

# Antimicrobial effect of calcium hydroxide as endo intracanal dressing on *Streptococcus viridans*

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## ABSTRACT

Calcium hydroxide had been used as the intra-canal dressing in endodontic treatment due to its high alkaline and antimicrobial capacity. It can also dissolve the necrotic tissue, prevent dental root resorption and regenerate a new hard tissue. The aim of this study was to determine the concentration of calcium hydroxide which had the highest antimicrobial effect on *Streptococcus viridans*. Samples were divided into 5 groups; each group consisted of 8 samples with different concentration of calcium hydroxide. Group I: 50%, group II: 55, Group III: 60%, Group IV: 65%, Group V: 70%. The antimicrobial testing was performed using diffusion method against *Streptococcus viridans*. The result of susceptibility test was showed by the inhibition zone diameter which measured with caliper (in millimeter). We analyzed the data using One-Way ANOVA test with significant difference 0.05 and subsequently LSD test. The study showed that calcium hydroxide with concentration 60% has the highest antimicrobial effect.

**Key words:** calcium hydroxide, *Streptococcus viridans*, antimicrobial effect

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## INTRODUCTION

Since calcium hydroxide applied in dentistry, it has been used for many purposes such as cavity liner; direct and indirect pulp capping; apexification; root resorption prevention; iatrogenic perforation; improvement in cavity base and root canal wall; treatment of horizontal and vertical root fracture; main substance of root canal paste (sealer) and root canal (filler); and irrigation material.<sup>1-3</sup>

Along with science development and modern technology, calcium hydroxide as intracanal dressing has been introduced in modern endodontic treatment to reach sterile root canal. It is due to the capability of calcium hydroxide to solve necrotic tissue; stimulate odontoblast activity and capable penetrate into accessories root canal and dentinal tubule so it would lower permeability of dentinal surface; capable to neutralize acid condition produced by osteoclast activity therefore it could prevent eliminated tissue from further damage; due to presence of calcium ion it could promote excessive exudates to dry and make permeability of capillary blood vessel decrease; and finally calcium hydroxide has very effective antimicrobial effect in endodontic treatment.<sup>1,4</sup>

Grossman *et al.*,<sup>5</sup> suggested that a medicine which is used as intracanal dressing must fulfill some requirements such as: germicide and fungicide; non irritated substance on periapical tissue; stable in solution; long antimicrobial effect; presence of blood, serum and derivate of tissue protein do not decrease the effect; low surface tension; does not change tooth color.

Tronstad *et al.*,<sup>6</sup> suggested that placing calcium hydroxide into root canal would increase pH, contribute alkalis environment in the adjacent tissue by diffusing hydroxyl ion through dentinal tubule. Increasing pH would make calcium hydroxide bactericidal and inhibit osteoclast activity. When calcium hydroxide is solved in sterile aquabidest, it would decompose in calcium ion ( $\text{Ca}^{2+}$ ) and hydroxyl ion ( $\text{OH}^-$ ). The occurrence of hydroxyl ion in solution would make the environment alkalis (pH = 12.5 at 37° C) and destroy bacterial membrane. That condition will killed bacteria.<sup>3,7</sup>

According to Siquira and Lopes<sup>8</sup> the antibacterial effect of calcium hydroxide used as intra-canal dressing is due to its ability to destroyed cytoplasmic membrane cell of bacterial, to denaturated protein and destroyed DNA of bacteria. The most common bacteria found in infected root canal is gram positive bacteria such as: *Streptococcus viridans* including *Streptococcus hemolyticus*, *Lactobacillus* and *Staphylococcus* and followed by gram negative bacteria and some fungi group.<sup>8,9</sup> Grossman *et al.*<sup>5</sup> found the domination of *Streptococcus α Hemolyticus* such as *Streptococcus viridans* (63%), *Staphylococcus albus* (17%), *Dipteroid bacilli* (6.5%), *Staphylococcus aureus*, *Bacillus proteus*, *Bacillus coli*. Saifudin<sup>10</sup> found *Streptococcus α hemolyticus* (76.6%) and anaerobe obligate bacteria (23.4%) infected root canal.

