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Research Report

Experimental comparative study and fracture resistance simulation with irrigation solution of 0.2% chitosan, 2.5% NaOCl and 17% EDTA

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ABSTRACT

Background: *Preparation in endodontic need irrigation materials as root canal debridement and disinfectant. However, irrigation materials is one of the factors that influence the tendency of fracture.* ***Purpose:*** *This study was aimed to see the resistance and fracture distribution if teeth irrigated with high molecular horseshoe crab chitosan at 0.2% concentration, 2.5% NaOCl solution and 17% EDTA solution in endodontic treatment with finite element method (FEM) simulation study and experimental studies.* ***Method:*** *Endodontic treatment performed on 28 maxillary premolars with group A: irrigation solution of 17% EDTA and 2.5% NaOCl solution; group B: 2.5% NaOCl irrigation solution; group C: 2.5% NaOCl irrigation solution and 0.2% chitosan solution; group D: 0.2% chitosan solution irrigation. Final restoration was done using prefabricated glass fiber post. Cementation of post using resin cement then restored with direct composite resin restorations. Pressure test was performed with a Universal Testing Machine with a speed of 0.5 mm/min until fracture occurred. A three dimensional finite element analysis was performed for total deformation, equivalent (von-mises) stress, and equivalent elastic strains.* ***Result:*** *Anova test showed significant differences in fracture resistance ($p<0.05$) in stroke difference between four groups. Based on the results of the analysis of post-hoc Bonferroni test and LSD, fracture resistance was significantly different between group A (1038.4±201.6) with group C (1515.6±243.3). Kruskal-Wallis test showed no significant difference in the distribution of fractures among the four groups ($p>0.05$). Statistical analysis showed no significant difference ($p>0.05$) between the results of experiment and FEM analysis results using the t-test.* ***Conclusion:*** *The results of this study demonstrated that there was effect of the use of high molecular 0.2% chitosan as a combined irrigation with NaOCl, but did not affect the fracture pattern distribution of endodontically treated teeth both experimentally and FEM analysis test.*

Keywords: *Irrigation solution; chitosan solution; fracture resistance; finite element method*

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INTRODUCTION

The post-endodontic teeth has a higher fracture risk than the vital teeth. The strength of post-endodontic teeth is directly proportional to the remaining healthy teeth structure and if the teeth structure is lost, the teeth fracture potential will be increasing.[1] The cause of fractures in post-endodontic teeth is multifactorial which are iatrogenic and noniatrogenic.[2] Some chemicals for endodontic irrigation are causing the changes in the chemical composition of dentin.[3] Endodontically trated teeth should have a good prognosis so it can function as a support to the final restoration.[1,2]

Chitosan as natural polysaccharide after cellulose that is obtained through deacetylation of chitin have biocompatible properties, bioadhesion and nontoxic for human cells.[4] Trimurni *et al*. cit. Pretty *et al.* [5] showed that chitosan blangkas (*Tachypleus gigas*) with degrees 84.20% deasetilisasi and a molecular weight of 893 000 Mv. is proven stimulate the dentinogenesis when it used as an

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ingredient of pulp capping. Chitosan liquid in hydrochloric acid produces dentin surface without the smear layer and contains collagen fiber[6] while Silva *et al*. showed that 0.2 % of chitosan is able to lift the smear layer compared to 15% of EDTA and 10% of citric acid.[6] Chitosan solution has a chelating properties which cause erosion of the dentine, yet it safe for intertubular dentin.[4]

Mechanical destructive tests such as fracture test is important to analyze the nature of biomechanics and dental restorative materials that will be studied when given the heavy load. This test has limited capacity to explain the stress-strain relationships on dental restorations complex.[7] The development of computer technology has increased the use of finite element method (FEM) in various fields of science, especially in the dentistry. FEM program can calculates the stress, strain and deformation in the three dimension view.[8] An analysis of FEM also obtains some informations such as distribution of internal pressure as compare to the experimental study. This study was aimed to find out the differences between NaOCl irrigation material that was combined with EDTA, and NaOCl that was combined with irrigation chitosan blangkas and the chitosan blangkas itself against the fracture resistance and fracture distribution after endodontic treatment using the experimental test and FEM analysis test.

