Effects of Irrigation Solutions on Root Canal Dentin

Paulina Grinkevičiūtė, DDS

Gintarė Leknickė, DDS

Greta Lodienė, DDS, PhD

Lithuanian University of Health Sciences
Odontology Faculty,
Department of Dental and Oral Pathology
Eivenių str. 2, LT-50161 Kaunas
Lithuania

Rita Kriūkienė, PhD
Research Associate
Lithuanian Energy Institute
Laboratory of materials research and testing
Breslaujos str. 3, LT-44403 Kaunas
Lithuania

Abstract

The aim of the study was to determine the irrigation solutions impaction on the root canal dentin micro-hardness and surface erosion. All irrigation solutions significantly decreased root canal dentin microhardness ($P < 0.05$). The greatest reduction was induced by Dual Rinse HEDP ($P < 0.05$), while 17% EDTA and 17% EDTA + 2.5% NaOCl showed a similar reduction with no significant difference ($P > 0.05$). SEM analysis did not show any erosion of the dentin surface in the randomly selected specimens. In this study, irrigation solutions have not induced root canal dentin erosion, but significantly reduced the dentin micro-hardness. The greatest reduction was induced by Dual Rinse HEDP.

Keywords: Vickers hardness test, dentin micro-hardness, sodium hypochlorite, EDTA, SEM.

1. Introduction

One of the purposes of endodontic treatment is to disinfect the root canal system, eliminate the microorganisms, their products and protect against the reinfection. The complexity of root canal system complicates the mechanical cleaning of the canal and the removal of the biofilm as well (Plotino *et al.* 2016). Irrigation solutions affect the chemical composition of dentin, which may have an impact on its physical properties (Pashley *et al.* 1985). Erosion around dentin tubules results from the effects of the irrigation solution on collagen (Wagner *et al.* 2017). When sodium hypochlorite (NaOCl) is used alone, and the collagen fiber is covered with hydroxyapatite, then the solution is limited to dentin. However, if a decalcification solution is used, hydroxyapatites are removed and thus collagen fibers are uncovered. The sodium hypochlorite solution then has a direct effect on the protein (collagen) and over a fairly short time causes significant collagen destruction, which could change the bending elasticity of the dentin (Grigoratos *et al.* 2001, Tartari *et al.* 2018). It was shown that 17% ethylenediaminetetraacetic acid (EDTA) and 2.5% NaOCl irrigation for 1 min. removes more Ca^{2+} ions from the root canal dentin than using 17% EDTA alone (Sayin *et al.* 2007). Due to the changes in organic and inorganic materials, micro-fluidity in the dentine may occur which could reduce the fracture resistance to fracture (Uzunoglu *et al.* 2012). Recently, etidronate (HEBP), a substance that prevents bone resorption has been used in medicine for patients suffering from osteoporosis or Paget´s disease and was suggested as substitute for traditional chelators due to fewer effects observed on dentin structure (Tartari *et al.* 2013). According to literature, etidronic acid has a less impact on dentin structure than EDTA (Ulosoy *et al.* 2017). EDTA was found to inhibit the ability of NaOCl to dissolve organic tissues, while 1-hydroxyethane 1,1-diphosphonic acid (HEDP) had a slight effect on this sodium hypochlorite property (Tartari *et al.* 2015). Using 2.5% NaOCl in combination with 9% etidronic acid, the smear layer is removed in a similar way to EDTA or citric acid (Gautschi *et al*. 2009). Thus, while treating dentin with HEBP and NaOCl, the latter's antibacterial properties are not lost and one universal irrigation solution is obtained (De-Deus *et al.* 2008). Solutions for the last canal irrigation prior to obturation are important for the root canal filling quality and the long-term tooth prognosis.

