

## ANTIMICROBIAL ACTIVITY OF DIFFERENT BIOACTIVE THERAPEUTIC PULP CAPPING MATERIALS WITH RELEVANCE TO THEIR BIOLOGICAL AND CLINICAL CONSIDERATION: A COMPARATIVE *IN VITRO* STUDY

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### ABSTRACT

**Background:** Pulp capping with an appropriate dental material aims to preserve the vitality of the tooth by acting as a barrier between the exposed pulp and the oral cavity thus protecting the dental pulp complex. The more the antimicrobial effect of this material, the less incidence of infection and inflammation of the pulp. The appropriate selection of biocompatible pulp capping will help proliferation and differentiation of the pulp cells with secondary dentin formation. **Objectives:** The antimicrobial activity of five different pulp-capping materials: Dycal, Urbical LC, TheraCal LC, mineral trioxide aggregate (MTA) and Biodentine was evaluated and compared. **Methods:** Using the agar disc diffusion test, *Streptococcus mutans* and *Lactobacillus* reference strains were streaked onto appropriate culture agar-plates. Paper disks were impregnated with each pulp capping material and placed onto the culture media and incubated at 37°C. The inhibition zone for each bacterial strain around different pulp capping materials was recorded and compared. The collected data were statistically analyzed using one-way ANOVA at  $p \leq 0.05$ . **Results:** For both types of bacteria, there was a significant difference between all groups. *Biodentine* group was significantly higher antibacterial activity and greater inhibition zone than other groups. **Conclusions:** Biodentine presented better antibacterial activity when compared with other tested materials in short and long-term period.

**KEY WORDS:** Antibacterial effect, Biodentine, calcium hydroxide, Urbical lc, dental materials, *Streptococcus mutans*, *Lactobacillus*, MTA, pulp capping.

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## INTRODUCTION

Pulp protection is the main concern when dealing with deep cavity. Pulp harm could occur during cavity preparation as results from the ingress of remaining microorganisms directly to the pulp through dentinal tubules. This dictates the necessity of using bioactive pulp capping materials under the permanent restorations that could provide dual action; a therapeutic activity to allow for pulp healing and an antibacterial activity to prevent microbial harm to the delicate pulp tissues and eventually preserving the pulp vitality and main source of tooth nutrition.<sup>[1]</sup> The more the antimicrobial effect of the pulp capping material, the less incidence of infection and inflammation of the pulp and eventually increasing the ability of the pulp cells to proliferate and differentiate for secondary dentin formation and pulp protection<sup>[2]</sup>.

The pulp protection procedures could be performed via direct or indirect pulp capping approaches. Many pulp capping materials are now available in the field of conservative dentistry. They were categorized into; calcium hydroxide based materials, calcium-phosphate compounds, calcium silicate based materials, resin based materials and mineral-trioxide aggregates based (calcium alumina silicate) ones.<sup>[3]</sup> Despite numerous investigations,<sup>[4-6]</sup> Indeed, the exact mode of action of calcium hydroxide is not fully understood, but its high pH likely contributes to its bactericidal properties, impacting microbial growth. Additionally, it's known for its ability to stimulate the formation of dentine bridges, aiding in dental treatments.. Dycal<sup>™</sup> (*Dentsply*) is the most popular calcium hydroxide based materials that is claimed to stimulate secondary dentin formation indicated for both direct and indirect pulp capping. Dycal from Denstply forms a protective base under cements, restorative materials and other base materials, stimulating the formation of secondary dentin.<sup>[1,4]</sup> It still have some clinical limitations as degradation

by etching and rinsing prior to restoration, and, it dissolves clinically within 1-2 years. It's crucial to address microleakage issues in dentine bridges under capping to prevent potential pulpal infections and necrosis. Regular monitoring and early intervention can maintain vitality of dental tissue<sup>[1]</sup> Urbical LC is a light-curing calcium hydroxide paste has proven to produce significantly higher compressive strength than conventional calcium hydroxides thus it was suggested that it provides better support and foundation under the permanent restorations and minimize the risk of filling fractures.<sup>[4]</sup>

