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In vitro evaluation of the effect of vinegar solutions on the microhardness of root dentin

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Abstract

Objective: This *in vitro* study evaluated the action of vinegar solutions on microhardness of root dentin.

Methods: Ten extracted human maxillary central incisors were sectioned transversely at the cement-enamel junction. The roots were fixed on cutting machine device and were cut on cervical region transversely in 2-mm sections. The second cervical section was divided into four parts. Each one quarter of section was fixed with wax in the center of a pre-fabricated acrylic disc. Four treatment groups were tested: group 1 – alcohol vinegar; group 2 – white wine vinegar; group 3 – EDTA (standard group); group 4 – distilled water (control group). The specimens were submitted to solutions application for 5 min. The measurements of microhardness (initial and post-treatment) were carried out using a Knoop microhardness tester. Data were analyzed by using Wilcoxon and Kruskal Wallis tests.

Results: The analysis of initial and final microhardness showed statically significant differences before and after application of the tested solutions alcohol vinegar, white wine vinegar and EDTA. Groups 1,2 and 3 presented a decrease in the microhardness values.

Conclusion: The solutions of alcohol vinegar and white wine vinegar, as well as the EDTA, reduced the microhardness of root dentin.

Keywords: Acid acetic; dentin; EDTA; hardness test; root canal irrigants

Avaliação *in vitro* do efeito de soluções de vinagre sobre a microdureza da dentina radicular

Resumo

Objetivo: Esse estudo *in vitro* avaliou a ação de soluções de vinagre sobre a microdureza da dentina radicular.

Métodos: Dez incisivos centrais superiores foram seccionados transversalmente na junção cemento-esmalte. As raízes foram fixadas no dispositivo de corte da máquina e foram cortados transversalmente na região cervical em secções de 2 mm. A segunda secção cervical foi dividida em quatro partes. Cada quarto da secção foi fixado com cera no centro de um disco de acrílico pré-fabricado. Os espécimes foram distribuídos em quatro grupos de tratamento: grupo 1 - vinagre de álcool; grupo 2 - vinagre de vinho branco; grupo 3 - EDTA (grupo padrão); grupo 4 - água destilada (grupo controle). Os espécimes receberam aplicação de soluções durante 5 min. A microdureza (inicial e pós-tratamento) foi medida utilizando um aparelho de microdureza, em escala Knoop. A análise estatística foi realizada através de testes Wilcoxon e de Kruskal Wallis.

Resultados: A análise de microdureza inicial e final mostrou diferença estatisticamente significativa antes e após a aplicação das solução de vinagre de álcool, vinagre de vinho branco e de EDTA. Os grupos 1,2 e 3 apresentaram uma diminuição dos valores de microdureza.

Conclusão: O vinagre de álcool e vinagre branco, bem como o EDTA, promoveram redução da dureza da dentina radicular.

Palavras-chave: Ácido acético; dentina; EDTA; testes de dureza; irrigantes do canal radicular

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Introduction

During the endodontic instrumentation using either manual or mechanized techniques, the dentin walls become covered by smear layer, a amorphous layer of inorganic and organic components as particles of calcified tissue and organic elements such as necrotic or viable pulp tissue debris, odontoblastic processes, microorganisms, blood cells and coagulated protein in dentinal tubules [1]. The presence of this layer on the dentin walls reduces the permeability, the medications diffusion and endodontic cements penetration into the dentin tubules, hampering the sealing of the obturation [2,3].

Differents solutions and combinations have been used to remove the smear layer, including EDTA, citric acid, phosphoric acid and MTAD, a mixture of tetracycline isomer (doxycycline), acid citric and detergent (Tween 80) [3-5]. The combination EDTA and sodium hypochlorite is most frequently used to remove the inorganic and organic components of the smear layer [6]. The chelating action of EDTA alter the mineral content of dentin inducing an adverse softening potential on dentin components and the NaOCl dissolves the organic portion. This chemical pre-treatments induce considerable changes in the morphology surface of dentine that may also exert effect in its physical, mechanical and chemical properties and consequently reducing the microhardness [6,7].

Several studies have shown the effect of these solutions on root dentin microhardness [8,9]. Furthermore, by reducing the microhardness, these solutions help during chemical-mechanical preparation, facilitating the access and action of endodontic instruments especially in narrow and calcified root canals [10]. Moreover, the changes caused by these solutions may alter the substrate for bonding [11]. A strong relationship exists between dentine microhardness and the respective bond strength [12]. Yoshiyama et al. [13] evaluated the bonding of self-etch and total-etch adhesives to carious dentin. They concluded that a fall in the bond strength values was accompanied by a decrease in Knoop microhardness values.