MATERIALS AND METHODOS

Twenty-eight of maxillary premolar that had been extracted for orthodontic purposes was suited to the inclusion criteria. The samples of this study were divided into 4 groups; (a) group A: teeth that were irrigated with 17% of EDTA and 2.5% of NaOCl solution; (b) group B: teeth that were irrigated with 2.5% of NaOCl solution; (c) group C: teeth that were irrigated with a solution of NaOCl 2.5% and 0.2% of chitosan solution, (d) group D: teeth that were irrigated with 0.2% of chitosan solution. The dental samples were planted on the gypsum then the pulp was opened by endo access bur (High speed) and root canal preparation was done by rotary instrument (protaper file), crown down system with X-Smart endomotor (Denstply, Switzerland) using irrigation solution according to the test group treatment. The irrigation itself was using irrigation needle -shaped one side-vented size 30G (Max-i-probe®, Dentsply, Switzerland). Root canal was dried by paperpoint and then canal obturation was done. Fiberglass post was put into a root canal using self adhesif Brezee resin cement (Pentron, USA), then followed by 20 second light cure. The teeth was restored by composite resin and polishing was done by enhance bur. Six samples of teeth was released from the gypsum block and immersed in artificial saliva for 24 hours for thermocycling process.[9] Six teeth were planted on self curing acrylic in a cut of 10 ml spuit pieces.

The process of pressure test was done to determine the strength of fracture resistance according to American Society for Testing and Material (ASTM E1434-00, 2006). The samples were placed in acrylic base and tested with pressing test (Torsee's Universal Testing Machine, Japan). The samples were pressed from the occlusal side of the teeth to the axis of teeth (zero degrees). Constant and slow pressure (not in the form of shockV/sudden pound) was given with direction speed of 0.5 mm/min until the fracture happened. The emphasis tool (Zig) made by metal, sized 5 x 5 x 0.3 cm, flattened shape with rounded edges. The load invoice was immediately recorded right after the fracture of sample in Newton (N), then fracture distribution also observed and recorded based on the location of the fracture.

The FEM test of this study was ANSYS program 14 by entering the data of modulus elasticity, poison ratio, density of each test material which is the preliminary decision stage (Table 1).[10] The next step was processing

Table 1. Mechanical character of FEM material test[10]

Material	Modulus Elastisitas	Poison Ratio	Density g/cm^3
Email	41.0 GPa	0.30 ν	2.97
Dentin	18.6 GPa	0.31ν	2.14
Fiber glass post	33 Gpa	0.28 ν	2.5
Resin composite	18.9 GPa	0.24 ν	2.09

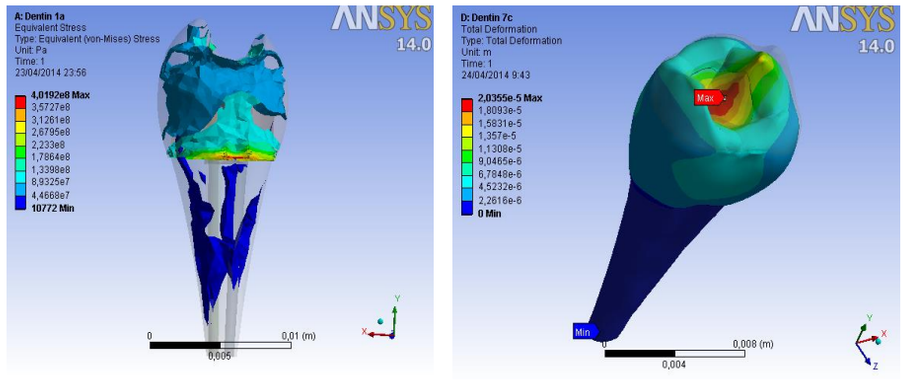

Figure 1. The stage of FEM analysis process of post endodontic maxillary premolar with glass fiberpost.

