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Tooth color change caused by photosensitizers after photodynamic therapy: An in vitro study☆,☆☆,☆☆☆,☆☆☆☆

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ABSTRACT

This study aimed to perform an in vitro evaluation of the effect of photosensitizers used in photodynamic therapy (PDT) on tooth color change when used in combination with conventional endodontic treatment. Forty extracted human mandibular premolars were accessed and underwent root canal therapy and PDT. Photosensitizers were used in accordance with the experimental groups: MB (n = 10) — PDT with Methylene Blue at 0.01%; TB (n = 10) — PDT with Toluidine Blue at 0.01%; MG (n = 10) — PDT with Malachite Green at 0.01%, at the concentration of 0.1 mg/mL; and PC (n = 10) — positive control, PDT with Endo-PTC cream stained with Methylene Blue at 25%. The samples were irradiated with 660-nm diode laser by means of a 330-μm-diameter optical fiber cable at a power density of 40 mW for 120 s. After light curing, the photosensitizers were removed from the specimens with 10 mL sodium hypochlorite at 1%. A reflectance spectrometer was used for evaluation of color prior to and 60 days after the experimental procedure based on the CIE L*a*b* system. According to ANOVA test, there were statistically significant differences between the experimental groups ($p = 0.003$). Tukey's test showed a significant difference between PC and TB ($p = 0.008$), as well as between MG and TB ($p = 0.009$). However, there was no statistically significant difference between PC, MG ($p = 0.957$) and MB ($p = 0.103$). It was concluded that the use of PDT as an adjuvant to root canal therapy, using different photosensitizers, led to color change in tooth structure.

1. Introduction

The maintenance of an aseptic chain and the elimination of microorganisms from the root canal system is the main condition for a successful root canal treatment. Several techniques have been developed to achieve this goal. One of them, photodynamic therapy (PDT), stands out for its relative convenience, safety [1,2,3,4] and excellent antibacterial potential, as demonstrated by in vitro [5,6,7,8] and in vivo studies [1,9]. It has been suggested as a good option to maximize the disinfection of the root canal [1,10].

PDT involves the use of a low-intensity light source, i.e. laser, associated with a photosensitizer, which consists of a nontoxic dye. When cured by a source of laser light at a specific wavelength (630 to 800 nm), the photosensitizer reacts directly with biomolecules or oxygen molecules generating cytotoxic products, such as free radicals (type I reaction) or highly reactive singlet oxygen (type II reaction) [11,12]. This reaction causes the rupture of the bacterial cell wall, leading to the death of the microbial cell [1].

Phenothiazine-based dyes, such as Toluidine Blue, Methylene Blue and Malachite Green, are currently the photosensitizers used most often in PDT, and they have produced favorable results [1,13,14,15]. However, because they are dyes, these photosensitizers can change the color of the tooth structure [13], compromising teeth esthetics and the mental well-being of patients [16].

In this context, although the antibacterial effectiveness of PDT has been extensively investigated, few studies have evaluated the effect of photosensitizers on tooth color change [4,13,14]. Thus, this study

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aimed to evaluate the effect of photosensitizers used in PDT on tooth color change.

2. Materials and Methods

2.1. Sample

Forty healthy, extracted human mandibular premolars with similar crown size were selected for this study. Cervico-occlusal and mesiodistal measurements of the crowns were performed with a digital caliper (Starrett Indústria e Comércio Ltda., Pinheirinho, SP, Brazil) with 0.01 mm accuracy. Teeth whose vestibular region of the crown differed by more than 10% from the mean were discarded. The selected teeth had intact vestibular surface, complete rhizogenesis and single root canal, as confirmed by periapical radiography. The experiment was approved by the Research Ethics Committee of the University (Certificate of Presentation for Ethical Consideration: 0141.0.107.000-09).

2.2. Preparation of Samples

The teeth were cleaned with Gracey 5-6 periodontal curettes (Golgran Ind. e Com. de Instrumental Odontológico Ltda., Brasília, DF, Brazil) and polished with a rubber cup and prophylactic paste (Herjos, Vigodent, Rio de Janeiro, RJ, Brazil) at low speed. Then, they were placed in Eppendorf tubes containing distilled water and kept in an oven for 72 h at 37 °C, until the laboratory procedures were started [17,18].

After this period, the apical foramen of each tooth was sealed with light-cured resin composite (Fill Magic, Vigodent, Rio de Janeiro, RJ, Brazil), and two layers of red nail polish (Colorama, São Paulo, SP, Brazil) were applied across the outer root surface in order to make the roots impermeable [5,19]. Subsequently, the coronary pulp chamber was accessed and the remaining layer of enamel and dentin of the vestibular surface was measured with a digital caliper [17,20].

