

The Effect of 2% Chitosan Oligosaccharides and 15% EDTA on Calcium Loss in the Root Canal

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ABSTRACT

Background: During instrumentation and irrigation, the composition of the dentin structure can be dissolved. Currently, the most widely used irrigation and chelating material are EDTA, which has the disadvantage of causing dentin erosion which reduces the hardness of the dentin, so that oligosaccharide chitosan is developed, which is more biocompatible and easier to manipulate as an irrigant material. This study aimed to evaluate whether there was a change in the concentration of calcium ions after treatment with chitosan oligosaccharide gel and EDTA gel using atomic absorption spectrophotometry. **Materials and Methods:** For 32 mandibular premolars, instrumentation, and irrigation were performed, and then chelation material was divided into two groups, namely the group with 2% chitosan oligosaccharide and 15% EDTA gel chelation material. Then the calcium ions in both groups will be measured using atomic absorption spectrophotometry. **Results:** There was a significant difference in calcium ion loss in root canal dentin after applying 2% chitosan oligosaccharide gel chelating agent and 15% EDTA gel at different times for 5 minutes, p -value = 0.021 ($p < 0.05$). **Conclusion:** 2% oligosaccharide chitosan can be a chelating agent when developed as a gel, affecting calcium ion loss. The 2% chitosan oligosaccharide resulted in the lowest average loss of calcium ions.

Keywords: chelating agent, 2% chitosan oligosaccharide gel, 15% EDTA gel, loss of calcium ions

ABSTRAK

Pendahuluan: Pada tahap instrumentasi dan irigasi dapat memengaruhi komposisi struktur dentin.. Saat ini bahan irigasi dan kelasi yang banyak digunakan adalah EDTA yang memiliki kekurangan yaitu menyebabkan erosi dentin yang mengurangi kekerasan dentin sehingga dikembangkan kitosan oligosakarida yang lebih biokompatibel dan lebih mudah dimanipulasi sebagai bahan kelasi. Tujuan penelitian adalah untuk mengevaluasi dan melihat apakah terjadi perubahan konsentrasi ion kalsium dengan menggunakan bahan kelasi saluran akar yaitu gel kitosan oligosakarida dan gel EDTA menggunakan spektrofotometri serapan atom. **Metode:** Penelitian eksperimental laboratorium dengan desain *pre and post test control group design*. Pada 32 gigi premolar mandibula dilakukan prosedur instrumentasi dan irigasi kemudian akan diberikan bahan kelasi dibagi menjadi 2 kelompok, yaitu kelompok dengan bahan kelasi gel kitosan oligosakarida 2% dan dengan bahan kelasi gel EDTA 15%. Kemudian akan diukur jumlah ion kalsium pada kedua kelompok menggunakan spektrofotometri serapan atom. **Hasil:** Ada perbedaan kehilangan ion kalsium pada dentin saluran akar yang signifikan setelah pengaplikasian bahan kelasi gel kitosan oligosakarida 2% dan gel EDTA 15% pada waktu yang berbeda selama 5 menit, nilai $p = 0,021$ ($p < 0,05$). **Kesimpulan:** kitosan oligosakarida 2% mempunyai potensi sebagai bahan kelasi bila dikembangkan sebagai gel dan memiliki pengaruh terhadap kehilangan ion kalsium. Bahan kelasi gel kitosan oligosakarida 2% menghasilkan nilai rerata kehilangan ion kalsium terendah.

