ANTIMICROBIAL EVALUATION OF DIFFERENT PULP CAPPING MATERIALS

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ABSTRACT

Objective: Three different pulp capping compounds, Dycal, Rootdent MTA, and Biodentine, were assessed for their antibacterial effectiveness against Streptococcus mutans and Enterococcus facialis microorganisms and were investigated by agar diffusion test. Materials and methods: A total of (10) sterilized Petri plates of agar were arbitrarily assigned into 2 groups conferring on the type of tested microorganisms applied (n=5); A1 and A2 for Streptococcus mutans and Enterococcus facialis, respectively. In each group, the experimented materials were classified into three major groups: I, II, and III corresponding to Dycal, Rootdent MTA, and Biodentine. Every major group was divided into three distinct subgroups based on the observation periods: (TB1), (TB2), and (TB3) observed after 1, 2, and 7 days, respectively, in order to examine the presence of growth inhibition zones (GIZs) that developed around pulp capping materials. Using a digital micrometer, the size of bacterial growth inhibitory zones was measured to the closest hundredth of a millimeter. Results: All tested materials exhibited antibacterial activity against both tested microorganisms. In Streptococcus mutans, in (TB1), the greatest diameter of bacterial GIZs was performed around Biodentine, followed by Rootdent MTA and Dycal, respectively. In Enterococcus facialis, the greatest diameter of bacterial GIZs was formed around Rootdent MTA at all evaluation periods, followed by Biodentine and Dycal, respectively. Conclusions: All the tested bioactive materials effectively inhibit the growth of both microorganisms. Rootdent MTA was more effective against Enterococcus facialis, Biodentine was more effective against Streptococcus mutans, while Dycal was the least effective material against both microorganisms.

KEYWORDS: Pulp capping, antimicrobial, growth inhibition zones, agar diffusion test.

INTRODUCTION

Necrosis and inflammation of the pulp may result from pulp exposure and subsequent infection. In such circumstances, root canal therapy may be required. The pulp-capping substances must function as a barrier to preserve the health of the injured pulp and avoid a need for root canal therapy. In addition, the direct pulp capping materials must be compatible with the touched pulp tissue and not produce inflammation, irritation, allergic response, or toxicity in the pulp. Microbiological effects of direct pulp-capping substances are among the most significant factors affecting the efficiency of the treatment. Due to the action of the substance, the microorganisms surrounding the pulp would be eliminated (¹).

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Bacterial contamination is the leading cause of failure. Microorganisms in deep lesions can induce significant inflammatory responses or even necrotic damage in the pulp. Consequently, minimizing pathogenic bacteria is essential for improving pulp capping treatment in cavities with deep lesions. Damage to the pulp may be caused by germs remaining in the dentine following cavity preparation. This deterioration necessitates using pulp-capping chemicals with antibacterial properties underneath the irreversible restorations (2).

Direct pulp-capping involves enclosing the pulp’s exterior surfaces to retain its viability and maintain its operational and biological activity. Widely acknowledged as the ultimate objective of capping substance is to trigger the cells of the pulp to create tough tissue. Numerous compounds, including Ca(OH)₂-based substances, Biodentine, and mineral trioxide aggregate (MTA), are often indicated to close the uncovered pulp inside the mouth (3).

Due to their tendency to liberate OH⁻ and Ca²⁺ ions upon breakdown, Ca(OH)₂-based compounds are the most common indirect and direct pulp-capping agents. It is hypothesized that they initiate a process in which undifferentiated pulp cells transform into odontoblasts. These odontoblasts subsequently build a barrier of hard tissue at the exposed pulp. The creation of healthy dentine in response to Ca(OH)₂ might not be related to the substance’s bio-inductive properties. It may be a defensive process initiated by the pulp in response to Ca(OH)₂’s irritating nature (4).

