Accepted Manuscript

Title: The comparison of penetration depth of two different photosensitizers in root canals with and without smear layer: An in vitro study

Author: Emad Kosarieh Sahar Khavas Arash Rahimi Nasim Chiniforush Norbert Gutknecht

PII: S1572-1000(15)30048-X
DOI: http://dx.doi.org/doi:10.1016/j.pdpdt.2015.11.005
Reference: PDPDT 715
To appear in: Photodiagnosis and Photodynamic Therapy

Received date: 20-9-2015
Revised date: 5-11-2015
Accepted date: 17-11-2015

Please cite this article as: Kosarieh Emad, Khavas Sahar Sattari, Rahimi Arash, Chiniforush Nasim, Gutknecht Norbert. The comparison of penetration depth of two different photosensitizers in root canals with and without smear layer: An in vitro study. Photodiagnosis and Photodynamic Therapy http://dx.doi.org/10.1016/j.pdpdt.2015.11.005

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
The Comparison of Penetration Depth of Two Different Photosensitizers in Root Canals with and without Smear Layer: An in vitro study

Emad Kosarieh 1, Sahar Sattari Khavas 2, Arash Rahimi 3, Nasim Chiniforush 4, Norbert Gutknecht5

1 DDS, MSc, Department of periodontics, Zanjan faculty of dentistry, Zanjan, Iran
2 DDS, MSc, Department of endodontics, Zanjan faculty of dentistry, Zanjan, Iran
3 DDS, MSc, Private practice, Karaj, Iran.
4 DDs, PhD candidate of laser dentistry, Laser Research Center of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
5 DDS, PhD, Aachen Dental Laser Center, University Aachen, Germany

Corresponding author: Nasim Chiniforush, DDS, PhD candidate of laser dentistry, Laser Research Center of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
Email:n-chiniforush@farabi.tums.ac.ir,
Tel/fax: 00982188994824, address: Laser Research Center of Dentistry, Dentistry Research Institute, Tehran University of Medical Sciences, Enghelab Ave, Tehran, Iran.
Highlights:

1) ICG can penetrate in deeper regions of the root canal wall.
2) The creation of new methods in root canal disinfection in order to improve the success rate of our treatment is necessary.
3) The usage of EDTA improved the mean of lateral penetration depth of ICG.
**Background:** The main objective of this study is to evaluate the penetration depth of suggested photosensitizers in the lateral wall of the human root canal.

**Materials & Methods:** Forty extracted single-rooted human teeth with straight canals that extracted for periodontal reasons were collected and stored in the sterile saline until employment in the experiment. Teeth were decoronated to a standard 12mm root segment using diamond disc. After instrumentation of specimens, the external root surface was sealed with two layers of nail polish to avoid environmental contamination. The apical foramen was subsequently closed with composite material. Teeth were divided randomly in two major groups consist of indocyanine green solution (ICG) and tolonium chloride solution (TCH) with and without EDTA in their subgroups. Specimens in all groups grooved longitudinally with a diamond disc and split in two halves with a stainless steel chisel. The measurements were done by the stereo microscope under 20X magnification in three zones of each specimen and the penetration depth of dye was measured.

**Results:** The results of this study showed that the mean of lateral penetration depth of ICG (224.04μm) was significantly (P<0.05) higher than TCH (70.15μm). Regarding to the influence of EDTA, in ICG group without consideration to the different regions, the usage of EDTA improved the mean of lateral penetration depth of ICG, but this improvement was not statistically significant (P>0.05).

**Conclusion:** Further to the findings of this study, it could be assumed that ICG could penetrate in deeper regions of the root canal wall. **Keywords:** Indocyanine green solution, Tolonium chloride solution, EDTA, Penetration depth.

**Introduction:**
The main purpose of current endodontic techniques is eliminating bacteria within the root canal system by using the combination of mechanical instrumentation and chemical irrigation. The removal of infected tissue, elimination of bacteria within the dentinal tubules and root canals, and prevention of recontamination after treatment are the main objectives of endodontic treatments(1). To achieve these objectives the treatment procedures for treatment of infected root canals should be included: mechanical cleaning and shaping (2), irrigation with antimicrobial agents, such as Sodium hypochlorite (NaOCl) and chlorhexidine, antibacterial dressing application, sealing of the root canals with a 3-dimensional obturation and placing a coronal seal(1,3).
It has been shown that residual bacteria are readily detectable in approximately one-half of teeth just before obturation (4). Our inability to eliminate bacteria from the infected root canals, leads to the requirement for retreatment and/or periradicular surgery in order to perform a successful treatment against persistent infections (5). There are some factors responsible for our inability to complete elimination of bacteria from the canals such as: complexities of the root canal system (4,6,7), inadequate instrumentation and missed canals (8).