The use of EDTA before root canal filling has been shown to improve the antibacterial properties of root canal sealers (Ulosoy et al. 2017). If sodium hypochlorite is used during the last irrigation, resistance to vertical root fracture is reduced (Uzunoglu *et al.* 2012). It is important to evaluate the duration of irrigation and concentration of solutions in order to minimize dentin erosion (Uzunoglu *et al.* 2012). Recently, Dual Rinse HEDP (Medcem GmbH, Switzerland) was introduced. According to the manufacturers, this single product is efficient for root canals irrigation due to physicochemical properties and reduces the probability of the occurrence of undesirable chemical reactions by mixing of irrigation solutions. Recently was found that irrigation with Dual Rinse HEDP significantly improved the push out bond strength of Biodentine to the root canal dentin (Paulson *et al*. 2018).

The aim of the study was to determine irrigation solutions impaction on the root canal dentin micro-hardness and surface erosion. The null hypothesis was that solutions used for the last root canal irrigation prior to obturation: 17% EDTA, EDTA in combination with 2.5% NaOCl and Dual Rinse HEDP reduce dentin microhardness and cause changes in the dentin surface structure.

2. Materials and methods

Lithuanian University of Health Sciences Bioethical Center has approved the research (No. BEC-OF-10).

2.1. Selection of teeth

41 single-rooted teeth were collected and placed in a 10% formalin solution until the experiment. Teeth were examined under a dental microscope (Carls Zeiss Meditec Inc., Göttingen, Germany) with 12.5x magnification to identify cracks, caries or resorptions. After examination, 28 teeth were selected for the experimental procedure. The surfaces of the roots were planed and washed with saline.

2.2. Specimens preparation

Teeth were decoronated at the CEJ level with a 0.2 mm disc (Yeti, Engen, Germany) with water cooling, horizontally sectioned in the apical third leaving a standard length of 10mm. The roots were cut along the axis with a 0.2 mm disc (Yeti, Engen, Germany) and divided into two parts. The 56 dentin specimens were examined under a microscope (Carls Zeiss Meditec Inc., Göttingen, Germany) with 12.5x magnification to identify any cracks. No cracks were observed. 30 mm diameter oval, plastic molds were filled up with self-polymerizing resin (Interdent, Celje, Slovenia). Specimens were horizontally fixed on the resin surface (Fig. 1). As the Vickers hardness test requires a smooth and polished surface, the samples were polished with a carborundum disk and felt (Picodent, Wipperfürth, Germany) using 0.05 μm aluminum oxide powder (Picodent, Wipperfürth, Germany) mixed with water.

Figure 1.Prepared samples.

2.3. Irrigation solutions usage

For the testing in this study 17% EDTA (i-dental, Vilnius, Lithuania), 2.5% NaOCl (Cerkamed, StalowaWola, Poland), Dual Rinse HEDP (Medcem GmbH, Weinfelden, Switzerland) were chosen. Fresh Dual Rinse HEDP solution prepared in a sterile container according to the manufacturer's recommendations. HEDP powder of 0.9 g mixed with 10 ml 2.5% NaOCl and stirred for 2 min. until dissolved.

Different final irrigation protocols for each group were provided as follows: C group (n=14) - control, dentin was exposed to saline for 1 min., E group (n=14) - dentin was exposed to 17 % EDTA for 1 min., ES group (n=14) - dentin was exposed to 17% EDTA for 1 min. + 2.5% NaOCl for 1 min. (1ml distilled water was used between the solutions)

and H group (n=14) - dentin was exposed to Dual Rinse HEDP solution for 3 min. After the experimental procedure all specimens were rinsed with 2 ml of distilled water.

2.4. Vickers hardness test

Dentin micro-hardness was determined by the Vickers hardness test. The specimens were mounted with a Vickers diamond indenter at 300 g load and a dwell time of 20 sec. The indentation was measured by a computer program using a 10x and 50x magnification optical microscope installed in the universal hardness measurement system (UH 250, Tukon, Germany). Three measurements (Fig. 2) were performed of each sample before and after the irrigation solution use and mean values were calculated (Fig. 3).

