DEMINERALIZING ACTION OF EGTA IN ENDODONTICS

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The demineralization of dentin obtained by treatment with a chelating agent ethylene diamminotetracetic acid (EDTA) or ethylene glycol-bis(β-aminoethyl ether)-N-tetraacetic acid (EGTA), is a dynamic process involving chelation and solubilization. The actions of the EDTA and EGTA on dentin are influenced by the pH. Increasing mM concentrations of EDTA or EGTA the equivalent pH decreases in a similar slope to 80 mM chelator concentration. Increasing the chelator concentration different data were obtained: with EGTA the pH decreases slightly while with EDTA goes back up to the initial values. After 80 mM, EDTA reduces the activity on the dentin, and EGTA continues to work at higher concentrations. We demonstrated that EGTA solubilized more of 60% of dentin while EDTA gives about 20% at the maximal of the solubility.

Biomechanical preparation of the root canal is an important phase in endodontic therapy. It is based on appropriate instrumentation and the use of irrigating and chelating solutions. These agents have the capacity to form complexes, called chelation complexes, binding metallic ions. The ethylenediamine tetra-acetic acid (EDTA) is considered the most active substance in this category, due to the value of the formation constant for calcium equal to log K= 10.7.

The use of a chelating agent may be useful to enhance cleanliness of the coronal and middle part of the root canal (1). The use of the chelating agent for a longer period does not increase its effect and therefore it is recommended to renew the solution every 15 minutes (2). Moreover, it has been shown that demineralization of the hard tissue is more effective at a neutral pH than acidic or alkaline pH (3-4). Several authors showed that during canal preparation, the cutting action of endodontic instruments produces smear layer (3, 5). It is widely accepted that the most effective method to remove this layer is to irrigate the root canals with EDTA solutions to dissolve inorganic debris and NaClO to dissolve organic tissue and bacteria (6-7).

A careful analysis of the chelating compound revealed than another chelator, ethylene glycol-bis (β-aminoethyl ether)-N-tetraacetic acid (EGTA), bind Ca^{2+} more specifically. It shows a constant formation for calcium equal to log K= 11. Calt and Serper demonstrated that EGTA, when compared with EDTA, was somewhat effective in removing the smear layer without inducing erosion (8). Another possible interpretation of these results stems from a consideration of calcium diffusion to the exchanger at different concentrations of EGTA. The Na, Ca exchanger is able to extrude calcium efficiently when free Ca^{2+} is in the range of several micromolar.

We observed that an EGTA and NaOCl combination does not cause erosion of the intertubular and peritubular dentin. However, this combination does not completely remove the superficial smear layer in the apical third, and in some of the dentinal tubular orifices it caused clogging (9-10).

The aim of this study is to compare the *in vitro* action EDTA and EGTA on demineralization of dentin powder, by a gravimetric method and whether the actions of the EDTA and EGTA at pH 7.12 on dentin influence the pH.

MATERIALS AND METHODS

The study was divided into two parts:

- The first part tested and compared the action of various solutions that contained EDTA or EGTA in 100 mg dentin powder, analyzing the dentin % solubilization in the process of demineralization;
- In the second part of the experiment the solutions the same 100 mg dentin powder was utilized, analyzing the influence of chelator concentration on the pH solution after 22 hours of incubation, with constant shaking to facilitate the contact between the chelating agent and dentin.

Action of the chelating agents over dentin powder. Extracted multirooted human teeth, with no decay, were collected and maintained in 2.5% sodium hypochlorite (NaClO). The pH was inactivated with distilled water 1000 times the initial volume. The enamel and cement, from each tooth, was removed using diamond burs and the teeth, now only with the dentin, were sectioned along their longitudinal axes. The residual pulp was removed with a K-file n.15 and the surfaces were cleaned with the toothbrush connected to a handpiece. Then the resultant pieces, placed in distilled water, were sent to a laboratory specialized in the preparation of ceramics and pulverized

Key words: dentin, chelant, EDTA, EGTA, pH

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0394-6320 (2007)

