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The Importance of Final Irrigation with Mineralolithic Effect Agents During Chemomechanical Treatment of Tooth Root Canal

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1. Introduction

In primary tooth root canal infections, the largest number of microorganisms can be found in main root canal. However, a considerable portion of infection is located deeper, in the lateral canals, apical ramifications and dentinal tubules (Hülsmann et al., 1997; Matuse et al., 2003; Živković et al., 2005). It is precisely those anatomic variabilities and physiological specificities of endodontic and periodontal tissues that make impeding factors in endodontic infection resolving (Gašić et al., 2003; Mitić et al., 2009; Chacker 1974; De Deus, 1975). All chemomechanical techniques of canal preparation leave considerable amounts of debris and smear layer (Živković et al., 2005; Mitić, 2008).

Smear layer is a layer of debris remaining on dentin during instrumentation, and consists of dentin particles, remnants of vital or necrotic pulp tissues, bacteria and their components (Nešković & Živković, 2009; Abdullah et al., 2005; Calt & Serper, 2002; Fouad et al., 2002; Jacinto, 2003; Love, 2001; Portenier et al., 2001, 2003; Spratt et al., 2001; Shabahang et al., 2003.)

In clinical practice, instrumentation and irrigation of canal within endodontic treatment is time-consuming and the most demanding treatment phase (Mitić et al., 2011; Živković et al., 2005; Morazin et al., 1994; Baumgartner & Mader, 1987).

Smear layer is an ideal medium for growth and proliferation of microorganisms, and therefore should be removed before the final root canal obturation to reduce the microorganisms present in the root canal, to improve the adhesion of root canal sealers to the root canal walls and to reduce the apical and coronal microleakage (Hülsmann et al., 1997; Takeda et al., 1999; Mitić, 2010.).
Medication aspect of chemomechanical root canal treatment involves the irrigation of root canal and removal of smear layer by the application of various preparations. The efficacy of irrigants is determined by numerous factors: concentration, pH value, root canal length, “age” of dentin tissue and time of application. One should bear in mind the fact that dentin tissue reduces the antimicrobial effect of various irrigants. Dentin hydroxyapatite possesses a buffering capacity, as it can donate protons, cause a pH change, and reduce the effects of various chemical agents when making contact with the dentinal wall (Haapasalo et al., 2000). The most frequently used irrigants with organolithic effects are sodium hypochlorite, hydrogen peroxide, chloramines, chlorhexidine. The final irrigants with mineralolithic effect are 17% NaEDTA, 10% citric acid, and the solution of recent date - MTAD (Biopure, Tulsa Dentsply, Tulsa OK, USA) – a combination of tetracyclines containing weak organic acids and anion-active substances (Torabinejad et al., 2002, 2003; Kando et al., 1991; Di Lenarda, 2000; Mitič et al., 2009; Yamaguchi et al., 1996; Haapasalo et al., 2005; Mitič, 2010).

The purpose of the present research was to analyze the surface of intracanal dentin after instrumentation and irrigation by organolithic agents (2.5% sodium hypochlorite, 3% hydrogen peroxide, 2% chlorhexidine) and final irrigation by mineralolithic effect solutions (17% NaEDTA, 10% citric acid and MTAD solution).

2. Methods

2.1. Materials

In the research, 145 freshly extracted single-rooted and double-rooted maxillary and mandibular human teeth were used. The teeth were extracted for orthodontic reasons in children of both sexes, aged 9-12 years.

The preparation of biomaterial involved storing of teeth in the sterile isotonic saline solution at 4°C, without the use of fixatives. All samples were prepared by one operator. The preparation of root canal was carried out by hand K-files, sized # 15-40 (Display, Maillefer, Ballaigues, Switzerland) and rotary instrumentation. Root canals were instrumented using a standard step-back technique, while the apex third was enlarged up to # 30. For canal irrigation, we used special irrigation needles with lateral perforations. They ensured an immediate contact between solution and intracanal dentin even in the apical region, improving thus the debridement of the entire root canal wall.

Teeth were divided into two groups. A control group (n=40) was divided into four subgroups (a, b, c, d) for the purpose of quantitative assessment of smear layer on the samples after manual and rotary root canal instrumentation without irrigation (a, b) and after rinsing with sterile saline solution solution (c, d) (positive control).

