Evaluation of 0.5% peracetic acid and 2.5% sodium hypochlorite on smear layer removal of root canal instrumented by three rotary systems

Ana Paula Martins GOMES¹, Laís dos Santos LISSI¹, Marcella Batista Pavanello COELHO¹, Eduardo Galera da SILVA², Frederico Canato MARTINHO¹, Maria Filomena Rocha Lima HUHTALA¹

1 – Institute of Science and Technology – UNESP – Univ Estadual Paulista – School of Dentistry – Department of Restorative Dentistry – São José dos Campos – SP – Brazil.

2 – Institute of Science and Technology – UNESP – Univ Estadual Paulista – School of Dentistry – Department of Social and Pediatric Dentistry – São José dos Campos – SP – Brazil.

ABSTRACT

Objective: The aim of this study was to evaluate the effect of 0.5% peracetic acid solution and 2.5% sodium hypochlorite solution on smear layer removal of root canal walls after rotary instrumentation. Material & methods: Sixty single-rooted human teeth with standardized length had their canals instrumented by three rotary systems (Biorace, MTwo and Endowave), varying the irrigation solution, as follows (n = 10): G1 - Biorace System + 0.5% peracetic acid solution; G2 - Biorace System + 2.5% sodium hypochlorite solution; G3 - MTwo System + 0.5% peracetic acid solution; G4 - MTwo System + 2.5% sodium hypochlorite solution; G5 - Endowave System + 0.5% peracetic acid solution; G6 - Endowave System + 2.5% sodium hypochlorite solution. After instrumentation, the roots were cleaved and the dentin walls were evaluated by SEM at x500 and x2000 magnification for assessing the cleaning of the root canals at the cervical, middle and apical thirds. The analyzed area was quantified according to the total number of tubules present and the percentage of open tubules at each region. Data were submitted to statistical analysis by ANOVA and Tukey tests, at the significance level of 5%. Results: There were no statistical significant differences among the experimental groups. In all groups the smear layer removal at cervical and medium thirds was higher than that of apical third. Conclusion: It was concluded that the cleaning obtained at the apical third was lower in all groups studied and neither the instrumentation technique nor the irrigating solution was able to promote total smear layer removal from root canal walls.

KEYWORDS

Peracetic acid; Root canal irrigants; Scanning electron microscopy; Sodium hypochlorite.

RESUMO

Objetivo: O objetivo deste estudo foi avaliar o efeito do ácido peracético a 0,5% e do hipoclorito de sódio a 2,5% na remoção da camada de smear nas paredes do canal radicular após instrumentação com diferentes sistemas rotatórios. Material e métodos: Foram utilizados 60 dentes humanos unirradiculares que tiveram seus canais instrumentados por três sistemas rotatórios (Biorace, MTwo e Endowave), variando a solução irrigadora utilizada, como segue (n = 10): G1 - Sistema Biorace + 0,5% de ácido peracético; G2 - Sistema Biorace + 2,5% de hipoclorito de sódio; G3 - Sistema MTwo + 0,5% de ácido peracético; G4 - Sistema MTwo + 2,5% de hipoclorito de sódio; G5 - Sistema Endowave + 0,5% de ácido peracético; G6 - Sistema Endowave + 2,5% de hipoclorito de sódio. Após instrumentação, as raízes foram clivadas e as paredes dentinárias foram avaliadas em MEV com aumento de 500x e 2000x nos terços cervical, médio e apical. A área analisada foi quantificada pelo número total de túbulos dentinários presente e pela porcentagem de túbulos abertos na região avaliada. Os dados relativos à porcentagem de túbulos abertos foram submetidos à análise estatística utilizando os testes de ANOVA e Tukey com nível de significância de 5%. Resultados: Não houve diferença estatisticamente significante entre os grupos experimentais. A remoção da camada de smear foi maior nos terços cervical e médio em relação ao terço apical. Conclusão: Não houve diferença estatisticamente significante entre os grupos experimentais. A limpeza obtida no terço apical foi inferior em todos os grupos avaliados.