In early 1900, a theory was found on focus infection using intracanal dressing with strong antimicrobial effect for root canal treatment. It contributes sterile root canal and periapical tissue and prevented the possibility of

spreading dangerous bacteria into the body.<sup>6</sup> Grossman *et al.*<sup>5</sup> and Suzuki *et al.*<sup>11</sup> stated that intracanal dressing could destroy pathogenic bacteria and kill microbial flora of infected root canal.

In general the clinical use of the 50% calcium hydroxide (50 gram calcium hydroxide powder in 100 ml sterile aquabidest) is based on factory procedure.<sup>12</sup> Hosoya *et al.*<sup>2</sup> and Estrela<sup>13</sup> used 44% and 38% calcium hydroxide with pH = 11.24 in their study to examine calcium ion (Ca<sup>2+</sup>) and hydroxyl ion (OH<sup>-</sup>) release in the adjacent apical of root canal for 3 days. Sjogren *et al.*<sup>14</sup> stated that calcium hydroxide paste is still effective as long as it is in root canal and shows antimicrobial force for seven days. Grossman *et al.*<sup>5</sup> reported that calcium hydroxide is strong disinfectant in root canal. In this study, pure calcium hydroxide was used not only because its cost is relatively cheap but also because it is availability. It is expected that pure calcium hydroxide would be able to be applied as intracanal dressing in endodontic treatment. The optimum concentration of calcium hydroxide as intra-canal dressing which has highest antimicrobial effect on *Streptococcus viridans* is still need to be studied further.

The purpose of this study was to determine the concentration of calcium hydroxide which has optimal antimicrobial effect against *Streptococcus viridans*. The advantage of this study is to determine concentration of calcium hydroxide applied as intracanal dressing, so optimal endodontic treatment could also be reached.

## MATERIAL AND METHOD

The study used the post test only controlled group design. The study was done at Dentistry and Oral Health Department of Dentistry Faculty, Airlangga University and antimicrobial test was done in microbiology laboratory of Dr. Soetomo General Hospital, Surabaya. The materials were pure calcium hydroxide powder (M2047, Merck Darmstad, Germany), sterile aquabidest (Kimia Farma), Media Brain Heart Infusion (BHI), Media chocolate agar, Normal saline (NaOCl 0.85%), *Streptococcus viridans* (isolated from the patient).

### Calcium hydroxide paste preparation

Calcium hydroxide paste was made by mixing calcium hydroxide powder with sterile aquabidest with concentration of 50%, 55%, 60%, 65%, and 70% until paste was formed. The mixture was made in appendorf tube, after mixed using sterile spatel cement for one minute the mixture was homogenized with vortex for 30 seconds.<sup>13</sup>

### Isolation of *Streptococcus viridans*

*Streptococcus viridans* was isolated from the patient's maxillary anterior teeth with the diagnosis of necrotic pulpa and periapical lesion. The procedure was done as follows; the working region was isolated by rubber dam and 70% alcohol was applied at the surface's tooth. Cavity entrance was made using sterile round bur and removed the pulp

wall. Sterile paper point was inserted into root canal for one minute, and then paper point was put into Brain Heart Infusion (BHI) and incubated in 37° C for 24 hours. The bacteria culture was re-inoculated by spreading it with ose into blood agar media. After 37° C incubation for 24 hours, the bacterial growth was examined using light microscope. The bacteria was re-cultured using chocolate agar plate and incubated 37° C for 24 hours to determine coccus gram positive bacterial growth (chain form,  $\alpha$  hemolytic). The identification was done by gram staining.

