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Original Research

Effect of Ethylene Diamine Tetra Acetic Acid and RcPrep to Microstrain of Human Root Dentin

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Abstract:

Background: Information about microstrain changes in human root dentine after application of chelating agent still limited. This result needed to determine the factors of microstrain which caused by non-instrumentation of human root dentine formation.

Materials and Methods: This experimental conducted in Department of Physic - Faculty of Nature and Mathematics Science, Syiah Kuala University. All 45 specimens randomly divided base on experiment solutions. All specimens were immersed for 15 min than powdered for X-ray diffraction (XRD) analysis. Grain size calculated using Scherer equation and microstrain using Hall-Plot.

Result: XRD patterns typically show apatitic and amorf crystallized. A crystal phase of ethylene diamine tetra acetic acid (EDTA) group known as CaOH, RcPrep group as fluorapatite, and Control as Ca-Cl, XRD pattern of EDTA confirmed as larger crystal than both control and RcPrep group. XRD pattern of RcPrep group confirmed as larger crystal than the control group. The average value of grain size indicates all treatment groups have larger grain size than control. Microstrain of the control group showed as a lower value than all treatment groups. Microstrain of EDTA group showed as the biggest value than RcPrep and control. Microstrain of EDTA and RcPrep indicate larger than control. Regression analysis of between control and both treatment groups were significant ($P < 0.05$). Microstrain of EDTA significantly greater than RcPrep. The relationships between EDTA to Control is weak ($R^2 = 0.37$) same as relationship of control and RcPrep ($R^2 = 0.37$).

Conclusion: Chelating agent application into root canal is either change structure or improving microstrain of apatite crystal. Concentration and exposure time of chelating agent may lead as the main factor increasing fracture risk of human root dentine. Application of RcPrep into human root dentine is better than EDTA in relation to decreasing of dentin fracture. EDTA has more capability to increase of microstrain of apatite crystal than RcPrep.

Key Words: Dentine, ethylene diamine tetra acetic acid, microstrain, RcPrep, X-ray diffraction

Introduction

Chelating agent is solutions consisting of a synthetic amino acid which assist widening root canal properly because it has the ability to softening hard tissue with low negative effect on the periapical tissues. The most common chelating agents used include ethylene diamine tetraacetic acid (EDTA) and EDTA + urea peroxide and Carbowax (RcPrep).[1]

The application of chelating agent into root canal is known to lead changes to human root dentine canal dimensions and capable to decompose body wall of human root dentine canal. The previous study stated chelating agents change human root dentine hardness along with the length of application of the chelating agent.[2,3] These events indicate the occurrence of increasing of microstrain in human root dentine. The increasing of microstrain identified capable to increasing fracture risk on human root dentine to lead improving hypersensitivity during mastication and in worst condition might tooth detached. Most serious cases of fracture should be treating by endodontic surgery. One of most famous treatment by endodontic approach is root resection.[4]

Information about microstrain changes in human root dentine after application of chelating agent still limited. Meanwhile, this initial information also needed to determine the factors of microstrain which caused by non-instrumentation of human root dentine formation.[5,6] Therefore, it is necessary to investigate the differences microstrain of human root dentine after chelating agents application.

Materials and Methods

This laboratory experimental conducted in Department of Physic-Faculty of Nature and Mathematics Science, Syiah Kuala University in January 2013. 15 extracted human premolars were sectioned at the cementoenamel junction using a diamond bur disc. Every group was then sectioned horizontally in ⅓ cervical, ⅓ middle, and ⅓ apical to have a total of 45 specimens. Based on the test solutions used, specimens were divided randomly into three groups: (1) The EDTA group: 0.05 mL of 17% EDTA for 15 min ($n = 15$), (2) the RcPrep group, 0.05 mL of 15% EDTA-urea peroxide-Carbowax

for 15 min (n = 15), and (3) the control group, 1 mL of 0.9% saline for 15 min (n = 15).