Canal irrigation is most commonly done by NaOCl. Its penetration into dentinal tubules is approximately 130 μm (9), whereas, scanning electron microscopy (SEM) studies described bacterial penetration up to 1100 μm into dentinal tubules (10). Meanwhile, it has cytotoxic and neurotoxic effects in extrusion into periapical area(11,12). Therefore, the creation of new methods in root canal disinfection in order to improve the success rate of our treatment is necessary. We need to develop non-invasive and non-toxic novel antimicrobial strategies that are more efficiently and faster than available antimicrobial agents and at the same time do not permit pathogens to easily develop resistance (13). One available alternative to current antimicrobial agents is lethal photosensitization (LP). The LP application to treat a disease is known as photodynamic therapy (PDT) (14).

PDT is based on the concept that a nontoxic photosensitizer (PS) can be preferentially localized in certain tissues and subsequently activated by light of the appropriate wavelength to generate singlet oxygen and free radicals, which are cytotoxic to cells of the target tissue (15) (Fig. 1). In biological systems, the lifetime of singlet oxygen and its radius of action are very short (<0.04s & 0.02μm respectively) (16), In the other words localization of the photosensitizer will define the site of initial cell damage resulting from PDT. Thus the reaction will be placed in a very limited space (localized response) and making it ideal for localized applications without any effect on distant cells or molecules(16,17). It means that the penetration depth of the photosensitizer in dentinal tubules and lateral canals will determine the killing effect of PDT on microorganisms. It has been shown that methylene blue and toluidine blue O are really effective photosensitizing agents for the inactivation of both gram-positive and gram-negative periodontopathic bacteria (17,18).

ICG is a fluorescent dye that is used mainly in medical diagnostics(19). Nowadays, its usage in dentistry as a photosensitizer is growing up because of its phototoxic effects in combination with the use of lasers.
Smear layer (SL) contains inorganic and organic substances that contain microorganisms, necrotic materials and odontoblastic processes fragments (20). It has been shown that effectiveness of irrigants and intracanal medicaments in disinfecting of dentinal tubules is diminished in the presence of “smear layer “ (21). It has been shown that after removal of smear layer, adhesion of obturation materials to the canal wall will be stronger (22,23). Other investigators showed that the penetration of sealers to the dentinal tubules was 10 to 80 μm after removal of the smear layer, whereas in cases with the intact SL, there was no penetration(24,25).Regarding the influence of smear layer on microleakage of root canal fillings several investigators have shown less dye leakage after removal of the smear layer(26,27) whereas, others have reported no significant effect of SL removal on the microleakage of root canals(28).
Up to now there is no study investigating the penetration depth of photosensitizers in the dentinal tubules or lateral canals. The present study is conducted to investigate the penetration depth of two kinds of photosensitizers and the influence of smear layer on that.

Materials and Methods:

Teeth collection
Forty extracted single-rooted human teeth (upper central incisors and upper canines) with straight canals that extracted for periodontal reasons were collected and stored in the sterile saline until employment in the experiment. All patients who their tooth was gathered for using in this study signed an informed consent that permits to use their teeth in this study.

Preparation of specimens
Teeth were decoronated to a standard 12mm root segment using diamond disc (Brasseler USA, Savana, GA). File measurement was taken at the point where the tip of a size # 15 kerr files (Maillefer Instruments SA, Switzerland) become visible at the apical foramen and 0.5mm will be subtracted to set the working length. Teeth were instrumented in a crown-down manner by a set of M2 rotary files (VDW GmbH, Germany) to achieve a master apical file size of M2# 40, 6% tapered at the working length. The cleaning was done with 10 ml of 2.5% NaOCl throughout the instrumentation sequence. The external root surface was sealed with two layers of
nail polish to avoid environmental contamination. The apical foramen was subsequently closed with composite material.

Teeth were divided randomly in four groups. In two groups, shaped canals were irrigated with 17% EDTA for 2 minutes followed by irrigation with normal saline to remove the smear layer and in other two groups; irrigation was done only by normal saline. Then specimens were sterilized by autoclaving for 15 minutes at 121 °C.

