

**A Comparative Confocal Microscopic Evaluation on Depth of Penetration into Dentinal Tubules Using Chloroquick, 10% Citric Acid and 17% EDTA**

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**Conflicts of Interest:** Nil

**Abstract**

**Aim:** To evaluate the depth of penetration of Rhodamine B dye into dentinal tubules using Chloroquick, 10% Citric acid and 17% EDTA under Confocal laser scanning microscope.

**Materials and Methods:** Thirty single-rooted human mandibular premolars with fully formed apices were selected. Root canal preparation was done with ProTaper Next, until size X3. Teeth were divided into three groups based on the irrigation protocol as Group 1: Chloroquick, Group 2:10% Citric acid, Group 3:17% EDTA. For fluorescence inspection, 5ml of labeled NaOCl was used for irrigating each canal and accompanied by manual dynamic agitation using master gutta percha points. Horizontal sectioning was performed into 1mm thick sections at apical third, middle third and coronal thirds and underwent examination by CLSM at 10x

magnification and 580nm wavelength. Statistical analysis was performed using Two-way ANOVA analysis to investigate the depth of penetration of groups.

**Results:** The results showed that coronal third exhibited the highest depth of penetration followed by middle third and least for apical third. 10% Citric acid at coronal third exhibited highest depth of penetration while Chloroquick showed least penetration in all three regions.

**Conclusion:** Chloroquick, 10% Citric acid and 17% EDTA promoted cleaning ability and penetration of dentinal tubules while 10% Citric acid group has exhibited higher penetration depth when compared to other groups.

**Keywords:** Depth of penetration, Rhodamine B dye, Chelating agents, Chloroquick, Citric acid, Confocal laser scanning microscope.

## Introduction

A successful endodontic therapy mainly relies on thorough chemomechanical debridement of pulpal tissue, dentin debris, and infective microorganisms. Irrigating solutions play a very large role in disinfecting the root canals. Chelating agents, such as Ethylenediaminetetraacetic acid (EDTA) and combinations of EDTA and NaOCl have been used to remove smear layer. They are used to improve chemomechanical debridement in root canal treatment by removing the smear layer by forming a coordinate bond with metallic ions of the organic compound. It prepares the root canal to receive the filling material for an efficient apical seal.<sup>3</sup>

Sodium hypochlorite (NaOCl) is one of the most commonly used endodontic irrigant because of its ability to destroy a broad spectrum of microbes, but it has some undesirable characteristics such as tissue toxicity, allergic potential and disagreeable taste, which has prompted researchers to look for other alternatives.<sup>9</sup>

EDTA is considered as the most effective chelating agent in endodontic therapy, showing the ability to very effectively remove the inorganic component, especially in the coronal and middle third of canal.<sup>9</sup> EDTA is mainly synthesized from Ethylenediamine (1,2- diaminoethane), Formaldehyde (methanal) and Sodium cyanide.<sup>1</sup>

Citric acid is used in concentrations ranging from 1- 40% in endodontic practice to remove smear layer and superficial smear plugs after root canal preparation. In operative dentistry, 10% Citric acid has been proposed as a mild etchant for dental hard tissue, particularly for dentinal conditioning and enhanced smear layer and smear plug removal.<sup>6</sup>

Chloroquick, which is a one-step irrigating solution containing 5% NaOCl and 18% Etidronic acid exhibit antimicrobial property and dissolution activity, thereby

helping in the removal of smear layer, eliminating microbes that are resistant to conventional endodontic irrigants and thereby providing sustained antimicrobial activity. Chloroquick stabilizes Sodium hypochlorite solution with buffer Hydroxyethane 1,1 diphosphonic acid (HEDP), detergent and system activator which reduces the surface tension.<sup>2</sup>

ProTaper Next was used in the present study which has a variable taper and with a rectangular cross section decentered. Protaper next files includes five conformation instruments with variable tapers. They are manufactured with the recent alloy of nickel-titanium, M-Wire, prepared by a special thermal process, which aims to improve flexibility and resistance to cyclic fatigue.<sup>9</sup>

On comparison of Scanning Electron Microscope with Confocal microscope, the latter has the advantage of providing detailed information regarding the presence and distribution of sealers or dental adhesives inside dentinal tubules in the total circumference of the root canal walls at relatively low magnifications 100× through the use of fluorescent Rhodamine-marked sealers.<sup>7</sup> The aim of the present study is to evaluate the depth of penetration of Rhodamine B dye into dentinal tubules using Chloroquick, 10% Citric acid and 17% EDTA under the Confocal laser scanning microscope (CLSM).