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stages which were model designing of the teeth, defining feature for each object material, performing meshing, load and support. After that, it was done with solve the model stage so that it will produce a post processing stage which contains a review of results and checked the validity of the results of the solution (Figure 1).

RESULTS

The result of fracture resistance test showed the differences of fracture stress in N for each treatment group. Based on the statistical test results One way ANOVA, obtained p value = 0.019 ($p<0.05$), which showed there was force difference between those four groups and based on analysis test result of Post–hoc Bonferroni, LSD and Tukey HSD obtained the difference fracture resistance which significantly different between group A (17% EDTA + NaOCl 2.5%) and group C (NaOCl 2.5% + 0.2% chitosan) with mean value of the highest fracture resistance in group C was 1515.6 N.

The occurred fracture distribution can be analyzed after the endurance test of the fracture by analyzing the location of the fractures in the whole sample. The location of the fracture pattern was divided into two categories; repairable when fractures occur in the crown, post and crown, and cervical (Figure 2). Irreparable for the fracture patterns in the middle root teeth, horizontal and vertical cracks until the root of the teeth (Figure 3).[11]

Figure 2. Fracture patterns of repairable (a) location of fractures in the crown and (b) location of fractures in the crown and post.

Figure 3. Fracture patterns of irreparable (a) location of fractures in the middle of the root and (b) location of vertical cracks along the root.

The result of Kruskal-Wallis test on the fracture pattern observation, data obtained value $p=0.392$ ($p>0.05$) indicating that there was no difference in the fractures distribution among the four groups or there was no difference of fracture pattern between irrepairable and repairable.

The results of FEM analysis showed: total deformation, equivalent (von-misses) stress, equivalent elastic strain for

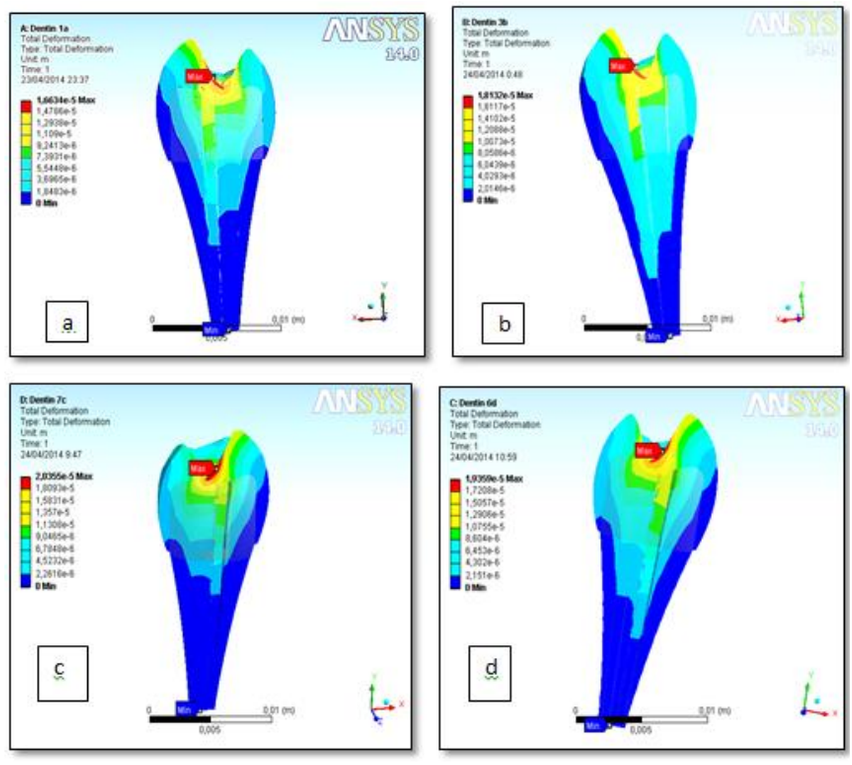

Figure 4. The result of total deformation of FEM test a) 17% of EDTA + NaOCl 2.5%; b) NaOCl 2.5%; c) NaOCl 2.5 % + 0.2 % of chitosan; d) 0.2% chitosan.