Dycal is a radiopaque Ca(OH)₂-based substance used in pulp-capping (indirect and direct). Its basicity (pH = 10) encourages the development of 2° dentine when it directly contacts the pulp (5). MTA is formed of Ca₃(PO₄)₂, silicon trioxide, bismuth, and calcium, as well as hydrophilic granules that become rigid in the presence of water. MTA was first offered as a material for perforation sealing and root-end repair. It has antibacterial properties due to its high PH. MTA is a biocompatible cement; when comes into touch with tissue fluid, hydroxyapatite crystals develop on top of it and close effectively. Nevertheless, it has some acknowledged downsides, like an increased price, a prolonged setting time, and the possibility of discoloration (6). Biodentine is an intriguing substitute for traditional Ca(OH)₂-based products. It provides benefits over pulp capping (direct type) and, in appropriately chosen situations, may aid in the lengthy preservation of tooth life (7).

MATERIALS AND METHODS

Chemicals

Three pulp capping materials were selected for this study; Dycal (Dentsply, Milford, DE, United States), Rootdent MTA (Spesifikasi, Techno Dent, Russia) and Biodentine (Septodont, SaintMaur-des-Fosses, France).

Study setting

The research was conducted on ten sterile Petri dishes (ten plates; five for each microorganism) at Assuite University, Department of Microbiology, Faculty of Medicine.

Study design

The study was carried out on microorganisms of Streptococcus mutans and Enterococcus facialis.

Sample preparation

Two microorganism strains were activated from stock culture and kept in Muller Hinton (MH) broth until usage. All microbial strains were cultured for 24 hours at 37 degrees Celsius in MH broth before being inoculated onto 15 milliliters of MH agar to obtain a McFarland turbidity level of 0.5, which equates to 10⁶ colony forming units on ml. The seeded sterilized (MH) was poured into 20cm² sterilized Petri plates and left to harden in an incubator at 37°C for 24 h (8).
Agar diffusion method

Using a sterile copper coil, three uniform cavities of 2 mm in depth with a diameter of 10 mm, corresponding to the three tested materials, were punched at equidistant sites in agar after 24 hours under aseptic conditions. The pulp capping ingredients instantly filled the voids after blending as instructed by the manufacturer. Subsequently, the plates were incubated at 37 degrees Celsius for a week.

Sample grouping

For each microorganism, the experimented pulp capping materials were classified into three main groups; Dycal, Rootdent MTA, and Biodentine, named as groups I, II, and III, respectively. Each main group was classified into three subgroups according to observation periods; (TB1), (TB2), and (TB3) observed after 1, 2, and 7 days respectively.

Observations

All specimens will be observed after one day (TB1), two days (TB2), and one week (TB3). The region of microbial GIZs around each capping material was determined in millimeters with an accuracy of 0.5 millimeters using a digital caliper. Measurement of the size of the inhibitory zone was determined using the expression: (diameter of halo - specimen diameter) × 1/2.3

Statistical analysis

A one-way ANOVA was used for the statistical examination of the average GIZs among the tested compounds. To compare diverse variables, the post hoc test was utilized. P <0.05 denoted a significant statistical variation among the studied groups.10

RESULTS

In Streptococcus mutans:

After 24 hours (TB1): the highest mean value and standard deviation of bacterial growth inhibition zones were recorded by Biodentine (9.89±0.95) followed by Rootdent MTA (9.57±0.46), while the lowest mean value and standard deviation were recorded by Dycal (6.03±0.17). There was a significant statistical variation among groups I, II, and III (p < 0.001). There was a significant statistical variation in (group I) compared to II and III with (p= 0.001 in both cases). Groups II and III exhibited no significant statistical variation (p=0.803).

After 48 hours (TB2): the highest mean value and standard deviation of the bacterial growth inhibition zones were recorded by Dycal (7.79±0.72), followed by Biodentine (7.77±0.38), while the lowest mean value was recorded by Rootdent MTA (6.85±0.63). The three groups did not differ significantly where (p = 0.165).

After 1 week (TB3): the standard deviation and highest mean value of the bacterial growth inhibition zones were recorded by Biodentine (5.77±0.80), followed by Dycal (5.72±0.69). In contrast, the lowest mean value was recorded by Rootdent MTA (5.61±0.45). None of the differences between groups I, II, and III were statistically significant (p= 0.955).

