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THE EFFECT OF CALCIUM HYDROXIDE PARTICLE SIZE ON DENTIN TUBULI HARDNESS

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ABSTRACT

Background: *Calcium hydroxide ($Ca(OH)_2$) is an effective root canal treatment, with calcium and hydroxyl ions effectively released on day 7. However, prolonged use can diminish dentinal tubule hardness and dissolve the hydroxyapatite crystals within them. Nanoparticle $Ca(OH)_2$ demonstrated superior antimicrobial activity compared to conventional $Ca(OH)_2$ because of its deeper penetration into the dentinal tubules..* ***Purpose:*** *This study aimed to evaluate the effect of particle size on dentinal tubule hardness.* ***Methods:*** *True experiment with fifteen premolars with one root canal, no caries, and apical closure were divided into three treatment groups: conventional $Ca(OH)_2$ (group 1, n=5), $Ca(OH)_2$ nanoparticles (group 2, n=5), and untreated (control group), n=5. All samples were incubated for 7 days, and hardness was measured using a micro-Vickers hardness tester at 1/3 of the root canal. Kruskal-Wallis test followed by Mann-whitney post hoc analysis was used to compare the mean microvickers hardness values among different groups. The level of significance was set at $p<0.05$.* ***Results:*** *The results showed that there was significant difference between conventional $Ca(OH)_2$ (73.00 ± 2.71) and $Ca(OH)_2$ nanoparticles (67.40 ± 0.62) p=0.01 and the control group (70.68 ± 1.70; p>0.05) and $Ca(OH)_2$ nanoparticles p=0.03.* ***Conclusions:*** *The use of $Ca(OH)_2$ nanoparticles as an intracanal medicament for 7 days reduced dentin microhardness, whereas conventional $Ca(OH)_2$ did not result in any change in microhardness. Particle size affects the hardness of dentinal tubules.*

Keywords: *Nanoparticle, Hardness, Medicament, Root Canal Treatment*

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INTRODUCTION

Root canal treatment is performed to maintain tooth function in the oral cavity. Root canal treatment aims to eliminate pathogenic bacteria in infected root canals to achieve healing in the periradicular area.[1]

Calcium hydroxide ($Ca(OH)_2$) has been demonstrated to be a highly effective medication for use inside the root canal.[2] This substance operates as an antimicrobial agent by releasing calcium ions (Ca^{2+}) and hydroxyl ions (OH^-), which breach the bacterial cytoplasmic membrane and disable bacterial lipopolysaccharide (LPS).[3] Maximum release of calcium ions (Ca^{2+}) and hydroxyl ions (OH^-) from conventional and nanoparticle $Ca(OH)_2$ intracanal medicaments occurred on day 7.[4] Several other studies have also shown that the use of $Ca(OH)_2$ for 7-14 days can reduce the incidence of recovery failure after root canal treatment and histologically can produce a significant reduction in inflammatory cells compared with one-time root canal treatment.[5] The use of $Ca(OH)_2$ as an intracanal medicament within 7 days proved to be efficient in eliminating the remaining bacteria after biomechanical preparation.[6] The length of time for using $Ca(OH)_2$ as a medicament is in line with the dissociation process of calcium ions, which on average takes 7-14 days in the root canal to obtain an optimal antimicrobial effect.[7]

However, prolonged exposure to $Ca(OH)_2$ in the root canal may decrease the hardness of the dentinal tubules, as it denatures or neutralizes acidic organic components such as phosphate and carboxylate groups in dentin.[8] This can disrupt the bond between collagen fibrils and hydroxyapatite crystals and result in weakened tooth structure, and increased susceptibility to root fracture.[9]

$Ca(OH)_2$ nanoparticles are particulate antibacterial agents that are less than 100 nm in size, whereas commercially available $Ca(OH)_2$ is 0.5-2 µm in size. Nanoparticles have higher antimicrobial activity than conventional $Ca(OH)_2$. The penetration of $Ca(OH)_2$ nanoparticles into the dentinal tubules is much deeper than that in the conventional form.[10] Calcium hydroxide nanoparticles have been developed to eliminate the limitations of conventional $Ca(OH)_2$. This study aimed to evaluate the effect of particle size on dentinal tubule hardness.