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each treatment group. Fracture distribution of the FEM analysis can be clearly seen by looking at the post process in equivalent (von-misses) stress based on the pattern of colours (red, orange, yellow, green, and blue). Red is the maximum points, while the blue colour is a minimum point. FEM analysis test, can be used for describing the simulation of strain and predicting the stress concentration area, which is a trigger point of fracture.[7,8]

The total value of highest deformation (red on the occlusal area) in this study was group C (NaOCl and chitosan) 2.0355 x 10^5 m and the lowest was group A (EDTA and NaOCl) 1.6634 x 10^5 m (Figure 4). The blue colour was the minimum point, which is the area that did not have a deformation change that occurred in the root area. The result of FEM equivalent (von-misses) stress at maximum point was sequentially listed from low to high as follows: group C 2.147 x 10^8 Pa; group D 2.076 x 10^8 Pa; group B 1.828 x 10^8 Pa; group A 1.720x 10^8 Pa.

DISCUSSION

Endodontically treated teeth have a higher fracture risk than the vital teeth. The post- endodontic teeth strength is directly proportional to the remaining healthy teeth structure and if the teeth structure is lost, the teeth fracture potential will increase. The cause of fractures in post-endodontic teeth is multifactorial which are iatrogenic and noniatrogenic.[2] From the perspective of biomechanics, fracture is a highly complex process which involves the formation and growth of micro crack and macro cracks. Micro crack can grows by the time and increases the concentrations of stress and tensile stress which is producing a microscopic plastic deformation in the end of that pressure concentration that leads on fracture on teeth structure[12]. The result of SEM examination showed that the micro crack can spread (propagation crack) for about 200 µm starting from the top of micro cracks. SEM features showed that the crack pattern was starting from microstructures namely tubules. Although, tubular did not have a big influence on the process of fracture or fracture pattern but, the cracks that occur continuously in peritubulus dentin can initiate the growth of the crack (sub crack). Sub crack initiated the main crack. Some chemicals used for endodontic irrigation are causing the changes in the chemical composition of dentin.[3] Endodontically treated teeth should have a good prognosis so it can function as a support to the final restoration.[1,2]

Chitosan as natural polysaccharide after cellulose that is obtained through deacetylation of chitin has biocompatible properties, bioadhesi and nontoxic for human cells. Furthermore, chitosan presents with biocompatibility, chelating capacity and also antimicrobial effects against a broad range of gram-positive and gram-negative bacteria as well as fungi.[4] Chitosan blangkas (*Tachypleus gigas*) with deasetilisasi degrees of 84.20% and a molecular weight of 893 000 Mv is proven the viability of pulp cell.[5] Chitosan blangkas is able to form a solid coagulum (sub base membrane) which is facilitating the attachment of pulp cells such as dentinoblast. Chitosan blangkas is able to form glucosamine - D when contacts to the pulp tissue that is easily accessing the cell proliferation so that the dentin genesis process occurs. Chitosan treatment improves the resistance of the dentinal surface to degradation by collagenase.[13] Silva *et al*. show that 0.2% of chitosan is able to lift the smear layer compared to 15% of EDTA and 10% of citric acid.[7] Pimenta *et al.* stated that the chitosan solution has a chelating property which causes erosion of the dentine, yet it safe for intertubular dentin.[4]

The result of this study indicated that after the fracture resistance test, it statistically showed the significant difference in all four groups, especially in group A (17% of EDTA and NaOCl 2.5%) and group C (NaOCl 2.5% and 0.2% of Chitosan). This result indicated that chitosan that was used as irrigation influenced the fracture resistance. Chitosan was able to inhibit the formation and growth of micro cracks and macro cracks since 0.2% of chitosan opened the dentinal tubules without changing the intertubular dentin as compared to the other group test (15% of EDTA and 10% of citric acid), so that caused a little erosion of dentin.[6]