Recently, there was a shift to calcium silicate materials as these materials are interesting but challenging. *MTA<sup>™</sup>* (well-root) is mineral trioxide aggregates that has been released, accepted and revealed widespread use in dentistry. Its main constituents were calcium and phosphorus in addition to other mineral oxides such as bisemous oxides that cause severe tooth discoloration. MTA as capping agent revealed the formation of reparative dentin. In addition to their antibacterial traits that were assigned to its release of calcium hydroxide on surface hydrolysis of the calcium silicate components<sup>[7,8]</sup>.

*Biodentine<sup>™</sup>* (Septodont) is a new fast-setting calcium silicate-based restorative material which is used as a dentine replacement material. It demonstrated to be very efficient for pulp capping, management of furcation perforation and retrograde filling as well owing to its great biocompatibility, the strong mechanical and physical properties and capability of inducing the apposition of the reactionary dentine. Moreover, being bismuth-free it doesn't induce tooth discoloration<sup>[8]</sup>.

*TheraCal LC* has been classified as a 4th generation light cured calcium silicate-based material released by the manufacturer for to be used as a pulp capping agent. The combination of polymerizable methacrylate monomers, Portland cement type III, polyethylene glycol

dimethacrylate, and barium zirconate suggests a versatile dental restorative material. The fact that it is well tolerated by immortalized odontoblast cells is a positive aspect for its biocompatibility.<sup>[9]</sup> All the different addressed formulations of these therapeutic pulp capping materials were claimed to have biochemical and bioactive properties so they were able to remineralize and reinforce the softened remaining thin dentin bridge and, minimize pulp inflammatory response at the exposure site, and stimulate reparative dentin formation.<sup>[1-9]</sup> However, their healing power is directly interrelated to their antibacterial properties to any prevent microbial harm during the healing process. So, detecting the best bioactive material in antimicrobial properties will be a guidance to know which pulp capping material of them is more effective in initiating and enhancing secondary dentin formation.

The aim of this in vitro study was to evaluate antibacterial activities of five different pulp-capping materials with different chemical formulations; Biodentine™, MTA (well-root), Dycal (dentsply), Urbical LC and TheraCal, against *Lactobacillus* and *Streptococcus mutans* by agar well diffusion method. The null hypothesis tested is there will be

no difference in antibacterial activity of the tested materials.

### Methods

This research protocol was registered in Faculty of oral and dental medicine, Ahram Canadian University. The research number is IRB00012819#73.

### Therapeutic pulp capping materials

Five pulp-capping materials were selected for this study: Dycal® (Dentsply), Urbical LC, TheraCal LC® (Bisco Inc), MTA (well root) and Biodentine® (Septodont) are showed in Fig 1. The materials descriptions, compositions and manufacturers are listed in table 1.

### Bacterial strain activation and preparation of culture media

They were prepared in strict compliance to manufacturers' instructions. The used bacterial isolates were *Streptococcus mutans* and *Lactobacillus* reference strains which were stored in brain heart infusion broth. *Streptococcus mutans* was cultured on blood agar (Oxoid, Rodano, Milano, Italy), while *Lactobacillus* was cultured on MRS *Lactobacillus* agar.