The second, experimental group (n=105) was divided into seven subgroups (A,B,C,D,E,F,G), in which process the samples from the subgroups A,B,C were rinsed only with solutions with organolithic effect, while the samples from the subgroups D,E,F and G, besides irrigation with organolithic solutions (2.5% sodium hypochlorite, 3% hydrogen peroxide, 2% chlorhexidine – 2ml), were finally rinsed by mineralolithic effect solutions (17% NaEDTA, 10% citric acid and MTAD - 2ml). The samples rinsed with 5.25% NaOCl and 17% NaEDTA served as (negative control).

After chemomechanical root canal preparation, teeth crowns were removed with a diamond disk at the cement - enamel junction. All the samples were irrigated with distilled water to
remove the superficial debris accumulated during cutting. Canals were dried with compressed air. Using the separation pliers, tooth roots were longitudinally grooved, into the mesial and distal halves. Each half of a sample was further fixed to a bed, coated with gold and viewed under the a scanning electron microscope JEOL-JSM-5300. The apex, middle and coronal thirds of all samples were analyzed; photomicrographs were taken at different magnifications.

According to the criteria specified by Hülsmann (1997), the smear layer of the root canal dentin was scored as:

- Score 1 - No smear layer, dentinal tubules open;
- Score 2 - Small amount of smear layer, several dentinal tubules open;
- Score 3 - Homogenous smear layer covering the root canal wall, only few dentinal tubules open;
- Score 4 - Dentinal wall completely covered by smear layer, no dentinal tubules open;
- Score 5 - Heavy, non-homogenous smear layer completely covering the root canal wall.

Statistical analysis included the comparison of the mean scores for the seven groups of analyzed samples; Kruskal Wallis nonparametric test was also used. Post hoc analysis was performed by Mann Whitney U test to determine single, intergroup differences among the mean scores.

For statistical analysis, Software SPSS 15.0 was used. Statistical significance was taken at p<0.05.

Diagnosis was established based on the anamnestic, objective examination and additional diagnostic methods (examination of vitality by electrotest and radiography). The patients were aged 29-56 years. All the examined teeth were diagnosed with primary apical periodontitis, with destructed tooth crowns but without fillings and prosthetic restorations. All interventions were performed maintaining a dry working field using a rubber dam.

Disinfection of crowns and cavities was done by 3% natrium hypochlorite.

Fig. 1. Tooth roots notched with diamand discs
Table 1. Experimental protocol for control group samples

<table>
<thead>
<tr>
<th>Subgroups/number of samples</th>
<th>Instrumentation</th>
<th>Irrigation during treatment</th>
<th>Final irrigation</th>
<th>Total time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Irrigants</td>
<td>Amount</td>
<td>Time</td>
</tr>
<tr>
<td>A/10</td>
<td>Hand K file</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>B/10</td>
<td>Rotary Ni-Ti files</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>C/10</td>
<td>Hand K file</td>
<td>Saline solution</td>
<td>5 x 2 ml</td>
<td>3 min</td>
</tr>
<tr>
<td>D/10</td>
<td>Rotary Ni-Ti files</td>
<td>Saline solution</td>
<td>5 x 2 ml</td>
<td>3 min</td>
</tr>
</tbody>
</table>
Table 2. Experimental protocol for group II (experimental) samples

<table>
<thead>
<tr>
<th>Subgroups/number of samples</th>
<th>Instrumentation</th>
<th>Irrigation during treatment</th>
<th>Final irrigation</th>
<th>Total time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Irrigants</td>
<td>Amount</td>
<td>Time</td>
</tr>
<tr>
<td>A/ 15</td>
<td>Hand K file</td>
<td>3%H₂O₂</td>
<td>5 x 2ml</td>
<td>3 min</td>
</tr>
<tr>
<td>B/ 15</td>
<td>Rotary Ni-Ti file</td>
<td>3%H₂O₂</td>
<td>5 x 2 ml</td>
<td>3 min</td>
</tr>
<tr>
<td>C/ 15</td>
<td>Hand K file</td>
<td>2,5% NaOCl</td>
<td>5 x 2 ml</td>
<td>3 min</td>
</tr>
<tr>
<td>D/ 15</td>
<td>Rotary Ni-Ti file</td>
<td>2,5% NaOCl</td>
<td>5 x 2 ml</td>
<td>3 min</td>
</tr>
<tr>
<td>E/ 15</td>
<td>Rotary Ni-Ti file</td>
<td>2,5% NaOCl</td>
<td>5 x 2 ml</td>
<td>3 min</td>
</tr>
<tr>
<td>F/ 15</td>
<td>Hand K file</td>
<td>3% H₂O₂ + 2% CHX alternatively</td>
<td>2 x 3 min</td>
<td>MTAD</td>
</tr>
<tr>
<td>G/ 15</td>
<td>Hand K file</td>
<td>3%H₂O₂</td>
<td>5 x 2 ml</td>
<td>3 min</td>
</tr>
</tbody>
</table>
Fig. 2. Sample prepared for evaporation