In Enterococcus facials:

After 24 hours (TB1): the highest mean value and standard deviation of bacterial growth inhibition zones were recorded by Rootdent MTA (8.86±0.55) followed by Biodentine (6.89±0.53), while the lowest mean value and standard deviation were recorded by Dycal (4.55±0.62). The three groups had a significant statistical variation (p<0.001). Also, a significant statistical variation was found when comparing group I with group II and group III, where (p<0.001) and (p = 0.006), respectively. In addition, a significant statistical variation was found between group II and group III (p = 0.013).

After 48 hours (TB2): the highest mean value and standard deviation of the bacterial growth inhibition zone were recorded by Rootdent MTA (9.46 ± 0.49) followed by Biodentine (7.73 ± 0.51), while the lowest mean value standard deviation
was recorded by Dycal (4.65 ± 0.71). There was a significant statistical variation between groups I, II, and III (p < 0.001). A significant statistical variation was found when comparing group I with group II and group III, where (p < 0.001) and (p = 0.001) respectively. Also, groups II and III showed a significant statistical variation (p = 0.025).

After 1 week (TB3): the highest mean value and standard deviation of the bacterial growth inhibition zone were recorded by Rootdent MTA (7.47 ± 0.57) followed by Biodentine (6.27 ± 0.28). In contrast, the lowest mean value standard deviation was recorded by Dycal (4.71 ± 0.51). There was a significant statistical variation between groups I, II, and III (p = 0.001). A significant statistical variation was found when comparing group I with group II and group III, where (p = 0.001) and (p = 0.016), respectively. Also, a significant statistical variation was found between groups II and III (p = 0.047).

**TABLE (1)** The mean and standard deviation (SD) values of Streptococcus mutans for different groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1 (Dycal)</th>
<th>Group 2 (Rootdent MTA)</th>
<th>Group 3 (Biodentine)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>After 24 hours (TB1)</td>
<td>6.03</td>
<td>0.17</td>
<td>9.57</td>
<td>0.46</td>
</tr>
<tr>
<td>After 48 hours (TB2)</td>
<td>7.79</td>
<td>0.72</td>
<td>6.85</td>
<td>0.63</td>
</tr>
<tr>
<td>After 1 week (TB3)</td>
<td>5.72</td>
<td>0.69</td>
<td>5.61</td>
<td>0.45</td>
</tr>
<tr>
<td>p-value</td>
<td>0.024*</td>
<td>&lt;0.001*</td>
<td>0.046*</td>
<td></td>
</tr>
</tbody>
</table>

*: significant (p<0.05)  ns; non-significant (p>0.05).

**TABLE (2)** The mean and standard deviation (SD) values of Enterococcus facialis for different groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1 (Dycal)</th>
<th>Group 2 (Rootdent MTA)</th>
<th>Group 3 (Biodentine)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>After 24 hours</td>
<td>4.55</td>
<td>0.62</td>
<td>8.86</td>
<td>0.55</td>
</tr>
<tr>
<td>After 48 hours</td>
<td>4.65</td>
<td>0.71</td>
<td>9.46</td>
<td>0.49</td>
</tr>
<tr>
<td>After 1 week</td>
<td>4.71</td>
<td>0.51</td>
<td>7.47</td>
<td>0.57</td>
</tr>
<tr>
<td>p-value</td>
<td>0.295ns</td>
<td>&lt;0.001*</td>
<td>0.012*</td>
<td></td>
</tr>
</tbody>
</table>

*: significant (p<0.05)  ns; non-significant (p>0.05)
FIG (1) Bar chart representing Streptococcus mutans for different groups.

FIG (2) Inhibition zones of pulp capping materials against Streptococcus mutans after 48 hours.

FIG (3) Inhibition zones of pulp capping materials against Streptococcus mutans after one week.

FIG (4) Bar chart representing Enterococcus facialis for different groups.