FIg. 1

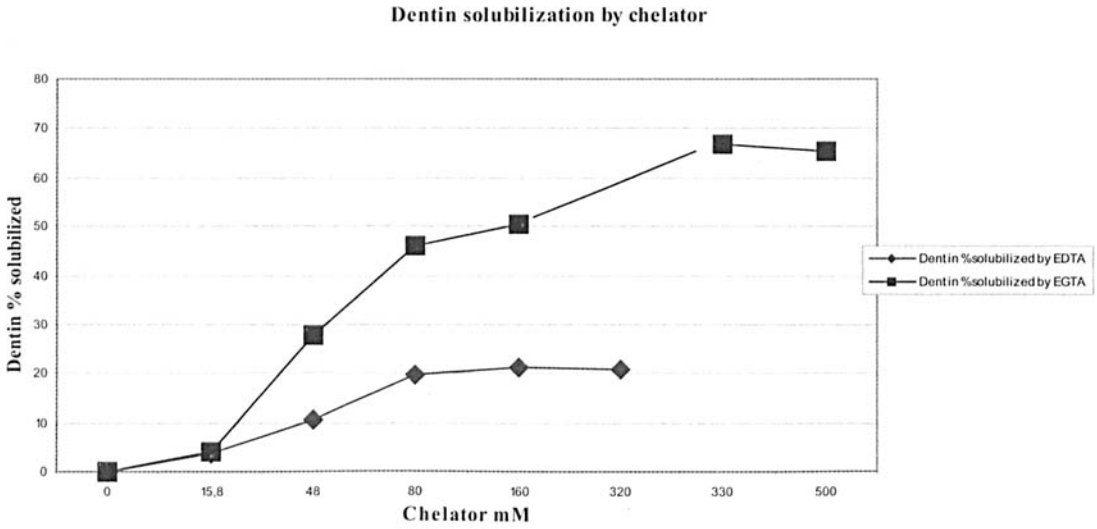
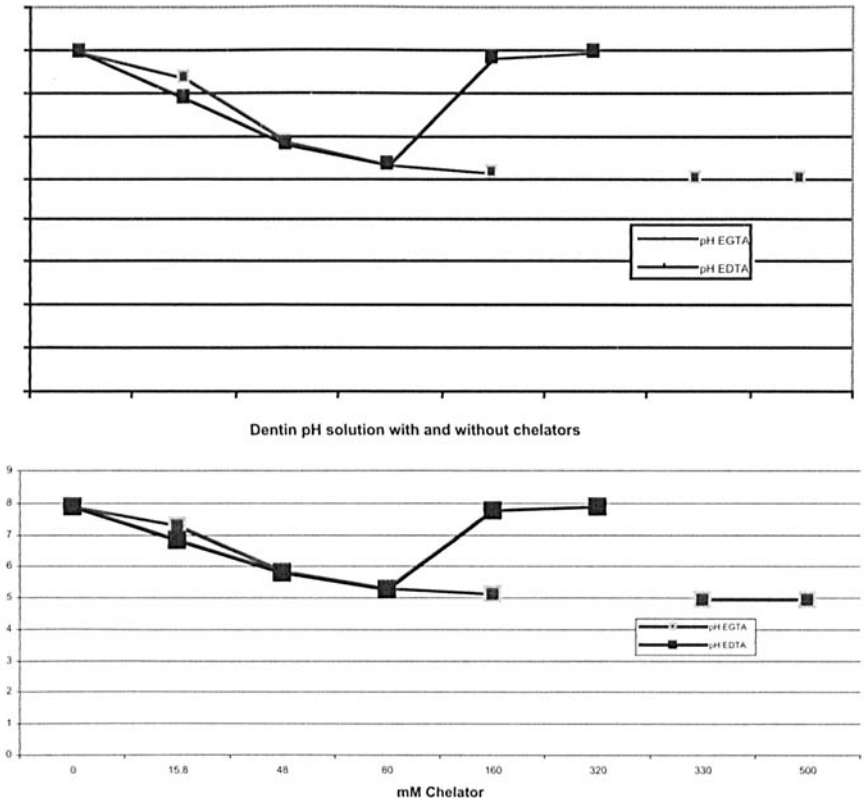


FIg. 2



by the action of a hydraulic pressure machine (Sundent strength of 8000Kg, pressure of 200 Bar) for 5 minutes, for 10 times. Then they were treated with an MMS machine to triturate the dentin, for a period of 40 minutes they were passed in a filter. The filter used was n.120 from Tecnotest, having a light magnification of 0.125 m/m. The optic microscopic observations demonstrated that it was an heterogeneous material with dimensions from 0.1 micron at 100 micron. The powdered dentin was treated with chloroform for 1 hour, to eliminate organic components; filtered in a filter for crystallization for 30 minutes and then dried in an electric oven, type Heraus at 100°C for 10 minutes.

The observations under the optic microscope of the dentin sample, after treatment with chloroform, revealed a material with heterogeneous particles with dimensions

varying from 0.1 to 50 micron. Using a precision scale, 11 samples of 100 mg each of dentin powder were weighted using the Gibertini E42 (Milano-Italy) and were placed in plastic tubes numbered progressively form 1 to 11.

Sample no.1 represented the control, therefore the dentin was immersed in aqueous solution without any type of treatment. The control sample was the difference between the initial and final weight without anything which constituted in a margin of error in our experiment.

The quantities of EGTA used were 15.8mM, 48mM, 80mM, 160mM, 330mM e 500mM, at a pH 7.12. The quantities of EDTA used were equivalent to 15.8mM, 48mM, 160mM, 320mM at pH 7.12. The prepared solutions were left to interact for about 22 h, with constant shaking to facilitate the contact between the chelating agent, the dentin and then each solution was immediately glass filtered for crystallization. Then the supernatant solutions from each sample were diluted to a final volume of 5 ml each to measure the pH analysis. Analysis of the pH was obtained using the pH meter Microprocessor HI9321 (HANNA instruments- Singapore). The filtered dentin pellet samples obtained after liquid removal were initially dried for 15 minutes at 80°C in an electric hot air oven, and at the same time shaken. Then they were subjected to various cycles of dehydration, about 45 minutes each, in a hot air oven at 50°C and then weighed. Each single experiment was repeated 3 times, at 24°C.