Fig. (1) Materials used in pulp capping

TABLE (1) The materials descriptions, compositions and manufacturers

Product name	Description	Composition	pH	Manufacturer
Dycal	Auto-cured calcium hydroxide	Two-paste system made of a base paste (1,3-butylene glycol disalicylate, zinc oxide, calcium phosphate, calcium tungstate, and iron oxide pigments) and a catalyst paste (calcium hydroxide, N-ethyl-o/p-toluene sulphonamide, zinc oxide, titanium oxide, zinc stearate, and iron oxide pigments)	9-11	Dentsply Tulsa Dental, Johnson City, TN, USA
Urbical	Light cured calcium hydroxide	Mixture of different dimethacrylates, calcium hydroxide, pigments, initiators, silicate fillers	10-12	Mixture of different dimethacrylates, calcium hydroxide, pigments, initiators, silicate fillers
TheraCal	Light cured calcium silicate	Light-curing, resin-modified calcium silicate filled liner single paste containing CaO, calcium silicate particles (type III Portland cement), Sr glass, fumed silica, barium sulphate, barium zirconate, and resin containing Bis-GMA and PEGDMA	10-11	Bisco Inc., Schamburg, IL, USA
MTA well root	Mineral trioxide aggregates	Powder containing type III Portland cement, bismuth oxide, tricalcium silicate, dicalcium silicate, and tricalcium aluminate tetracalcium aluminoferrite	12	Vericom, South Korea
biodentine	Dentine substitute	Powder: tricalcium silicate, dicalciumsilicate, calcium carbonate and oxide filler, iron oxide shade, and zirconium oxide. Liquid: calcium chloride as an accelerator and a hydrosoluble polymer that serves as a water reducing agent	12	BiodentineTM (Septodont, St. MaurdesFossis, France)

### Agar well diffusion method

Agar well diffusion method was used to evaluate the antimicrobial activity of the five pulp-capping materials. In this method, the agar plate surface was inoculated by spreading  $1 \times 10^7$  cells/ml of an overnight culture of each strain over the entire agar surface at 37°C. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100  $\mu$ L) of the pulp-capping materials was introduced into the well. Then, agar plates were incubated under aerobic condition with 5% CO<sub>2</sub> for *Streptococcus mutans*

and under anaerobic condition for *Lactobacillus* for 24-72 hours. The pulp-capping material diffuses in the agar medium and inhibits the growth of the microbial strain tested. The diameter of the halo formed around the agar well (inhibition zone) was measured after 24 h, 48 h and 72 h.<sup>[10]</sup>

### Statistical analysis:

Numerical data was represented as mean and standard deviation (SD) values. Shapiro-Wilk's test was used to test for normality. Homogeneity of variances was tested using Levene's test. Data showed parametric distribution and variance

homogeneity so they were presented as mean and standard deviation values and were analyzed using one-way ANOVA followed by Tukey’s post hoc test for comparison between different groups and repeated measures ANOVA followed by Bonferroni post hoc test for comparison between different intervals. The significance level was set at  $p \leq 0.05$  for all tests. Statistical analysis was performed with R statistical analysis software version 4.1.3 for Windows. <sup>[11]</sup>

**RESULTS**

For both types of bacteria, as revealed from table 2 and figures 2,3,4, there was a significant difference between all groups ( $p < 0.001$ ). Regarding *Streptococcus mutans* count recorded at 24 and 72 hours, Biodentine group was significantly higher than other groups, TheraCal group was significantly higher than groups with lower mean values and Dycal group was significantly higher than Urbical and MTA well groups ( $p < 0.001$ ). At 48 hours, streptococcus mutans count at Biodentine group was significantly higher than other groups and value recorded at TheraCal group was significantly higher than groups with lower mean value ( $p < 0.001$ ). Regarding *Lactobacillus* count recorded at 24 hours, Biodentine group was significantly higher than other