FIG (5) Inhibition zones of pulp capping materials against Enterococcus facialis after 48 hours.

FIG (6) Inhibition zones of pulp capping materials against Enterococcus facialis after one week.
DISCUSSION

Pulp capping and pulpotomy operations depend heavily on pulpal tissue’s recovery capacity, age, periodontal health, stage of root development, size of exposure and its traumatic or carious character, and microbiological contaminations all have a significant impact on the success rate of the pulp capping procedure (11). Direct pulp capping is an alternative to extraction or endodontic treatment that includes inserting a biocompatible substance on pulp tissue that has been accidentally exposed due to trauma or intervention. The therapy aims to preserve healthy pulp tissue by preventing bacterial infiltration of the pulp and beginning the creation of a dentine bridge at the exposure location (12). In pulp capping failure, bacteria are the principal etiological agents. S. mutans and E. faecalis were included in the current investigation because S. mutans has a substantial impact on both the initial pulpal lesion and subsequent pulpal disease. In addition, E. faecalis is more likely to be detected in instances of endodontic treatment failure due to resistance (13).

The antimicrobial properties of dental cements have been thoroughly examined. Due to its antibacterial qualities and ability to trigger enzymatic processes that result in the creation of a dentine bridge, Ca(OH)₂ is still regarded as the standard material for pulp capping. The antibacterial action of Ca(OH)₂-based materials is dependent on the ionization process that releases hydroxyl ions and raises the pH. A microorganism’s cellular membrane enzymes may be reversibly or permanently inactivated by increased pH (14).

The agar diffusion and direct contact tests have been used to investigate the antibacterial impact of dental materials (15). The agar diffusion test was employed in this investigation because it directly compares various materials against the same tested microorganism, but it does not discriminate between the materials’ microbiostatic and microbicidal capabilities (16).

The bacterial plates containing the tested materials were incubated at 37°C in anaerobic conditions to simulate the oral temperature and condition for one week and evaluated after 24 h, 48 h, and one week. The diameter of bacterial GIZs was measured by a digital caliper 24 h after the tested materials were assumed to reach their final chemical structures, then at 48 h and one week as the release of active ingredients from the tested materials is still possible after setting (17).

All tested materials exhibited antibacterial activity against both tested microorganisms at all evaluation periods. In Streptococcus mutans, at 24 h evaluation period, the greatest diameter of bacterial GIZs performed around Biodentine, Rootdent MTA, and Dycal, respectively. The difference between Dycal and each of Biodentine and Rootdent MTA was statistically significant, while the difference between Biodentine and Rootdent MTA was statistically non-significant. The difference between the three tested materials was statistically non-significant at 48 h and one week. In Enterococcus facialis, at all evaluation periods, the greatest diameter of bacterial GIZs was formed around Rootdent MTA, Biodentine, and Dycal, respectively. The difference between all tested groups was statistically significant.

This result agrees with the study performed by Taha Ozyurek et al. (10). They reported that Biodentine produced significantly greater inhibition zones against E. coli and S. mutans than MTA-Angelus. MTA-Angelus inhibited P. aureus and E. faecalis more effectively than Biodentine. Furthermore, the results are in agreement with the study performed by Mine Koruyucu et al. (18). They reported that in studying the antibacterial activity of MTA, Biodentine, and Dycal against the Enterococcus faecalis; the maximum bacterial growth inhibition zone was observed around MTA, Biodentine, and Dycal, respectively, in samples freshly mixed and aged for 24 h. In samples collected after one week, the antibacterial activity of MTA and Biodentine was shown to be comparable to each
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other and greater than that of Dycal. Biodentine’s antibacterial efficacy maintained its normal levels during the one-week period, but MTA’s antibacterial efficacy declined and matched Biodentine’s levels. Likewise, research conducted by Madani et al. (19), reported that the bacterial cell count of Enterococcus faecalis, Escherichia coli, Streptococcus mutans, and Candida albicans was significantly lower in MTA-based sealer compared to Ca(OH)$_2$-based sealer.

