Evaluation of methylene blue penetration capacity in root dentin of human teeth with and without sonication: a pilot study

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ABSTRACT

Introduction: As the need for complementary antimicrobial therapies arises, photodynamic therapy (PDT) shows promising results in the inactivation of microorganisms inside root canals. **Objective:** The aim of this study was to analyze the penetration capacity of a photosensitizer, i.e., methylene blue, inside the dentin at cervical, middle and apical levels of root canals of human teeth, while comparing different applications of the solution. **Methods:** The sample included 20 single-root pre-molars, distributed into four groups, which were subjected to chemical and mechanical procedures with rotary instruments and HCT20 irrigation. Each group received its own photosensitizer application. The action time of the substance inside the root canal was five minutes: Group 1 = 2% methylene blue; Group 2 = 2% methylene blue with 0.125% sodium lauryl sulfate; Group 3 = 2% methylene blue with sonication; Group 4 = 2% methylene blue 2% with 0.125% sodium lauryl sulfate and sonication. All teeth were cross-sectioned at the cervical, middle and apical levels, then observed under a surgical microscope. **Results:** Results were subjected to statistical analysis using ANOVA, Levene and Tukey tests ($p < 0.05$). Mean penetration ranged between 0.55 mm and 0.75 mm at the cervical third; 0.30 mm and 0.48 mm at the middle third; and 0.17 mm and 0.24 mm at the apical third. **Conclusion:** Results showed that no matter which solvent is used, and whether or not sonication is implemented, under the tested conditions there were no significant statistical differences among groups.

Keywords: Endodontics. Photochemotherapy. Methylene blue. Dentin permeability. Detergents. Tooth root.

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Introduction

Photodynamic therapy (PDT) is a treatment modality that uses light to activate a photosensitizing agent in the presence of oxygen. Exposure of photosensitizer to light results in the formation of oxygen species, such as singlet oxygen and free radicals, causing local damage and cell death.[1] It is a fast and safe process used to kill cells, and the same principle has been used to eliminate microorganisms.[2]

PDT is a treatment modality that has been in constant development in several medical specialties since 1960.[3] Initially, the technique was developed as an alternative for the treatment of cancer.[4] However, the emergence of antibiotics against resistant pathogenic bacteria led to a great research effort to find alternative antibacterial therapies, in which the bacterium would not develop resistance easily.[5] Currently, PDT has been widely studied as an alternative for inactivation of microorganisms, and may be used in chronic periodontitis and sinusitis, as well as in Dermatology and Ophthalmology.[4]

In Dentistry, photodynamic therapy use has rapidly increased. The application of this therapy in cases of oral cancer, periodontal infections, and endodontic treatment is currently being extensively studied and shows promising results.[1,6,7] In Endodontics, although no consensus has been reached on a standard protocol for the incorporation of PDT during root canal treatment,[8] the therapy has proven important in conjunction with conventional endodontic treatment in the elimination of microorganisms that remain viable in the root canal system.[7,8]

Despite the high antimicrobial potential of PDT as adjunct to Endodontics, the technique has limitations that still require improvement. Studies show that deficiency in the penetration capacity of the photosensitizer inside the dentin is related to potential failures of therapy.[9,10]

The purpose of this study is to evaluate the penetration capacity of methylene blue photosensitizer in dentin tubules. Association *versus* non-association of the photosensitizer with an anionic detergent, and the application of the solution according to the conventional technique recommended for PDT and increased by sonication, at the cervical, middle and apical levels in roots of human teeth are compared.

Material and methods

Twenty single-root premolars with complete root formation and straight roots, obtained at the Dental Clinic of *Hospital Universitário de Brasília*, were selected. Teeth had been clinically referred for extraction to preserve patient's oral health. All teeth were kept in an oven at 37 °C in 100% moisture. This study was approved by the Research Ethics Committee of School of Health/UnB, under registration CAAE #54656316.1.0000.0030.

Access and preparation of crown chamber were performed using diamond bur #1012 (KG Sorensen) and Endo-Z drill (Maillefer), respectively. The direct visual technique was used to determine the Actual Tooth Length (ATL), in which K-file #10 (Maillefer - Dentsply) reaches the apical foramen. ATL measurement was performed with cursor adjustment from the crown reference point to the tip of the file at the root apex and with the aid of a millimeter ruler (Angelus). The canal was explored using the same file used in patency. In preparing the cervical segment (ATL / 2), the canal was instrumented up to Gates Glidden #3 drill, thus achieving a 0.90-mm enlargement. In preparing the middle-apical segment, a #35 K-file was positioned in the 2ATL/3 portion, and after conformation provided by this file, Gates Glidden #2 drill reached the same length. Surgical preparation of the apical segment was performed with NiTi instruments (K3XF - SybroEndo) at ATL-1, with expansion of 200 micrometers starting from the anatomical diameter of the canal at this length. Irrigation of root canals was carried out with HCT20 using a Luer-Lock syringe (5 mL at each file change) and a NaviTip 25-mm needle (Ultradent™). For the 20 teeth, the last irrigation was enhanced with sonication (Sonic Borden 2000N - KAVO™) of solution, using E1-Irrisonic (Helse™) tip for 60 seconds.

