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# *In vitro* evaluation of methylene blue removal from root canal after Photodynamic Therapy

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ABSTRACT

In Endodontics, photosensitizers' such as methylene blue and toluidine blue have been used in Photodynamic Therapy due to their positive results. However, they can stain the dentin from the root canal after Photodynamic Therapy (PDT). The present *in vitro* study aimed to evaluate different stain removal protocols from root canal after PDT using methylene blue (MB) dye. After mechanical preparation of the root canal of 40 uni-radicular human teeth, PDT was performed using 0,01% MB with parameters of 3 min of pre-irradiation and a diode laser irradiation emitting at 660 nm, 40 mW, 4 min, 9.6 J. After PDT, different protocols of MB removal were performed: Group 1 − control (0.9% saline solution); Group 2 − sodium hypochlorite (2.5% NaOCl); Group 3–17% ethylenediamine tetraacetic acid (EDTA); Group 4 − passive ultrasonic irrigation (PUI); The color of the dentin of the root canal was measured, before, immediately after the PDT and immediately after the cleaning using a spectrophotometer. The ΔE values found were statistically compared using the ANOVA and Tukey's tests ($\alpha = 0.05$). All the treatments lead to some cleaning of root canal after PDT, however, none of the treatments tested completely removed all staining caused by MB photosensitizer of the root canal. Among the treatments tested, PUI and Hypochlorite 2.5% promoted greater cleaning, with no statistically significant difference between them. In conclusion, within the protocols tested in the present study, no treatments were able to completely remove MB staining of the root canal after PDT.

## 1. Introduction

In Endodontics, the majority of failures are related to residual microorganisms which are resistant to chemical-mechanical preparation or intracanal medication [1]. New methods of microbial decontamination have been tested in Endodontics [2]. Photodynamic Therapy, also known as PDT, has been described with promising results in intracanal bacteria reduction, thus eliminating resistant microorganisms and reducing the chances of failure [2,3].

In PDT, a photosensitizer is applied to the tissue and is activated by a light at a specific wavelength. In the presence of oxygen, this light is absorbed by the photosensitizer [2,3]. The absorption of light by the photosensitizer results in the transfer of energy to oxygen, leading to the formation of reactive oxygen species, such as singlet oxygen and other free radicals [4]. Such molecules are capable of damaging proteins, lipids, nucleic acids and other microbial cellular components. It is essential that the photosensitizer absorb the light, so that the reaction of release of singlet oxygen and free radicals occurs [4]. In Endodontics, the photosensitizers derived from phenothiazine, such as methylene blue and toluidine blue, have been frequently used for PDT [2,3].

Methylene blue has been used as a target for microorganisms related to endodontic infections. Due to its hydrophilic nature, accompanied by low molecular weight and positive charge, it allows passage through protein-porin channels in the outer membrane of gram-negative bacteria [2,3,5]. Methylene blue interacts predominantly with anionic lipopolysaccharide macromolecules, participating in the process of photosensitization [2,3,5]. However, the photosensitizers derived from phenothiazine are dyes and thus, they end up staining the dental structure [6], which may compromise aesthetics. Few studies have evaluated the level of dental staining and aesthetic damage caused after the use of these photosensitizers [6–8]. The novelty of the present study is to test different protocols, using saline, sodium hypochlorite (2.5%

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NaOCl), 17% ethylenediamine tetraacetic acid (EDTA) and passive ultrasonic irrigation (PUI) to remove methylene blue of root canals.

Costa et al. [7] evaluated by reflectance spectroscopy the color change in the coronal portion of premolar teeth after PDT using dyes derived from phenothiazine. The authors reported a significant color change 60 days after PDT when dye washing with 10 ml of 1% sodium hypochlorite was considered. The studies of Carvalho et al. [6] and Figueiredo et al. [8] evaluated several techniques for stain removal after the use of methylene blue solution and were successful in removing the dye with protocols containing 2.5% sodium hypochlorite.

However, it is worth mentioning that in all the mentioned studies the color change analysis was performed outside of the tooth samples, in the region of the dental crown, and not directly in the dentin of the root canal. Thus, it is important to know the ideal method for stain removal after the use of the methylene blue photosensitizer, by directly analyzing the results in dentin of the root canal.

This *in vitro* study aims to evaluate different protocols (saline, sodium hypochlorite 2.5%, EDTA, PUI) for the effective removal of methylene blue dye in root canal dentin after PDT.

## 2. Material and methods

After the approval by the Ethics Committee of the School of Dentistry of the University of São Paulo (CAAE: 61716516.3.0000.0075), a total of 40 uniradicular human teeth were selected.

### 2.1. Preparation of samples

Cleaned samples underwent a clinical and radiographic selection process that included: the presence of single root canal, complete apicigenesis and absence of calcifications, resorption and previous endodontic treatment. Samples that did not meet the cited criteria's were excluded.