RESULTS

Action over the dentin powder. The results obtained from the action of the EDTA and EGTA over dentin powder are shown in Figure 1. All the results were corrected from the control value. A quantity of EDTA equivalent to 15.8 mM solubilized 3.5% of dentin powder; 48 mM of EDTA solubilized 10,5% of the utilized dentin. A concentration of EDTA in the order of 160mM is able to chelate 21.2% of dentin powder. Values of 320mM of EDTA can obtain a demineralization of 20.7% of the dentin powder. Analyzing the data related to EGTA, it was possible to observe that in low concentration (15.8mM) this already permits the solubilization of 4.17% of dentin.

Concentration equal to 48mM of EGTA demineralizes 26.67% of dentin powder. Increasing the concentration to 80mM, the EGTA solubilizes 46.12% of the material. 50.52% of the dentin powder is chelated by 160mM of EGTA. It reached a value of solubilization of 66.86% and 65.47% with concentrations of EGTA, 320mM and 500mM respectively.

pH analysis. The analysis of the pH, was the same (7,12) for all the concentration of EDTA and EGTA used to analyze the solubilization of the 100 mg of dentin powder and are shown in Figure 2. The pH results obtained by analyzing the filtration liquid are very interesting. The dentin control solution reached pH 7.90. After demineralization with 15.8 mM of EDTA, the pH of the solution was 6.83. For the EDTA 48mM pH 5.80. were obtained For the EDTA 80 mM pH 5.29were obtained. In the concentration of 160mM, the pH became 7.78, while for the solution equivalent to 320 mM pH reached 7.90. In respect of the sample treated with EGTA, pH 7.30 was obtained for the concentration of 15.8mM; 5.87 for the concentration of 48mM; 5.30 for the quantity of EGTA equal to 80mM; pH 5.13 for the concentration 160mM; pH 4.94 for the concentration 33mM e pH 4.94 for the concentration 500mM.

DISCUSSION

The demineralization of dentin obtained by treatment with chelating agents EDTA or EGTA, is a dynamic process that can be explained with the results of two different chemical reactions: chelating action of EDTA and EGTA; and solubilization of the material after an acid-base reaction. Many authors have already indicated the chelating ability of EDTA is optimized in a basic pH and it is good in neutral conditions. The action of the EDTA on the dentin is influenced by the pH. The pH values used in our study, 7,12 , are in accordance with the ones mentioned in the literature (3-4). Our results show that by increasing mM concentration of EDTA or EGTA the equivalent pH decreases following a similar curve till 80 mM chelator concentration. Increasing the chelators concentration different data were obtained: with EGTA the pH decreases slightly while with EDTA goes back up to the start values (Fig. 2). After 80 mM, EDTA reduces the activity on the dentin, and EGTA continues to work at higher concentrations. The effects of EGTA and EDTA on the removal of smear layer demonstrated that EGTA was somewhat effective in the removal of the smear layer without inducing any erosion on the canal wall (8-11). We demonstrated that EGTA solubilized more of 60% of dentin while EDTA gives about 20% of the solubility at the end of the process (Fig. 1). On the other hand, the solubility of the dental materials (hydroxyapatite) increases with the reduction of the pH, reaching the optimum around an acid pH. In basic conditions or neutral, the process of chelation works best, it also releases H^+ ion in the solution, with the decrease of the pH and creates a limiting factor to the process of chelation. With decreasing pH the process of solubilization is established, caused by the release of H^+ ion from the EDTA. These ions in the solution react with the hydroxyapatite from the dentin, causing solubilization. The difference between the initial weight and the final weight of the sample of dentin treated with EDTA, confirm the findings previously reported in the literature, that EDTA has a demineralizing effect on dentin. The analysis of variation in weight, related to the use of EGTA, also confirms that, besides the demineralizing effect, it also has greater efficacy, from

the greater quantity of solubilized dentin. In our study, to obtain the maximum chelating action and to overcome the limiting factors of the demineralization caused by the variables in time and dimension of the particles, we used a material composed of heterogeneous dimensions and a maximum incubation time of 22 hours for the solution,. This time was chosen because it has been demonstrated that the greatest effect of EDTA over the walls of the radicular canals was at a maximum period of 24 hours (13). A known solution of EDTA can only dissolve a determined quantity of dentin that is in contact with, when all chelating ions utilized have reacted, an equilibrium is reached, therefore the chelating effect is limited and it is not possible to obtain other dissolution (14-15). In conclusion, we find that increasing mM concentration of EDTA or EGTA the equivalent pH decreases following similar curves till 80 mM chelator concentration, increasing the chelator concentration different data were obtained for the two chelator: EGTA keeps on decreasing slightly while EDTA goes back to the initial values. After 80 mM, EDTA reduces the activity on dentin, and EGTA continuous to work better at higher concentrations. We demonstrated that EGTA solubilized more of 60% of dentin while EDTA gives about 20% of the solubility at the end of the process. We know that EGTA significantly decreased substrate adherence capacity of macrophages (17). These data suggest that EGTA could be a useful means in the preparation of root canal and an important phase in endodontic therapy.

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