After instrumentation of root canals, teeth were circumferentially identified with graphite pencil at the heights where cross-sections would be made. Subsequently, roots were waterproofed with the use of colorless nail polish (Miracle Nail™). For application of methylene blue, teeth were fixed in #7 pink wax (Wilson™), which served as support base.

Teeth were randomly divided into four groups according to the solvent used to make the photosensitizing solution, and according to the use or not of sonication inside root canals.

- Group 1 (AM) = 2% methylene blue, conventional application.
- Group 2 (AMT) = 2% methylene blue combined with 0.125% sodium lauryl ether sulfate (Pharmacotechnics – Brasília, Brazil), conventional application.
- Group 3 (AMSon) = 2% methylene blue, with sonication.
- Group 4 (AMTSon) = 2% methylene blue combined with 0.125% sodium lauryl ether sulfate (Pharmacotechnics – Brasília, Brazil), with sonication.

Root canals were filled up to the pulp chamber using a Luer-Lock syringe (3 mL) and a NaviTip 25-mm needle (Ultradent™). Solution action time inside root canals was five minutes.

In groups in which application was conventional (Groups 1 and 2), root canals were filled and without further interventions the recommended time until the next step was allowed to elapse. In Groups 3 and 4, root canals filled with methylene blue were subjected to sonication throughout the entire period. Thereafter, all canals were dried by aspiration and absorbent paper cones (Tanari™).

All roots were immediately cross-sectioned using a thin double-sided diamond disk (KG Sorensen) at pre-established locations: cervical, intermediate (mean root length) and apical (1 mm from root apex). The three cross-sections of roots were fixed in sheets of #7 pink wax.

Specimens were then analyzed under a surgical microscope (CEMAPO™ L860) with focal magnification of 40 x 12.5, and photographed by a photo-coupled system (DSC-W510 Sony™). Measurements were recorded by a single operator using a digital caliper (Mitutoyo™).

For each specimen, the following results were obtained:

- Number of stained surfaces.
- Penetration value, expressed in mm, of each stained surface (a mean value was obtained from two different points on the same surface).

Sample calculation was not necessary in order to define the number of specimens per group, given that this was a pilot study that will serve as foundation for future research.

Results

Descriptive statistical techniques included obtaining statistical measures of mean and standard deviation. For analysis of variance, Levene test was performed. Inferential statistical techniques involved the application of one-way ANOVA.

In the event of significant differences, Tukey paired comparisons were used with $p < 0.05$ significance level.

Analysis of cervical section results (Table 1) shows that in the AM group, the reach of photosensitizer in dentin mass was higher than in all other groups. Statistically, there was significant difference between AM and AMT groups ($p < 0.05$).

In comparing the results at the middle section level (Table 2), the AMTSon group showed the highest mean penetration, while the AMT group showed the lowest one. There was statistically significant difference between AM and AMT groups, as well as between AMT and AMSon groups ($p < 0.05$).

At the apical section level (Table 3), despite mean variation from 0.17 mm to 0.24 mm between groups, no significant statistical difference was found ($p = 0.115$).

By inter-relating Tables 1, 2 and 3, it becomes obvious that mean infiltration was higher for the cervical section, followed by middle section, and lower for the apical section.

In another analysis comprehensively comparing tested groups (i.e. without differentiating the dental thirds) (Table 4), results showed no significant statistical difference between the nature of the solvent agent and methylene blue and/or sonication ($p = 0.368$).

Previous Levene test showed variance was homogeneous in all cases.

In analyzing the percentage of stained surfaces (Table 5) at cervical and middle levels, values reached 100%, while at the apical level this percentage was around 50%, with a mean of 2.25 surfaces stained.

Figures 1, 2 and 3 show a microscopic view of teeth at the cervical, middle and apical sections under 40 x 12.5 focal magnification.

Table 1. Mean and standard deviation of infiltration measures at the cervical level (expressed in mm).

Statistics	Intervention			
	AM	AMT	AMSon	AMTSon
Mean	0,75[A]	0,55[B]	0,59[AB]	0,61[AB]
Standard Deviation	± 0,12	±0,16	±0,03	±0,09

AM: 2% methylene blue; AMT: 2% methylene blue with sodium lauryl sulfate; AMSon: 2% methylene blue with sonication; AMTSon: 2% methylene blue with sodium lauryl sulfate and sonication. A-A: No statistical difference was found between groups; B-B: No statistical difference was found between groups; A-B: Statistical difference was found between groups.