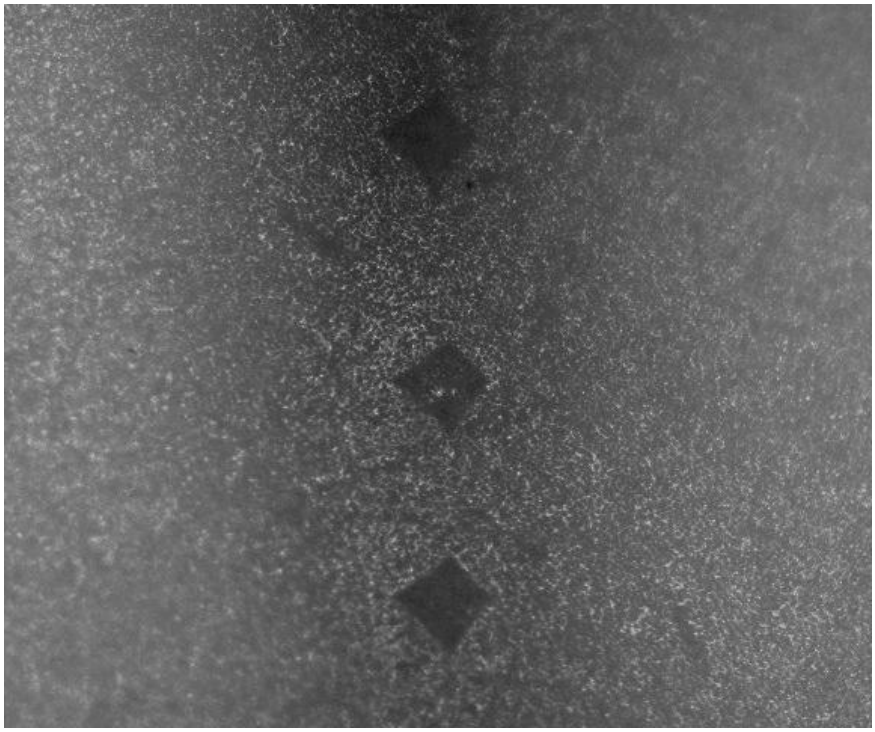

Figure 2. Indentations marked in the mid-root region.

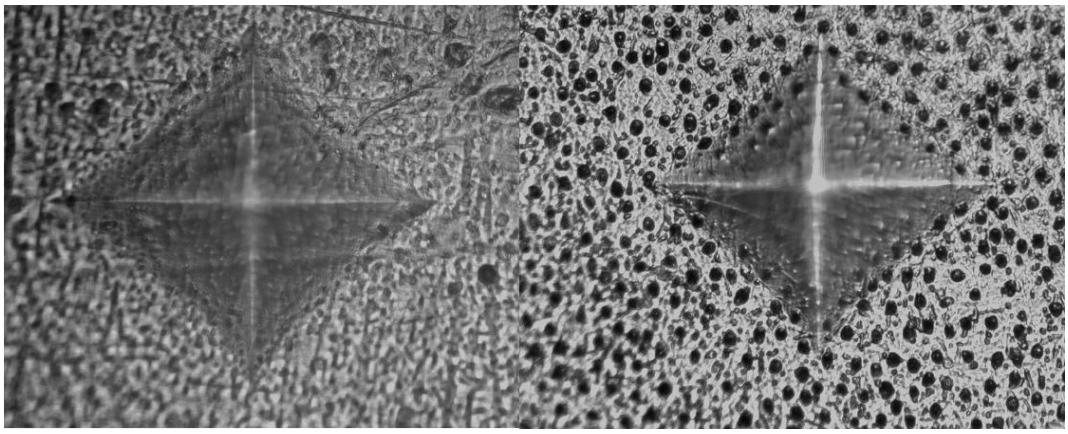

Figure 3.Indentation before and after Dual Rinse HEDP irrigation.

