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Comparative Analysis of Photosensitizer Penetration Depth in Root Canal Debridement for Endodontic Disinfection

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Introduction: Microbial agents play a crucial role in periapical lesions and despite mechanical preparation, presence of persistent bacteria in root canal system is a challenge. Photodynamic therapy offers a debridement method, utilizing photosensitizers such as Curcumin, Indocyanine Green (ICG), and Methylene Blue (MB). This study aimed to assess and compare the penetration depth of these photosensitizers on the lateral surface of the root canal. **Materials and Methods:** The crown of 30 single-rooted teeth were separated by a diamond disc. The canals were prepared using a rotary system and were rinsed with 10 mL of 2.5% NaOCl. In order to remove the smear layer debris, 17% EDTA was placed in the root canal for 1 min, then rinsed with NaOCl and saline. The teeth were sterilized by autoclave and randomly assigned to three groups that filled with curcumin, ICG, or MB. Subsequently, they were incubated for 10 min and dried up by paper. Longitudinal sections were cut, and penetration depth of the photosensitizers in coronal, middle, and apical sections were measured using a stereomicroscope. **Results**: Curcumin demonstrated a higher average penetration depth (3000 µm) than MB, and MB showed higher penetration depth than ICG. Significantly different penetration depths were observed in pairwise comparisons among all three groups ($P<0.005$). **Conclusion**: Curcumin with its superior average penetration depth, emerges as a promising choice for effective root canal disinfection in endodontic treatments. Consideration of these findings may enhance the selection of photosensitizers in clinical applications.

Keywords: Curcumin; Indocyanine Green; Methylene Blue; Photodynamic Therapy; Photosensitizers

Introduction

Microbial agents are the main cause of pulp disease and periapical lesion [1]. The complex anatomy of the root canal have made it impossible to completely debride the canals by mechanical preparation and irrigation using intracanal drugs between sessions [2]. It has been observed that conventional endodontic treatments are not successful in complete elimination of microbes, and it appears that the bacteria persist in the root canal after root canal treatment [3]. Sodium hypochlorite is the most common solution for cleaning the canals, penetrating 130 microns into dentinal tubules [4], while the penetration depth of microbes is more than 1100 microns [5], and these substances are cytotoxic and neurotoxic if they reach the periapical area [6, 7].

Furthermore, the penetration depth of chlorhexidine is 100 microns despite the absence of toxic effects [8]. Failure in endodontic treatments causes a need for repeated treatments and periradicular surgeries [9]. One of the supplementary methods of chemo mechanical debridement is the use of photodynamic therapy with different photosensitizers that provides greater penetration depth and disinfection ability [10, 11]. In this process, light at a specific wavelength is used to activate light-sensitive materials (in the presence of oxygen).

The transfer of energy from activated photosensitive materials to available oxygen increases the formation of activated

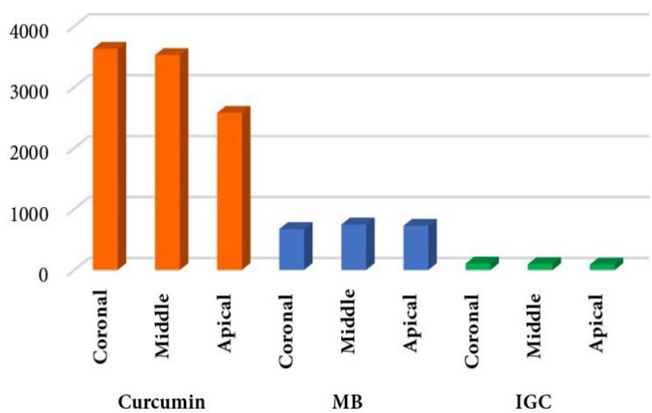

Figure 1. Mean (SD) of penetration depth of curcumin, MB, and ICG in different areas of the root canal

oxygen species and singlet oxygen, which have the ability to kill microorganisms by destroying the main cellular molecules, including proteins, membrane lipids and nucleic acids [12]. The previously conducted studies have investigated photodynamic therapy and the use of light sensitive materials to clean the root canals; nonetheless, due to the lack of information regarding the depth of penetration of different photosensitizers, this study aimed to compare the penetration depth of indocyanine green (ICG), curcumin, and methylene blue (MB) in the lateral wall of the root canal of human teeth.

