EFFECT OF EDTA AND CITRIC ACID SOLUTIONS ON MINERAL CONTENT AND MICROHARDNESS OF ROOT CANAL DENTIN
(AN IN VITRO STUDY)

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INTRODUCTION

Irrigation is one of the most important aspects of root canal preparation. It could help to clean those areas of the root canal system that could not be directly planed by instruments. The ideal irrigant has a number of functions: lubrication, debridement, antibacterial effect and removal of debris and smear layer. Unfortunately, there has not been found an irrigant up till now that could perform all functions above. So, we usually use two or three kinds of irrigants alternatively in clinic to get the most perfect cleaning effectiveness.

There are many different types of root canal irrigants that are used in root canal treatment. The mostly widely used were sodium hypochlorite (NaOCL) and ethylene diamine tetra-acetic acid (EDTA) solution. Each kind of irrigant has its own efficiency NaOCL can only dissolve organic matter and has no effect on inorganic matter that mostly existed in the root canal system. EDTA can only remove the inorganic matters of the smear layer. NaOCL and EDTA were usually alternatively to get a clean root canal wall. (1)

The effect of chelating agents used during root canal treatment had been examined with a variety of methods. Microhardness measurements, micro radiographic assessments and, most often, electron microscopic investigations had been carried out to evaluate the efficiency of these agents in removing smear layer and demineralizing and softening root dentin.

Citric acid with different concentrations has been recommended for root canal irrigation. This irrigating solution promotes smear layer removal, dentin wall cleaning and root canal disinfectant.

It has been reported that these types of chemical agents caused alterations in the chemical structure of human dentin and changed the calcium/phosphorous (Ca/P) ratio of the dentin surface. The alterations in Ca/P ratio may change the original ratio between organic and inorganic components that in turn change the permeability, solubility characteristics of dentin and may also affect the adhesion of dental materials to hard tissues.

REVIEW OF LITERATURE

1- Effect of EDTA, citric acid and other chelating agents on microhardness of root canal dentin:

Lewinstein and Rotstein (1992)[2] examined the effect of 90% trichloracetic acid on the microhardness and surface morphology of human dentin and enamel. Intact extracted human teeth were sectioned and embedded in acrylic resin. Each tooth was grinded and highly polished exposing a flat surface of dentin and enamel. The teeth were treated with 90% trichloracetic acid for 30, 60 and 90 s. Vicker's microhardness of the dentin and enamel was assessed for each tooth before and after each treatment. In addition the surface morphology of a trichloracetic acid treated tooth was examined via SEM. The results showed that 90% trichloracetic acid caused a second order type reduction of the microhardness, as well as structural changes in both dentin and enamel.

Lewinstein et al. (1994)[3] examined The effect of 30% hydrogen peroxide and a paste of sodium perborate mixed with hydrogen peroxide at different temperatures and time intervals on the microhardness of human enamel and dentin. Intact extracted human teeth were sectioned, embedded in acrylic resin, polished, and divided into four test groups related to surface treatment. The groups were 30% hydrogen peroxide at 37 degrees C, 30% hydrogen peroxide at 50 degrees C in an illuminated chamber, a paste of sodium perborate mixed with hydrogen peroxide at 37 degrees C, and a paste of sodium perborate mixed with hydrogen peroxide at 50 degrees C in an illuminated chamber. Teeth treated with distilled water at either 37 degrees C or 50 degrees C served as controls. The results indicated that treatment with 30% hydrogen peroxide reduced the microhardness of both enamel and dentin. This reduction was statistically significant after 5-min treatment for the dentin and after 15-min treatment for the enamel. Treatment with sodium perborate mixed with hydrogen peroxide did not alter the microhardness of either the enamel or dentin at the tested temperatures and time intervals. It is therefore suggested that the use of high concentrations of hydrogen peroxide for bleaching purposes should be limited. Sodium perborate appears to be a less damaging bleaching agent.