2.5. Scanning electron microscopy

Photographs were taken of randomly selected specimens from each group by scanning electron microscope (SEM) (Carl Zeiss EVO MA10, Göttingen, Germany) using 2000x magnification, EHT=10-20x.

2.6. Statistical analysis

Statistical analysis of the data was conducted using the SPSS 17.0 program. The Kolmogorov-Smirnov test was performed to verify the normality of the data.

Inferential statistical analysis was performed using a Kruskal-Wallis and Mann-Whitney test to detect a significant difference between the groups. The Wilcoxon test was designed to examine the dependent groups. The level of significance was set at $P < 0.05$.

3. *Results*

Root canal dentin microhardness alteration before and after use of the irrigation solutions is shown in Table 1. All irrigation solutions significantly decreased root canal dentin micro-hardness compared to saline (control group) ($P < 0.05$). The greatest reduction was induced by Dual Rinse HEDP ($P < 0.05$) while 17% EDTA and 17% EDTA + 2.5% NaOCl showed a similar reduction with no significant difference ($P > 0.05$) (Fig. 4).

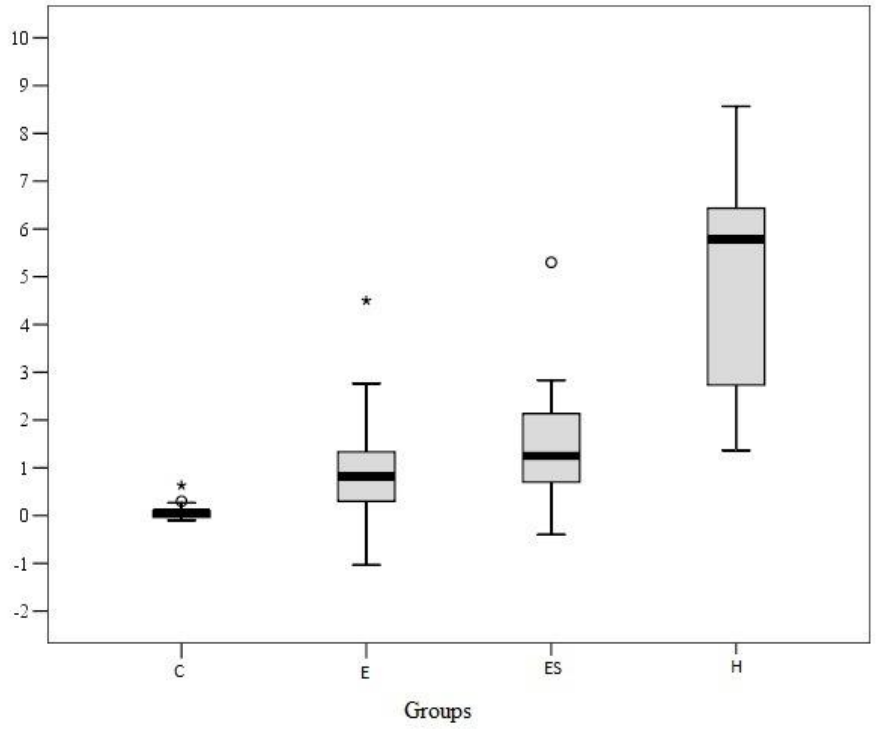

Figure 4. The mean of micro-hardness change observed among experimental groups.

Group	Mean (SD)	
	Before	After
C(Saline)	52,89 (2,93) [a]	52,80 (2,91) [a]
E(17% EDTA)	52,55 (5,40) [a]	51,55 (5,66) [b]
ES(17% EDTA + 2,5% NaOCl)	52,99 (2,73) [a]	51,42 (2,48) [b]
H(Dual Rinse HEDP)	54,79 (4,38) [a]	49,74 (3,61) [c]

Table 1.Values of root canal dentin micro-hardness before and after irrigation.SD, standard deviation; Different superscript letters indicate significant differences (analysis of variance, $P < 0.05$).No pathological changes in the dentin surface were observed in SEM images. Erosion of the peritubular and intertubular dentin was not observed in either group (Fig. 5).