Fig. 3. Placing samples on appropriate supporter

3. Result

The results obtained in this study are presented in tables 1-7 and figures 1-15. In the first group of control samples subgroups (A, B, C, D), it was observed that root canal walls were covered with substantial amounts of dentin debris, where smear layer completely closed the openings of dentinal tubules. Such presentation of dentin surface is described in literature as “bark tree”. Eight samples were scored 5, while two samples were scored 4.

In the experimental group, the poorest results were obtained after the irrigation of walls with organolithic effect solutions. The most favorable outcome of the procedure was observed in the group where canal irrigation during instrumentation was done with organolithic effect solutions, and final irrigation with mineralolithic effect irrigating agents, in which process the most optimal combination of irrigation solutions was 3% H₂O₂ + 2% CHX + MTAD (1.10±0.31), which is a good choice of irrigants in endodontic clinical practice.
Statistical analysis showed that the experimental group treated with MTAD as the final irrigation had significantly cleaner walls compared to control group samples (p<0.001). The analysis of results showed that there was statistically significant difference in the mean scores among the examined groups of samples ($\chi^2=50.674$; p<0.001). The lowest score, and therewith the most favorable outcome, was found in the group F (Tab. 2). Mann Whitney U test determined statistically significant differences in scores among the groups, and the best results were obtained in the teeth in which MTAD was used as the final irrigation (p<0.001).

<table>
<thead>
<tr>
<th>Group/subgroup</th>
<th>N</th>
<th>Chemomechanical treatment</th>
<th>5</th>
<th>4</th>
<th>3</th>
<th>2</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/A</td>
<td>10</td>
<td>Manual treatment without irrigation</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I/B</td>
<td>10</td>
<td>Engine driven treatment without irrigation</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I/C</td>
<td>10</td>
<td>Manual treatment + Saline solution</td>
<td>3</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I/D</td>
<td>10</td>
<td>Engine driven treatment + Saline solution</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. Values of quantitative estimation of smear layer and dentin debris for group I (control group) samples – subgroups A, B, C and D

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>$\bar{X}$</th>
<th>SD</th>
<th>95% C.I.</th>
<th>Min</th>
<th>Max</th>
<th>Sig.</th>
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</thead>
<tbody>
<tr>
<td>I/A</td>
<td>10</td>
<td>4.80</td>
<td>0.42</td>
<td>4.50-5.10</td>
<td>4</td>
<td>5</td>
<td>A</td>
</tr>
<tr>
<td>I/B</td>
<td>10</td>
<td>4.90</td>
<td>0.32</td>
<td>4.67-5.13</td>
<td>4</td>
<td>5</td>
<td>B, C</td>
</tr>
<tr>
<td>I/C</td>
<td>10</td>
<td>4.30</td>
<td>0.48</td>
<td>3.95-4.65</td>
<td>4</td>
<td>5</td>
<td>B</td>
</tr>
<tr>
<td>I/D</td>
<td>10</td>
<td>4.20</td>
<td>0.79</td>
<td>3.64-4.76</td>
<td>3</td>
<td>5</td>
<td>A, C</td>
</tr>
</tbody>
</table>

A (I/A vs I/D); B (I/B vs I/C); C (I/B vs I/D);

Table 4. Comparison of values of quantitative estimation of smear layer and dentin debris in group I (control samples - subgroups A, B, C and D: ANOVA test

By comparing the quantitative estimation values obtained for smear layer and dentin debris in the group I (control) samples – subgroups A, B, C and D using the ANOVA test, a statistically significant difference was found in the mean values among the groups I/A and I/D, I/B and I/C, I/B and I/D.