groups and Dycal group was significantly higher than groups with lower mean values ( $p < 0.001$ ). At 48 hours, values recorded at different groups was significantly different from each other ( $p < 0.001$ ). At 72 hours, Biodentine group was significantly higher than other groups, and Dycal group was significantly higher than groups with lower mean values and Urbical group was significantly higher than MTA well root group ( $p < 0.001$ ). For intragroup comparison of *Streptococcus mutans* count, there was a statistically significant difference between different intervals in all groups ( $P < 0.05$ ) except for Biodentine group ( $p = 459$ ). For TheraCal, Urbical and MTA well root groups, 24 hours interval showed significantly lower value than other intervals ( $p < 0.001$ ). For Dycal group, 72 hours interval showed significantly higher value than 24 hours interval ( $p < 0.001$ ). For intragroup comparison of *Lactobacillus*, there was a significant difference between different intervals in Urbical and Dycal groups ( $p < 0.05$ ). For both groups, 72 hours interval showed significantly higher value than 24 hours interval ( $p < 0.001$ ). Other groups showed no statistically significant difference between different time intervals ( $p > 0.05$ ). Photographs showing the inhibition zones of the different pulp-capping materials as determined by agar well diffusion method; are presented in Fig. 2.

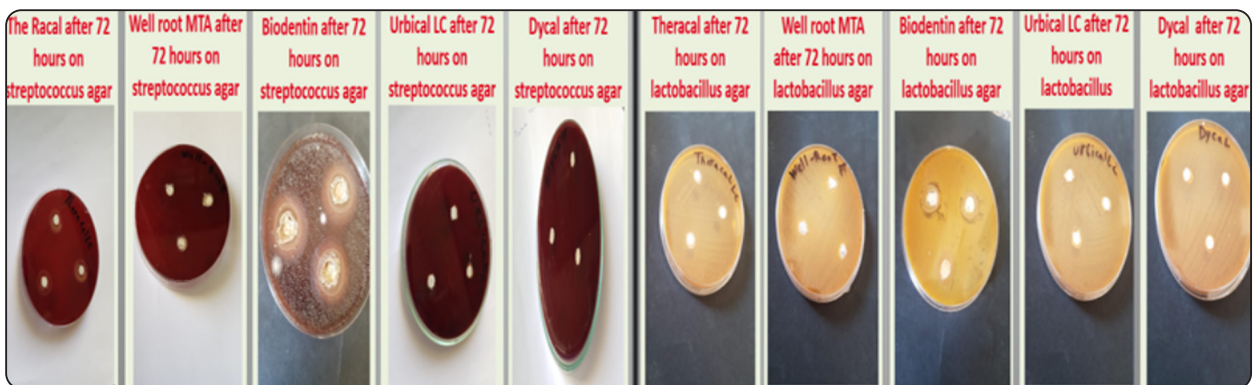


Fig. (2): Inhibition zones of different pulp capping materials



TABLE (2) Mean, Standard deviation (SD) values of the inhibition zone for different groups

Bacteria	Time	The inhibition zone (mean±SD)					p-value
		TheraCal	Urbical	Dycal	MTA well root	Biodentine	
<i>Streptococcus mutans</i>	24 hours	15.23±1.23 <sup>Bb</sup>	10.56±0.43 <sup>Db</sup>	13.05±0.77 <sup>Cb</sup>	11.06±0.32 <sup>Db</sup>	30.54±0.76 <sup>Aa</sup>	<0.001*
	48 hours	17.07±0.38 <sup>Ba</sup>	12.91±0.77 <sup>Ca</sup>	14.01±0.89 <sup>Cab</sup>	12.61±0.56 <sup>Ca</sup>	31.14±2.28 <sup>Aa</sup>	<0.001*
	72 hours	17.82±0.29 <sup>Ba</sup>	12.08±0.23 <sup>Da</sup>	15.42±0.50 <sup>Ca</sup>	12.22±0.14 <sup>Da</sup>	31.09±0.16 <sup>Aa</sup>	<0.001*
	p-value	0.003*	<0.001*	0.009*	<0.001*	0.459ns	
<i>Lactobacillus</i>	24 hours	11.05±0.58 <sup>Ca</sup>	11.25±0.20 <sup>Cb</sup>	14.12±0.73 <sup>Bb</sup>	10.54±0.64 <sup>Ca</sup>	20.24±0.47 <sup>Aa</sup>	<0.001*
	48 hours	11.13±0.07 <sup>Da</sup>	12.02±0.21 <sup>Ca</sup>	15.12±0.56 <sup>Ba</sup>	10.25±0.30 <sup>Ea</sup>	20.51±0.75 <sup>Aa</sup>	<0.001*
	72 hours	11.09±0.94 <sup>CDa</sup>	12.42±0.25 <sup>Ca</sup>	15.61±0.15 <sup>Ba</sup>	10.16±0.60 <sup>Da</sup>	20.64±0.60 <sup>Aa</sup>	<0.001*
	p-value	1ns	<0.001*	0.026*	1ns	1ns	