## Materials and Methodology

Thirty human single canal mandibular premolar teeth with fully developed root apices were included in the study. Teeth with the presence of canal curvatures, apical fractures, previous obturation were excluded from the study. The teeth were cleaned of attached tissue, calculus, washed and debrided with 3% NaOCl and stored in normal saline solution until use. Decoronation was performed for root standardization at 15mm length using a diamond disc with a straight handpiece. A stainless steel 10K file was inserted in each root canal to check

apical patency, and then 1mm was subtracted from the mentioned length to obtain a working length of 14mm.

Chemo-mechanical preparation was performed by Protaper Next system upto X3 of the apical diameter 0.3mm and taper 7%. Irrigation was proceeded with 2ml of 3% NaOCl following each instrument shift using a 30 gauze side vented needle for one minute. The exterior part of each root was covered with wax apically to prevent the irrigant from dripping through apex.

### **Irrigation Groups**

Canals were irrigated with 5ml of distilled water after instrumentation and the roots were divided into three groups based on final irrigation protocol with different irrigating solutions as follows,

**Group 1:** Irrigation was done with 10ml of CHLOROQUICK for 1 minute. (n=10)

**Group 2:** Irrigation was done with 10ml of 10% CITRIC ACID for 1 minute. (n=10)

**Group 3:** Irrigation was done with 10ml of 17% EDTA for 1 minute. (n=10)

### **Confocal Laser Scanning Microscope**

Before performing fluorescence inspection under Confocal laser scanning microscopy, irrigation was done by using 2.5ml of 3% NaOCl to be mixed with 2.5ml of 0.1% Rhodamine B dye to obtain a total 5ml of labeled NaOCl for irrigating each canal. A 30 gauze side vented needle was used and it was accompanied by manual dynamic agitation using the master gutta percha points at value of 100 strokes for approximately one minute. Horizontal sectioning was performed using a straight handpiece with diamond disc into 1mm thick sections at apical third, middle third and coronal third from the anatomical apex and the sections were mounted onto glass slides. The sectioned samples were divided into coronal, middle and apical third regions into three groups i.e., Chloroquick group, 10% Citric acid group and 17%

EDTA group respectively with a total of 30 samples in each group.

The tooth specimens underwent examination by Confocal Laser Scanning Microscope at a magnification of 10x with an emission wavelength of 580nm. The penetration of the irrigants is superior in the coronal third followed by middle third region and least in the apical third region.

### **Statistical Analysis**

Statistical analysis was performed using Two-way ANOVA analysis to investigate the depth of penetration of groups with different irrigation protocols. Pair wise comparisons with mean depth of penetration was done by Tukey's multiple posthoc procedures and the significance level was adjusted to  $p < 0.05$ . A significant difference was observed between the Chloroquick, 10% Citric acid and 17% EDTA groups with respect to depth of penetration.

### **Results**

Among all groups, 10% Citric acid exhibited highest penetration depth and followed by 17% EDTA and least depth of penetration was exhibited by Chloroquick group. When all the three regions (Coronal, Middle and Apical) were compared coronal third exhibited the highest depth of penetration followed by middle third and least for apical third. Amongst all groups, 10% Citric acid at coronal third exhibited highest depth of penetration and 17% EDTA at apical third exhibited least depth of penetration (graph 1, Figure 1).

### **Discussion**

The success of endodontic treatment depends largely on cleaning and shaping, disinfection, and 3D obturation. An age-old adage is that: "Files shape while irrigants clean." The ultimate aim of root canal treatment is to eradicate all debris and microorganisms from the root canal system to prevent recontamination. The complex anatomy of the root canal system makes it impossible to

completely disinfect the root canal using instrumentation alone.<sup>7</sup>

Chelation is a chemical procedure involving formation of a heterocyclic ring compound that contains at least one metal cation or hydrogen ion in the ring.<sup>7</sup> Mechanical preparation should be accompanied by solutions used for irrigation to ensure the cleanliness of the intracanal surface and eradication of infection.<sup>10</sup>

In the present study, single canaled mandibular premolars were used as they are almost straight canals and may facilitate the flow and penetration of the irrigant in the apical third.<sup>17</sup> The selection of storage solution is important to preserve microelastic tissue properties. The specimens were stored in normal saline solution (containing 0.9% Sodium Chloride) until use, which resulted in a progressive decrease in the acoustic impedance in dentin by up to 70%. The specimens were decoronated at the Cemento enamel junction as it represents the anatomic limit between crown and root surface and is the area of junction of the cementum and enamel at the cervical region of the tooth.<sup>18</sup>