Kata kunci: bahan kelasi, gel kitosan oligosakarida 2%, gel EDTA 15%, kehilangan ion kalsium

1. Introduction

It has been reported that some chemicals used for endodontic irrigation are capable of causing changes in the inorganic chemical composition and mineral composition of the root canal dentin. Tartari (2013) showed that the ability of EDTA to remove the smear layer, especially inorganic components, affects the chemical structure of root canal dentin, which causes changes in the $\text{Ca}^{2+}:\text{PO}_4^{3-}$ ratio in root canal dentin.¹

Root canal dentin is a mineralized tissue. Most of the constituents of dentin are inorganic components. Calcium (Ca) and phosphorus (P) contained in hydroxyapatite crystals are the main

inorganic components of dental hard tissue. Continuous demineralization will form small pores or porosity on the surface of tooth enamel so, which can cause the dissolution of calcium minerals.²

The chelating properties of the irrigating material can remove inorganic dentin substances, and the demineralization effect causes the loss of dentin mineral ions, one of which is the loss of calcium ions. Any change in the calcium ion ratio can change the original proportion of organic and inorganic components, shifting the dentin composition's microhardness, permeability, and solubility properties.³ The degree of mineralization and the amount of hydroxyapatite in the dentinal intertubular substance are essential factors in determining the intrinsic hardness of the dentinal structure. Therefore, the loss of calcium ions due to demineralization causes a decrease in the dentin's microhardness, weakening the tooth structure. This increases the risk of fracture, which can affect the success of root canal treatment.⁴ The reduction of calcium ions on the surface of the root canal dentin can also significantly reduce the sealer and adhesive ability of some adhesives.^{2,3} The most commonly used irrigating and chelating agents are NaOCl and EDTA. However, both materials have the disadvantage of being toxic and can cause dentition erosion. Therefore, chitosan was developed. Chitosan is a natural polysaccharide obtained from chitin deacetylation and has biocompatible, bioadhesive, and non-toxic properties to human cells.⁵

However, high molecular chitosan has drawbacks. The high molecule is difficult to manipulate because it is not soluble in water, has a high viscosity, and tends to agglomerate with protein at high pH. This is because high molecular chitosan has a long polymer chain structure, and high molecules make it difficult to use in material preparation and manipulation.⁶ The development of water-soluble chitosan oligosaccharides (COS) is currently being carried out to overcome the difficulties in material preparation and manipulation of the chitosan used.⁷ This study aims to evaluate and see whether there is a change in the concentration of calcium ions using root canal chelation material, namely oligosaccharide chitosan gel and EDTA gel, at different times using atomic absorption spectrophotometry.

2. Material and Methods

This experimental study was carried out using a posttest-only control group design. Ethical clearance was approved by the Ethical Research Committee of Health Research Universitas Sumatera Utara (USU) No. 198/KEPK/USU/2022.

2.1. Preparation of Chitosan Oligosaccharides

COS (Chitosan Oligosaccharides) powder was obtained from the Center for Innovative Excellence, University of North Sumatra. A 2% COS gel was made by dissolving 1 gram of chitosan oligosaccharide powder into 50 ml of distilled water and then stirring until homogeneous with a magnetic stirrer for 10 minutes. Next, a 2% hyaluronic acid was made by dissolving 1 gram of hyaluronic acid powder into 50 ml of distilled water and then stirring until homogeneous with a magnetic bar for 30 minutes to form a hydrogel preparation. Then, 30 ml of 2% hyaluronic acid was mixed with 2 mL NaCl while gradually dropping 14 mL of COS 2% solution with a dropper and stirred until homogeneous for 24 hours and then refrigerated for 24 hours.⁸

2.2. Root Canal Preparation

Sample preparation by measuring working length by measuring the size of each tooth using a caliper, then subtracting 1 mm to get the working distance. The sample is cut at the cemento-enamel junction using a disc with the help of a blade. Samples were rinsed with 5 ml of 2.5% NaOCl solution for 1 minute. Then explore the root canal using K-files #08, #10, and #15 until the channel feels loose. Then, the root canal was prepared using the crown down technique using a rotary instrument with an endo motor (Saeshin, Korea) and file i3 gold (Denjoy, China). Root canal preparation was started using a flaring file (white ring, taper 10, diameter 0.2 mm; length 16 mm). Then every file change, the sample was irrigated with 2 ml of 2.5% NaOCl solution for 1 minute, followed by an ultrasonic activator. Preparation continued with file 20/.04 (yellow ring, taper 04, diameter 0.2 mm; length 21/25 mm). Irrigation again with 2 ml of 2.5% NaOCl for 1 minute and preparation with a 20/.06

File (yellow ring, taper 06, diameter 0.2 mm; length 21/25 mm). Then the root canal was irrigated again with 2 ml of 2.5% NaOCl for 1 minute and prepared with a 25/.06 file (red ring,

taper 06, diameter 0.25 mm, length 21/25 mm) for the entire length of the work with an up motion and down. The sample was irrigated with 5 ml of 17% EDTA solution for 1 minute, then activated using an ultrasonic activator. Next, the root canals were rinsed with saline and dried with paper points. Then the samples were divided into two groups: Group I: 16 dental models were applied with 15% EDTA gel measured by AAS at 5, 10, 15, and 20 minutes. Group II: 16 dental samples were used with 2% oligosaccharide chitosan gel, measured by AAS at 5, 10, 15, and 20 minutes.⁹

2.3. Loss Calcium Ion Evaluation

Assessment of the release of calcium ions using Atomic Absorption Spectrophotometry (AAS) begins with appropriate sample preparation, where the sample is placed in an oven at 110°C for 1 hour and then stored in a desiccator for 15 min. Then put in the machine and burned at 450 ° for 8 hours until it becomes ash. Then 5 mL of a 1000 ppm calcium standard solution was pipetted, and distilled water was added to the mark. Then prepared, a standard solution of calcium 0.1: 0.2: 0.3: 0.4: 0.5. Calcium standard solutions 2 ml, 4 ml, 6 ml, 8 ml, 10 ml, from 10 ppm calcium standard solution, each pipette into a 50 ml volumetric flask. Then, distilled water up to the mark limit was added to obtain a calcium concentration of 0.1: 0.2: 0.3: 0.4: 0.5 ppm in a 25 mL volumetric flask. They were then assessed with AAS with a wavelength (λ) of 422.70 nm. The measurement results were then plotted against the concentration to obtain a calibration curve of the standard metal calcium (Ca) solution.¹⁰

2.4. Statistical Analyses

The data will be processed and analyzed using the Statistical Package for the Social Sciences (SPSS). The Shapiro-Wilk test was conducted to determine whether the data were normally distributed. The data obtained were tested for normality with the Shapiro-Wilk test p-value <0.05. Because the data were not normally distributed, the analysis used a non-parametric statistical test, namely the Kruskal-Wallis and Mann-Whitney tests.

3. Result and Discussion

Table 1 shows the Kruskal-Wallis assay. In group I, there is no significant difference in the number of calcium ions lost between 5 minutes, 10 minutes, 15 minutes, and 20 minutes, with p-value = 0.954 ($p > 0.05$). In group II, there was a significant difference in the amount of calcium ion lost between 5 minutes, 10 minutes, 15 minutes, and 20 minutes, p-value = 0.003 ($p < 0.05$).

Table 1. Statistic results with Kruskal Wallis analyses

Groups	N	Calcium Loss (Mean $\pm$ S.Dvt)				p-value
		5 Min	10 Min	15 Min	20 Min	
I	16	0.025 $\pm$ 0.144	0.2490 $\pm$ 0.461	0.3992 $\pm$ 0.4539	0.3740 $\pm$ 0.4313	0.954
II	16	0.018 $\pm$ 0.004	0.0259 $\pm$ 0.146	0.144 $\pm$ 0.219	0.082 $\pm$ 0.009	0.003

Table 2. Statistic results with Mann-Whitney analyses

Groups	N	Calcium Loss (Mean $\pm$ S.Dvt)			
		5 Min	10 Min	15 Min	20 Min
I	16	0.025 $\pm$ 0.144	0.2490 $\pm$ 0.461	0.3992 $\pm$ 0.4539	0.3740 $\pm$ 0.4313
II	16	0.018 $\pm$ 0.004	0.0259 $\pm$ 0.146	0.144 $\pm$ 0.219	0.082 $\pm$ 0.009
p-value	32	0.021	0.248	1.000	1.000

Table 2 shows the Mann-Whitney test, which offers the value of $p = 0.021$ ($p < 0.05$). There is a significant difference in the average value of the loss of calcium ions between group 1 and group 2 at 5 minutes measurement, obtained p value = 0.248 ($p > 0.05$). There was no significant difference in the mean value of the amount of calcium ion loss between group 1 and group 2 at 10 minutes measurement, obtained p value = 1.00 ($p > 0.05$), there was no significant difference in the mean

value of the amount of ion loss. There was no significant difference in the mean value of the amount of calcium ion loss between group 1 and group 2 at the 20-minute measurement.

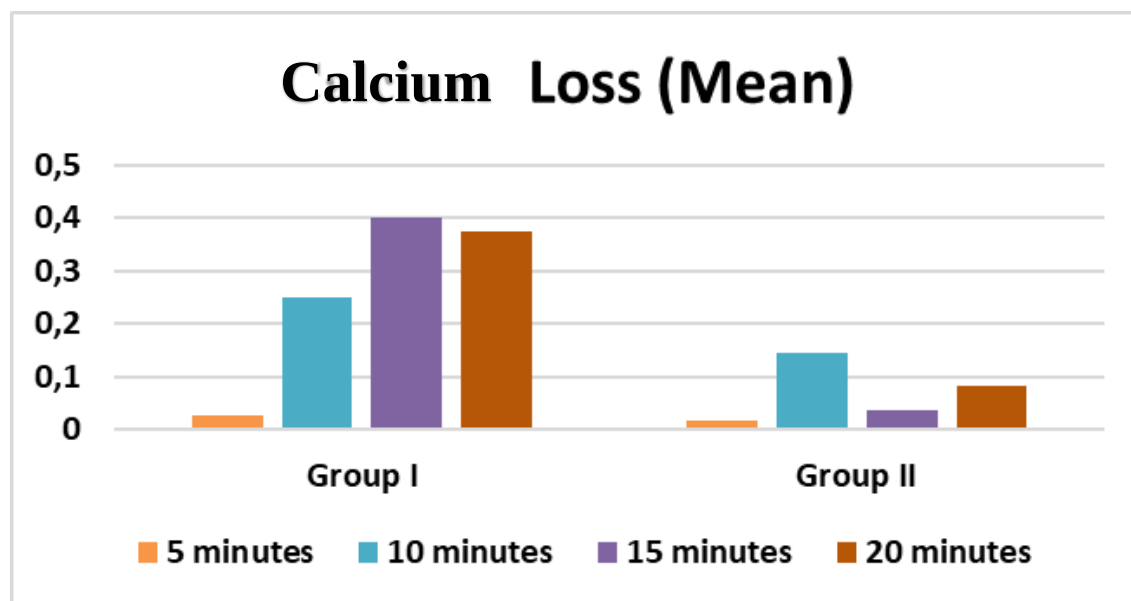


Figure 1. Calcium loss differences. Group I (15% EDTA gel) and Group II (2% chitosan oligosaccharide gel)

In this study, each sample was prepared and irrigated using 2.5% NaOCl and 17% EDTA, the gold standard for root canal irrigation. Then the model will be split in a buccolingual direction for later treatment. After treatment, the calcium ion loss was measured and analyzed using the atomic absorption spectroscopy (AAS) method. This method is very suitable for analyzing substances at low and fast concentrations. AAS works based on the evaporation of a sample solution, and then the metal is converted into free atoms. The atom absorbs radiation from a light source emitted from a cathode lamp containing the element to be determined. The amount of radiation absorption is then measured at specific wavelengths according to the type of metal. This study used a wavelength (λ) of 422.70 nm to analyze calcium.¹¹