Different superscript letters indicate a statistically significant difference within the same horizontal row \*; significant (p ≤ 0.05) ns; non-significant (p>0.05)

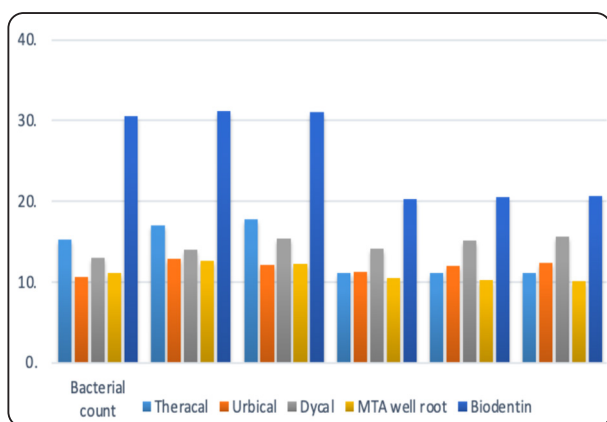


Fig. (3) Bar chart showing average bacterial count for different groups

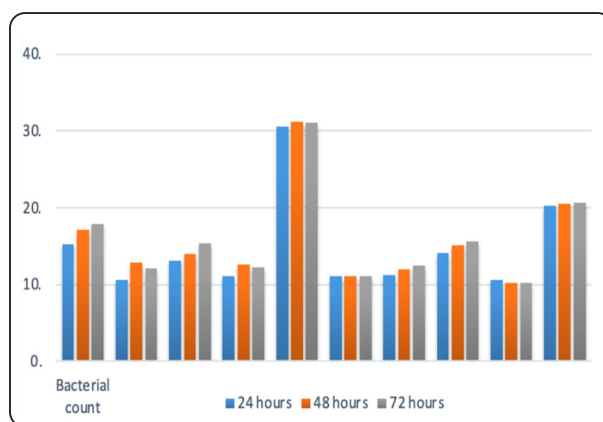


Fig. (4) Bar chart showing average bacterial count for different follow up intervals

DISCUSSION

The incidence of infection and inflammation of the pulp decreased by increasing the antimicrobial effect of the pulp capping material, and eventually increasing the ability of the pulp cells to form secondary dentin and preserve the pulp vitality.<sup>[1,2]</sup> So, assessment of the antimicrobial properties of different pulp capping materials will help us to know which pulp capping material of them is more effective in initiating and enhancing secondary dentin formation. Since Few studies in the literature

discussed the antimicrobial effect of newly emerged pulp capping materials,<sup>[2-9]</sup> so the present study aimed to evaluate the antimicrobial properties of newly emerged materials and to compare their antimicrobial effects with old materials by using the agar well diffusion test (ADT) to study the antimicrobial activity of five different pulp-capping materials: Dycal (Dentsply, Germany), Urbical LC (Voco, Germany), TheraCal LC (Bisco, USA), mineral trioxide aggregate (MTA)(well-root, UK) and Biodentine (Septodont, France).