3.1. Ultrastructural presentation of intracanal dentin
3.1.1 Ultrastructural presentation of intracanal dentin surface after manual treatment without irrigation

After tooth root canal treatment using the hand driven instruments, canal walls were covered with large particles of dentin debris and smear layer of irregular surface found at all levels of intracanal dentin. The size of dentin debris particles was 2-6 μm. Dentinal tubule
openings were not visible; dentin debris with a „tree bark“ configuration was observed at the level of the apical third.

Fig. 4. SEM micrographs of coronal (K), middle (S) and apical (A) thirds of canal walls after manual treatment using K files without irrigation. Massive accumulation of dentin debris with large particles, with the underlying smear layer. Dentinal tubules invisible

3.1.2 Ultrastructural presentation of intracanal dentin surfaces after engine driven treatment without irrigation

Intracanal engine driven treatment of tooth root canal using NiTi instruments, without irrigation, leaves massive accumulation of dentin debris, with the underlying smear layer of thicker, more compact structure having large dentin particles distributed throughout the root canal. Dentinal tubules are completely closed. In the apical regions, there is a large amount of dentinal debris in the form of „plug“.

Fig. 5. SEM micrographs of coronal (K), middle (S) and apical thirds(A) of the canal walls after engine driven treatment using NiTi files without irrigation. Massive accumulation of dentinal debris

3.1.3 Ultrastructural presentation of intracanal dentinal surfaces following manual treatment using saline solution

After manual root canal treatment with K files and irrigation with saline solution during instrumentation, a smaller amount of superficial debris can be noted as well as the presence of inhomogeneous smear layer in all root regions. Only a few dentinal canal openings can be seen, completely or partially covered with smear layer and debris (coronal and middle root regions), while the apical region contains larger amounts of surface debris and smear layer.
3.1.4 Ultrastructural presentation of intracanal dentin surfaces after engine driven treatment and irrigation with saline solution

After intracanal engine driven treatment using NiTi instruments and irrigation during instrumentation with saline solution, smaller particles of dentinal debris and abundant smear layer can be seen.

3.1.5 Ultrastructural presentation of intracanal dentin surfaces after the irrigation with 3% H₂O₂ and final irrigation with 2.5% NaOCl

After manual treatment and irrigation during instrumentation using 3% H₂O₂ and final irrigation with 2.5% NaOCl, it can be noted that larger amount of debris in the coronal
The smear layer was removed; however, the smear layer is present. The open dentinal tubules show irregular shape and diameter. In the middle and apical thirds of the coronal region considerable amounts of surface debris attached to smear layer can be observed, which largely blocks the openings of dentinal tubules. There is a lack of mineralolithic effect, which results in impure intracanal dentin surface.

3.1.6 Chlorhexidine (CHX) as intracanal irrigants
Chlorhexidine is gluconate salt, and as an intracanal irrigants it is used in the form of bisbiguanide. This biocide has prolonged antibacterial efficacy at pH - 5.5-7.0. Ultrastructural presentation of intracanal dentinal surfaces after the irrigation with 3% H$_2$O$_2$ and final irrigation with 2% CHX.

After the manual root canal treatment and irrigation during instrumentation using 3% H$_2$O$_2$ and final irrigation with 2% CHX, the largest part of debris was removed. However, small particles of the smear layer, sized 1-2 µm, can be noted in all the samples, which results in not so smooth surface of the intertubular dentin. Dentinal tubule openings are not clearly limited, and inside the tubules some small particles of precipitate can be seen, produced during the reaction between hydrogen peroxide and chlorhexidine.

Having observed the dentinal wall from the coronal to the apical third, it was found that the amount of the smear layer was increased from the middle towards the apical third. In the middle third, the openings of dentinal tubules are of uneven diameter and irregular shape. The largest quantity of the smear layer in the form of „plug“ is in the apical third, wherein the created precipitate is incorporated into the dentinal tubules. In the apical third, the openings of dentinal tubules cannot be seen.