METHODS

This study is a type of laboratory experimental research approved by the Health Research Ethics Committee of the Faculty of Medicine, Padjadjaran University (ethical number 222/UN6).KEP/EC/2023. The samples were maxillary first premolars with the following criteria: intact roots, no fractures or caries, perfectly closed apical teeth, and one root canal. Fifteen tooth samples were divided into three groups: conventional $Ca(OH)_2$ treatment (group 1, n=5), $Ca(OH)_2$ nanoparticles (group 2, n=5), and untreated control group (n = 5). The tooth was then cut transversely at the cemento-enamel junction (CEJ) using a carborundum disk, leaving a ±12 mm root canal section.

The tooth was then placed on a damp sponge to retain the moisture of the tooth. A #15 K-file needle was inserted into the root canal until it was visible through the apical foramen, and then subtracted 1 mm from the root length to obtain the working length. Irrigation was performed using a solution of 2.5% NaOCl, NaCl, and 17% EDTA with lubrication of as much as 3 ml in each root canal. Preparations using S1 needle files, which are designed to shape the coronal 1/3 section of the tooth root canal, are irrigated again. Preparations with the S2 file to shape and widen the middle 1/3 of the root canal and irrigate again. Preparations with F1 files up to the root canal length can optimize the final shape of the apical 1/3 section and irrigation again. The preparation was continued until needle file F3 reached its working length. Irrigation to remove the smear layer was performed using 5 ml of 2.5% NaOCl for 3 min and 5 ml of saline solution (0.9% NaCl). Root canals were dried using paper points.

Conventional $Ca(OH)_2$ (Merck) and $Ca(OH)_2$ nanoparticles were synthesized using the same method as that in a previous study.[11,12] The nanoparticles were dissolved in distilled water (Milli-Q) at a water/powder ratio of 0.8. The resulting paste was applied to the prepped root canal.

After that, incubation was carried out using Phosphate Buffered Saline (PBS) solution for 7 days, followed by observation to measure the hardness of dentinal tubules using a microvickers hardness tester (Buehler). Each sample was applied with a load of 50 g for 10 seconds until 3 indentations were obtained on each sample.

RESULT

This study was conducted to evaluate the effect of particle size on the hardness of dentinal tubules. All data obtained were tabulated as mean and standard deviation. Based on Fig 1. The results of the dentinal tubule microvickers test in the $Ca(OH)_2$ nanoparticle group had hardness values ranging from 66.7 VHN to 68.3 VHN with an average of 67.4 VHN. Meanwhile, the microvickers test results on dentinal tubules with conventional $Ca(OH)_2$ have hardness values ranging from 70 to 77 VHN, with an average of 73 VHN. According to the results of this study, 1 week after intracanal medicament application, the mean microhardness values after application of conventional $Ca(OH)_2$ were the highest among all groups.

Figure 1. **Microvickers test results on dentinal tubules**

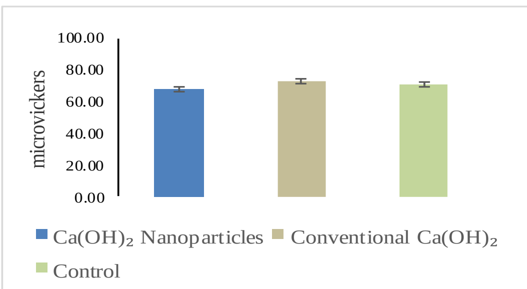

Table 1. showed the results on dentinal tubules with distilled water had hardness values ranging from 69.7 VHN to 73.7 VHN with an average of 70.68 VHN. The Kruskal-Wallis non-parametric test showed a p-value of 0.005, indicating that there was no significant difference between the groups.

Table 1. Hardness value of dentinal tubules of all groups on day 7

Groups	Mean ± SD	*p*
$Ca(OH)_2$ Nanoparticles	67.40 ± 0.62	
Conventional $Ca(OH)_2$	73.00 ± 2.71	0.005
Control	70.68 ± 1.70	

Kruskal-Wallis test, Post-hoc test Mann-Whitney: nano vs conv p<0,05; nano vs conv p<0,05 ; conv vs control p=0,2 .

