Antimicrobial activity of calcium hydroxide, calcium oxide, and mineral trioxide aggregate paste against α-Hemolytic *Streptococcus*

Eveline Yulia Darmadi¹, Diana Soesilo²
¹Division of Conservative Dentistry, Faculty of Dental Medicine, Universitas Ciputra, Surabaya, Indonesia
²Department of Conservative Dentistry, Faculty of Dentistry, Universitas Hang Tuah, Surabaya, Indonesia

**ABSTRACT**

**Background:** The bacteria mostly found in the root canals are aerobic and facultative anaerobic bacteria, such as α-Hemolytic *Streptococcus*. Calcium hydroxide has alkaline characteristics with pH of 12.5, and antimicrobial properties. Calcium oxide is hydrophilic and has a smaller molecular weight than calcium hydroxide. Mineral trioxide aggregate (MTA) has alkaline properties, good biocompatibility, stimulates healing and inhibits bacterial growth. **Purpose:** The purpose of this study was to examine the differences of antimicrobial activity between calcium hydroxide, calcium oxide, and mineral trioxide aggregate (MTA), also to find which of these materials has the highest antimicrobial activity. **Methods:** The design of this study was a post-test only control group design. The study subjects were divided into 4 groups. K (-) was control group without any treatment, P1 was treated with calcium hydroxide, P2 was treated with calcium oxide, and P3 was treated with MTA. Each group consisted of 7 samples. The α-Hemolytic Streptococcus were spread on Mueller Hinton agar. Antimicrobial test was using diffusion method and diameter of inhibition zones were measured with caliper. **Results:** Average inhibitory zone of each sample was P1 (30.9643 mm ± 0.7431), P2 (35.2357 mm ± 0.7099), P3 (28.6 mm ± 1.5532). Tukey HSD test showed significances results between samples P1 and P2 (p=0.001), P1 and P3 (p=0.002), P2 and P3 (p=0.001). **Conclusion:** The highest antimicrobial activity against α-Hemolytic *Streptococcus* was calcium oxide followed by calcium hydroxide and MTA.

**Keywords:** antimicrobial activity, calcium hydroxide, calcium oxide, mineral trioxide aggregate, α-Hemolytic *Streptococcus*

Correspondence: Eveline Yulia Darmadi, Division of Conservative Dentistry, Faculty of Dental Medicine, Universitas Ciputra, Citraland CBD Boulevard, Sambikerep, Surabaya 60219, Indonesia. E-mail: eveline.darmadi@ciputra.ac.id

**INTRODUCTION**

Previous researches have been done to seek materials with potent antibacterial activity.¹⁻³ These materials may come from marine resources.⁴⁻⁵ The materials may also come from other natural resources.⁶⁻⁸ The bacteria most often found in root canals are anaerobic, aerobic and facultative anaerobic bacteria, such as α-Hemolytic *Streptococcus*. The α-Hemolytic *Streptococcus* is one of bacteria in the root canals which is gram positive and has the form of a coccus with long chains.⁹ Root canal bacteria adhere to the dentin walls or penetrate the dentin tubules. Bacteria attached to dentin are easier to kill than in dentin tubules, but bacteria in dentin tubules can also be killed with a paste that has antimicrobial properties.¹⁰

One of the ingredients often used in root canal treatment is calcium hydroxide. Calcium hydroxide paste was introduced in the field of dentistry in 1920 as a treatment and filling of root canals. Calcium hydroxide is also an ingredient used for direct or indirect pulp capping treatment, prevention of root resorption, root perforation, root fracture and apexification.¹¹ Although calcium hydroxide may affect the viability of cells, it can regulate anti-inflammatory cytokine.¹²⁻¹⁴ Treatment with calcium hydroxide can cure periapical infections, cease pus formation, reduce pain, has a pH of 12.5 so it is alkaline, and also has antimicrobial properties. Calcium hydroxide releases hydroxyl ions which can cause damage to the cytoplasmic membrane of bacteria, then the process of protein denaturation occurs, and inhibits DNA replication of bacteria, thereby inhibiting bacterial growth. Calcium hydroxide paste can inhibit the growth of *Streptococcus mutans, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Bacillus subtilis, Enterococcus faecalis, and Porphyromonas gingivalis*.¹⁵⁻¹⁷ Persistent infections sometimes occur and are one possibility due to the presence of germs in the dentin tissue. Bacteria and their products are also found in many dentinal tubules and accessory canals; therefore, research was developed until the discovery of the calcium oxide technique based on biological and chemical principles.¹⁰
Materials and Methods

Tools used in this research were sterilized using a dry heat sterilizer. The α-Hemolytic Streptococcus bacteria were transferred in BHIB (Brain Heart Infusion Broth) media with a loop. Then 0.1 cc was taken with a micropipette, taken into 25 ml of Mueller Hinton Agar medium, then spread evenly over the Mueller Hinton Agar medium with a spreader, then onto the medium 4 wells were made in each petri dish by placing a platinum ring with a diameter of 5 mm and a 9 mm height. The ring is placed using tweezers heated with spiritus burner. The first well was the control group (without treatment), the second well was filled with calcium hydroxide paste, the third well was filled with calcium oxide paste, and in the fourth well was filled with MTA. Samples were incubated at 37°C for 24 hours.