### Antibacterial examination

Suspension was done by taking colonies of *Streptococcus viridans* and its culture media using ose, and then mixed by normal saline (NaCl 0.85%) until turbidity equal to standard Mc. Farland 0.5. One ml of *S. viridans* suspension was taken and put into petridish containing Muller Hinton agar media and spread using sterile spreader. Wells were made at the surface of agar media by placing platinum ring with diameter of 6 mm and 9 mm height. Twenty five  $\mu$ l of Calcium Hydroxide with concentration of 50% (group I), 55% (group II), 60% (group III), 65% (group IV), 70% (group V) were put into the wells in Muller Hinton agar media using pipette and incubated at 37° C for 24 hours. The inhibition zone was measured using caliper (0.5 accuracy; in millimeter).<sup>16</sup>

## RESULT

The mean and standard deviation of inhibition zone for 50%, 55%, 60%, 65%, and 70% calcium hydroxide against *Streptococcus viridans* showed on table 1 and figure 1. The result showed that 60% of calcium hydroxide had the highest inhibition zone comparing to other groups.

**Table 1.** The mean and standard deviation of inhibition zone calcium hydroxide in various concentration against *Streptococcus viridans* (mm)

Concentration	N	$\bar{X}$	SD
Group I 50%	8	14.6250	1.1877
Group II 55%	8	14.8750	1.6421
Group III 60%	8	15.7500	1.4880
Group IV 65%	8	13.1250	1.260
Group V 70%	8	11.350	1.1877

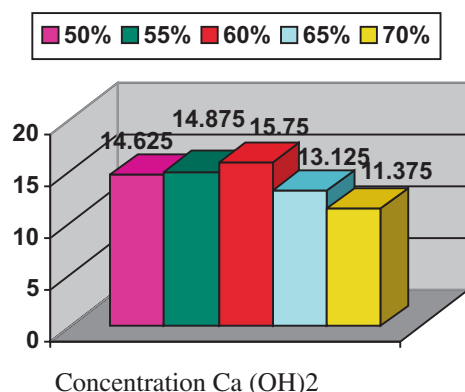
Note: N = Number of samples,  $\bar{X}$  = Mean of inhibition zone, SD = Standard deviation

Statistical analysis using One Direction ANOVA test with significance level  $p = 0.05$  was done to determine the difference of inhibition zone of calcium hydroxide against *Streptococcus viridans*. The statistical result showed that there was a significant difference of inhibition zone of calcium hydroxide in various concentration against *Streptococcus viridans* ( $p < 0.05$ ). Least Significant

**Table 2.** The result of LSD test in diameter of inhibition zone of calcium hydroxide in various concentration against *Streptococcus viridans*

Concentration	Group I	Group II	Group III	Group IV	Group V
Group I	---	0.712	0.102	0.032*	0.000*
Group II		---	0.201	0.013*	0.000*
Group III			---	0.000*	0.000*
Group IV				---	0.013*
Group V					---

\*) significant difference ( $p < 0.05$ )

**Figure 1.** Mean of inhibition zone for 50%, 55%, 60%, 65%, and 70% calcium hydroxide against *Streptococcus viridans*.

Difference (LSD) test was done to determine the difference of diameter of inhibition zone (table 2).

## DISCUSSION

An intra-canal dressing is necessarily given prior to root canal preparation in endodontic treatment to sterilize root canal either from anaerobe or facultative anaerobe bacteria. The dominant bacterial found in root canal are *Streptococcus viridans* which is facultative anaerobe. Calcium hydroxide used as an intracanal dressing in endodontic treatment because it has an alkalis pH (12.5) and high anti microbial effect.<sup>5,17,22</sup> The antimicrobial effect of material or drugs can be examined by sensitivity tests against bacteria, such as dilution or diffusion method.