It was reported that cultivable microorganisms usually infect the deeper layers of carious dentine most commonly *Lactobacillus species* and some *Streptococcus mutans*. So in the present study, it was seen beneficial to assess the antibacterial efficiency of newly emerged materials like fast-setting calcium silicate-based effective and biocompatible pulp capping material [Biodentine™] and light cured calcium silicate-based material [TheraCal LC] in comparison to old and gold standard routinely used calcium based pulp capping materials like Dycal and Urbical against these pathogens. The antimicrobial characteristics of the dental materials have been tested using two different assays; Direct contact test (DCT) and agar diffusion test (ADT). The DCT is a reproducible and quantitative method simulating the contact of the root repair materials in the root canal with the microorganism<sup>[12,13]</sup>. ADT has the advantage of the availability for different bacterial species. It depends on the solubility and diffusion capacity of the tested materials and the medium used, molecular weight, the shape and size of the active antimicrobial agent, as well as load and concentration of the tested materials<sup>[13]</sup>.

In the current study it was intended to select different therapeutic pulp capping materials with different chemical formulation covering wide range of almost the different classification of pulp capping materials so it could be possible to compare their antibacterial properties related to their chemical composition. Moreover, it was addressed in the literatures that the selected materials having a biochemical and bioactive properties for successful healing process and inducing reparative dentine formation<sup>[14,15]</sup>. Fortunately the current study proved that all tested pulp capping materials showed bacterial inhibition zones with different diameters. It was found interesting to reveal carefully the interpretation of these findings. The most important factor was the influence of pH on growth, metabolism and cell division of microorganism. All the tested materials contain or produce Calcium hydroxide during their biochemical reaction creating an alkaline hydroxyl environment [16,17]. In the current study the null hypotheses was rejected

as all tested pulp capping materials react differently based on their chemical formulation and viscosity.

The antibacterial activity of capping materials has been intensively tested in previous studies. Calcium hydroxide has been commonly used in pulp capping due to its antimicrobial properties and ability to promote dentin bridge formation. Calcium hydroxide maintains the vitality of tooth pulp by promoting dentin bridge formation, exhibits antibacterial properties and has a low cytotoxicity, enhancing its effectiveness as a pulp capping agent<sup>[1,3-9,18]</sup>.

In the current study, regarding calcium hydroxide auto-cured and light cured groups (Dycal and Urbical), the result revealed a significantly high value at 24 and 72 hours. In *Streptococcus mutans* the result showed that Dycal group was significantly higher than Urbical and MTA (well-root) groups ( $p < 0.001$ ). While in *Lactobacilli*, Dycal group at 24 hours was significantly higher than other groups ( $p < 0.001$ ). For Dycal group, 72 hours interval showed a significantly higher value than 24 hours interval (For TheraCal, Urbical and MTA (well-root) groups) ( $p < 0.001$ ). This result might be attributed to the fact that calcium hydroxide causes a superficial coagulation of the pulp tissue on which it is placed inducing dentine formation and repair<sup>[18]</sup>. The success rates with calcium hydroxide on carious exposures are highly variable, unpredictable and often unsuccessful. This could be attributed to the antibacterial activity of Calcium hydroxide-based materials which depends on loss of cellular cytoplasmic membrane integrity by releasing hydroxyl ions that causes an increase in pH. However, some calcium hydroxide-based materials were inefficient in inhibiting some facultative aerobic and anaerobic bacteria. It was reported that calcium hydroxide lack adhesive and sealing abilities due to poor physical properties and easy dissolution over time. In addition to presence of multiple "tunnel" defects and formation of porosities under calcium hydroxide pulp caps. It has relatively low flexural, compressive strength, wear resistance and adhesion properties. These properties are not as critical for its use in pulp capping<sup>[5, 13]</sup>.

Seltzer and Bender,<sup>[2]</sup> stated that calcium hydroxide when used as a pulp capping or a pulpotomy agent had two undesirable side effects which was attributed to the possibility of eventual complete calcification of the tissue in the root canal leading to difficult subsequent endodontic therapy. In addition to persistence of induced inflammation causing internal resorption<sup>[18,9]</sup>.