Saleh and Ettman (1999)[4] studied the effect of endodontic irrigation solutions on microhardness of root canal dentin. Combination of 3% H_2O_2, 5% NaOCL and 17% EDTA solutions were used in this study. The specimens were subjected to Vickers microhardness tester. Eighteen freshly extracted maxillary incisors were used in this experiment. The prepared roots were divided equally into two groups each of nine roots. Each root was sectioned transversely into cervical, middle and apical segments. The three sections of each root were separately mounted in a metal chuck with acrylic resin. The coronal dentine surfaces of the root segments were polished. The microhardness of the dentine was measured for the purposes of control data at 500 microns and 1 mm from the pulpo-dentinal interface. The canal portions in the root segments included in the first group were irrigated with 3% H2O2 and 5% NaOCl solutions used alternatively, while 17% EDTA solution was the irrigation used in the second group. They concluded that both H_2O_2/NaOCL and 17% EDTA irrigating solutions significantly reduced the microhardness of root canal dentin.

Akisue et al. (2000)[5] evaluated the effect of 17% EDTA and 25%citric acid solution in the microhardness of the dentin. Sixty five dentin fragments coming from the medium third of 4 superior canine teeth were immersed in the solutions to be tested by periods as described following: Group 1: physiologic solution (control), Group 2: 17% EDTA solution for 3 min, Group 3: 17% EDTA solution for 15 min, Group 4: 25% citric acid solution for 3 min, Group 5: 25% citric acid solution for 15 min. The microhardness values was measured using a carl-zeiss microhardness tester unit. The 25%citric acid showed more reduction in microhardness than 17%EDTA and 15 minutes period of time resulted in more reduction in microhardness.

Cruz-Filho et al. (2001)[6] evaluated the effect of 15% EDTAC, 1% CDTA and 1% EGTA on microhardness of root canal dentine. Five newly extracted maxillary incisors were sectioned transversely at the cemento-enamel junction, and the crowns were discarded. The roots were embedded in blocks of high-speed polymerized acrylic resin and cut transversely into 1-mm sections. The second slice of the cervical third of the root of each tooth was sectioned and divided into four parts. Each part was placed on an acrylic disc that was used as a base for microhardness measurement. Fifty microliters of 15% EDTAC, 1% CDTA, or 1% EGTA were applied to the dentin surface. Deionized and distilled water was used as control. The microhardness values were measured with vicker's microhardness 50 gm for 15 s. All three irrigating solutions reduced microhardness of root canal dentin with no statistical significant difference.

Da cruz-Filho et al. (2002)[7] evaluated the effect of 1%,3% and 5% EGTA on microhardness of root canal dentin of the cervical third of human teeth. Five newly extracted maxillary incisors were sectioned transversely at the cementoenamel junction, and the crowns were discarded. The roots were embedded in blocks of high-speed polymerized acrylic resin and cut transversely into 1-mm sections. The second section of the cervical third of the root of each tooth was sectioned and divided into four parts. Each part was placed on an acrylic disc that was used as a base for microhardness measurement. Fifty microliters of 1% EGTA, 3% EGTA, or 5% EGTA were applied to the dentin surface. Deionized and distilled water was used as control. Dentin microhardness was then measured with a load of 50 g for 15 s.

Results showed that the three concentrations of the chelating solution EGTA significantly reduced dentin microhardness when compared with water and there was a statistically significant difference among the three concentrations of the solution.

White et al. (2002)[8] determined that calcium hydroxide, mineral trioxide aggregate and sodium hypochlorite caused a change in the force required to fracture root dentin. Ten bovine central and lateral incisors were machined using various saws and drills to produce a cylinder of dentin with a 6.0-mm outer diameter 3.5-mm inner diameter and a length of 10 mm. The canal sides of the sections were then placed into Petri dishes containing a 1-mm depth of calcium hydroxide, mineral trioxide aggregate, sodium hypochlorite and physiologic saline (control). The shear was tested by using an Instron machine. A 32% mean decrease in strength was discovered for calcium hydroxide, a 33% decrease in strength for mineral trioxide aggregate, and a 59% decrease for sodium hypochlorite. They concluded that root dentin was weakened after 5 weeks of exposure to calcium hydroxide, mineral trioxide aggregate, or sodium hypochlorite.