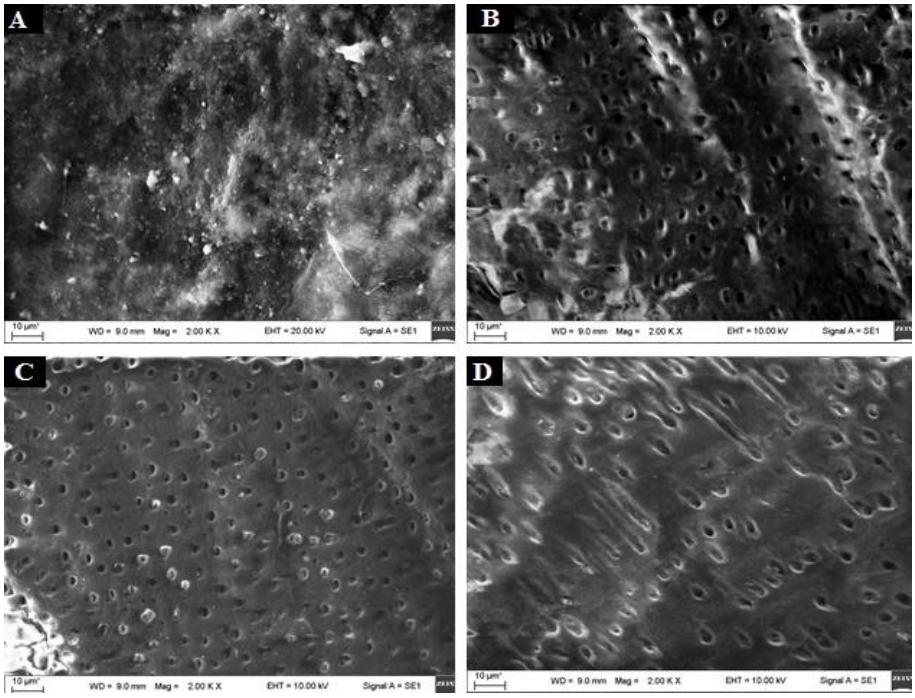

Figure 5.Scanning electron microscopy images of samples (2000 x). A (C group) - smear layer of the dentine. Uncovered dentin tubules, no visible erosion in all groups: B (E group), C (ES group), D (H group).

4. Discussion

The long-term prognosis of endodontic treatment depends on the quality of the instrumentation, disinfection and obturation of the root canal system. During irrigation, both coronal and root dentin are affected by irrigation solutions, what may have an impact on the physical and chemical properties including the dentin microstructure. Ideal irrigation solution should remove both the organic and inorganic parts in all thirds of the canal without damaging the dentine (Lofti *et al*. 2012). In this study, root canal dentin was not pretreated with instruments and NaOCl trying to avoid decrease in micro-hardness or causing the erosion because the purpose of research was to evaluate the effect on dentin of final irrigation solutions.

Widely used Vickers hardness test was chosen in this study because of its ability to assess the changes in human hard tissue due to chemical exposure (Kara *et al*. 2015, Ballal *et al.* 2010, Baldasso *et al.* 2017). This test could be used with all materials and test specimens as the procedure covers the entire hardness range and ensures indentation very clear and easy measure.

Micro-hardness of the dentin differs depending on the tooth area and number of tubules (Pashley *et al.* 1985). In order to obtain the most accurate results, in previous studies, dentin micro-hardness was measured before and after the use of irrigation solutions (Kara *et al*. 2015, Ballal *et al.* 2010, Aranda-Garcia *et al.* 2012, Dineshkumar *et al.* 2013). However, there are similar studies comparing micro-data only with the control group and not with pre-study data (Baldasso *et al.* 2017, Saghiri *et al*. 2012). In this study, dentin micro-hardness was measured before and after the use of irrigation solutions and compared to the control group with no effect.