Agar diffusion method was chosen based on several reasons. First, this method could be used for activity evaluation of antimicrobial medicine which the concentration had been determined. Second, the time needed to analyze the inhibition zone was relatively short. Third, the equipments were simple and easily obtained. Fourth, the cost was relatively inexpensive, and the most important was the method could determine the bactericidal effect of medicine by observing inhibition zone occurred in agar media. This method is generally used to examine anti microbial effect of materials including material of endodontic treatment.<sup>17,18</sup>

Di Fiore,<sup>19</sup> Siqueira and Uzeda,<sup>18</sup> Gomes *et al.*<sup>20</sup> stated that the result of anti microbial test using agar diffusion method depends on several aspects: the size of material molecule, solubility and diffusibility of material in agar media, medicine sensitivity, source of bacterial (strain of species colony), the number of inoculated bacteria, pH of substrate in plate, agar viscosity, condition of agar storage, incubation time and metabolic activity of bacteria. The higher the solubility and diffusibility of material against the media the bigger inhibition zone will be.

Fifty percent of calcium hydroxide is the lowest concentration. The result of previous study showed that the viscosity of calcium hydroxide mixed with sterile aquabidest similar to paste. In this case, it is similar with clinical use in which the ratio between calcium hydroxide powder and sterile aquabidest with 50% concentration. Gomes *et al.*<sup>20</sup> using 50% concentration of calcium hydroxide paste proved that Gram negative anaerobe bacteria was more sensitive to calcium hydroxide than Gram positive anaerobe facultative bacteria.

Sixty percent concentration of calcium hydroxide showed mean of inhibition zone was 15,7500 mm (Table 1) which the highest antimicrobial effect comparing to the other groups. It might due to ion hydroxyl released ( $\text{OH}^-$ ) from calcium hydroxide. Ion hydroxyl ( $\text{OH}^-$ ) is highly free radical oxidant shows strong reactivity against bacterial cell.<sup>21</sup> Effect of ion hydroxyl ( $\text{OH}^-$ ) is very reactive and quickly combines itself with lipid, protein and nucleate acid resulting lipid peroxides. It would increase membrane permeability of bacterial cells, and followed by protein denaturation, inactivating enzyme and DNA destruction which kill the bacteria.<sup>1,12</sup> In this concentration calcium hydroxide has not reached the saturation point so calcium hydroxide is still capable to diffuse into Muller Hinton agar culture media which has been exposed by *Streptococcus viridans*.

Table 2 showed the antimicrobial test result of calcium hydroxide against *Streptococcus viridans*. Calcium hydroxide 55%, and 60% concentration compare to group 50%, 60% concentration compare to 55%, did not show significant difference. It might due to viscosity of the mixture and the number of released hydroxyl ion was the same. In 65% concentration compare to 50%, 55%, and 60% concentration of calcium hydroxide, and also 70% concentration comparing to 50%, 55%, 60%, 65% calcium

hydroxide showed significant difference in inhibition zone against *Streptococcus viridans*. It might due to the viscosity of calcium hydroxide powder and sterile aquabidest has reached saturation point in 65% and 70% concentration, consequently, hydroxyl ion is difficult to release into agar media resulting the decrease of number of hydroxyl ion and small inhibition zone. The diffusibility and solubility of calcium hydroxide in agar media was very low due to high viscosity of material. Safavi and Nakayama<sup>7</sup> suggested that the effect of water insoluble solvent would decrease the affectivity of calcium hydroxide. It is also proved by Suzuki<sup>11</sup> by mixing calcium hydroxide and pure glycerin or propylene glycol (including calcium hydroxide vehicle). Both materials are non-polar therefore it does not show inhibition zone of bacteria. Since the solution has reached saturation point, so, hydroxyl ion (OH<sup>-</sup>) can not diffuse into agar culture media. The number of hydroxyl (OH<sup>-</sup>) which has been released will be less and the anti microbial effect will decrease. There is other factor such as buffer capacity of culture media which lowering the pH. It makes the antimicrobial of calcium hydroxide reduce.<sup>23,26</sup> The study showed that calcium hydroxide with 60% concentration showed the highest antimicrobial effect compared to calcium hydroxide with 50%, 55%, 65%, and 70% concentration.

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