In two groups, (EDTA group and non-EDTA group) TCH solution (PACT, Cumdente GmbH, Germany) and in other two groups, ICG solution (EmunDo, A.R.C. laser GmbH, Germany) were used. In all groups before filling the root canals with photosensitizers, they dried again with paper cone, afterwards filled with suspected photosensitizer and allowed to incubate for 10 minutes. After that the root canals dried again with paper cone. Therefore, our groups were as follows:

- Group A: TCH solution in root canals without the smear layer.
- Group B: TCH solution in root canals with the smear layer.
- Group C: ICG solution in root canals without the smear layer.
- Group D: ICG solution in root canals with the smear layer.

**Stereoscopic microscopy**

In this study, we used Nikon SMZ1500 stereo microscope (Nikon, Japan) for measurement of penetration depth of suggested photosensitizers in the lateral wall of root canals. Specimens in all groups grooved longitudinally with a diamond disc (Brasseler USA,) and split in two halves with a stainless steel chisel and one of them, which contained more root canal borders was chosen for measuring the penetration depth of dye in the lateral wall of the canal. All measurements were done by one of my colleagues who was blinded to this study using the stereo microscope. The measurements were done under X20 magnification in three zones of each specimen:

- Coronal zone: 4mm coronal part.
- Middle zone: 4mm middle part.
- Apical zone: 4mm apical part.

In each zone four measurements were done and the mean of them was considered as penetration depth value at that site. The microscope was equipped by its own software and all measurements were done by that.
Statistics:
Achieved data was evaluated by descriptive statistic methods via statistic software SPSS 16. For evaluation of the influence of different variables include: group (ICG & TCH), region (Apical, middle & coronal) and EDTA (with or without) as independent variables and lateral penetration depth of photosensitizer as dependent variables, the multi variable of ANOVA test was used. In this study, the P value < 0.05 was considered as significant. Normalized distribution of collected data was assessed by Kolmogorov-Smirnov test.

Results:
Achieved data (Table.1) showed that the mean of lateral penetration depth without consideration to the different regions and presence or absence of EDTA, in ICG group was 224.04 μm whereas, in TCH group was 70.15μm (diagram. 1,2) and the difference between them was statistically significant (P<0.001). The means of lateral penetration depth of ICG in all regions were higher than TCH (diagram.3) without consideration to the presence or absence of EDTA and this difference was statistically significant (P<0.001).

The pictures of specimen in TCH and ICG groups with stereomicroscope (X20 magnification) were shown in Fig.1 and 2.
In both groups without consideration to the presence or absence of EDTA, the mean of lateral penetration depth in coronal part was higher than middle, and in middle part was greater than the apical part but in ICG group, the differences between coronal and middle parts and middle and apical parts were not statistically significant (P>0.05) whereas, the difference between coronal and apical parts was statistically significant (diagram. 3). In TCH group the differences between coronal and middle and coronal and apical parts were statistically significant whereas between middle and apical was not (diagram. 3).
Regarding to the influence of EDTA, in ICG group without consideration to the different regions, the usage of EDTA improved the mean of lateral penetration depth of ICG, but this improvement was not statistically significant (P>0.005). But in the TCH group, the mean of lateral penetration depth of TCH into the lateral wall of the canal was significantly improved by EDTA usage (P=0.004). With consideration to
the different regions, in ICG group in all regions using of EDTA improved the mean of penetration depth of dye, but the differences were not statistically significant. In TCH group, usage of EDTA improved the mean of penetration depth significantly (P<0.001) in coronal part whereas, in the middle and apical parts only improved the dye penetration, and differences were not statistically significant.

Discussion:
There are some factors responsible for the permeability of dentin to the small and large molecules, solutions and bacteria such as: number and type of bacteria, exposure time, and presence or absence of smear layer(1), surface tension, molecular size, osmotic and hydrostatic pressure. According to the Pashley and Livingston(29), increasing in molecular size led to decrease in the permeability coefficient in human root canal dentin. In their study dentin permeability was decreased 100 fold after 19 fold increasing in molecular radius. Although regarding fluoride and chlorhexidine, their permeability were much lower than expected according to their molecular weight or size, suggesting their bond to the dentin. Like that study in our study regardless of lower molecular weight of TCH in comparison to the ICG, the mean penetration depth of ICG was significantly higher than TCH in all regions (P<0.001) suggesting the bonding of TCH to the dentin. In this study neither permeability coefficient, osmolality nor the surface tension of suggested solutions were not assessed.

In all groups, the penetration depth of photosensitizers decreased from coronal part of root segments to the apex without consideration to the presence or absence of EDTA.