Table 2. Mean and standard deviation of infiltration measures at the middle level (expressed in mm).

Statistics	Intervention			
	AM	AMT	AMSon	AMTSon
Mean	0,43[A]	0,30[B]	0,41[A]	0,48[AB]
Standard Deviation	± 0,07	±0,06	±0,06	±0,10

AM: 2% methylene blue; AMT: 2% methylene blue with sodium lauryl sulfate; AMSon: 2% methylene blue with sonication; AMTSon: 2% methylene blue with sodium lauryl sulfate and sonication. A-A: No statistical difference was found between groups; B-B: No statistical difference was found between groups; A-B: Statistical difference was found between groups.

Table 3. Mean and standard deviation of infiltration measures at the apical level (expressed in mm).

Statistics	Intervention			
	AM	AMT	AMSon	AMTSon
Mean	0,24[A]	0,20[A]	0,23[A]	0,17[A]
Standard Deviation	± 0,05	±0,05	±0,05	±0,04

AM: 2% methylene blue; AMT: 2% methylene blue with sodium lauryl sulfate; AMSon: 2% methylene blue with sonication; AMTSon: 2% methylene blue with sodium lauryl sulfate and sonication. A-A: No statistical difference was found between groups.

Table 4. Mean and standard deviation of general infiltration measures for each group (expressed in mm).

Statistics	Intervention			
	AM	AMT	AMSon	AMTSon
Mean	0,43[A]	0,30[A]	0,41[A]	0,48[A]
Standard Deviation	± 0,66	±0,27	±0,60	±0,98

AM: 2% methylene blue; AMT: 2% methylene blue with sodium lauryl sulfate; AMSon: 2% methylene blue with sonication; AMTSon: 2% methylene blue with sodium lauryl sulfate and sonication. A-A: No statistical difference was found between groups.

Table 5. Number of stained surfaces at each section level.

Section	Intervention				Mean
	AM	AMT	AMSon	AMTSon	
Cervical	4	4	4	4	4
Middle	4	4	4	4	4
Apical	2,2	2,6	2	2,2	2,25

AM: 2% methylene blue; AMT: 2% methylene blue with sodium lauryl sulfate; AMSon: 2% methylene blue with sonication; AMTSon: 2% methylene blue with sodium lauryl sulfate and sonication.

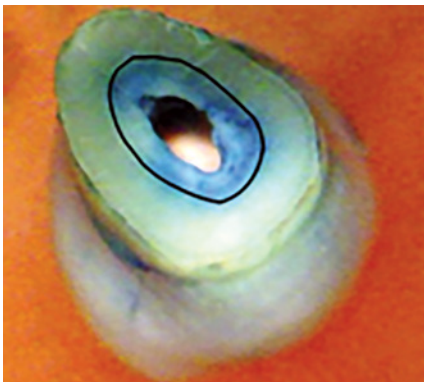

Figure 1. Microscopic view of cervical section.

Figure 2. Microscopic view of middle section.

Figure 3. Microscopic view of apical section.

Discussion

Given its proven efficacy in eliminating microorganisms and its selective antimicrobial action, photodynamic therapy (PDT) emerges as powerful adjunct to antimicrobial therapy,[11] particularly at a time when bacteria have been increasingly resistant to antibiotics.

Despite technical-scientific advances presently observed in Endodontics, the number of failures in endodontic treatment caused by persistence of microorganisms inside dentinal tubules and microspaces of root canals remains vast. PDT emerges in Endodontics as adjunct to surgical treatment of root canals, showing enormous potential for reduction of bacteria.[12-16]

Soukos et al[13] concluded that PDT can be developed as adjunct to eliminate residual bacteria in the root canal system after conventional endodontic treatment.[13]

Garcez et al[16] showed that the combination of conventional endodontic treatment with PDT was more effective in reducing bacteria than each treatment separately. Additionally, this combination was much more effective in reducing the level of intracanal bacterial repopulation after 24 hours.

Regarding photosensitizers, phenothiazine derivatives (methylene blue and toluidine blue) have been widely used in research involving PDT. At low concentrations, they do not produce cytotoxic action, with the dose required for bacterial death being less than the dose required to cause damage to cells. This study employed 2% methylene blue to achieve a visible dye effect that could be possible to quantify with the use of a surgical microscope.

Nevertheless, some limiting factors of the technique may influence the efficacy of PDT treatment. Difficulty of the photosensitizing agent to completely penetrate the dentinal mass of root canals can lead to low antimicrobial efficacy of the technique.[9,10]

In addition, a study addressing dentinal permeability of root canal shows its great importance to Endodontics, since dentinal canaliculi may harbor microorganisms caused by pulp infection.[17]

Permeability is a characteristic of dentin structure, and is dependent on factors such as the number and diameter of dentinal tubules, thickness of dentin, and the presence or absence of smear layer and other precipitates.[18,19]

Siviero et al[19], in their quantitative and diametral analysis of dentinal tubules, showed the cervical third presented a greater number of dentinal tubules and larger diameters, followed by middle and apical thirds.