The repeated penetration of the apical foramen with a file of proper size during instrumentation prevents the accumulation of debris in this area leaving the foramen unblocked and this is described as apical foramen patency. Therefore, establishing apical patency is leaving the apical foramen accessible which is free from dentin chips, pulp fragments and other debris.<sup>20</sup> A stainless steel 10K file was inserted in each canal to check apical patency in the present study. According to Ronaldo Arouja (2006), the most commonly accepted working length is 1mm short of the apex, which means that 1mm of the root canal will not be instrumented and thus will not be cleaned. Thus, for the standardized root length of 15mm, a working length of 14mm was determined and radiographically confirmed.<sup>19</sup> The first three files of

ProTaper Next rotary files manufactured with the NiTi alloy, M-Wire. The overall sequence is not always necessary to use, as the diameter of the tip of last rotary instrument that we use in each canal was determined by the smallest diameter of the apical constriction. So, these files were used in present study till X<sub>3</sub> (apical diameter 0.3mm, taper 0.07), which was in accordance to study done by Gema Fernandez-Pazos et al.(2018).<sup>9</sup>

In the present study, an irrigating needle of 30 gauge was used to penetrate till the apical part of the root canal. It was found that the irrigant could flow just 1mm in depth beyond the tip of the needle. Therefore, the needle tip was located at a length of 1mm shorter than the working length in our study.<sup>10,17</sup> In the present study, specimens were irrigated between each instrument with 2ml of 3% NaOCl, which is in accordance to study done by Saied Samar et al. (2021), as Sodium hypochlorite has shown reduction in the mechanical resistance of dentin and could not bring out the inorganic component of the smear layer.<sup>17</sup>

As irrigants lack in removing the smear layer completely from all parts of the canal, the coronal, middle, and apical thirds of the canal were separately studied to test the efficiency of the irrigants in smear layer removal.<sup>16</sup>

Covering the apex of the root using wax was essential so that the solution could not drip out from the canal through the apical foramen. Canals have been rinsed using 5ml of distilled water after being instrumented with Protaper Next files to remove any residual sodium hypochloride solution remnants within the specimens.<sup>10</sup>

In the present study, Chloroquick irrigating solution was used in the first group which is a combination solution of stabilised 5% Sodium hypochlorite solution with buffer and 18% Etidronic acid with a detergent and system activator along with other excipients remove smear layers by eliminating microbes which are resistant to

conventional endodontic irrigants and thereby provide sustained antimicrobial activity by reducing the surface tension.<sup>3</sup>

Chloroquick solution proceeds with chelating irrigation protocol because of the better opening of dentinal tubules which were covered by smear layer.<sup>4</sup> This is useful on the inorganic as well as organic part of smear layer at the same time.<sup>6</sup> In the second group, 10% Citric acid irrigating solution was used as it has lower molecular weight, so it can easily penetrate deeper into dentinal tubules than EDTA, the highest molecular weight chelator. The pH of the Citric acid solution becomes a more important factor in terms of the demineralization than that of concentration.<sup>8</sup>

In the present study, penetration of 3% NaOCl labelled with 0.1% Rhodamine B dye was utilized as an indicator for evaluating the ability of the various irrigating solutions to remove the smear layer through tracing the dye through the dentinal tubules under Confocal microscope. Miftakul Munir et al.(2017), stated that the addition of NaOCl will reduce the peak absorbance of Rhodamine B. This is due to NaOCl liberates Iodine (I<sub>2</sub>) which fades color of Rhodamine B and also proportional to the rising of NaOCl concentration.<sup>22</sup>

Manual Dynamic Activation (MDA) technique was used in the present study with Master GP points as it effectively eliminates the smear layer and ultimately results in clean canals in the apical area and this was in accordance to a study done by Pierre Machtou (2018).<sup>21</sup>

In the present study, on intra group comparison there was a statistically significant difference among all the three regions i.e. coronal, middle and apical regions with each other. Coronal third region has exhibited highest depth of penetration when compared to middle and apical third regions. Least penetration depth was exhibited by apical third region among all the three regions. Pair wise

comparisons with mean depth of penetration was done by Tukey's multiple posthoc procedures and the significance level was adjusted to  $p < 0.05$ . These results were in accordance with the study done by Ballal NV et al. (2010), Aydin ZU et al. (2019), Saied Samar et al. (2021).<sup>11,15,17</sup>