Materials and Methods

In this experimental study, 30 single and straight-rooted maxillary incisors and canines of patients aged between 20-40 years were used. Teeth that were extracted due to periodontal disease were kept in sterile saline. The crowns of the teeth were separated by a diamond disc, and root fragments of 12 mm in length were obtained. To obtain the working length of each tooth, a file size 15 was used and taken to the apical foramen of the tooth. The working length of the canal was considered 0.5 mm less than the file length. Preparation of the canals was performed using the BioRace rotary system (FKG Dentaire, La Chaux-de-Fonds, Switzerland) with crown-down single length method.

The final size of the file preparation was #40, 0.04 taper, and the teeth were rinsed with 10 mL of NaOCl with a concentration of 2.5% between the files. Finally, the final rinse was performed with saline, and to remove the remains of the smear layer, 17% EDTA was placed in the canal for 1 min and then washed with NaOCl for 2 min; subsequently, the canal was washed again with saline. The outer surface of the teeth was sealed with two layers of nail polish to prevent pollution, and the apical foramen was closed by composite. The teeth were sterilized by autoclave for 15 min at 121°C and randomly assigned to three groups.

In each group, the canals were first dried with a paper cone; thereafter, in each group, the canals were filled with ICG (0.1 mg/mL perio green®, curcumin (5.0 mg/mL; Adonis Gol Daru, Shiraz, Iran), or MB (50 µg/mL; Merch, Darmstadt, Germany). They were incubated for 10 min, and the canals were then dried again with the help of a paper cone. The samples in all groups were longitudinally grooved by a diamond disc and divided by a chisel. The penetration depth of photosensitive materials was measured by a stereo microscope (Nikon SMZ1000; Nikon Corp, Tokyo, Japan). These measurements were made with 20× magnifications in three areas of each tooth sample: Coronal part: 4 mm, Middle part: 4 mm, and Apical part: 4 mm.

Four measurements were made in each area, and their average was selected as the penetration depth of the light-sensitive material.

Data were analyzed with SPSS software (SPSS version 16.0, SPSS, Chicago, IL, USA) using descriptive statistical methods. In this study, *P*-value less than 0.5 was considered significant and normal distribution of data was evaluated by Kolmogorov Smirnov-test. Since the data distribution was not normal, the Mann Whitney test was used.

Results

The results of this study demonstrated that the average penetration depth of curcumin is more than that of MB, and the penetration depth of MB is more than that of ICG. Moreover, in the comparison of the penetration depth of two-by-two materials, a significant difference was detected in all three groups ($P<0.05$) and in comparing the penetration depth of three substances in different sections of the lateral wall, curcumin had the highest penetration rate significantly in the coronal section of the root wall compared to other middle and apical areas and compared to other substances.

Since the data were not normally distributed, the nonparametric Mann Whitney test was used instead of ANOVA. (Figure 1 and Table 1).

Table1. A two-by-two comparison of the penetration depth of light-sensitive materials in the root canal. In all three groups of the study, the difference was significant ($P<0.05$)

Two-by-Two Comparison	Mean (SD)	*P*-value
ICG-MB	10.200 (4.442)	0.022
ICG-Curcumin	21.129 (4.112)	0.000
MB-Curcumin	10.929 (4.112)	0.008

Discussion

This study assessed the penetration depth of three photosensitizers on the lateral surface of the root canal. Curcumin had superior average penetration depth, and seemed an effective root canal disinfection in endodontic treatments.

Today, the most effective detergent used to irrigate the canal is sodium hypochlorite, which has a penetration depth of 130 micrometers in dentinal tubules [4] and has no effect on bacteria, such as *Enterococcus faecalis (E. faecalis)* which has a greater depth of penetration [5]. Moreover, in some cases, for more debridement, intracanal drugs, such as calcium hydroxide, are used between sessions. They prolong the treatment but have no effect on *E. faecalis* (due to having a hydrogen pump and biofilm formation and resistance to the alkalinity of calcium hydroxide) [13, 14]. Therefore, we must use supplementary methods for the complete debridement of root canals [2].