All specimens were prepared for the determination of microstrain of root dentin using X-ray diffraction (XRD) analysis. Grain size calculated using equation:

$$D = \frac{K\lambda Rad}{\beta r \cos\theta}$$

Where, D = Grain Size, K = Shape factor constant (0.9), λ = Wavelength of the X-ray (1.5405600), cos θ = cosine of half the 2θ angle, and βr = Broadening of diffraction line measured at half its maximum intensity (radians). The microstrain of root dentin calculated using equation:

$$K = \frac{B_r \cos\theta}{\sin\theta}$$

Wher w, ŋ = Microstrain; B_r = Full width at half maximum in radian; θ = Diffraction angle. Furthermore, Microstrain value statically analyzed using regression test with each variabel (EDTA and RcPrep).

Results

All patterns were typically apatitic and amorf crystallized, although some peaks of homogenous apatites were irregular curves. In general, all specimens showed similar patterns with the main character looks at the highest intensity in 2θ = 31°-32°. Crystal phase of control showed as Ca-Cl, EDTA group showed as CaOH, and RcPrep group showed as fluorapatite. General similar pattern of each group as demonstrate in Figure 1.

The XRD pattern of EDTA confirmed as larger crystal than both control and RcPrep group. XRD pattern of RcPrep group confirmed as larger crystal than Control group. Data normality test using Shapiro-Wilk using Microsoft Excel Analyze-it version 3.90.5 revealed data is normal ($P > 0.05$). The average value of grain size calculated using the Scherrer equation indicates all treatment groups have larger grain size than control. The largest of grain size crystals founded in EDTA group. Grain size of each group as demonstrate in Table 1.

The Microstrain of the control group showed as a lower value than all treatment groups, while, microstrain value of EDTA group showed as the biggest value than control and RcPrep. The average value of microstrain indicates all treatment groups larger than control. The microstrain values of each group as demonstrate in Table 2.

Regression analysis of between control and treatment groups were significant ($P < 0.05$). The microstrain was significantly greater in 17% EDTA when compared to 15% RcPrep. The relationships between EDTA application effect to Control in the category of low and positive relation ($R^2 = 0.37$). Regression analysis between microstrain of control and RcPrep also confirmed low and positive relation ($R^2 = 0.37$). Regression of each microstrain demonstrate in Table 3.

Discussion

The apatite group is a group of similar isomorphous hexagonal phosphate minerals members were traditionally known as fluorapatite, chlorapatite, and hydroxylapatite.[7,8] It is well established by XRD analysis that the mineral constituent of human root dentine is essentially chlorapatite, and that chlorapatite has a structure differing only in small details from that of the well-crystallized mineral hydroxylapatite and fluor-apatite.[9] The same pattern as displayed in 2θ with high intensity which indicate all specimens is same groups in apatite crystal. Each HA crystal is arranged by cell unit in lattice arrangement of Ca and P, and also the lattice arrangement of O and H.[7] Moreover, we suggest chlorapatite is the main structure of human root dentine. The main difference, and in fact, the only fully established one, between the two structures, is that the hydroxyapatite has a slightly larger unit cell than fluor-apatite or chlorapatite. Similar with this result, Elliot was stated that apatites have the general formula, $Ca_{10}(PO_4)_6X_2$ where X is typically F (fluorapatite, FAp), OH (hydroxyapatite, OHAp), or Cl (chlorapatite, ClAp).[7]

As a displayed on result, the pattern of all specimen displayed irregular curve and amorphous phases. Dentine is one of the hard tissues in human body. This hard tissue formed by apatite crystal. It differs with enamel which has more percentage of inorganic. Dentin arranged by composition between inorganic and organic is 70%, organic 20%, and water 10%, whereas enamel is the hardest tissue in the human body and consists of approximately 96% inorganic minerals, 1% organic materials,

Table 1: Grain size of each specimen.

Grain size		
Control (nm)	**EDTA (nm)**	**RcPrep (nm)**
8.2	8.9	8.8

EDTA: Ethylene diamine tetra acetic acid

Table 2: Microstrain of each specimen.