The results of the present study were in accordance with study done by Gu Hiran et al. (2017) and Aydin., et al.(2010), which stated that the tubules are larger and more densely packed within the coronal and middle thirds of the roots, and tubular sclerosis begins within the apical area, which may partly account for the lower penetration of irrigant in the apical 2mm region.<sup>14</sup>

Maira Prado et al. (2011), in his study observed that smear layer removal using 17% EDTA and 10% Citric acid and found that these solutions were equally efficient and more effective in the middle and cervical thirds compared to apical third region. 17% EDTA and 10% Citric acid were equally efficient, but 17% EDTA was more effective in the cervical third when compared to middle and apical thirds.<sup>12</sup>

In the present study highest depth of penetration was observed in the coronal region followed by middle third region and least depth of penetration was observed in the apical region in all the groups compared i.e. Chloroquick, 10% Citric acid and 17% EDTA groups. These results are in accordance with a study done by Carmen Llana., et al. (2015).<sup>13</sup>

Deepest penetration point was measured from the canal wall to the point of maximum dye penetration in  $\mu\text{m}$ .<sup>50</sup> Confocal microscopy can preserve the integrity of the samples as it doesn't need processing and therefore it decreases the artifacts. It can help in scanning up to  $10\mu\text{m}$  below the surface of the sample.<sup>10</sup> Thus, CLSM was preferred and selected for the present study. By using CLSM, the surface below the smear layer can be

scanned and analyzed. All the specimens were mounted onto glass slides and examined using a confocal laser microscope at a wavelength of 580nm and 10x magnification, with a zoom oil lens.<sup>13</sup>

### Limitations

1. The present study is an in-vitro study performed on extracted teeth hence in the in-vivo, angulation and inclination of the teeth present in the oral cavity might produce varying results in the root canal preparation.
2. In the present study, teeth with only straight canals have been included and the instrumentation has been restricted to three files. Further research can be done on curved canals using newer file systems.
3. Furthermore, surface tension, temperature and time of application of the tested chelating agents should be investigated.

### Conclusion

The penetration of the irrigants is superior in the coronal third followed by middle third and least in the apical third sections. 10% Citric acid exhibited highest depth of penetration in the coronal and apical third regions, while 17% EDTA exhibited highest penetration depth in the middle third region respectively. Chloroquick exhibited least depth of penetration when compared to 10% Citric acid and 17% EDTA groups.

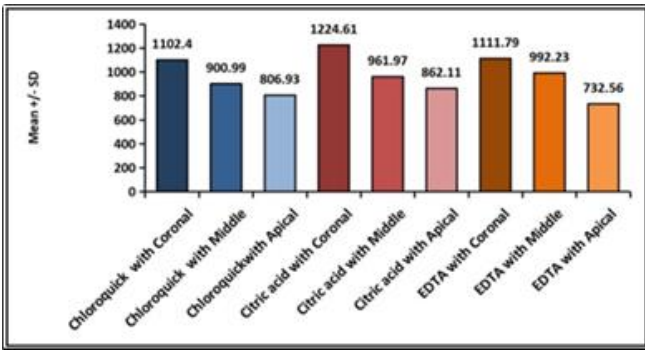
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**Legend Graph and Figure**



Graph 1: Comparison of interactions of three groups (Group 1, Group 2, Group 3 and three regions (Coronal, Middle, Apical) with Depth of penetration

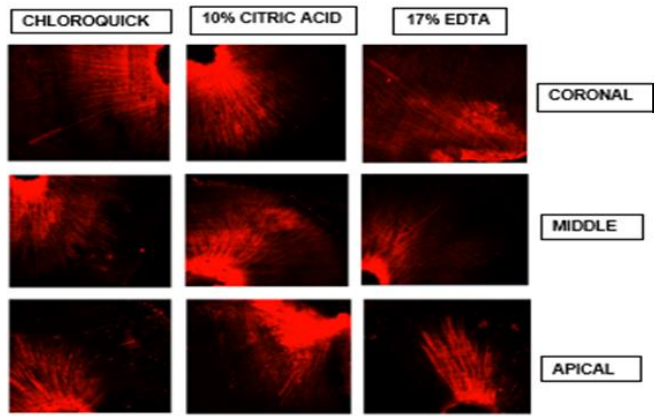


Figure 1: CLSM images representative of dye penetration into dentinal tubules for each group at the coronal, middle and apical

Figure 1: