Original Article

Determining the best anti-microbial properties of dental cements used for pulp capping procedures using deep dentinal carious material

Soumya Makarla[1], Reshma Venugopal[1], Radhika M. Bavle[1], Arul K. Selvan[2], Sudhakara S. Muniswamappa[1], Rathidevi Dinesh[1]

Departments of [1]Oral and Maxillofacial Pathology and [2]Microbiology, Krishnadevaraya College of Dental Sciences and Hospital, Bangalore, Karnataka, India

Abstract

Introduction: The exposure of the healthy pulp in cases of deep dentinal caries (DDC) that contain carious microorganisms can be prevented by placing a layer of pulp capping agent on the affected dentin. The cements used for pulp-capping should also ensure good anti-microbial properties. The present study was carried out to detect the antimicrobial efficacy of the commonly used cements by culturing the samples directly from DDC.

Aims: To determine the efficacy of dental cements in the growth inhibition of microorganisms involved in DDC using direct contact anaerobic culture test.

Method: 100 samples of DDC were collected in RTF. Ten microliters of the specimen containing RTF was incubated in thioglycolate broth consisting of 1 mm^3 cement blocks of GIC, $CaOH_2$, ZnOE and MTA anaerobically for 24 hours. This was further sub-cultured using selective media for streptococcus mutans, lactobacillus and bifidobacterium. Growth inhibition was measured by calculating the number of CFUs and statistically analysed using ANOVA and Tukey's post-hoc tests.

Results: Tests showed variation in the anti-microbial effects of the cements and was highly significant at $P < 0.001$. Bifidobacterium showed most number of CFUs. MTA was the most effective pulp capping agent exhibiting 87.13% reduction in microbial growth, followed closely by ZnOE (84.6%).

Conclusion: A conservative approach to treat DDC is the need of the hour which calls for the use of pulp capping cements of good antimicrobial efficacy. The current study revealed bifidobacterium to be the most prevalent in DDC and the cement that would best inhibit the mixed culture growth was MTA followed closely by ZnOE.

Keywords: Anaerobic culture technique, bifidobacterium, calcium hydroxide, deep dentinal caries, direct contact test, glass inomer, lactobacillus, mineral trioxide aggregate, pulp capping, streptococcus species, zince oxide eugenol

Address for correspondence: Dr. Reshma Venugopal, Department of Oral and Maxillofacial Pathology, Krishnadevaraya College of Dental Sciences and Hospital, Bangalore - 562 157, Karnataka, India.
E-mail: reshmav132@gmail.com

Submitted: 06-Apr-2021, **Revised:** 19-Oct-2022, **Accepted:** 16-Dec-2022, **Published:** 21-Mar-2023

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.jomfp_109_21

How to cite this article: Makarla S, Venugopal R, Bavle RM, Selvan AK, Muniswamappa SS, Dinesh R. Determining the best anti-microbial properties of dental cements used for pulp capping procedures using deep dentinal carious material. J Oral Maxillofac Pathol 2023;27:239.

INTRODUCTION

The human teeth are predominantly affected by dental caries, a microbial disease. The teeth can be restored when the caries process involves enamel and dentin. Once its proximity to the pulp is high or it involves the pulp, root canal treatment that involves removal of infected pulp, laborious pulp sacrificing procedure has to be performed.

When the caries process reaches close to pulp but has still not infected the pulp, a procedure termed as pulp capping is done. Here dental cements are placed over the remaining affected dentin or on the pin point exposed pulp and sealed with temporary restorative material. The cavity is re-entered after a months' time to check if hard dentin has been formed and then once the dentin bridge is formed the cavity is restored with permanent restoration.[1]

The main features which allow the healing of pulp and formation of the dentin bridge include: ability of the material to stimulate remineralization, prevent carious microorganisms from progressing i.e., an antimicrobial action and proper sealing to prevent ingress into pulp. Antibacterial efficacy of the pulp capping cement (PCC) is essential to prevent the microbial growth and halt the progression of caries.[2,3]

Various studies have been executed using commercially available pure bacterial strains to test the antibacterial efficacy of PCC. The present study was done using the sample of the final carious material that contains mixed microbial strains as against the pure strains used in other studies.