### 2.2. Study groups

To ensure randomization, teeth were numbered and randomly assigned to the following groups (n = 10): Group 1 − control (0.9% saline solution); Group 2 − sodium hypochlorite (2.5% NaOCl); Group 3–17% ethylenediamine tetraacetic acid (EDTA); Group 4 − passive ultrasonic irrigation (PUI).

### 2.3. Biomechanical preparation of root canals

Initially, access surgery was performed with diamond spherical drills coupled to the high rotation motor. After localization of the root canal the pulp chamber and cervical third of the root canal were washed with 2 ml of 2.5% NaOCl. The channel was scanned with K-file # 10 files (Dentisply Maillefer®, Rio de Janeiro, RJ, Brazil). The working length (WL) was determined 1 mm below the apical foramen. After WL determination, the root canal was washed with 2 ml of 2.5% NaOCl using a 31G Navitip needle (Ultradent®, USA).

The root canal instrumentation was performed according to the manufacturer's recommendation. The Reciproc system (VDW®, Munich, Germany) was used. The instrument R50 was used and the equipment was adjusted to make reciprocating movements. The instrument was inserted into the root canal with in-and-out movements, not exceeding the limit of 3–4 mm. Between each movement a #10 file was inserted into the WL to check the patency. The kinematics was repeated at least 3 times until the WL was reached. Between each insertion of the instrument into the root canal the instrument was cleaned with a gauze slightly moistened in alcohol as well as root canal was irrigated with 2 ml of 2.5% NaOCl.

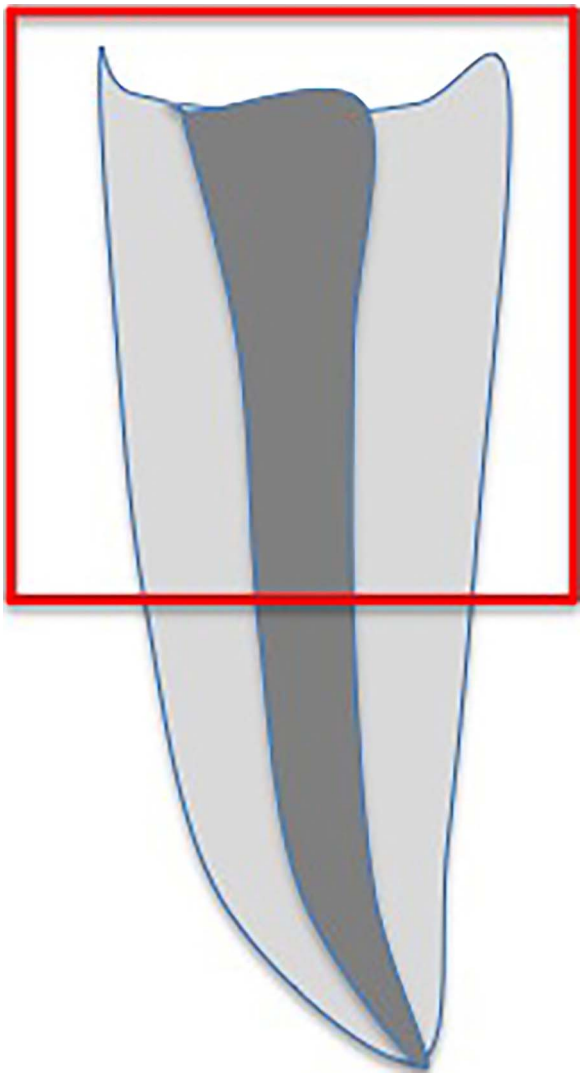

**Fig. 1.** Area of color analysis (5 × 7 mm) in spectrophotometer.

### 2.4. Final sample preparation

After instrumentation, the tooth was sectioned in 2 hemi halves using a diamond faceted disc (7010 − KG Sorensen) coupled to a handpiece. After cutting, the samples were washed in an ultrasonic cleaner (Digital Ultrasonic Cleaner CD-4820, Kondortech®, Sao Carlos, Brazil) immersed in 17% EDTA for 1 min, to remove possible debris from the sample surface.

### 2.5. Color analysis

Quantitative color measurements were performed on an area of 5 × 7 mm, measured from the cervical border of the sample (Fig. 1). The measurement was performed by a spectrophotometer (Konica Minolta CM3700A, Konica, Japan), according to the parameters of the CIELab system (Comissione Internationale de L'clairage L*, a*, b*). The reflection measurement was used as standard. The source of illumination was provided by a light with wavelength of 400–700 nm, illuminant D65, standard observer of 2 degrees, with black background. The reading area of the mask used was rectangular and 1 flash per measurement was used. Each point was measured three times by the same trained researcher, and an average of the three values was obtained.

The values of color change obtained from the coordinates L*, a* and b* after the treatments were subtracted from baseline and the color change was measured by the mean values of ΔE, obtained by the