The coronal surface structure of calcium hydroxide-induced bridges was examined by light microscope and scanned by electron microscope by Schroder and Granath. They found collagen bundles similar to those found in normal predentine surrounding tubular openings. It has been found that alkaline phosphatase and lactic dehydrogenase activity was relatively inhibited by saturated calcium and barium hydroxide depending on the pH level<sup>[18,19]</sup>.

MTA has been proposed as a suitable material for pulp capping, on the basis of its biological properties. In the current study, MTA results reveal that: intragroup comparison of both *Streptococcus mutans* and *Lactobacillus* count, MTA (well-root) groups showed significantly lower value in 24 hours interval than other intervals ( $p < 0.001$ ). This could be explained by the fact that MTA stimulates the differentiation and proliferation of pulp cells and facilitates the formation of a more structured mineralized barrier. MTA is currently considered the “gold standard” material, despite the long time required for manipulation and the need for moisture during setting<sup>[20-24]</sup>.

In this study, Biodentine results in both *Streptococcus mutans* and *Lactobacillus* count recorded at all intervals were significantly higher than other groups. This might be attributed to its inherent alkaline pH which was able to control the growth of *S. mutans* and *Lactobacillus*. In addition, during hydration of tricalcium silicate present in Biodentine, the calcium hydroxide is produced enhancing the antimicrobial and anti-inflammatory properties of the material<sup>[25,26]</sup>. While in other groups, there was a statistically significant difference between different intervals in all groups

( $P < 0.05$ ) regarding results of *Streptococcus mutans* count.

The principal advantages of Biodentine over MTA are its shorter setting time (12 min) and greater viscosity. These properties make Biodentine suitable for pulp-capping material, compatible with the cell recruitment and a dentine substitute. Shayegan *et al.*, assessed the pulpal response of primary pig teeth against Biodentine. They found that Biodentine encourages regeneration of hard tissue and has bioactive and sealing properties without pulp inflammation response or necrosis<sup>[17,21]</sup>.

This study reveals that regarding *Streptococcus mutans* results recorded at 24 and 72 hours, TheraCal group was significantly higher than other groups except Biodentine. This could be explained by the fact that TheraCal LC has a slower reaction rate and lower calcium ion release than Biodentine. In addition, TheraCal calcium-releasing ability was reported to be significantly less than Biodentine. An alkaline pH does not favor bacterial survival and proliferation. Intragroup comparison of *Streptococcus mutans* results for TheraCal group, 24 hours interval showed significantly lower value than other intervals ( $p < 0.001$ ). This may be attributed to the advantageous properties of TheraCal LC, such as its alkaline pH, protective lining, and lower solubility compared to Dycal, ProRoot MTA, and Biodentine in dental applications.<sup>[9,17,20-24]</sup>

## CONCLUSIONS

Considering the limitation of the current study associated to an in-vitro study design, presence of other pulp capping materials in the market, and assessing only 2 types of bacteria using only ADT for testing antimicrobial activity, it could be suggested that Biodentine showed the highest antibacterial activity than other pulp capping materials used in this study and it could be considered more effective in enhancement of secondary dentin formation.

## List of abbreviations:

Not applicable



### Clinical Relevance:

Detecting the best bioactive material in antimicrobial properties is a guidance to select the most effective pulp capping material in initiating and enhancing secondary dentin formation. Biodentine is effective bioactive pulp capping material with high antibacterial activity than other pulp capping materials and could be considered more effective in enhancement of secondary dentin formation in deep carious lesions and traumatic exposure for a better prognosis.

### RECOMMENDATIONS

Further studies should be done with more pulp-capping materials having antimicrobial activities. This research team are planning to do further studies on the same pulp capping materials used in this study to correlate between their antimicrobial activities and their potential to enhance dental pulp stem cells proliferation and odontogenic differentiation & subsequent dentin bridge / secondary dentin formation.

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