The major cariogenic species are acidogenic and acid tolerant species particularly include Streptococcus mutans, Lactobacillus and Bifidobacterium species.[4] The study involves the use of selective culture media for these 3 caries causing organisms obtained from the deep dentinal caries (DDC) samples.

Some authors and studies are of the opinion that the softened dentin that contains microbes present in DDC may not further the decay process but creates an acidic environment, which may lead to loss of pulpal vitality. Complete removal of this softened dentin is also not recommended as it might lead to pulp exposure and also decrease the stability of the restoration placed in such a deep cavity. Therefore, to preserve the pulp rather than resorting to endodontic treatment, suitable PCC with good antimicrobial properties act as an effective alternative.[1]

Numerous materials have been used for pulp capping. Calcium hydroxide (CaOH2) has been used as 'gold standard' since its first description by Zander in 1939. It aids in reparative dentin formation by inducing cellular differentiation, extracellular matrix formation and subsequent mineralisation.[3]

Disadvantages were observed on long term usage of CaOH2 which included tunnel defects in newly formed dentin and gradual disintegration of the material over time. To overcome these disadvantages, other materials like glass ionomer cement (GIC) and more recently mineral trioxide aggregate (MTA) are being used as PCC.[3]

According to a survey by Northwest Precedent group, and also a similar survey conducted by our Department among dental practitioners, four cements are routinely used in clinical practice as pulp capping agents with none being popular.[5] Our survey results showed the most commonly used cements to be CaOH2 in the form of Dycal cement, GIC, Zinc oxide eugenol cement (ZnOE) and MTA. Hence the present study was carried out using these four cements.

Aims and objectives

To determine the efficacy of various dental cements in inhibiting the growth of microorganisms involved in DDC. This was carried out by culturing the microorganisms involving the DDC using selective media followed by assessment of the anti-microbial action of dental cements based on their inhibitory action.

MATERIALS AND METHODS

The study was approved by Institutional Ethical Committee on 13/07/2016. A total of 100 samples (n = 100) of deep dentinal caries (DDC) were considered for the study. Reduced transport fluid (RTF) was used to transport the sample to the laboratory and for storage. Thioglycolate liquid broth with hemin and vitamin K, selective culture media for Streptococcus mutans, Lactobacillus and Bifidobacterium species were used to culture the specific bacteria.

Sample collection method

The sample which is the final carious material removed before the placement of the restoration was collected under aseptic conditions using a rubber dam around the tooth involved [Figure 1]. A spoon excavator of 1 mm diameter was used to collect the sample and was placed in 1 ml of RTF. The medium was stored at 4 degree Celsius. At the time of the experiment, the RTF with the sample

was vortexed before use to disperse the microorganisms into the fluid.

Culture method

A modified direct contact test was employed to test the anti-microbial efficacy of the various cements employed against the micro-organisms causing dentinal caries.

Ten microliters of each of this sample was placed in one ml of thyioglycolate broth with hemin and vitamin K which is a standard anaerobic liquid media. Five such vials were made. One vial contained only the thioglycolate broth without any block of cement and was taken as a positive control. The second, third, fourth and fifth contained 1 mm3 cement block of GIC, CaOH2 in the form of Dycal cement, MTA and ZnoE respectively in thioglycolate broth [Figure 2]. After 24 hours of anaerobic incubation the vials were turbid [Figure 3], 10 microliters from each of the five vials were streaked on to three selective media designated for lactobacillus (lactobacillus agar), streptococcus mutans (Mitis salivarius agar with bacitracin and potassium tellurite) and bifidobacterium species (Bifidobacterium agar) which are commercially available.