These results are corroborated by Whittaker and Kneale,[20] who assessed the number of dentinal tubules in root thirds and found the latter to be larger in the crown region, significantly decreasing in size towards the tooth apex.

Corroborating previous studies,[19,20] results showed a positive relationship between dye penetration and permeability of dentinal tubules in each segment, since mean penetration of photosensitizer was higher in the cervical third, followed by middle and apical thirds.

This showed the likelihood of PDT exerting its strongest action on microorganisms in the dentinal tubules of cervical and middle regions.

Although the use of EDTA has proven effective at the end of root canal instrumentation, in this study, the authors followed treatment and instrumentation protocol adopted in the Dentistry course of *Universidade de Brasília*, that is, exclusive use of HCT20 for irrigation.

Under conditions of lower dentin permeability, as in the apical region of root canal, chemical modification of the solution (2% methylene blue + 0.125% sodium lauryl sulfate) enhanced by sonication hindered photosensitizing penetration.

There may be a limit to dentin permeability that may or may not favor the use of sonic enhancement with the purpose of acting inside dentinal tubules.

In order to determine penetrability of a solution inside root canals, surface tension is an important factor that must also be taken into account. It is a known fact that the lower the surface tension of a solution, the greater its ability to penetrate the irregularities of the canal wall.[21]

In this study, the AM group achieved more significant penetration compared to the AMT group, both at the cervical and middle levels. Although surface tension of methylene blue with 0.125% sodium lauryl sulfate was not checked, result shows the detergent did not increase penetration power in that case.

This result might have been related to the sample, since in this investigation the age of samples was not considered as an inclusion criterion. It is also known that an increase in mineralization of dentinal tubules takes place over the years, thus reducing dentin permeability.[19,22,23]

In another comparison, the AM group obtained greater dentinal penetration than in groups in which sonication (AMSon and AMTSon) was used at the cervical level, although there was no significant difference. It is possible that mechanical sonic action inside root canals does not have sufficient power to increase penetration of solutions in the dentinal tubules.

In comparing sonic *versus* ultrasonic irrigation, Sabins et al[24] found passive ultrasonic agitation produced much more significant canal cleaning than did sonic irrigation.

Costa et al,[25] in a comparative study of root canal cleaning with manual and ultrasonic instrumentation, concluded that ultrasonic instrumentation is more effective than manual instrumentation in eliminating dentinal magma. At the apical third level, it is also effective despite dentin magma remnants, which occur in lower quantity when using ultrasound.

Feller et al[26] studied *in vitro* the change in dentinal root permeability due to methylene blue penetration by comparing manual *versus* ultrasound canal preparation. The study showed that permeability was significantly higher in the cervical third when ultrasound was used. However, in the apical third, this difference was only arithmetical.

Perhaps the use of PUI (passive ultrasonic irrigation) may increase its penetration into dentinal tubules by mechanical leverage over the substance.

Nevertheless, despite the fact that the literature already shows ultrasonic agitation to be superior to sonic one, the choice for sonication in this study aimed to analyze the cost-benefit of these instruments for the general practitioner and dental students.

Results did not show any significant difference when comparing AMT groups, and both sonications (AMSon and AMTSon) at the cervical level.

At the middle level, when comparing AMT and AMSon groups, the use of sonication showed greater power in the penetration of photosensitizer than the association of methylene blue with sodium lauryl sulfate.

Although it favored photosensitizer penetration in groups subjected to sonication, the use of detergent seems to have hindered its penetration in all groups.

In another analysis, results of tested groups were compared integrally, i.e., without differentiating dental segments. No statistically significant difference was found among groups.

Thus, it can be inferred from these results that the use of sonication and chemical modification during preparation of the solution did not contribute to improving penetrability of photosensitizer.

As regards the number of surfaces stained in each segment, unlike cervical and middle segments, in which all surfaces were stained, a mean value of 2.25 stained surfaces was observed in the apical segment. This phenomenon can be justified by greater difficulty in cleaning the foraminal region when compared to cervical and middle segments, even when using sonication.[27-29] Given greater mineralization of smear layer,[30] methylene blue cannot penetrate the dentin tubules.

Conclusions

Based on the results achieved, the authors concluded that:

- Penetration of methylene blue is higher at the cervical level, followed by middle and apical levels.
- There is no significant difference in penetration of photosensitizer between conventional application and application enhanced by sonication.
- There is no significant difference in penetration of photosensitizer when comparing the use of 2% methylene blue alone or associated with 0.125% sodium lauryl sulfate.

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