2.3. Chemical–Mechanical Preparation of Root Canals

The root canals of the selected teeth were prepared with the crown-down technique using Gates-Glidden drills (Dentsply Maillefer®, Rio de Janeiro, RJ, Brazil) nos. 1, 2 and 3 in the cervical and middle thirds and instrumentation with one size 40 k-file (Dentsply Maillefer®, Rio de Janeiro, RJ, Brazil). The specimens were irrigated during instrumentation with 10 mL sodium hypochlorite at 1% (Biodynamics, Ibiporã, PR, Brazil), followed by EDTA at 17% (Biodinâmica, Ibiporã, PR, Brazil) and final irrigation with saline solution at 0.9% (Laboratório Tayuyna Ltda., São Paulo, SP, Brazil). The irrigation with sodium hypochlorite at 1% and EDTA at 17% was performed with the objective of removing the smear layer and increase dentin permeability for better penetration of the photosensitizer [6]. After preparation, the canals were dried with absorbent paper cones (#40) (Tanariman Industrial Ltda., Manaus, AM, Brazil).

2.4. Color Analysis of Dental Crowns by Photoreflectance

The teeth were subjected to evaluation of color change using a spectrometer (Model HR2000, Ocean Optics, Orlando, USA), composed of an integrating sphere (Model ISP-REF, Ocean Optics, Orlando, USA) and an optical fiber cable (Ocean Optics, Orlando, USA). Barium sulfate (Vetec Química Fina Ltda., Rio de Janeiro, RJ, Brazil) was placed in a plastic container and used as reference material (white) for total reflection. The dark pattern was obtained by using the integrating sphere uncovered.

After that, measurements were performed for each specimen. For this reason, the light beam of the sphere was directed at the central portion of the vestibular surface of each sample and the values L*, a*, b * provided by the device were recorded. Ten measurements were taken for each specimen, and the end result was the mean of these values. Each mean separately provided the results of coordinates L*, a* and b* as recommended by the Commission Internationale de l'Eclairage (CIELAB-CIE-1976, Vienna, Austria). L* indicates brightness and varies between 0 for black and 100 for white; a* determines the amount of red (positive values) or green (negative values), and b* displays the amount of yellow (positive values) or blue (negative values).

2.5. Photodynamic Therapy (PDT)

After chemical–mechanical preparation and color analysis, the samples were randomly divided into four experimental groups according to the photosensitizers (PS) used, namely: MB (n = 10) — PDT with Methylene Blue at 0.01% (Chimiolux, Belo Horizonte, MG, Brazil); TB (n = 10) — PDT with Toluidine Blue at 0.01% (Farmácia Fórmula & Ação, São Paulo, SP, Brazil); MG (n = 10) — PDT with Malachite Green at 0.01% (Farmácia Fórmula & Ação, São Paulo, SP, Brazil), at a concentration of 0.1 mg/mL; and PC (n = 10) — positive control, PDT with Endo-PTC cream stained with Methylene Blue at 25% (Farmácia Fórmula & Ação, São Paulo, SP, Brazil).

2.6. Parameters of Laser Irradiation

The root canal was filled with the respective PS, with aid of a sterile syringe (MB, TB and MG groups) or a size 15 k-file (PC group). There was a 5-min pause (pre-irradiation time) before irradiation with Twin InGaAlP Diode Laser (MMOPTICS, Sao Carlos, SP, Brazil), previously calibrated by the manufacturer [21,22]. The following parameters of irradiation were used: wavelength = 660 nm, power density = 40 mW, continuous emission mode, energy density = 120 J/cm^2 and irradiation time = 120 s.

An optical fiber cable with 330 m in diameter (MMOPTICS, São Carlos, SP, Brazil) was used for the irradiation of light. The fiber cable was introduced inside the root canal to reach the working length (WL), at a distance of 1 mm from the radiographic apex. Helical movement was performed from the apical to the cervical region in order to ensure a uniform distribution of light within the canal [6].

After light curing, the PS was removed from the specimens by means of irrigation with 10 mL sodium hypochlorite at 1%. In all groups, the cable was dried with absorbent paper and, finally, the coronary access was sealed with white gutta-percha points (DFL, Rio de Janeiro, RJ, Brazil) and resin-modified glass ionomer cement (Vitro Fil LC, DFL, Rio de Janeiro, RJ, Brazil).

2.7. Reading of Color Change

After 60 days, the samples were again analyzed for color change using the spectrometer. The coordinate values of L*, a* and b* of each sample were reduced from the initial value (baseline) and color change was measured by the mean ΔE value, represented by the equation: $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]\ 1/2$, where $\Delta L = L1 - L0$; $\Delta a = a1 - a0$; and $\Delta b = b1 - b$ [18,23,24,25].

2.8. Data Analysis

The data were analyzed using one-way ANOVA. This analysis was complemented by multiple comparisons of the means by Tukey's test at a significance level of 5%.

3. Results

Figs. 1, 2 and 3 indicate the means of the values obtained by coordinates L*, a* and b*, before (baseline) and 60 days after PDT in each sample. The values of ΔE (color change) can be seen in Fig. 4.

The results of ANOVA (one-way ANOVA) showed significant differences between the photosensitizers tested ($p = 0.003$). According to Tukey's multiple comparison test (Table 1), there was no significant difference between the groups PC and TB ($p = 0.008$), as well as between