Fig. 9. SEM micrographs of the coronal (K), middle (S) and apical (A) thirds of the canal wall after the treatment and irrigation with 3% H$_2$O$_2$ and final irrigation with 2% CHX solution. Only small particles of dentinal debris can be partly seen as well as the smear layer on intertubular dentin; precipitates present inside the dentinal tubules

3.1.7 Ultrastructural presentation of intracanal dentin surfaces after treatment, irrigation with 2,5% NaOCl and final irrigation using 17%Na EDTA
Intracanal dentin surfaces in all regions, after the treatment and irrigation with 2,5% NaOCl and final irrigation with 17% NaEDTA solution, show preserved and clean structural dentin surface, open dentinal tubules of regular and even lumen.
Fig. 10. SEM micrographs of canal walls after treatment and irrigation with 2.5% NaOCl and final irrigation using 17% NaEDTA solution. Dentinal debris and smear layer completely removed. Dentinal tubules clearly open, of regular shape; preserved, smooth dentinal structure.

3.1.8 Ultrastructural presentation of intracanal dentin surfaces after treatment and irrigation with 2.5% NaOCl + 10% citric acid

Citric acid (10%) is efficient in removing the smear layer from the root canal walls and complete cleansing of the canal system, and, therefore, can be used as a final irrigant during endodontic treatment. In order to avoid aggressive etching and potential erosion of dentin, the time of citric acid action must be limited from 20 seconds to 1 minute. The basic problem when applying the citric acid in the intracanal irrigation is the acidity of solution and a possibility of accidental contact with the mouth cavity soft issues. Combined application with NaOCl can bring about sudden neutralization, pH changes and releasing the chloride.

Fig. 11. SEM micrographs of intracanal dentine after irrigation with 2.5% NaOCl and 10% citric acid in duration of 60 sec. Small dentin particles present with the underlying smear layer. Dentinal tubules open, of uneven diameter and shape. Because of chelating Ca+ from dentin, formations of calcium citrate are produced.
gases. Higher concentration of citric acid can chelate Ca$^{2+}$ from dentin and cause the formation of calcium citrate crystals in the root canal. Industrial products are: 19% citric acid solution – canal Clean (Ognapharma, Italy); 10% citric acid solution – Citric acid solution (Ultradent) for canal application, Cetrimide.

3.1.9 Ultrastructural presentation of intracanal dentin surface after the irrigation with 3% H$_2$O$_2$ i 2% CHX and final irrigation with MTAD solution

After the canal system instrumentation and irrigation with 3% H$_2$O$_2$ i 2% CHX and final irrigation with MTAD solution in duration of 1 minute, the results obtained are ideal in the coronal, middle and apical thirds of the tooth root canal. Dentinal debris and smear layer complexly removed. Dentinal tubules open, of regular shape and even diameter (coronary third 3.5 µm, middle third 2.5-3 µm, apical third 2-2.5 µm). Dentinal structure preserved.

Fig. 12. SEM presentation of coronal third of intracanal dentin after manual treatment and irrigation with 2% CHX + 3% H$_2$O$_2$ and final irrigation with MTAD solution

Fig. 13. SEM presentation of middle third of intracanal dentin after manual treatment and irrigation with 2% CHX + 3% H$_2$O$_2$ and final irrigation with MTAD solution
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Fig. 14. SEM presentation of apical third of intracanal dentin after manual treatment and irrigation with 2% CHX + 3% \( \text{H}_2\text{O}_2 \) and final irrigation with MTAD solution

<table>
<thead>
<tr>
<th>Group/subgroup</th>
<th>N</th>
<th>Irrigants</th>
<th>5</th>
<th>4</th>
<th>3</th>
<th>2</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>II/A</td>
<td>15</td>
<td>3%H(_2)O(_2)+2,5% NaOCl</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>II/B</td>
<td>15</td>
<td>3%H(_2)O(_2)2% +CHX</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>II/C</td>
<td>15</td>
<td>2,5%NaOCl+17%NaEDTA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>II/D</td>
<td>15</td>
<td>2,5%NaOCl</td>
<td>4</td>
<td>4</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II/E</td>
<td>15</td>
<td>2,5%NaOCl +10% citric acid</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>II/F</td>
<td>15</td>
<td>3%H(_2)O +2%CHX +MTAD</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>I/G</td>
<td>15</td>
<td>3%H(_2)O + MTAD</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15</td>
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</table>

Table 5. Values of quantitative estimation of smear layer and dentinal debris for the samples of group II (experimental) – subgroups A, B, C, D, E, F and G

