Diffusion of calcium ions in one-third of root canals after administration of high molecular Chitosan nanohydroxyapatite medicament (In Vitro)

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ABSTRACT Calcium hydroxide is currently the gold standard medicament. Still, it has the disadvantage of leaving a residue on the surface of the root canal wall. Hence, it can interfere with the setting time and adhesion of the sealer material to the root canal wall. This study aims to determine the ability of calcium ion diffusion in one-third of the root canal after administration of nanohydroxyapatite medicament with a different vehicle than calcium hydroxide. This study used 24 mandibular premolars extracted, decorated, and prepared, then grouped into four treatment groups. Based on the paired T-test, the study results showed no significant effect of calcium ion diffusion in group B (p = 0.739) on the 7th and 14th days. The ANOVA test results showed no significant difference in calcium ion diffusion between groups A, B, and C, on the 7th day of testing (p=0.773) and the 14th day (p=0.661). In conclusion, there was no effect and difference in the diffusion of calcium ions in the apical third after administration of medicament nanohydroxyapatite vehicle bidistilled water and nanohydroxyapatite vehicle nanochitosan compared to calcium hydroxide on the 7th and 14th day.

KEYWORDS: diffusion, calcium hydroxide, ion calcium, nanohydroxyapatite, nano chitosan, vehicle

INTRODUCTION

Endodontic treatment aims to eliminate bacteria in the root canal, promote healing of periapical lesions to prevent apical periodontitis.1,2 The success of endodontic treatment depends on the principles of the endodontic triad, namely cleaning, shaping, and obturation.3 One-third of the apical root canal is most of the lateral canal, which is 73.5% and has many ramifications and canals' accessory.4 Difficult to reach this area with instrumentation, requiring root canal medicaments to eliminate bacteria in the root canal and the periapical area.5

Calcium hydroxide is the current gold standard medicament.6,7 Calcium hydroxide has broad-spectrum antibacterial activity and can maintain long-lasting antibacterial properties, destroy necrotic tissue debris, stimulate hard tissue formation, and heal periapical tissue.6,7 The disadvantage is that it leaves a residue of 20-45% on the surface of the root canal wall, even after irrigation with saline, NaOCl, or EDTA, thereby interfering with the setting time of the ZOE (zinc oxide eugenol) and resin-based sealer materials as well as the adhesion of the sealer to the wall of the root canal.2 Calcium hydroxide is a strong base. Consequently, it can denature the carboxylate and phosphate groups which cause the weakening of the dentin structure, thereby reducing the microhardness value of dentin.8

Hydroxyapatite is a major component of bones and teeth.9,10 Natural sources are clamsHELLS (nacre), mammal bones, coral reefs, and eggshells.11,12 Pearl shell nacre is a natural composite material consisting of more than 95% inorganic materials, calcium carbonate in the form of aragonite crystals, and 1-5% organic matter.13 Marpaung Y's research regarding the content of nacre ingredients, which consists of calcium content (95.04%), so it is known that nacre powder is a calcium-rich material.14

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Chitosan is a natural polysaccharide obtained from chitin. Chemically, chitin is composed of copolymers of (1-4)-2-amino-2-deoxy-β-D-glucan and (1-4)-2-acetamide-2-deoxy-β-D-glucan. Chitosan is biocompatible, biodegradable, bioadhesive, nontoxic, high bioactivity, low solubility, capable of forming gels, anti-inflammatory, and excellent antibacterial activity. Calcium hydroxide and nacre (nanohydroxyapatite) are known to have calcium ions (Ca2+) and hydroxyl ions (OH-). Calcium ions play a role in the healing and repair of periapical tissues. The hydroxyl and calcium ions produced will diffuse through the dentinal tubules, lateral canals, and accessories to reach the external surface of the root canal and periapical.

Related to the description above, the purpose of this study was to determine the difference in the diffusion ability of calcium ions in one-third of the root canal after administration of the medicament nanohydroxyapatite vehicle bidistilled water, nanohydroxyapatite vehicle nanochitosan, compared to calcium hydroxide.

**MATERIALS AND METHODS**

The type of research used is an experimental laboratory with a post-test-only control group design. This research was conducted at the Department of Dental Conservation, Faculty of Dentistry, USU, Chemistry Laboratory of FMIPA USU, and USU Integrated Research Laboratory. It used 24 mandibular premolar samples extracted for orthodontic purposes and divided into four groups.