A test group (17% of EDTA and NaOCl 2.5%) was a test group with the lowest fracture resistance since NaOCl 2.5% was able to affect the composition and structure of dentin thus, affecting the mechanical properties of dentin due to degradation of the organic components of dentin. The changes of physical properties of dentin occur because the changes of organic and inorganic phase dentin so, that the dentine surface was rougher on the canal wall due to the demineralised dentin which causes loss of mechanical strength. The reduction of organic matter after NaOCl irrigation can lead mechanical changes. Irrigation of 2.5% and 6% NaOCl for 5, 10, and 20 minutes was decreasing the microhardness dentin for about 500 µm.[14] The changes of dentin in the mechanical characteristics and biomechanical response variations caused the post-endodontic teeth becomes fracture.[2] The combination of EDTA and NaOCl caused a progressive dissolution of dentine extending to peritubular and intertubular area.[7] EDTA solution can dissolved the dentin tissue by reacting with inorganic compounds. In this study, dentin was irrigated with 17% of EDTA for 10 ml for 1 and 10 minutes and followed by NaOCl 5% for 10 ml. In 1 minute group of EDTA smear layer was effectively removed, where as in the 10 minutes group of EDTA there was too much demineralization in the area peritubular and intertubular dentin.[14] Sayin *et al*.[3] proved that the use of EDTA, either alone or in combination with NaOCl, significanly reduces the microhardness of dentin root.

NaOCl actually impede the attachment of dentin with the resin-based material. Insertion of fiber post needs a resin cement. NaOCl is a strong oxidizing material, it can alters the surface characteristics of root canal dentin becomes oxygenated. The residue of free oxygen affects the

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interfacial polymerization. The gap that occurs between the luting cement channel walls which can lead to micro cracks along the post rooms that affect the shape of the pattern of fracture occurrence.[14]

Fracture distribution showed that there was no significant difference in the fracture pattern p=0.2 (p>0.05) between the treatment groups. The reason of this result was the use of glass prefabricated fiber post system that has a modulus of elasticity which is similar to the dentin so, that possibly distributing the evenly pressure while the teeth were receiving loads of mastication. The characteristics of fiber post had a similarity to resin luting materials, core materials and dentin so, that the concept of a monoblock system which produces a homogeneous pressure distribution was created. The concept of monoblock system showed the ability of fiber material as the bondable material which is strengthen the teeth after being treated.[15]

The development of computer technology has increased the use of FEM in various fields of science, especially in dentistry. FEM program can calculates the stress, strain and deformation in the three dimension view.[7] An analysis of FEM also obtains some information such as distribution of internal pressure as compare to the experimental study.

FEM analysis is very useful for indicating the pressure distribution and researching the new material to reduce the failure risk and fracture of restorative material and teeth structure. FEM analyzes the changes of strain distribution materials of teeth structure after the placement of the post, core, and final restoration.[9] Fracture resistance of pure titanium post is almost the same and the best stress analysis (FEM test) while compared to the commercial post.[9] In addition, the FEM analysis more easily compare the biomechanical response even with the addition of various test parameters, such as FEM which analyzes the distribution of strain on the premolars with different contact point that analyzes different stress distribution in the post-endodontic teeth along with other different posts.[16,17]

The result of statistical analysis of this study showed that there was no difference between the experimental test's results and the results of FEM analysis with p=0.642 (p>0.05) by t-test. The FEM analysis were also showed that 0.2% chitosan solution as an alternative irrigation material affected the pressure distribution. This result was caused by the ability of chitosan as an antibacterial, chelating agent can remove the smear layer, less debris extrusion, not dentin eroding, capable for shaping the collagen fiber and has a D-glucose so it has the ability for reinforcing the dentin with the canal materials, thus increasing system adhesive.[13]

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