGIA DE ARARAQUARA - UNESP

completa de equipamentos de segurança e proteção. Como benefícios tem-se o estudo de um possível efeito adverso (manchamento) de uma nova terapia com
ação antimicrobiana.

Comentários e Considerações sobre a Pesquisa:

Trata-se de uma pesquisa importante uma vez que trabalhos que investigam a possibilidade de manchamento das estruturas dentais e de materiais restauradores pelos agentes fotossensibilizantes são escassos.

Considerações sobre os Termos de apresentação obrigatória:

Os termos estão adequados. Não há TCLE uma vez que os dentes serão obtidos no banco de dentes da FOAr.

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:

Os pesquisadores responderam à pendência, incluindo os riscos e documento referente à devolução de remanescentes dentários.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

Protocolo APROVADO em reunião de 13 de maio de 2014.

ARARAQUARA, 13 de Maio de 2014

Assinado por:
Mauricio Meirelles Nagle
(Coordenador)

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Araraquara, 05 de Março de 2015.

CAMILA CRUZ LORENZETTI