PALAVRAS-CHAVE

Ácido peracético; Hipoclorito de sódio; Irrigantes do canal radicular; Microscopia eletrônica de varredura.
INTRODUCTION

The irrigant solutions used during biomechanical preparation should display antimicrobial and tissue solvent activity, lubrication and root wall cleaning capacity, enabling a better action of intracanal medication and adequate bonding of the filling material to dentinal walls [1]. Sodium hypochlorite solution, at different concentrations, is one of the most employed solutions in Endodontics because of low superficial tension, capacity of dissolving organic matter, and deodorizing, bleaching, lubricating and antimicrobial actions.

However, during biomechanical preparation, even using proper irrigant solutions and instruments, a residual layer, so-called smear layer, is formed and composed by debris, organic matter and microorganisms adhering to root canal walls, hindering the opening of the dentinal tubules. This layer must be removed with the aid of auxiliary chemical substances [2], thus resulting in the increasing of the dentinal permeability [3,4] and greater bonding of endodontic cements to root canal walls [5].

Many irrigant solutions have been employed to remove the smear layer, such as citric acid, MTAD, phosphoric acid, RC-Prep, maleic acid, sodium citrate, acetic acid [6-8], however, EDTA is the chelating acid most used to remove this residual layer. EDTA, when used for a longer time may cause excessive peritubular and intertubular dentinal erosion and decreasing root dentine microhardness [9-11].

Peracetic acid is a candidate to be used as alternative irrigant solution. This acid shows antimicrobial activity even at low concentrations [12]. In Endodontics, 2.25% peracetic acid has been recently investigated as final irrigant solution in association with NaOCl [13]. Such substance was capable of removing smear layer and chelating the calcium ions similarly to 17% EDTA. Other recent researches have reported that low-concentration of peracetic acid (0.5%) can be used in the disinfection of gutta-percha points [14] and in smear layer dissolution [15].

The instruments and the instrumentation technique together with the irrigant solutions account for the cleaning and shaping of root canals, resulting in a more efficient endodontic treatment. Two decades ago, the instruments had limitations regarding to flexibility. Some researchers evaluated the use of nickel-titanium alloys for the construction of endodontic instruments which at that time were made of stainless steel [16]. Consequently, a new generation of nickel-titanium instruments appeared which allowed the development of new and promising rotary systems [17-19].

Until now, an ideal irrigant solution aggregating all characteristics required for promoting root canal cleaning and disinfection is not available. Therefore, the search for an alternative irrigant showing antimicrobial activity and smaller irritating potential to be associated with new rotary endodontic instruments is desirable because it can enable great advancements in root canal preparation and disinfection.

MATERIAL & METHODS

Selection, storage and preparation of the teeth

This study was submitted and approved by the Ethical Committee in Research of the Science and Technology Institute of São José dos Campos – UNESP (protocol number 043/2011 – PH/CEP).

Sixty single-rooted human teeth, freshly extracted due to orthodontic or periodontal reasons were used. The teeth were cleaned, autoclaved and kept in deionized water until the moment of their use aiming to maintain them hydrated and to decrease the risks of cracks and fractures during biomechanical preparation. The teeth were radiographed (at labial-lingual and mesial-distal directions), selected and equally distributed among the study groups according
to their similar anatomical morphologies (single root canal, without marked curvature, lack of both calcifications and internal/external root resorptions).

The tooth crowns were removed with the aid of double-face diamond discs (Microdent Micro Usinagem de Precisão Ltd., São Paulo, Brazil) to obtain root remnants with 16±0.5 mm in length. The specimens were also standardized regarding to the foramen diameter of root canals. For this purpose, a K file instrument was inserted into the canal until its visualization in the apical foramen. It was employed roots whose foramen diameter was correspondent to size 15 to 20 K files. The roots were equally distributed among the experimental groups according to the foramen diameter.