<table>
<thead>
<tr>
<th>Group/subgroups</th>
<th>N</th>
<th>( \overline{X} )</th>
<th>SD</th>
<th>95% C.I.</th>
<th>Min</th>
<th>Max</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>II/A (3%H(_2)O(_2)+2,5%NaOCl)</td>
<td>15</td>
<td>2.66</td>
<td>0.89</td>
<td>2.16-3.16</td>
<td>1.00</td>
<td>4.00</td>
<td>A,B,C,D,E,F</td>
</tr>
<tr>
<td>II/B (3%H(_2)O(_2)2%+CHX)</td>
<td>15</td>
<td>2.73</td>
<td>0.88</td>
<td>2.24-3.22</td>
<td>1.00</td>
<td>4.00</td>
<td>A,G,H,I,J,K</td>
</tr>
<tr>
<td>II/C (2,5%NaOCl+17%NaEDTA)</td>
<td>15</td>
<td>1.20</td>
<td>0.41</td>
<td>0.97-1.42</td>
<td>1.00</td>
<td>2.00</td>
<td>A,G,L</td>
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<tr>
<td>II/D (2,5%NaOCl)</td>
<td>15</td>
<td>3.80</td>
<td>0.86</td>
<td>3.32-4.27</td>
<td>3.00</td>
<td>5.00</td>
<td>C,H,L,M,N,O</td>
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<tr>
<td>II/E (2,5%NaOCl+10% citric acid)</td>
<td>15</td>
<td>1.26</td>
<td>0.45</td>
<td>1.01-1.52</td>
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<td>2.00</td>
<td>D,I,J,M</td>
</tr>
<tr>
<td>II/F (3%H(_2)O+2%CHX+MTAD)</td>
<td>15</td>
<td>1.06</td>
<td>0.25</td>
<td>0.92-1.20</td>
<td>1.00</td>
<td>2.00</td>
<td>E,J,N</td>
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<tr>
<td>II/G (3%H(_2)O+MTAD)</td>
<td>15</td>
<td>1.00</td>
<td>0.00</td>
<td>1.00-1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>F,K,O</td>
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</tbody>
</table>

A (II/A vs II/B); B (II/A vs II/C); C (II/A vs II/D); D (II/A vs II/E); E (II/A vs II/F); F (II/A vs II/G); G (II/B vs II/C); H (II/B vs II/D); I (II/B vs II/E); J (II/B vs II/F); K (II/B vs II/G); L (II/C vs II/D); M (II/D vs II/E); N (II/D vs II/F); O (II/D vs II/G)

Table 6. Comparison of quantitative estimation values of smear layer and dentinal debris on the samples of group II (experimental) – subgroups A, B, C, D, E, F, and G: ANOVA

By comparison of the quantitative estimation values of smear layer and dentinal debris on the samples of group II (experimental) – subgroups A, B, C, D, E, F and G, using the ANOVA test, it was found that the values obtained in the group II/A are statistically
significantly different from the results obtained in all other groups. Mean value obtained in the group II/A was statistically significantly lower compared to the values in the groups II/B and II/D. The values obtained in the group II/B are statistically significantly higher compared to the values obtained in the groups II/C, II/E, II/F and II/G, but lower than the values obtained in the group II/D. The values of group II/d are statistically significantly higher than the values obtained in all other groups. The values of groups II/F and II/G are lower compared to the values obtained in the groups II/A, II/B and II/D.

3.2 Analysis of antimicrobial effect with MTAD in infected canal system using PCR technique

With the aim to determine the antibacterial efficacy of MTAD solution using the PCR method, several most common endopathogenic microorganisms were identified in the

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Number of infected root canals</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before therapy</td>
<td>After therapy</td>
<td>( \chi^2 )</td>
<td>( p )</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggregatibacter</td>
<td>8</td>
<td>32</td>
<td>4</td>
<td>16</td>
<td>1.72</td>
<td>0.185</td>
</tr>
<tr>
<td>Prevotella intermedia</td>
<td>9</td>
<td>36</td>
<td>0</td>
<td>0</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Porphyromonas gingivalis</td>
<td>4</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Tanerella forsythenis</td>
<td>6</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>15</td>
<td>60</td>
<td>5</td>
<td>20</td>
<td>8.17</td>
<td>0.004</td>
</tr>
<tr>
<td>Treponema denticola</td>
<td>7</td>
<td>28</td>
<td>3</td>
<td>12</td>
<td>1.96</td>
<td>0.161</td>
</tr>
</tbody>
</table>

Table 7. Prevalence of microorganisms in infected root canals prior and after MTAD therapy

Fig. 15. Prevalence of microorganisms in infected root canals before and after therapy with MTAD

* - \( p < 0.05 \); ** - \( p < 0.01 \)
infected canal system (*Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Tanerella forsythensis, Prevotella intermedia, Treponema denticola* and *Enterococcus faecalis*) before and after the irrigation with MTAD solution. Analysis of results and estimation of antibacterial efficacy of MTAD solution gives the clinical reference to this final irrigant in the treatment of infected root canals.