The results were done by observing and calculating the area of the inhibition zone. Measurement of the inhibition zone was done by measuring the diameter of the inhibition zone using a caliper which has an accuracy of 0.05 mm.
Table 1. The p value of the results of the Shapiro Wilk test and Levene test of the inhibition zone against α-Hemolytic Streptococcus

<table>
<thead>
<tr>
<th>Sample Group</th>
<th>Shapiro Wilk test</th>
<th>Levene test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium hydroxide</td>
<td>p = 0.993 *</td>
<td></td>
</tr>
<tr>
<td>Calcium oxide</td>
<td>p = 0.758 *</td>
<td>p = 0.139 *</td>
</tr>
<tr>
<td>MTA</td>
<td>p = 0.815 *</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Tukey HSD test results for the inhibition zone against α-Hemolytic Streptococcus

<table>
<thead>
<tr>
<th>Sample Group</th>
<th>Calcium hydroxide</th>
<th>Calcium oxide</th>
<th>MTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium hydroxide</td>
<td>-</td>
<td>p = 0.001 *</td>
<td>p = 0.002 *</td>
</tr>
<tr>
<td>Calcium oxide</td>
<td>-</td>
<td>-</td>
<td>p = 0.001 *</td>
</tr>
<tr>
<td>MTA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

zone measurements against α-Hemolytic Streptococcus have normal data distribution. Meanwhile, the results of the homogeneity of variance test using the Levene test statistical test have a p value > 0.05. This showed that the three groups of inhibitory zone measurements for α-Hemolytic Streptococcus had homogeneous variances.

The One-Way ANOVA test was carried out to determine differences in inhibition zones against α-Hemolytic Streptococcus between groups which obtained p value = 0.001 (p < 0.05). This shows that there was a significant difference in the zone of inhibition against α-Hemolytic Streptococcus between the three groups. To determine the level of significance between the three groups, the Tukey HSD statistical test was used. The results obtained were as shown in Table 2 that the results of the difference test between all groups had a p value <0.05. This showed that there was a significant difference in the zone of inhibition against α-Hemolytic Streptococcus between the three groups.

DISCUSSION

Calcium oxide is hydrophilic and alkaline. The binding reaction of water with calcium oxide forms calcium hydroxide. Release of hydroxyl ions occurs, neutralizing the acidic pH of bacteria, which can cause damage to the cytoplasmic membrane of bacteria, causing lipid peroxidation and inhibiting the work of enzymes that play a role in the growth and metabolism of bacteria in the cytoplasmic membrane in tissues through the activity of alkaline phosphate. This enzyme is important for inhibiting the work of bacterial enzymes. The release of hydroxyl ions causes protein denaturation, inhibits DNA replication, lyses necrotic tissue and releases carbon dioxide, thus inhibiting bacterial growth. Calcium oxide paste contains zinc oxide, where zinc oxide also has bacterial inhibitory power by damaging cell proteins and bacterial enzymes.11,21

Calcium hydroxide paste has inhibitory power against α-Hemolytic Streptococcus bacteria due to the release of hydroxyl ions, which will come into contact with the bacteria resulting in hydrolysis of the polysaccharide lipids from the bacteria, this will increase the permeability of the bacterial cell membrane, then the process of protein denaturation, inactivation of enzymes and inhibiting DNA replication occurs, so, it will inhibit the growth of bacteria.22 The bacterial inhibitory power of calcium hydroxide paste is directly influenced by the release of hydroxyl ions. Calcium hydroxide can inhibit bacterial growth because it is bacteriostatic. The release of hydroxyl ions inactivates bacterial lipopolysaccharide. Bacterial lipopolysaccharide possesses wide range of effects to the periapical tissue destruction.23,24 The inactivation of bacterial lipopolysaccharide reduces the local inflammatory response, increases the solubility of necrotic tissue, and creates an alkaline environment around the tissue.25

Mineral trioxide aggregate paste has a similar inhibitory reaction to calcium hydroxide. The release of hydroxyl ions is obtained from calcium oxide which comes into contact with water to form calcium hydroxide which then releases calcium ions and hydroxyl ions.26,27 With the release of hydroxyl ions, it will damage the cells cytoplasmic membrane, then a protein denaturation process occurs, thereby inhibiting bacterial DNA replication which will inhibit bacterial growth.28

The inhibitory power of mineral trioxide aggregate paste was smaller than calcium oxide paste and calcium hydroxide paste, because inhibiting bacterial growth was obtained from the release of hydroxyl ions resulting from calcium dissolution. The calcium content in paste containing calcium oxide is 71.4%; in paste containing calcium hydroxide of 54.11%; and MTA paste by 50%. This content affects pH in releasing hydroxyl ions. The greater the calcium ions that are released, the greater the hydroxyl ions that are produced.29

Things that can influence the way antimicrobial properties work are the speed of dissociation and diffusion of a drug, membrane permeability, buffer capacity, pH and hydroxyl ions of a drug, and viscosity. These factors also influence the size of the obstacle zone.30 Drugs that easily experience diffusion and dissociation will produce high bacterial inhibition power and produce large inhibition zones. The mechanism of action of antimicrobial drugs is to inhibit bacterial cell synthesis, inhibit the formation of bacterial cell walls, inhibit protein wall synthesis, inhibit bacterial cell nucleic acid synthesis and disrupt bacterial permeability.31,32 This study is preliminary where complexity of the root canal system that affect the efficacy of these materials in application is not considered. Further studies are suggested with advanced techniques or more complex root canal systems.

In conclusion, there were differences in the inhibitory activity among calcium oxide, calcium hydroxide, and MTA paste against α-Hemolytic Streptococcus. Calcium oxide paste had the greatest inhibitory activity compared to calcium hydroxide paste and MTA paste against α-Hemolytic Streptococcus, while calcium hydroxide paste has a greater inhibitory activity compared to MTA paste against α-Hemolytic Streptococcus.
REFERENCES