The specimens had their apexes closed with resin composite (Z100, 3M do Brazil Ltd., Campinas, São Paulo, Brazil) to prevent the irrigant solution extravasation through the foramen during root canal preparation. The working length was determined visually by introducing a K file instrument into root canal until its tip was seen at the apical foramen. Then, 1 mm was subtracted from that measurement.

**Division of the experimental groups**

The solutions of 2.5% NaOCl and 0.5% peracetic acid were prepared by a Manipulation Pharmacy (Terapêutica, São José dos Campos, SP, Brazil). The roots were divided into 6 experimental groups according to the irrigant solution used. Each group was subdivided into 3 subgroups according to the rotary system employed (Biorace, MTwo and Endowave), as seen in Chart 1.

**Protocol of root canal instrumentation and irrigation**

The opening of the root canals was prepared by increasing order of size #2 and #3 Gates-Glidden drills (Dentsply/ Maillefer Ind. Com. Ltd., RJ, Brazil). The rotary instruments were driven by Motor X Smart (Dentsply/ Maillefer Ind. Com. Ltd., RJ, Brazil), with reduction of 16:1, speed from 280 to 350 rpm and torque of 1 to 3 N.cm, according to the manufacturer's instructions for each system at working length of 15mm. During the preparation, the instruments sequence was employed according to the manufacturer's instructions, as follows: Biorace System: #BR0, #BR1, #BR2, #BR3, #BR4, #BR5; MTwo System: #10.04, #15.05, #20.06, #25.06; Endowave System: #35.08, #30.06, #25.06.

All canals were irrigated with the aid of NaviTip needles (Ultradent, South Jordan, UT) introduced at 1mm short of working length (14 mm). At every instrument change,

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>IRRIGANT SOLUTION</th>
<th>ROTARY SYSTEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>0.5% peracetic acid</td>
<td>Biorace</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(FKG Dentaire La Chaux-de-Fonds, Switzerland)</td>
</tr>
<tr>
<td>G2</td>
<td>2.5% NaOCl</td>
<td>Biorace</td>
</tr>
<tr>
<td>G3</td>
<td>0.5% peracetic acid</td>
<td>MTwo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(VDW, Munich, Germany)</td>
</tr>
<tr>
<td>G4</td>
<td>2.5% NaOCl</td>
<td>MTwo</td>
</tr>
<tr>
<td>G5</td>
<td>0.5% peracetic acid</td>
<td>Endowave</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(J Morita, Kyoto, Japan)</td>
</tr>
<tr>
<td>G6</td>
<td>2.5% NaOCl</td>
<td>Endowave</td>
</tr>
</tbody>
</table>
irrigation was executed with 5 mL of the irrigant solution studied. After the completion of the biomechanical preparation, all canals were filled with ethylenediaminetetraacetic acid (EDTA – Iodontec, Porto Alegre, RS, Brazil) at 17%. The final irrigation was executed with 10 mL of saline solution (Glicolabor Indústria Farmacêutica Ltd., Ribeirão Preto, São Paulo, Brazil). Following, the root canals were dried with the aid of absorbent paper points (Tanari, Manaus, Amazonas, Brazil) and the access cavity was sealed with Cavit (ESPE GMBH, Seefeld, Germany). Next, two longitudinal grooves were executed on the labial and lingual surfaces of the roots with the aid of double-face diamond disc (Microdont Micro Usinagem de Precisão Ltd., São Paulo, Brazil) in order to guide cleavage. Caution was taken not to reach root canal. The roots were externally washed and cut in two halves (mesial and distal), to be analyzed through SEM.

Preparation and analysis of the specimens for SEM

The specimens were fixed in 2.5% glutaraldehyde, buffered in 0.1M sodium cacodylate pH 7.4 for 12 h at 4 ºC. Elapsed this period, they were washed in buffered 0.2M sodium cacodylate pH 7.4 for one hour, changed three times, immersed in distilled water for one minute and then dehydrated by ascending grades of alcohol (25%, 50%, 75% for 20 minutes each; 95% for 30 min; 100% 60 min).