Vinegars are composed by acetic acid (about 5% in concentration) [14]. Some studies have shown that vinegars are able to remove smear layer and open dentinal tubules, increasing the permeability and decrease the microhardnesss of the most superficial root canal dentin layer [10,14,15]. Zandim et al. [15] observed in vitro that alcohol, apple, rice, white wine and balsamic vinegar were able to remove smear layer and exposure of dentinal tubules, of these, the balsamic vinegar was associated with less removal of smear layer.

The acetic acid used in the vinegar production, gives the possibility of a new alternative solution. Thus, this study aimed to evaluate the effect of different solutions of vinegar on the microhardness of root dentin.

Methods

Ten extracted human maxillary central incisors were sectioned transversely at the cement-enamel junction and the crowns were discarded. The roots were fixed by cutting machine device and the cervical region was cut transversely in 2-mm sections. The second cervical section was divided into four parts. Each to which was fixed with wax in the center of a pre-fabricated acrylic disc with the cervical side facing up. For the sectioning, an electric cutting machine Mecatome P100 was used (Presi, Grenoble, France) with water-cooled diamond disc (Presi, Grenoble, France).

The surface to be analyzed was submitted to sandpapering and polishing using an Aropol 2V polisher (Arotec, São Paulo, Brazil) with 600- and 1200-grit sandpapers. For the final polishing, a self-adhesive polishing cloth (Tex Met 1500, PSA backed, Buehler, Lake Bluff, Illinois, USA) together with a diamond paste (Metadi Diamond Suspension 1 micron- Blue Color Polish Spray – Water Base nº 40-6530, Buehler, Lake Bluff, Illinois, USA) was used. After the polishing with the diamond suspension, the specimens were immersed in detergent (Ultramet Sonic Cleaning Solution, Buehler, Lake Bluff, Illinois, USA) and submitted to ultrasonic agitation.

The hardness test was carried out using a microhardness tester HMV-2 (Shimadzu, Tokyo, Japan) with a Knoop penetrator. Initially, at a distance of 1.500 µm from the root canal, a reference indentation, was made using a load of 100 g for 15 s to facilitate the localization of indentations before and post-treatment. Then, at 500 µm from the reference indentation, with a load of 25 g for 15 s, five parallel indentations were made, separated from each other by a distance of 100 µm. The area selected for the indentations must not have potential irregularities and must allow other indentations to be made, post-treatment, at 100 µm from the first ones using a load of 25 g for 15 s.

Prior to application of the solutions, the endogenous pH at room temperature of each solution was determined by a digital pH meter (pH meter DPMH-2, Digimed, São Paulo, Brazil). After calibration of the apparatus, 50 ml of each solution was transferred to a glass of polyethylene, being performed immersion of the electrode, followed by reading and recording.

The samples were selected according to Knoop hardness. Considering that the average Knoop dentin hardness is 68±3 [16], the samples with an average of 10% above or below this value were excluded from the research. Forty samples were randomly assigned for each experimental group: Group 1 – alcohol vinegar; Group 2 – white wine vinegar; Group 3 – 17% EDTA; Group 4 – distilled water. The samples were treated with 50 µl of the experimental solution for a period of five minutes. Table 1 presents the solutions tested in this research according experimental groups.

Immediately after the solutions application, the samples were washed with distilled water and dried with paper towels and then resubmitted to the determination of microhardness. To the determination of microhardness, the new indentations were carried out at a distance of 100 µm from the initial ones.

Table 1. Descriptive table of solutions tested in the research according to experimental groups.

Group – Experimental solution	Composition	Tested Volume	Brand and Manufacture
1 – Alcohol vinegar	Acetic fermented of alcohol and water	50 µl	Minhoto, Ind. Reunidas Raymundo da Fonte S.A.
2 – White wine vinegar	Acetic fermented of White wine and water	50 µl	Minhoto, Ind. Reunidas Raymundo da Fonte S.A.
3 – 17% EDTA	Disodium ethylenediaminetetraacetic acid	50 µl	Inodon, Inodon Ltda.
4 – Distilled water	–	50 µl	–

Statistical analysis was performed by using non-parametrics tests at 5% significance level with the MatLab® software package. To analyse of the paired dates, before and after the treatment with the tested solutions, we used Wilcoxon Test. To determine statistical differences among the non-paired data, we used Kruskal Wallis test.

Results

The pH values of solutions were 5.28 (alcohol vinegar), 2.76 (white wine vinegar), 5.89 (17% EDTA) and 5.28 (distilled water).

All experimental groups showed initial Knoop hardness statistically similar, with no statistically significant difference (Kruskal Wallis test, P=0.7268). After the application of the experimental solutions, it was observed statistically significant difference among groups (P<0.001).