Microstrain		
Control	**EDTA**	**RcPREP**
0.895698047	0.979657791	0.902449062

EDTA: Ethylene diamine tetra acetic acid

Table 3: Regression of microstrain.

Description	Regression statistics EDTA versus control	Regression statistics RcPREP versus control
R^2	0.37	0.38
Coefficient value (F_{hit})	10.12	10.32
Relationship (T_{hit})	3.18	3.21

Relationship is significant if $t_{hit} > t_{table}$ and relationship is significant if $F_{hit} > T_{table}$. EDTA: Ethylene diamine tetra acetic acid

and 3% water.[10,11] Although both tissues are comprised of a calcium apatite mineral phase and a protein component, they differ to overall structure include crystal size and shape, the nature of the proteins present, and the relative proportions of mineral and protein components. Differences in structural organization and composition give rise to mineralized tissues with different properties that are well-suited for their intended biological purpose.[12,13] Similar with this statement, our result has found the grain size of these hexagonal-member apatites with the formula $Ca_5(PO_4)_3$ X (X: Cl, OH, F) as found in this experiment: D (nm)=8.2; 8.9; 8.8 for chlorapatite; hydroxylapatite, and fluorapatite. The result has suggest, chlorapatite is main structure of human root dentine, application of both chelating agents EDTA and RcPrep into root canal will change size, structure and microstrain of crystal human root dentine.

Apatite crystal is $Ca_5(PO_4)_3(X)$ which X is able to substitute by other element. This substitution changes of lattice arrangement of cell unit which improving grain size of apatite. The apatite lattice is very tolerant of substitutions, vacancies, and solid solutions, for example, X can be replaced by other elements[14] Thus, every cell units of apatite suggested ion Ca position on the hexagonal corner to formed calcium column. Ca position is perpendicular to C-axis. Ca position is also on cell central canal which formed Ca triangles. Spaces between Ca columns were placed by two ions PO_4 on the hexagonal side. The F ion position is higher than OH position on C-axis.[13] The XRD data of human root dentin which applied to RcPrep have slimmer peak than other group. This found suggest human root dentin which applied to RcPrep have better crystallinity. Calderyn,[12] was reported that OH position on C-axis between ¼-⅓, while ion F position is ¼ above the Ca triangle. Application of RcPrep may cause a remineralization in dentine crystals by F ion and affect crystal more strength than EDTA application.

The main difference, and in fact, the only fully established one, between the two structures is that the hydroxyapatite has a slightly larger unit cell than that of the fluorapatite. These confirm as human root dentin which applied to RcPrep achieved dimensions close to enamel whereas has better crystallinity than dentine. The previous study also stated 20% of 85% organic of dentine is arranged by collagen.[14,15] This organic as confirm in XRD diffractogram displayed as graph or curve pattern is more amorf than enamel. This fact also confirms every mineral have specific diffractogram. Otherwise, EDTA known has the function to clean the root canal as well as bleaching agent which commonly used in dentistry, but EDTA proved reducing mineral content of human root dentine. Application of EDTA may cause a reduction in the calcium crystals and increasing percentage of microstrain. These results are consistent with Calt and Serper which states, chelating agents have ability to demineralized human hard tissue.[16]

The increasing of microstrain which imply by EDTA which properly known has capability to dissolving calcium which is found in the building blocks of dentin. Calcium pulled