The samples were incubated anaerobically in the anaerobic jar and commercially available anaerobic Gaspak for a further 24 hours. Methylene blue chemical indicator was used to check the anaerobic conditions [Figure 4].

The growth of the organisms was compared in each of the media through counting the colony forming units (CFUs) [Figure 5a-c] and further analysed with statistical software package IBM® SPSS® Version 23 for Windows. (SPSS Inc., IBM Corporation, NY, USA). One way Analysis of variance (ANOVA) test was done to identify the statistical differences between the different cements used and also between the growths of the organisms subjected to each of the cements. The differences were considered as statistically significant at level ($p < 0.05$).

Tukey's post-hoc test was used for pair-wise association between the averages when the ANOVA test was significant.

OBSERVATIONS AND RESULTS

In this study, the 24-hour anaerobic growth samples were sub-cultured using media specific for three main microorganisms identified in DDC i.e., lactobacillus, streptococcus and bifidobacterium species compared with growth on broth (control, with no inhibitory cement component).

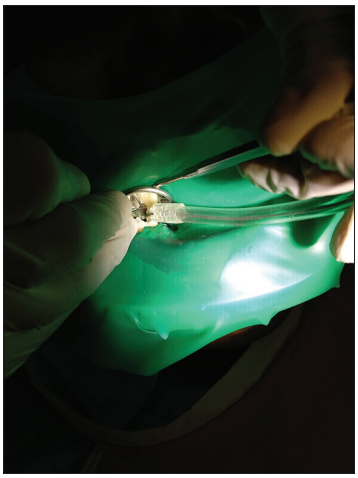


Figure 1: The last carious material from the deep dentinal caries collected using a spoon excavator under aseptic conditions

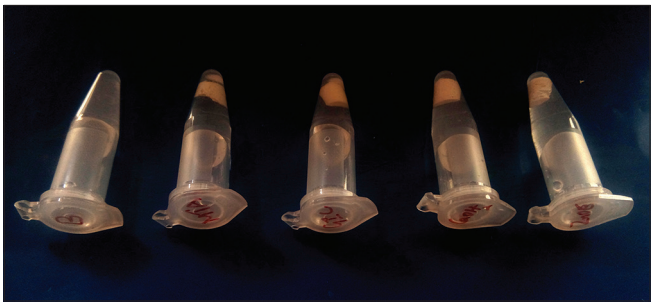


Figure 2: Vials; one without cements and four of the vials with one cubic millimetre of MTA, GIC, CaOH2 (Dycal), ZnOE cement blocks in thioglycolate liquid broth before incubating with carious sample

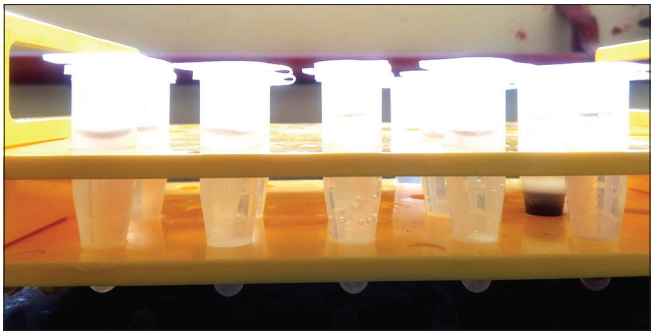


Figure 3: Turbidity in the vials after 24 hours of anaerobic incubation determining the growth of organisms

The anti-microbial effect of each of the cement was analysed by the growth of bacterial colonies on their specific selective media by counting the number of CFUs [Figure 5a-c].

One-way ANOVA test showed considerable variation in the anti-microbial effects between the different cements and the micro-organism groups which was highly statistically significant at $P < 0.001$ [Table 1].

The table indicates that the greatest reduction in microbial growth was induced by exposure to MTA cement. An

overall reduction in growth of 81% in Bifidobacteria species, 94% reduction in Lactobacillus species and 86% reduction in Streptococcus mutans was observed. ZnOE was also highly effective in growth reduction showing 79% reduction of Bifidobacteria, 86% reduction of Lactobacilli and 88% growth reduction of Streptococcus mutans (slightly more than MTA also).