The hardness of dentinal tubules treated with $Ca(OH)_2$ nanoparticles was the lowest of all the groups. Dentinal tubules treated with conventional $Ca(OH)_2$ had the highest hardness value, exceeding that of untreated dentinal tubules or the control. These results suggest that the hardness of dentinal tubules is significantly affected by particle size, with an application duration of seven days. The results imply that the hardness of dentinal tubules is significantly affected by particle size, as both treated $Ca(OH)_2$ nanoparticles and untreated tubules exhibited comparable hardness values.

DISCUSSION

$Ca(OH)_2$ intracanal medicaments release calcium and hydroxyl ions. Both ions are responsible for damaging the bacterial cytoplasmic membrane and inactivating bacterial lipopolysaccharides.[13] The antimicrobial effect of $Ca(OH)_2$ results from the release of hydroxyl ions by increasing pH. Such an alkaline environment is formed and is not suitable for bacteria to live in.[14] The results of this study showed that there was significant difference in the results of the dentinal tubular microvickers test measured on day 7 with different $Ca(OH)_2$ particle sizes between the conventional $Ca(OH)_2$ group and the $Ca(OH)_2$ nanoparticle group. Similar results in previous studies have shown a significant increase in fracture resistance in samples treated with $Ca(OH)_2$ after 1 week of incubation. This is thought to be due to the penetration ability of $Ca(OH)_2$ into dentin resulting in further increased fracture resistance.[15] As in other studies it is often expressed that after 5 weeks the hardness of the dentinal tubules begins to drop significantly and weakens the tooth causing the fracture resistance of the tooth to decrease.[16]

The low hardness of the dentinal tubules of the $Ca(OH)_2$ nanoparticle group on day 7 may be due to the high antimicrobial activity of the material through the high release of hydroxyl ions (OH^-), resulting in a significant increase in pH, which causes the neutralization of acidic organic components in the dentinal tissue. The neutralization of acidic organic components in dentin tissue can result in the breaking of bonds between hydroxyapatite crystals and collagen fibrils, which can lead to a reduction in the density of dentin structure.[10] The hardness of dentinal tubules can be affected by the diameter and number of dentinal tubules available, the lower these two things, the lower the dentinal hardness. Root dentin has a lower number of tubules than coronal dentin, and the number will continue to decrease from the cervical to the apical direction. The cervical to apical dentin also has a lower density of dentinal tubules compared to other sections.[17] The organic matrix contains phosphate and carboxylate groups that act as binding agents between hydroxyapatite and collagen fibers.[15] The use of alkaline intracanal medicaments such as $Ca(OH)_2$ can neutralize the organic components of dentin tissue, which act as binding agents between hydroxyapatite crystals and collagen fibrils, resulting in a weakening of dentin hardness, making teeth more susceptible to fracture.[18]

$Ca(OH)_2$ nanoparticles have potential as intracanal medicaments. This is because of its ability to effectively penetrate and eliminate bacteria during both short- and long-term exposure. Previous studies have shown a notable negative correlation between dentin microhardness and tubular density.[12,19] $Ca(OH)_2$ nanoparticles have potential as intracanal medicaments.

The application of $Ca(OH)_2$ to dentinal tubules has been extensively studied for its effects on mechanical properties. Studies have shown that prolonged application of $Ca(OH)_2$ can lead to a decrease in the fracture toughness and microhardness of dentin, ultimately weakening the tooth structure.[9] Similarly, short-term application of $Ca(OH)_2$ has also been shown to decrease the tensile strength of dentin.[20] Other studies have shown that the longer the duration of $Ca(OH)_2$ application, the more significant the decrease in fracture toughness, with SEM characterization showing cracks on the dentin surface and FTIR characterization showing a lower mineral-to-matrix ratio in roots exposed to $Ca(OH)_2$ for a prolonged period of time.[21] These findings collectively suggests that while $Ca(OH)_2$ may have antibacterial properties, its effects on the mechanical properties of dentinal tubules, such as tensile strength and elastic modulus, may compromise the structural integrity of the tooth over time. In our study, which is one of the few studies conducted on human teeth, the results clearly showed that intracanal application of different particle sizes of $Ca(OH)_2$ in the root canal for 7 days significantly reduced the hardness of dentinal tubules, which may result in tooth fracture. Further studies to evaluate the duration of the application effect on the mechanical properties are required based on the initial findings.

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