This finding could be explained by the greater number of dentinal tubules in coronal parts in comparison to the apex region which are supported by previous studies that showed that the number of dentinal tubules decreased from coronal to the apical parts (30,31).

Carriganetal.(31) in 1984 showed that the mean number of tubules in cervical and mid-root dentin were 254300 & 234060 respectively whereas in the apex area was 49140. They suggested that the greater number of tubules in coronal parts could be
responsible for rapidly increasing of bacteria through the coronal dentin (32). Meanwhile, these findings are in accordance with the findings of another study that was done by Paque and his coworkers(33) which showed statistically significant decreasing of dye penetration from coronal to the apical parts of root canals. Previous studies regarding permeability specifications of dentin to radioactive albumin and tritiated water confirmed the restriction effect of SL on surface area available to different size (small & large) molecules (in comparison to the amounts achieved after its removal) and the bacterial penetration into the pulp (34,35). Meanwhile, it has been shown that the huge increase in the amount of filtration between unetched dentin and dentin acid-etched for 5 seconds was related to the removal of SL100. Pashley et al. (36)in an in vitro study showed that SL was responsible for 86% of resistance to movement of fluid through the dentin. Fogel and Pashley in 1990 showed 50% reduction of root dentin conductivity in the presence of smear layer(33).In accordance with the mentioned studies in this study in TCH group removing the SL led to increase the penetration depth significantly (P=0.004). In ICG group removing of SL resulted in increasing the penetration depth but the difference was not statistically significant (P=0.7). This finding is comparable to the findings of Foster et al. in 1993(37) in which EDTA and NaOCl were used for removing of the smear layer and dressing of root canals was done by calcium hydroxide. They showed the minimal effect of smear layer removing on the distribution of hydroxide ions from root canals. On the other hands Paque F.(33) and his co-workers in 2006 showed that the dye penetration into the dentin in root canals that instrumented endodontically was independent of smear layer presence or absence but, was related to the function of tubular sclerosis. Tubular sclerosis is a physiologic phenomenon starting from the third decade of life in the apical part of the root and progresses coronally(38). In our study, the age of patients whose teeth were gathered was unknown but as the teeth came from the patients suffering from periodontal diseases, it could be assumed that most patients were over 30-years-old. Therefore, the tubular sclerosis was a factor that may inhibit the influence of EDTA in penetration depth of ICG.

**Conclusion:**
These findings showed us a higher penetration depth of ICG in comparison to the TCH. Meanwhile, this amount was higher than that had been reported in previous studies regarding the penetration depth of NaOCl. It could be assumed that ICG can
access to the microorganisms in deeper parts of the root canal wall. Therefore, it could be a good alternative for TCH for using in PDT.

References:


Fig. 1: The specimen in TCH group under the stereo microscope with X20 magnification.

Fig. 2: The specimen in ICG group under the stereo microscope with X20 magnification.
Diagram 1: Histogram related to the lateral penetration depth of ICG (in microns) without consideration to the regions or presence or absence of EDTA.

Diagram 2: Histogram related to the lateral penetration depth of TCH (in microns) without consideration to the regions or presence or absence of EDTA.
Diagram 3: Error bar related to the comparison between the mean of lateral penetration depth (in microns) of ICG and TCH in different regions. The lateral penetration depth in all regions was significantly higher in ICG group in comparison to the same region in TCH group.
Table 1: The mean of penetration depth of ICG & TCH in different regions in presence or absence of EDTA in microns.

<table>
<thead>
<tr>
<th>Group</th>
<th>Region</th>
<th>EDTA</th>
<th>Mean</th>
<th>Std. Error of Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICG</td>
<td>Apical</td>
<td>Yes</td>
<td>142.94</td>
<td>24.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>136.85</td>
<td>40.02</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>Yes</td>
<td>208.81</td>
<td>27.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>207.89</td>
<td>41.35</td>
</tr>
<tr>
<td></td>
<td>Coronal</td>
<td>Yes</td>
<td>370.51</td>
<td>76.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>277.21</td>
<td>36.28</td>
</tr>
<tr>
<td>TCH</td>
<td>Apical</td>
<td>Yes</td>
<td>39.60</td>
<td>10.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>19.69</td>
<td>6.17</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>Yes</td>
<td>76.56</td>
<td>13.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>42.32</td>
<td>5.66</td>
</tr>
<tr>
<td></td>
<td>Coronal</td>
<td>Yes</td>
<td>163.20</td>
<td>23.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>79.51</td>
<td>13.88</td>
</tr>
</tbody>
</table>