Among other disinfection methods of the root canal system, we can refer to the use of intracanal drugs between sessions, low-power laser, generating heat to destroy bacteria, and Photodynamic therapy. In this process, a specific wavelength of light is used to activate light-sensitive materials. The energy from the activated photosensitive material is transferred to the available oxygen and produces activated oxygen, which destroys the microorganisms [11, 15]. These light-sensitive substances include curcumin (maximum absorption at 430 nm wavelength), MB (maximum absorption at 670 nm wavelength), and ICG (maximum absorption at 810 nm wavelength). The ICG is a green fluorescent material with the mechanism of mostly photothermal therapy [16]. This material is used to measure blood volume, cardiac output, and liver function.

Today, in dentistry, it is common to use this material together with laser due to its phototoxic effects [10]. Kosarieh *et al.* [17] compared the depth of penetration of two light-sensitive substances, ICG, and tolonium chloride (TCH), in the root canals of teeth. A total of 40 single-rooted teeth were assigned to two groups and bisected longitudinally with a diamond disc, and the penetration depth of the material was checked in three areas (coronal-middle-apical). The lateral penetration depth of ICG was 224.04 micrometers, and in agreement with the results of our study, the penetration depth of ICG was obtained at 300 micrometers, and both studies indicate a relatively low penetration depth of ICG in the lateral tubules of teeth.

Beltes *et al.* [10] investigated the antimicrobial effect of photodynamic therapy with ICG and diode laser in *E. faecalis* colony reduction. In this research, planktonic suspension of *E. faecalis* was prepared, and according to the results, the photodynamic therapy group with the use of laser and ICG had the lowest number of colonies after 24 h among other groups of the study. In this research, the germicidal effect of ICG was investigated; nonetheless, the depth of its penetration was not assessed. The second light-sensitive substance examined in this research was MB. This substance has been used for nine centuries as a light-sensitive substance, and its maximum absorption is at 670 nm wavelength. It is used in photodynamic therapy to destroy Gram-positive and Gram-negative bacteria [14].

In a study by Souza *et al.* [18], the antibacterial effect of photodynamic therapy was investigated with MB and toluidine blue (TB) on *E. faecalis* in single-rooted teeth. According to the obtained results, PDT with MB or TB has no effect on canal disinfection, which is contrary to our study due to the proven antimicrobial effects of MB and its relatively high penetration depth (600 μm). In a study by Raymond *et al.* [19], a group of teeth was disinfected with chemo-mechanical therapy and washing, while another group was disinfected with PDT and MB. Consistent with the findings of the present study, the number of remaining bacteria in the PDT group was less than in other groups, and the results indicated the antimicrobial property of MB. Curcumin is the third light-sensitive substance obtained from turmeric and has various therapeutic properties, including anti-inflammatory, antibacterial and antiviral, as well as very strong photochemical effects that can act as a photosensitive substance [16, 20].

In their study, Praveenkumar *et al.* [21] assessed the antimicrobial power of curcumin against endodontic bacteria. After culture, the bacteria were transferred to the BHI bath and incubated, then they were exposed to different concentrations of curcumin. The results of this research demonstrated the germicidal effect of curcumin on all endodontic microbes except *E. faecalis*; nonetheless, the penetration depth of this substance was not investigated. According to our research on the dentin of the extracted human teeth, the penetration depth of curcumin in dentin tubules is the highest, and it is suitable for disinfection of canals in endodontics, methylene blue has a relatively high penetration depth in dentin tubules and is useful in endodontic treatments. The penetration depth of indocyanine green is low, and it is not suitable for endodontic treatment. According to previous studies, it is used for the treatment of gum diseases to reduce the sulcus fluid flow rate (SFFR) under the name "Perio Green" [22]. in this research the method of measuring the penetration depth was done by stereo microscope which is a traditional method to calculate and observe the penetration depth, comparing the use of confocal laser scanning microscope (with high accuracy and specificity), which is a limitation of our study. Another limitation of our research was the lack of using agitation method within the canal to enhance the distribution and penetration of disinfection agents and photosensitizers.

Conclusion

As evidenced by the obtained results, the average penetration depth of curcumin (3000 µm) is higher than that of the two other photosensitive substances; therefore, it can be suitable for the disinfection of canals in endodontic treatments.

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Conflict of interest
None.

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Authors' contributions
Seyedeh Saba Sharifzadeh, collecting the data and updating the paper. Mehrnaz Gerami Amin performing the experiment. Nahid Moezzi Ghadim: supervising the experiment. Mahta Fazlyab, supervising the experiment. Arash Azizi, conceiving, supervising and writing the paper. All authors. Writing-Review & Editing: All authors.

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