Ari et al. (2004)[9] evaluated the effect of 0.2% chlorhexidine gluconate on the microhardness and roughness of root canal dentin compared with widely used irrigating solutions. Ninety, mandibular, anterior teeth extracted for periodontal reasons were used. The crowns of the teeth were removed at the

CEJ. The roots were separated longitudinally into two segments, embedded in acrylic resin, and polished. A total of 180 specimens were divided into 6 groups of 30 teeth at random according to the irrigation solution used: group 1: 5.25% NaOCl for 15 min; group 2: 2.5% NaOCl for 15 min; group 3: 3% H2O2 for 15 min; group 4: 17% EDTA for 15 min; group 5: 0.2% chlorhexidine gluconate for 15 min; and group 6: distilled water (control). Each group was then divided into 2 subgroups of 15 specimens: groups 1a, 2a, 3a, 4a, 5a, and 6a were submitted to Vickers microhardness indentation tests; groups 1b, 2b, 3b, 4b, 5b, and 6b were used for determination of the roughness of root dentin The results indicated that all the irrigation solutions except chlorhexidine significantly decrease microhardness of dentin; 3% H_2O_2 and 0.2% chlorhexidine gluconate had no effect on roughness of root canal dentin. 0.2% chlorhexidine gluconate seems to be an appropriate endodontic irrigation solutions because of its harmless effect on the microhardness and roughness of root canal dentin.

Eldeniz et al. (2005)[10] evaluated the effect of citric acid and EDTA solutions on the microhardness and the roughness of human root canal dentin. Forty five human teeth sectioned longitudinally (90 halves) and specimens were randomly divided into three groups of 30 each and were treated as follow: (a) one molar (19%) citric acid for 150s followed by NaOCL 5.25%; (b) 17% EDTA for 150s and raised with 5.25% NaOCL;

(c) rinsed with distilled water and several as control. Three groups are then divided into 2 subgroups of 15 specimens each the specimen in the first subgroup subjected to Vicker's testing whereas the second subgroup underwent surface roughness testing. The conclusion was 10 mL 17% EDTA for 150s followed by 10 mL of 5.25% NaOCL for 150s for 19% citric acid followed by 5.25% NaOCL. Both were effective in removal of smear layer and resulted in decrease microhardness due to surface changes and increase mineral loss and also increase surface roughness.

De Deus et al. (2006) [11] evaluated the effect of 10% buffered citric acid, EDTA and EDTAC solutions on the microhardness of human root canal dentin. The samples were randomly divided into three groups according to the chelating agent employed as follows: group 1: EDTA 17%, Group 2: EDTA C 17%, Group 3: Citric acid 10%. Dentine microhardness was then measured with a load of 50 g for 15 s. At the beginning of the experiment, reference microhardness values were obtained for samples without any etching. The same samples were then exposed to 50 microL of the chelator solution for 1, 3 and 5 min. They found that microhardness decreased with increasing time of application of chelating solutions. There was no significant difference between EDTA and EDTA C after 5 min. citric acid caused significantly less reduction in microhardness. It was concluded that overall, citric acid was least effective in reducing dentin hardness, while EDTA had the strongest effect.

Qing et al. (2006)[1] evaluated the cleaning effect of root canal walls using strong acid electrolytic water (SAEW) as a root canal irrigant and investigated the influence of SAEW on the root canal dentin by micro-hardness test. Forty-three single-rooted, single-canaled teeth were instrumented using standard step-back technique with K-files. Irrigation was performed using distilled water, 5.25% NaOCl and 3% H_2O_2, SAEW and 15% EDTA solution in five groups. Samples were examined under SEM and subjected to Vickers microhardness test machine. Results showed that the root cleaning effects of the combined use of SAEW and NaOCl as root canal irrigants were equivalent to those in the group with NaOCl and 15% EDTA. There was no decrease in hardness of dentin when SAEW was used for 1min under ultrasonic vibration.