One of the most critical aspects of root canal treatment is the cleaning and shaping the root canals. Residual smear layer due to instrumentation will result in repeated accumulation of bacteria, especially in narrow root canals. Previous research explained that the use of chelation material was believed to be able to help remove the smear layer on one-third of the roots, which had a complex anatomy because the area was difficult to reach by irrigation materials.¹² In this study, 15% EDTA gel was generally used as a chelating agent.⁵ In the Kruskal Wallis test, p-value = 0.954 ($p > 0.05$), there was no significant difference in the measurements of 5, 15, 10, and 20 minutes. In using 15% EDTA gel, there was an increase in lost calcium ions at 5 minutes, 10 minutes, and 15 minutes and a decrease in calcium ions lost at 20 minutes.

Poggio (2015) stated that the use of 15% EDTA alone or with 2.5% NaOCl was able to significantly reduce the microhardness of dentin in the first 5 minutes and did not show significant changes after use for more than 10-15 minutes. Following the chelating effect of 15% EDTA, where the increase in organic matter exposed to the root dentin surface after the demineralization action, the dentin organic matrix can act as a limiting factor in the dissolution of inorganic components, one of which is Ca^{2+} ions, thereby reducing the decalcification action of chelating agents over time.¹³ In a previous study, the use of EDTA as a root canal chelation agent reacted with calcium ions in the apatite crystals, which caused changes in the dentin microstructure and could affect the strength of the tooth structure. In this study, an alternative chelating material was used, which is expected to maintain the physical properties of the teeth naturally, one of which has the effect of low calcium loss.¹⁴

This study used 2% oligosaccharide chitosan gel as an alternative chelation material derived from natural ingredients, which is expected to have potential as a chelating agent and affect calcium ion loss in root canal dentin. Based on the Kruskal Wallis statistical test in the table, $p = 0.003$ ($p < 0.05$). The 2% chitosan oligosaccharide gel chelating agent affects calcium ion loss in root

canal dentin. Chitosan oligosaccharides have a low molecular weight and have advantages over other types of chitosan, which are water-soluble. Chitosan oligosaccharides have a chemical structure similar to chitosan, except for the molecular weight and degree of deacetylation. Therefore, chitosan oligosaccharides have properties similar to chitosan, namely having a high chelating effect, non-toxic, biocompatible, and bioadhesive. The chelation material is believed to assist in preparing narrow and calcified root canals and remove organic and inorganic substances from the smear layer.^{15, 16}

In this study, the chelating material with the lowest average calcium ion loss was the chitosan oligosaccharide gel chelating material at 5 minutes measurement of 0.0181 ± 0.0045 ppm. Previous studies also reported that 0.2% chitosan resulted in higher microhardness and lower surface roughness of root canal dentin than 17% EDTA. Research by Hosseini S et al. (2016) showed that the penetration of chitosan nanoparticles into root canals was more effective than EDTA and NaOCl.¹⁷ However, it was found that one of the drawbacks of chitosan in previous studies was that it was difficult to manipulate because of its insoluble nature and high viscosity. This is due to the structure of chitosan as a polysaccharide with a long chain and high molecular weight, so its use is limited to both the food and beverage industry.¹⁸ There was no significant difference in the group using chitosan oligosaccharide gel at 10, 15, and 20 minutes. Still, if you look at the graph, the mean value from 10 to 15 minutes to 20 minutes there was a considerable difference in the mean value where at 10 minutes, the loss of calcium ions was as much as 0.1440 ± 0.2194 ppm, at 15 minutes 0.0376 ± 0.0091 ppm and at 20 minutes 0.0826 ± 0.0095 ppm. And statistically, the loss of calcium ions in the 2% chitosan oligosaccharide group had a significant difference between the 5 minutes time measurements.