Ca (OH)$_2$’s antibacterial action depends on its alkalinity (pH = 10), and its antibacterial activity is connected to the release of hydroxyl ions in an aqueous environment (20). Hydroxyl ions are extremely reactive free radicals with a wide range of biomolecules. Because of its high and indiscriminate reactivity, this free radical seldom diffuses away from its source. In contrast, MTA is composed of 50-75% calcium oxide and 15-25% silicon dioxide by weight. These components were combined to form tricalcium silicate, di-calcium silicate, tri-calcium aluminate, and tetra-calcium aluminoferrite. The production of a silicate hydrate gel and Ca(OH)$_2$ results from the hydration of the cement. As a result, MTA and Ca(OH)$_2$ may share a similar mode of action against bacteria (21). In Biodentine; the powder comprises tricalcium silicate, calcium carbonate, and zirconium oxide as a radio pacifier, while the liquid comprises calcium chloride as a setting accelerator and water as a reducing agent. The calcium silicate may interact with water, causing the cement to set and solidify. The hydration of tricalcium silicate results in the formation of hydrated calcium silicate gel, Ca(OH)$_2$, and unreacted tricalcium silicate. Because of the high alkaline pH of the surrounding environment when it dissolves, the Ca(OH)$_2$ formed by tricalcium silicate hydration has antibacterial and anti-inflammatory characteristics. Over time, the pH level remained steady at roughly 11-12. The hydroxyl ions produced from Biodentine develop their mechanism of action. Hydroxyl ions are highly active molecules that reversibly or irreversibly inactivate the cellular cytoplasmic membrane enzymes, inhibiting microorganisms’ growth (22).

The results of this study are in disagreement with the study performed by Aditi Subodh Jain et al. (23), they reported that inhibition zones performed around Biodentine against S. mutans and E. faecalis were significantly larger than the zones performed around MTA. The differences in the used materials and corresponding halo diameter may be the cause as in the study performed by Aditi Subodh Jain et al. They used MTA (Dentsply, Tulsa Dental, OK, USA) with corresponding halo diameters of (4 mm width x 4 mm depth). While in this study, the used material was Rootdent MTA; (Spesifikasi Techno Dent -Russia.) with corresponding halo diameters of (10 mm width x 2 mm depth). In disagreement with the study performed by Saeed Asgary et al. (24), they reported that the antimicrobial effect of Ca(OH)$_2$ against S. mutans and E. facialis was superior to that of MTA. The differences in the used materials and corresponding halo diameters may be the cause, as in the study performed by Saeed Asgary et al., they used Ca(OH)$_2$ (Sealapex, Keer, Orange, CA, USA) and ProRoot MTA (Dentsply Tulsa Dental, OK, USA) with corresponding halo diameters (4 mm width x 2 mm depth). While in this study, the used materials were Dycal and Rootdent MTA (Spesifikasi Techno Dent -Russia.) and 10 mm width x 2 mm depth corresponding to halo diameter. This disagrees with the study by Claudio Poggio et al. (25), they reported that; Ca(OH)$_2$-based materials presented a higher antibacterial activity than MTA-based products against Streptococcus salivarius, Streptococcus sanguis, and Streptococcus mutans strains. The paper disc impregnated with the tested material in the agar diffusion test may be the cause of the difference, as in this study, the agar diffusion test was used with uniform cavities cross bonding to the tested materials. Also, in dis accordance with the study performed by Torabinejad et al. (26), they did not find an antibacterial effect of MTA against any of the strictly anaerobic bacteria. However, as shown by the results of this study, it is possible that the highly alkaline (pH =12) MTA affords antimicrobial activity even in anaerobic conditions.
CONCLUSIONS

From the current study, it could be concluded that; all the tested bioactive materials are effective in inhibiting the growth of both microorganisms. Root dent MTA was more effective against Enterococcus facialis, and Biodentine was more effective against Streptococcus mutans, while Dycal was the least effective material against both microorganisms.

REFERENCES