Then, the specimens were completely dried in filter paper for 48 hours and prepared for SEM analysis (JSM – 840A Jeol, Tokyo, Japan). For this purpose, the specimens were mounted in metallic stubs with the aid of conductive carbon tape and silver glue. Their metallization was carried out by a thin gold layer (200Å) in a high-pressure evaporator (DV-502-Denton NJ). For each specimen, electronic microscope was placed at a distance of about 25 mm, and an area of 200 μm² was irradiated with a voltage of 15V for 100 s, with an electron beam penetration of 1 μm in depth.

The cleaning of root canal walls was proved by verifying the presence or absence of the smear layer at cervical, medium and apical thirds. Areas representing each analyzed root third were selected and photographed at x500 and x2000 magnification. The images obtained at x 2000 magnification were transferred to image software (Paint for Windows) which enabled the counting of the opened and closed dentinal tubules. The data related to the opened tubules, per mm³, were submitted to statistical analysis.

Study design

This study followed a 2 x 3 factorial scheme, that is, the two study factors were the irrigant solution (two levels) and the instrumentation type (three levels). The experimental unit was the human tooth and the 60 teeth were randomly assigned regarding to the six experimental conditions or groups. The response variable was the percentage of opened dentinal tubules through SEM image analysis.

The obtained data were submitted to descriptive and inferential statistics. Descriptive statistics comprised the calculation of the means and standard deviations. Inferential statistics consisted of the application of two-way analysis of variance (ANOVA), in which the factor “thirds” was considered as repeated factor. To localize the statistical differences among groups, Tukey test was applied (5%).

RESULTS

Descriptive statistics

Descriptive statistics about the smear layer removal according to the percentage of the total number of opened tubules verified in the images is expressed in Table 1.
Table 1 - Mean and standard deviation (SD) of the percentage (%) of opened dentinal tubules in the groups evaluated.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Mean (%)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 - Biorace + 0.5% peracetic acid</td>
<td>10</td>
<td>29.30</td>
<td>17.39</td>
</tr>
<tr>
<td>G2 - Biorace + 2.5% NaOCl</td>
<td>10</td>
<td>36.01</td>
<td>17.64</td>
</tr>
<tr>
<td>G3 - Mtwo + 0.5% peracetic acid</td>
<td>10</td>
<td>33.01</td>
<td>19.79</td>
</tr>
<tr>
<td>G4 - Mtwo + 2.5% NaOCl</td>
<td>10</td>
<td>46.60</td>
<td>20.88</td>
</tr>
<tr>
<td>G5 - Endowave + 0.5% peracetic acid</td>
<td>10</td>
<td>32.37</td>
<td>19.13</td>
</tr>
<tr>
<td>G6 - Endowave + 2.5% NaOCl</td>
<td>10</td>
<td>38.68</td>
<td>23.19</td>
</tr>
</tbody>
</table>

Table 2 displays the mean and standard deviation values within each root third for each experimental group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Thirds</th>
<th>Cervical</th>
<th>Medium</th>
<th>Apical</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 - Biorace + 0.5% peracetic acid</td>
<td></td>
<td>32.82 ± 14.61</td>
<td>34.86 ± 22.78</td>
<td>20.21 ± 10.00</td>
</tr>
<tr>
<td>G2 - Biorace + 2.5% NaOCl</td>
<td></td>
<td>40.49 ± 22.80</td>
<td>38.95 ± 8.47</td>
<td>28.59 ± 18.02</td>
</tr>
<tr>
<td>G3 - Mtwo + 0.5% peracetic acid</td>
<td></td>
<td>36.17 ± 17.06</td>
<td>35.09 ± 20.15</td>
<td>27.76 ± 22.77</td>
</tr>
<tr>
<td>G4 - Mtwo + 2.5% NaOCl</td>
<td></td>
<td>38.27 ± 13.33</td>
<td>57.99 ± 13.33</td>
<td>44.07 ± 25.90</td>
</tr>
<tr>
<td>G5 - Endowave + 0.5% peracetic acid</td>
<td></td>
<td>46.61 ± 19.55</td>
<td>18.58 ± 11.91</td>
<td>31.71 ± 11.66</td>
</tr>
<tr>
<td>G6 - Endowave + 2.5% NaOCl</td>
<td></td>
<td>46.09 ± 19.57</td>
<td>36.25 ± 20.83</td>
<td>33.16 ± 19.70</td>
</tr>
</tbody>
</table>

Table 3 - Mean and standard deviation (SD) of the percentage (%) of opened dentinal tubules in the groups evaluated.