Marending et al. (2007)[12] assessed the impact of different irrigation sequences of NaOCL (2.5%; total exposure time, 24 min) and EDTA (17%; 3 min) on the elastic modulus and flexure strength of standardized human root dentin bars (n=11) per group. Exposures to EDTA (3 min), NaOCL (24 min) and water were used as control treatments. Results were evaluated by 3 point bending tests; modulus of elasticity and flexures strength values were compared between groups. They concluded that 24 min exposure to NaOCL; caused significant drop in flexures strength compared with water or EDTA treated controls whereas the elastic modulus remained unaffected. In contrast, the short exposure to EDTA was clinically recommended which did not affect mechanical dentin parameters under investigation, regardless of the irrigant sequence that was used.

Sayin et al. (2007)[13] evaluated the effect of single and combined use of EDTA, EGTA, EDTA plus EDTAC, Tetracycline – HCL and NaOCL on the microhardness of root canal dentin. The specimens were embedded into autopolymerizing acrylic resin leaving root canal dentin exposed. The microhardness at the apical, mid root and cervical levels of root canal dentin were evaluated by Vicker's microhardness test. After that the specimens treated with single (test solution only) or combined (test solution followed by 2.5% NaOCL) for 5 minutes post-treatment microhardness values were obtained. Pre and post treatment values were compared. The results were that all treatment regimens except distilled water significantly decrease the microhardness of root canal dentin. The single and combined use of EDTA decreased the microhardness of root canal dentin significantly more than all other treatment regimens compared with their single treatment version. All combined treatment regimens decreased the mean microhardness values significantly. The conclusion was that the use of EDTA alone or before NaOCL resulted in the maximum decrease in dentin microhardness. However, for combined treatment regimens, subsequent use of NaOCL levels the statistical differences between the regional microhardness values obtained after treatment with EGTA, EDTAC, and tetracycline HCL.

Saghiri et al. (2009)[14] evaluated the relation between erosion and microhardness of root canal dentin after irrigation with 2.6%NaOCl, 17%EDTA followed by 2.6%NaOCl for (5min), 17%EDTA followed by NaOCl for (1min), MTAD (5min), 2%chlorhexidine (5min) and saline (control). Pre- and post- treatment microhardness values was measured at depth 100µm and 500µm also amount of dentin erosion was measured by Scanning electron microscopic analysis. The results was 17%EDTA followed by 2.6%NaOCl (5min) showed most erosive effect with least reduction in microhardness at depth 100µm whereas MTAD showed most reduction in dentin microhardness and least erosive effect on dentin. They concluded that erosion was not the main factor in decreasing the dentin microhardness, whereas the amount of irrigant was the main cause.

Singh et al. (2009)[15] determined the effect of EDTA, EDTAC solutions, RC-Prep and *Biopure* MTAD on the Coronal, Middle and Apical root canal dentine. 40 samples were divided in to four groups (n=10). Group I samples were treated with 17% EDTA for 1 minute, Group II samples were treated with EDTAC for 1 minute (n=10),Group III samples were treated with RC-Prep for 1 minute, Group IV samples were treated with BioPure MTAD for 2 minutes and 5 minutes respectively. Results showed that there was no statistically significant difference in the microhardness reduction in the coronal, middle and apical third of the root canal dentin when treated with 17% EDTA, EDTAC, RC-Prep and BioPure MTAD. In all the four groups, microhardness of the root canal dentin was reduced. BioPure MTAD was least effective in reducing the microhardness of root canal dentine and 17% EDTA had the maximum effect.

Ballal et al. (2010)(16) evaluated the effect of 7% maleic acid and 17%EDTA solutions on microhardness and surface roughness of human root canal dentin. The samples were embedded longitudinally into acrylic resin. The microhardness values were measured using Vickers microhardness tester. And surface roughness was measured by roughness tester (Surtronic, Leicester, England). They concluded that maleic acid reduced microhardness as EDTA but increased surface roughness more than EDTA.

Rai et al. (2011)(17) evaluated the effect of decalcifying agents on the microhardness of root canal dentin by two ways in vitro and in vivo study. The decalcifying agents were 15% EDTAC liquid, 15% EDTA paste, 10% EDTA and 10%citric