The research procedure is begun with sample preparation. The sample was cleaned using an ultrasonic scaler. Next, the teeth were decorated at the cementoenamel junction using a diamond bur disc, with a standardized length of each tooth of approximately 15 mm. Then, cleaning and shaping were performed, starting with root canal exploration using K-file #08, #10, and K-file #15. Negotiation and determination of root canal glide path with K-file #10 up to as many files can fit in. The working length is reduced by 1 mm from the size of the decorated tooth (±15 mm). Root canal preparation using the crown down technique using the instrument i3 gold rotary file (Dentjoy, China). Practice with 350 rpm speed, started with a flaring file, file 20#, file 20#06, then file 25#06 up to all processes. Each file changed a sum up of 2ml of 2.5% sodium hypochlorite solution as root canal irrigation using spuit one side vented 30-Gauge. Before the final irrigation, irrigation was carried out using saline. Last irrigation was carried out with 2 ml of 17% EDTA solution for 1 minute, and the root canals were rinsed with 5ml of saline to remove the EDTA precipitate. The root canals were dried using paper points.

The procedure for preparing nanohydroxyapatite medicament was begun with 5 grams of nanohydroxyapatite (nacre) powder placed on a petri dish, next 5ml of bidistilled water was dropped. Stirred it using a stir bar until turned in a nanohydroxyapatite paste form. Lastly, could you put it in a closed container?

The preparation of 0.2% chitosan nanohydroxyapatite medicament is begun with the preparation of 0.2% chitosan nanoparticles solution. A total of 0.2gr of powdered chitosan (Chitosan Medan) from Prawn Shell, in 100ml of 1% acetic acid solution, was stirred until homogeneous with a magnetic stirrer at a speed of 800 rpm for 2 hours. After that, the chitosan solution was dripped with 0.5% of Natrium tripolyphosphate (NaTPP 0.5%) 20 drops while stirring slowly for ±30 minutes to make it homogeneous. The addition of 0.5% NaTPP was carried out to make the solution's surface smooth. The answer was put into an ultrasonic bath for 1 hour to break the chitosan particles into nanoparticles.

Furthermore, the manufacture of nanohydroxyapatite medicament with a nano chitosan vehicle. 5gr of nanohydroxyapatite powder was placed on a petri dish, then 5ml of 0.2% chitosan nanoparticle solution was added. Stir using a stir bar until well blended into a paste. Store the nanohydroxyapatite paste with the nano chitosan vehicle in a closed container.

Procedure for preparing calcium hydroxide medicaments. Commercial calcium hydroxide (viopaste) 2gr in a syringe equipped with a disposable tip, endo-stop, and a tip cap.

The medicament placement of the root canal procedure was started with the teeth randomly divided into 4 groups, each consisting of 6 teeth. Group A was given nanohydroxyapatite medicament with a distilled water vehicle. Group B was given nanohydroxyapatite medicament with 0.2% nano chitosan vehicle using a lentulo spiral. Group C was given calcium hydroxide paste medicament with a syringe, and group D was dental samples after cleaning and shaping without medicament. Then the cavity was closed with a moist cotton pellet and filled with glass ionomer cement (GIC).

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Procedure for preparing calcium hydroxide medicaments. Commercial calcium hydroxide (viopaste) 2 gram in a syringe equipped with a disposable tip, endo-stop, and a tip cap. The root canal medicament placement procedure started with the teeth randomly divided into four groups, each group consisting of 6 teeth. Group A was given nanohydroxyapatite medicament with a distilled water vehicle. Group B was given nanohydroxyapatite medicament with 0.2% nano chitosan vehicle using a lentulo spiral C was given calcium hydroxide paste medicament with a syringe, and group D was dental samples after cleaning and shaping without medicament. Then the cavity was closed with a moist cotton pellet and filled with glass ionomer cement (GIC).

Furthermore, each tooth was coated with nail polish except for the apical third to close the lateral canal of the tooth. The samples were stored separately in a plastic tube with a lid containing 20 ml of bidistilled water and stored in an incubator at 100% humidity and 37°C. The recording of the diffusion of calcium ions was carried out on samples with different analysis times, the 7th day and the 14th day. The process of testing the number of calcium ions using Atomic Absorption Spectrophotometric (AAS).