<table>
<thead>
<tr>
<th>Variation source</th>
<th>Sum of squares</th>
<th>Degrees of freedom</th>
<th>Quadratic mean</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>4500.1</td>
<td>5</td>
<td>900.0</td>
<td>2307</td>
<td>0.078122</td>
</tr>
<tr>
<td>Group Error*R</td>
<td>19853.0</td>
<td>47</td>
<td>422.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>2426.5</td>
<td>3</td>
<td>1213.3</td>
<td>3.5087</td>
<td>0.033914*</td>
</tr>
<tr>
<td>Groups *Thirds</td>
<td>5390.9</td>
<td>18</td>
<td>539.1</td>
<td>15590</td>
<td>0.131175</td>
</tr>
<tr>
<td>Error</td>
<td>32504.0</td>
<td>94</td>
<td>345.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Groups <em>R</em>Thirds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Inferential statistics

The results from two-way ANOVA for repeated measurements applied to the experimental groups comprising the instruments associated with the irrigant solutions and root thirds are seen in Table 3.

Table 4 - Formation of groups of same performance in terms of smear layer removal according to Tukey test (5%) for the factor “root third”

<table>
<thead>
<tr>
<th>Root third</th>
<th>Mean ± SD</th>
<th>Statistical grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical</td>
<td>39.96 ± 19.57</td>
<td>A</td>
</tr>
<tr>
<td>Medium</td>
<td>37.60 ± 20.83</td>
<td>A</td>
</tr>
<tr>
<td>Apical</td>
<td>30.61 ± 19.70</td>
<td>B</td>
</tr>
</tbody>
</table>

* Values followed by equal letters did not show statistically significant differences (p > 0.05)

Figures 1 to 6 exhibit the SEM images of cervical third of each experimental group.
Figure 1 - Photomicrograph of the cervical third after Biorace rotary instrumentation and irrigation with 0.5% peracetic acid (Group 1 - x2000 magnification).

Figure 2 - Photomicrograph of the cervical third after Biorace rotary instrumentation and irrigation with 2.5% sodium hypochlorite (Group 2 - x2000 magnification).

Figure 3 - Photomicrograph of the cervical third after MTwo rotary instrumentation and irrigation with 0.5% peracetic acid (Group 3 - x2000 magnification).

Figure 4 - Photomicrograph of the cervical third after MTwo rotary instrumentation and irrigation with 2.5% sodium hypochlorite (Group 4 - x2000 magnification).
DISCUSSION

The obtained results in Table 1 showed the greatest percentage of opened dentinal tubules in group 4 (2.5% NaOCl + Mtwo), but without statistically significant differences among groups (Table 3). There were statistical differences among root thirds, in which Tukey test exhibited that the apical third was statistically significant different from the cervical and medium thirds regarding to the percentage of open dentine tubules (Table 4).

In this study, Mtwo rotary system was used in group 4 presenting the highest percentage of opened dentinal tubules. Mtwo rotary system has excellent results regarding to preparation and time amount according to some studies [17,18,20,21]. In addition to keep the canal curvature, this system displays an excellent cleaning capacity [22].

Mtwo system associated with 2.5% sodium hypochlorite behaved satisfactorily, obtaining 46.60% (in average) of opened dentinal tubules (Table 1). On the other hand, groups 1 (Biorace + 0.5% peracetic acid) and 2 (Biorace + 2.5% NaOCl) obtained an average of 29.30% and 36.01%, respectively. These results can be explained by the fact that Biorace system has a triangular convex cross section and a narrower nucleus diameter which results in smaller debris accumulation inside root canal, enabling that the irrigation/aspiration process is efficient in removing the smear layer produced during biomechanical preparation [23].