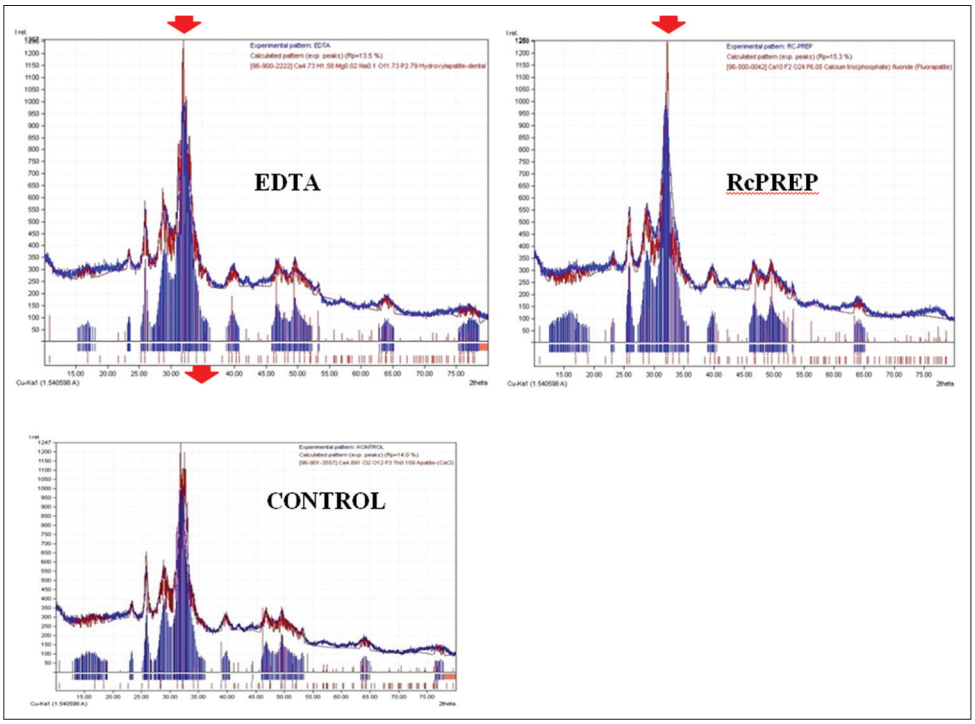

Figure 1: All specimens showed similar patterns with the main character looks at the highest intensity (↓) in 2θ = 31°-32°. X-ray diffraction (XRD) pattern of RcPrep group confirmed as better crystal than both control and ethylene diamine tetra acetic acid (EDTA) group. XRD pattern of EDTA group confirmed as better crystal than control group.

out from crystal arrangement consequently affects crystal structure. Microstain that occurs in dentin due to changes in the structure or position of atoms will influencing root dentin resistance to accepting mechanical forces.[17] A large grain size of crystal in dissolving dentine and microstrain value, automatically increasing of susceptibility of dentine to fracture. Conversely, both grain size and microstrain of fluorapatite as an element which resistance to acid will constant to withstand the mechanical forces.

The results of this study indicate that the application of EDTA is increasing of susceptibility of human root dentine to fracture. Similar with this statement, analyzed data between microstrain controls when compare to EDTA and RcPrep, statistically showed the significant effect and positive relationships. In other word, the increasing in microstrain is affected by both chelating agents application. In line with the results, analysis by regression confirms the relationship of chelating agent to improving fracture-risk is positive and weak. It indicates chelating agent application both EDTA and RcPrep predicted have 37-38% factor which able to increasing of fracture-risk of human root dentine. Concentration and exposure time of chelating agent may lead to the main factor to increasing fracture risk of human root dentine.

In addition, chelating agent application into root canal will change the structure and improving of microstrain. Compilation of microstrain between treatment groups to chlorapatite as control showed application EDTA increasing microstrain on crystal up to 9% while application of RcPrep only 1%. Tsukamoto *et al*. stated that the crystallinity is strongly influenced by the crystal size and microstrain.[18]

Microstrain obtained from the small size of microstructures on a fluorapatite root dentine was lower up to 1% while has larger grain size compared to normal [Table 2]. Based on this result, application of RcPrep into human root dentine is better than EDTA in relation to decreasing of dentin fracture.

Conclusion

Chelating agent application into root canal is either change structure or improving microstrain of apatite crystal. Concentration and exposure time of chelating agent may lead as the main factor increasing fracture risk of human root dentine. Application of RcPrep into human root dentine is better than EDTA in relation to decreasing of dentin fracture. EDTA has more capability to increase of microstrain of apatite crystal than RcPrep.

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