The research data obtained were processed, computerized, and analyzed using the normality test using the Shapiro-Wilk test to determine the data were normally distributed. Then a paired T-test was performed to investigate the comparison of the quantity of calcium ion diffusion at seven days and 14 days based on groups A, B, and C. Then the one way ANOVA test with a significance level of α=0.05 to determine the comparison of the quantity of calcium ion diffusion between group A, B, and C at the 7th day and the 14th day.

RESULTS

The results showed that the highest mean value was found in the vehicle aqueous calcium hydroxide group with a value of 0.066 ppm at the 7th day and 0.130 ppm on the 14th day. Testing the research sample obtained data as listed in Table 1.

Table 1. The average value of calcium ion quantity on the 7th day and the 14th day. based on groups A, B, and C

<table>
<thead>
<tr>
<th>Group</th>
<th>7 Days</th>
<th>14 Days</th>
<th>R</th>
<th>T-paired</th>
</tr>
</thead>
<tbody>
<tr>
<td>nanohydroxyapatite vehicle bidistilled water</td>
<td>0.065±0.073</td>
<td>0.052±0.036</td>
<td>-0.013</td>
<td>0.829</td>
</tr>
<tr>
<td>nanohydroxyapatite vehicle nanochitosan</td>
<td>0.035±0.028</td>
<td>0.042±0.011</td>
<td>0.007</td>
<td>0.739</td>
</tr>
<tr>
<td>Calcium hydroxide vehicle aqueous</td>
<td>0.066±0.065</td>
<td>0.130±0.137</td>
<td>0.064</td>
<td>0.286</td>
</tr>
</tbody>
</table>

The results of the paired T-test (Table 1) showed that in group A, the p-value = 0.829 (p>0.05), which means that there is no significant effect of the quantity of calcium ion diffusion after administration of the medicament nanohydroxyapatite vehicle nanochitosan between the 7th day and the 14th day. In group B, the p-value = 0.739 (p>0.05), which means no significant effect of calcium ion diffusion after administration of the medicament nanohydroxyapatite vehicle bidistilled water between the 7th day and the 14th day. In group C, the p-value = 0.286 (p>0.05), meaning no significant effect of calcium ion diffusion after administration of calcium hydroxide medicament between the 7th and 14th days.
The effect of natural silver modified with Zeolite

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Figure 1. Graph of the average calcium ion quantity between groups against the measurement time interval of the 7th day and the 14th day

![Graph showing calcium ion diffusion](image)

Table 2. Comparison of calcium ion quantity between groups at the 7th day and the 14th day

<table>
<thead>
<tr>
<th>Group</th>
<th>one way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 Day</td>
</tr>
<tr>
<td></td>
<td>X ± SD</td>
</tr>
<tr>
<td>nanohydroxyapatite vehicle bidistilled water</td>
<td>0.065±0.073</td>
</tr>
<tr>
<td>nanohydroxyapatite vehicle nano chitosan</td>
<td>0.035±0.028</td>
</tr>
<tr>
<td>Calcium hydroxide vehicle aqueous</td>
<td>0.066±0.065</td>
</tr>
</tbody>
</table>

Based on the one-way ANOVA test (Table 2), the result of p-value = 0.773 (p>0.05) on the 7th day and p-value = 0.661 (p>0.05) on the 14th day, it was concluded that there was no difference in diffusion ability. Significant calcium ions were found in one-third of the root canals after administering the medicament nano-hydroxyapatite vehicle bidistilled water, nanohydroxyapatite vehicle nano chitosan, and calcium hydroxide on the seventh and fourteenth days.

DISCUSSION

This study indicates that the administration of nanohydroxyapatite medicament with vehicle bidistilled water, nanohydroxyapatite medicament with vehicle of nano chitosan, and calcium hydroxide is no significant difference in the quantity of calcium ion diffusion in general. It is due to smear layers on one-third of the apical root canal. The presence of smear layers is possible because the instrument used is a rotary that cut more dentin and dynamic manual agitation irrigation techniques. The use of spuit one side vented 30-gauge. This irrigation technique is challenging to reach one-third of the apical root canal. Its part is impenetrable and has more fins, isthmus, cul-de-sac, lateral and accessory canals, and ramifications. The use of spuit one side vented 30-gauge makes the pressure of the irrigant flow rate is not optimally distributed over the entire surface of the root canal.15

In Table 1, it is known that there was a decrease in the value of calcium ion diffusion in the nanohydroxyapatite group with vehicle bidistilled water, at 0.013 ppm on the 14th day. Statistical tests using paired T showed no effect of calcium ion diffusion in one-third of the root canals after administration of nanohydroxyapatite medicament with vehicle bidistilled water on the 7th and 14th days.