The Wilcoxon test not revealed statistically significant differences between initial and final microhardness on control group (distilled water). However, statistically significant difference was observed between initial and final microhardness of the groups white wine vinegar, alcohol vinegar and EDTA (Table 2).

Table 2. Average (AVG) Knoop hardness and standard deviations (SD) of initial and final Knoop hardness; comparison of microhardness before and after the application of tested solutions.

Irrigation solution	n	Initial knoop hardness (AVG±SD)	Final knoop hardness (AVG±SD)	*P*-value
White wine vinegar	10	67.55±3.81	49.51±4.79	0.002
Alcohol vinegar	10	69.22±2.45	47.12±3.57	0,.002
EDTA	10	67.27±4.78	46.53±3.81	0.002
Distilled water	10	67.66±3.12	65.97±4.63	0,108

Discussion

The efficiency of the chelating agents in the removal of the smear layer, demineralization and reduction of microhardness can be analyzed through various methods such as Atomic absorption spectrometry, scanning electron microscopy [17], digital optical microscopy [18] and microhardness tests [8-10]. The microhardness test is widely used to classify materials and compare changes in their properties, being a simple, effective and well defined methodology. Also, it is a nondestructive method, and can be used sequentially on the same dentin specimen, a property fundamental to the analysis of microhardness before and after the application of experimental solutions [19].

Hardness tests measure hardness in terms of deformation of the specimen by penetration of a standard-shaped point applied by a specified machine. These tests have long used various types of indenters as Vickers and Knoop. The Knoop microhardness uses a pyramidal-diamond indenter [20]. Our purpose in this study is evaluates *in vitro* the action of vinegar solutions on microhardness of root dentin by Knoop hardness test. The Knoop indenter possesses certain advantageover other similar hardness measuring tools [20]. The chief characteristic of the Knoop hardness test is its sensitivity to surface effects and textures [21,22]. For a given load, the Knoop impression depth is less than the half the depth of impression caused by Vickers, so the Knoop hardness is able to measure the hardness of materials extremely fragile [23].

The present study has compared the action of EDTA, a chelating agent widely used, with alcohol vinegar and white wine vinegar on microhardness of root dentin. Other studies evaluated the properties of vinegars on dentin [10,14,15] however, there is no study in the literature evaluating the effect of alcohol and white wine vinegars on microhardness of root dentin.

The results obtained in this study indicate that the EDTA, as well as the alcohol and white wine vinegars, were able to reduce the microhardness of the root dentin. This finding corroborates with previous studies that have shown that EDTA significantly decreases the root canal dentin microhardness [8,9]. Cruz Filho et al. [10] evaluated the effect of different chelating solutions on the microhardness of the most superficial dentin layer from the root canal lumen and observed that apple vinegar, acetic acid, and malic acid had a similar reducing effect on microhardness to each other and smaller than that of EDTA and citric acid.

The considerable reduction in dentin microhardness achieved by the alcohol and white wine vinegars, observed in this study, may indicate that these solutions have an effect on the structural components of dentin. Spano et al. [17] showed using atomic absorption spectrophotometry that apple vinegar removed amounts of calcium ions from the root canal similar to 5% malic acid, and 5% acetic acid. These solutions were less effective than 10% citric acid and the EDTA resulted in the greatest concentration of calcium ions.

The efficacy of a chelating agent is dependent on the time for which it remains in the root canal, since the solution does not act immediately when placed in contact with the dentin, requiring some minutes to obtain a chelating effect [24]. Prado et al. [4] observed that 37% phosphoric acid, 17% EDTA and 10% citric acid at 30 seconds were not effective for removing the smear layer. In 1 minute, the phosphoric acid solution showed better results than the others substances evaluated. In 3 minute, all the substances worked well in the middle and cervical thirds although phosphoric acid solution showed excellent results even in the apical third. Also, EDTA has a self-limiting action, since one molecule chelates one mol of metal ion. Over time there is a reaction with calcium ions, neutralization and loss of chemical action, it thus requires constant renewal [25]. For this reason, in this study the solutions were renewed over the specimen surface during the five minute period allowing the time required for an evaluation of its chelating activity.

Conclusions

The alcohol and white wine vinegars, as well as the EDTA, were effective in reducing the dentin microhardness, and were shown to be promising alternative chemical substances for use in Endodontics. However, although the objective of this study was achieved, it is fundamental that more research be carried out in order to investigate the other physico-chemical and biological properties of these vinegars. Further studies are needed to evaluate the depth of demineralization and erosion caused by this solutions and its influence on root canal filling.

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