Groups 5 (Endowave + 0.5% peracetic acid) and 6 (Endowave + 2.5% NaOCl) had 32.37% and 38.68% (in average) of opened dentinal tubules (Table 1). EndoWave system has contact points alternated throughout its cutting surface but with a design that maintains these alternated points centralized, limiting the flow and reducing the torque required. The surface finishing of NiTi instrument is obtained through electropolishing, minimizing the failures and possible defects of the alloy. This results in increasing of the strength, preventing its fracture. In this present study, it could be
verified that when the specimens instrumented by Biorace and Mtwo system were compared with those prepared by Endowave systems, the results were similar without statistically differences (Table 3).

The mechanical cleaning seems to be insufficient to assure disinfection, which leads one to conclude that the irrigant solution must have antimicrobial properties to achieve a satisfactory biomechanical preparation [24]. In this sense, the action of sodium hypochlorite at high concentrations has been already proved [25,26].

According to the study of Naenni et al. [27], none tested substance was capable of dissolving organic matters as efficient as sodium hypochlorite, including peracetic acid at 10%. The use of peracetic acid has been observed in some Dentistry areas [13-15,28-31] and especially in Endodontics, it has been employed at concentrations of 0.5% and 2% as chelating and disinfection agent of gutta-percha points [13-15].

Lottanti et al. [13] and Salvia et al. [14] reported the efficacy of 2% peracetic acid as disinfection agent of gutta-percha points and chelating agent, respectively. On the other hand, De-Deus et al. [15] searched for a less toxic concentration to be used as chelating agent and found that 0.5% peracetic acid was efficient.

As far as we are concerned, peracetic acid has not been used as irrigant solution, making difficult the comparison of our results with those of prior studies. Notwithstanding, it is known that this acid has antimicrobial capacity at low concentrations, even in the presence of organic matter [32]. According to Lottanti et al. [13], peracetic acid can be kept within root canal after instrumentation to associate the disinfection action with that of smear layer dissolution.

In this present study, the apical third exhibited the smallest amount of opened dentinal tubules, proving to be difficult to clean by the clinical protocols used for this purpose. According to Table 4, the apical third showed only 30.61% of opened dentinal tubules. This result is in agreement with many studies on smear layer removal, in which the apical third is the most difficult to clean [33-36].

Therefore, based on the analysis and discussion of the results, further studies are necessary to verify the use of peracetic acid as irrigant solution since its chemical, physical and biological properties have not been totally known yet.

**CONCLUSION**

According to the results obtained, it can be concluded that:

a) The removal of smear layer was similarly achieved in all groups, without statistically significant differences among the irrigant solutions and rotary systems tested;

b) The cleaning at the apical third was worse than that of the cervical and medium thirds in all groups evaluated, regardless of either the irrigant solution or the instrumentation technique;

c) Neither technique nor the irrigant solution was capable of promoting the total smear layer removal from root canal walls.

**ACKNOWLEDGMENTS**

The authors are not affiliated with any of the companies related to this study or its sponsors. The authors are grateful to Ivan Balducci for providing assistance with the statistical analysis of the results. This study was supported by FAPESP (Process 2011/22022-0).
REFERENCES


Evaluation of 0.5% peracetic acid and 2.5% sodium hypochlorite on smear layer removal of root canal instrumented by three rotary systems

Gomes APM et al.


Ana Paula Martins Gomes
(Corresponding address)
Institute of Science and Technology Department of Restorative Dentistry
Avenida Engenheiro Francisco José Longo, 777, Jardim São Dimas São José dos Campos, SP, Brasil, CEP 12245-000
e-mail: paula@fosjc.unesp.br

Date submitted: 2014 Jan 10
Accept submission: 2014 Feb 18