Nanohydroxyapatite derived from pearl oyster's nacre is a calcium-rich material. Still, calcium derived from nanohydroxyapatite may bind to nanohydroxyapatite in peritubular and tubular
dentin so that calcium ions do not diffuse well out of the root canal. It causes low calcium ion diffusion results in this study. According to Hashmi et al.'s research, it was stated that there is a layer of ions consisting of phosphate, calcium, calcium phosphate, and chitosan on the surface/sub-surface of dentin after conditioned dentin with chitosan-hydroxyapatite nano-complex. The presence of bonds between calcium ions and other ions with the dentinal surface allows for an increase in the mechanical integrity of the dentin at the root of the tooth.16

Based on research conducted by Saez et al., analyzing the increase in pH and diffusion of calcium ions over time on calcium hydroxide with distilled water, the enormous diffusion of calcium ions lasted between 30 and 60 days.17 Nanohydroxyapatite with vehicle aqueous will cause calcium and hydroxyl ions to decompose rapidly. It quickly diffuses in the dentinal tubules, lateral canal, and apical foramen and is more soluble when in contact with tissue. Calcium hydroxide with vehicle aqueous has the rapid release of calcium and hydroxyl ions. It is desirable in time-visit situations requiring short-term disinfection.

The statistical test results using paired T showed insignificant results in the nanohydroxyapatite group with nano chitosan vehicle. It is because there is not too much difference in the average value of calcium ion diffusion at 7 days and 14 days, meaning that there is a difference in the increase in calcium ion diffusion, slightly, therefore the statistical test obtained insignificant results.

Based on the average value of calcium ion diffusion, there was an increase in the value of calcium ion diffusion at 0.007 ppm on the 14th day. It means that there is the ability to diffuse calcium ions in one-third of the root canals after placement of medicaments with nano chitosan vehicles between the 7th and 14th. Calcium ions derived from nanohydroxyapatite bind to nanohydroxyapatite on the dentin surface. The addition of nano chitosan, which has chelating properties, causes the release of calcium ions to increase gradually and continuously.

CONCLUSION

In this study, it was seen that there was a continuous increase in the diffusion of calcium ions after administration of nanohydroxyapatite medicament with nano chitosan of 0.007 ppm on the 14th. Based on the results of statistical tests using the paired T-test in this study, there was no significant effect of calcium ion diffusion on the nanohydroxyapatite group with the nano chitosan vehicle (p=0.739) on the 7th and the 14th day. The ANOVA test showed that there was no significant difference in calcium ion diffusion between each group with a value (p=0.773) at the time of testing on the 7th day and (p=0.661) at the time of testing on the 14th day.

Following Ginting’s research (2019), it stated that the diffusion of calcium ions in the calcium hydroxide medicament group with a chitosan vehicle showed continuous and gradual release of calcium ions over time. It ends because of the ion exchange effect and chelating properties of chitosan, which func18 Ballal et al.’s research also concluded that chitosan used as a calcium hydroxide vehicle showed the release of calcium ions from calcium hydroxide, which controlled for an extended period.19 The nano chitosan vehicle was able to increase the viscosity of the nanohydroxyapatite paste and the nature of the nanochitosan which had a lower solubility, causing the chitosan nanohydroxyapatite medicament to be in contact for longer on the root canal wall compared to the aqueous vehicle. It will cause the calcium and hydroxyl ions to decompose gradually and continuously.

In the Calcium hydroxide vehicle aqueous group, statistical test results using paired T showed no significant effect of calcium ion diffusion in one-third of the root canals after administration of calcium hydroxide medicament (Viopaste) on the 7th and 14th day. The average value of calcium ion diffusion has increased, it is because the calcium hydroxide paste used has an aqueous vehicle, which is water-based calcium hydroxide.

The research conducted by Pranab et al. showed that the calcium hydroxide group with vehicle aquabidest, the initial release of high calcium ions in a short duration, followed by a decrease in their number within measurement time.20 The combination of calcium hydroxide with aqueous vehicle will increase the synergism of each component. Properties of the material because it produces reactive oxygen and the occurrence of deprotonation. Aqueous-type vehicles will cause calcium and hydroxyl ions to decompose quickly and dissolve more easily when contact with tissues.
REFERENCES