The application time and concentration of chelating agents influence calcium ions' loss.^{5,6} In the study of Silva et al. (2012) showed that 0.2% chitosan applied for 3 minutes to root dentin showed a cleaning ability similar to 15% EDTA on its chelating capability of removing inorganic smear layers.¹⁶ Although the exact mechanism is not yet known, it is believed that this is due to the adsorption properties, ion exchange effects, and chelating properties that function to form complexes of chitosan substances with metal ions. The chitosan oligosaccharide dissolved in citric acid could remove the smear layer significantly with minimal erosion after immersion for 5 minutes.¹⁷ Tooth tissue was used as a sample. This uncontrolled variable may cause significant differences in the measurements of 5, 10, 15, and 20 minutes.¹⁹

Based on Table 2, the results of statistical testing using Mann-Whitney found a value of $p = 0.021$ ($p < 0.05$) at 5 minutes measurement where there is a difference in the number of calcium ions lost in root canal dentin after the application of 15% EDTA gel and 2% chitosan oligosaccharide gel. At the 5 minutes measurement, the p -value = 0.248 ($p > 0.05$) at the 10-minute height showed that there was no difference in the amount of calcium ion lost in the root canal dentin after the application of 15% EDTA gel and 2% chitosan oligosaccharide gel at 10 minutes measurement. Meanwhile, at 10 minutes and 20 minutes, the value of $p = 1.00$ ($p > 0.05$) showed that there was no difference in the number of calcium ions lost in the root canal dentin after the application of 15% EDTA gel and 2% chitosan oligosaccharide gel in the measurement of 15 minutes or 20 minutes.

The relationship between the concentration of the chelating agent and the time of application seems important because high-concentration solutions applied over a long period can cause dentin surface roughness.²⁰ This follows the research of Mittal (2018) where a 0.2% chitosan solution, even in such a low concentration, could remove the smear layer and gave statistically similar results to a solution with a higher concentration of 15% EDTA.²¹ The use of chitosan oligosaccharide gel at a concentration of 2% in this study follows previous research by Ernani (2015), who stated that the use of a low concentration of chitosan for 5 minutes was the most suitable combination for use on root dentin.²² Chelating effect of 0.2% chitosan compared to other solutions tested, related to its beneficial properties and low concentration, showed that this low chitosan concentration was preferred for dentin decalcification.²³ The ability of EDTA to solubilize Ca^{2+} in root canal dentin was demonstrated by the large amount of smear layer removed during instrumentation. Although the loss of calcium ions after the application of chitosan oligosaccharides is not fully known, the mechanism in their work. The chemical modification of chitosan into chitosan gel could increase the adsorption power because the gel form has a larger pore volume.¹⁹ Due to their high adsorption properties and good chelating properties, chitosan oligosaccharides can form complexes between substances and metal ions.²⁴

This study found that the data were not normally distributed in the EDTA and chitosan treatment groups based on the Shapiro-Wilks test. It shows that the loss of calcium ions can be influenced by other factors that cannot be controlled in this study. In addition to application time, concentration, and volume, other factors that can affect the amount of calcium ions in root canal dentin are the thickness of the root canal dentin surface, the size of the dentinal tubules, and the anatomy of the root canal system. Kinds of variations become a challenge.¹⁹

4. Conclusion

When developed as a gel, the 2% oligosaccharide chitosan can be a chelating agent, affecting calcium ion loss. Based on Kruskal Wallis statistical assay was a significant difference in the amount of calcium ion loss at 5, 10, 15, and 20 minutes, p -value = 0.014 ($p < 0.05$). Also, Mann-Whitney analyses showed a significant difference in calcium ion loss in root canal dentin after the application of 2% chitosan oligosaccharide gel and 15% EDTA gel at a measurement time of 5 minutes, p -value = 0.021 ($p < 0.05$).

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Authors Contribution

Contribution	Trimurni A	Geovani G
Concepts or ideas	√	√
Design	√	√
Definition of intellectual content	√	
Literature search		√
Experimental studies	√	
Data acquisition	√	√
Data analysis	√	
Statistical analysis	√	√
Manuscript preparation	√	√
Manuscript editing	√	√
Manuscript review	√	√



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