By comparing the frequency of occurrence of certain bacteria in root canals, before and after the irrigation with MTAD solution, statistically significant decrease of *Prevotella intermedia* (36% vs 0%), *Tanerella forsythenis* (24% vs 0%) and *Enterococcus faecalis* (60% vs 20%) was found. The presence of other bacteria was also decreased, but not statistically significant.

### 4. Conclusion

Based on the results obtained by SEM and statistical data processing, it can be concluded that the final irrigation of root canal system with mineralolithic effect irrigations must be a mandatory part of endodontic protocol. The best results and outstanding efficacy were demonstrated with MTAD solution. In combination with CHX and H$_2$O$_2$, it completely removes the smear layer from root canal walls, where the dentin surface structure remains preserved and openings of dentinal tubules are of even diameters and regular shapes. All mineralolithic solutions for final irrigation are used in duration of one minute, as longer exposure of dentin to these agents can bring about unwanted erosive changes and compromise the entire endodontic procedure. By regular use of final irrigating agents, complete efficacy in removal of smear layer from root canal system could be achieved.

### 5. Clinical recommendations

- When performing the manual and endogine driven instrumentation of the root canal, dentinal debris and smear layer are produced at all the levels of the intraradix region; they are not different in respect to the amount but presentation and structure.
- Saline solution applied as an irrigant exerts only the mechanical effect of removal and partial evacuation of debris.
- Irrigation by using organolithic agents alone cannot completely remove the smear layer.
- The combination of organolithic with mineralolithic agents has shown as the most efficient in the removal of smear layer at all the levels of the intraradix region.
- The combined application of hydrogen peroxide (and chlorhexidine) during instrumentation and final irrigation with MTAD solution in duration of 1 minute results in complete removal of the smear layer.
- MTAD solution as the final irrigant meets all the standards for good irrigant proscribed by the endodontic protocol, which means that it preserves the structure of dentine, removes the smear layer and possesses the satisfactory antimicrobial properties.
- After the chemomechanic treatment and irrigation of the root canal using the MTAD solution, statistically significant decrease of *Enterococcus faecalis, Prevotella intermedia* and *Tanerella forsythenis* was found, while in cases of *Treponema denticola, Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* the antibacterial efficacy of MTAD solution was considerable but not statistically significant.
- MTAD solution, used as the final intracanal antiseptic in duration of 1 minute, efficiently removes the smear layer, in the case of which the intracanal structure
remains intact and morphologically unchanged, eliminating thus the majority of microorganisms.

6. References


Jacinto, RC.; Gomes, BP.; Ferraz, CC.; Zaia AA. & Filho FJ. (2003). Microbiological Analysis of Infected Root Canals From Symtomatic Teeth with Periapical Periodontitis and the Antimicrobial Susceptibility of Some Isolated Anaerobic Bacteria. Oral Microbiology and Immunology, Vol. 18, pp. 285-292. ISSN 0902-0055


The Importance of Final Irrigation with Mineralolithic Effect Agents During Chemomechanical Treatment of Tooth Root Canal


Oral health care in pediatric dentistry deals with complete oral health, including preventive aspects for children right from their conception to adolescence, encompassing all the spheres of dentistry including various specialties. It also includes planning a preventive program at individual and community levels. The current research interests in oral health care include studies regarding the role of stem cells, tissue culture, and other ground-breaking technologies available to the scientific community in addition to traditional fields such as anatomy, physiology, and pharmaceuticals etc of the oral cavity. Public health and epidemiology in oral health care is about the monitoring of the general oral health of a community, general afflictions they are suffering from, and an overall approach for care and correction of the same. The oral health care-giver undertakes evaluation of conditions affecting individuals for infections, developmental anomalies, habits, etc. and provides corrective action in clinical conditions. The present work is a compendium of articles by internationally renowned and reputed specialists about the current developments in various fields of oral health care.

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