When using EDTA, the recommended time for removing the smear layer is 1 min. (Gautschi *et al.* 2009). In this study, the dentin surface had been irrigated by 17% EDTA for 1 min. what significantly reduced the dentin micro-hardness. This result is in agreement with other studies (Baldasso *et al.* 2017, Patil et al. 2011, Dineshkumar *et al.* 2012). Calt *et al.* 2002 concluded, that EDTA solution should be applied for a maximum of 1 min. to avoid dentin erosion and found out that EDTA produces erosive effect in both peritubular and intertubular dentine, if exposition time is longer than 10 min. However, in most of the previous studies, EDTA was used longer than one minute, usually 3-5 min. or even 15 min., making the changes of dentin hardness more obvious (Patilet al. 2011, Lofti *et al*. 2012, Kara *et al*. 2015, Baldasso *et al.* 2017). Saha *et al*. (2017) used EDTA 17% for 15 min., resulting in very significant micro-hardness change ranging from 56.88 ± 1.48 VHN to 43.12 ± 2.51 VHN.

Sodium hypochlorite is associated with a significant decrease in micro-hardness in *in vitro* studies (Kara *et al*. 2015, Baldasso *et al.* 2017, Dineshkumar *et al.* 2012). In other study was found that EDTA significantly reduced dentin micro-hardness and caused erosion when the final irrigation with 2.5% NaOCl was performed (Garcia et al. 2013).

Greater dentin microhardness decrease after EDTA irrigation following NaOCl compare to EDTA use alone was obtained in this study but the difference was not significant. These results could be due to time the irrigation solutions affected the dentin - no longer than 1 min.

The effect of Dual Rinse HEDP on the dentin micro-hardness and dentin surface has not been studied before. In this study, the Vickers hardness test showed the greatest effect of this solution on dentin micro-hardness reduce compared to other irrigators. These results could be due to the long working time of the solution - 3 min., which is recommended by the manufacturers. These results are in agreement with Tartari *et al.* (2015) study, where HEBP (editronic acid) significantly reduced dentin micro-hardness, although irrigation solutions interacted longer, HEBP did not interact with NaOCl at the same time and Knoop micro-hardness test was used instead of Vickers.

In this study, after evaluation the surfaces by SEM no dentin erosion was observed in the tested samples. However, there are contradictory results in the literature. NaOCl causes erosion of the dentin surface when used after the action of demineralizing solutions (Ballal *et al.* 2010). In Wang *et al.* (2016) SEM study was found that sodium hypochlorite (3%, 5%) use after EDTA for 1 or 5 min. induced dentin erosion. After removing the hydroxyapatite layer, the collagen collapsed immediately after sodium hypochlorite use (Baldasso *et al.* 2017). Saghiri *et al*. (2009) found that after 5 minutes of using EDTA and NaOCl for 5 min. or 1 min, erosion could be observed inside and around the dentin tubules but after 5 min. erosion was more prevailing and this difference between groups was statistically significant. In other study, dentinal tubules erosion was promoted by using 17% EDTA and 2.5% NaOCl for 3 min. (Aranda-Garcia *et al.* 2013) but in this study, erosion of the dentin was not observed in either group. The contradictory results could be due to the time irrigation solutions affected the dentin. The time when irrigation solutions affect the dentin surface may be a crucial factor in the development of dentin erosion.

5. Conclusions

Irrigation solutions have not induced root canal dentin erosion, but significantly reduced the dentin micro-hardness. The greatest reduction was induced by Dual Rinse HEDP.

Conflict of interest

The authors have stated explicitly that there is no conflict of interests in connection with this article.

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