CaOH2 (Dycal) showed 50% reduction of Bifidobacteria, 64% reduction of Lactobacilli and a good 76% reduction in S mutans CFUs.

Figure 4: Ten microliters of the turbid growth streaked onto the selective culture media and incubated anaerobically using commercially available Gaspaks

GIC was about 47% effective against Bifidobacteria; but had the least effect on Lactobacilli and S mutans with only 3-4% and 14% growth retardation respectively.

On colony counting, we observed that the highest number of CFUs were of the Bifidobacterium species, followed by Lactobacillus. Streptococcus mutans showed the least number of colonies [Graph 1].

The maximum growth inhibition was observed with MTA followed by ZnOE while CaOH2 exhibited moderate inhibition of CFUs and GIC had the least antimicrobial properties.

Intergroup comparison of mean CFU of Bifidobacteria showed statistically significant difference with highest CFU in broth and least CFU in MTA group [Table 2 and Graph 2].

Individual pair-wise comparison of the mean CFU for Bifidobacteria species was significant in most cases except for comparison between GIC and Dycal and MTA and ZnOE [Graph 2 and Table 3].

The intergroup comparison between the mean CFUs of Lactobacilli was significant at value of $P < 0.006$ [Table 4 and Graph 3]. Broth showed the highest number of CFUs and MTA the lowest.

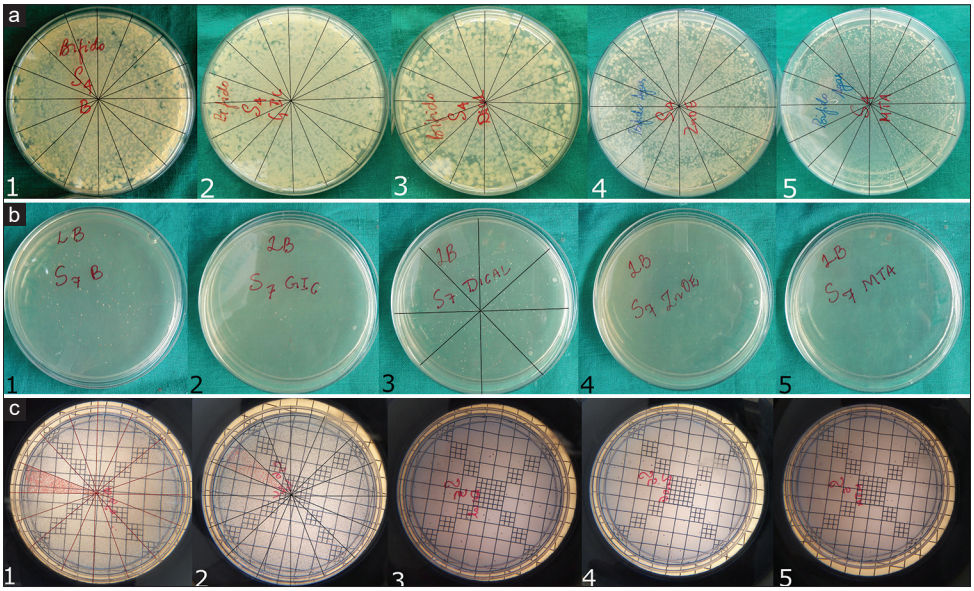


Figure 5: (a) Bifidobacterium culture media; (b) Lactobacillus Agar Media; (c) Mitis Salivarius Agar. 1: Positive control without exposure to any of the cements labelled as B, 2: Culture growth and colony counting of the carious sample exposed to GIC cement, 3: Culture growth and colony counting of the carious sample exposed to CaOH2 (Dycal) cement, 4: Culture growth and colony counting of the carious sample exposed to ZnOE, 5: Culture growth and colony counting of the carious sample exposed to MTA

Table 1: Comparison of reduction in colonies of various micro organisms

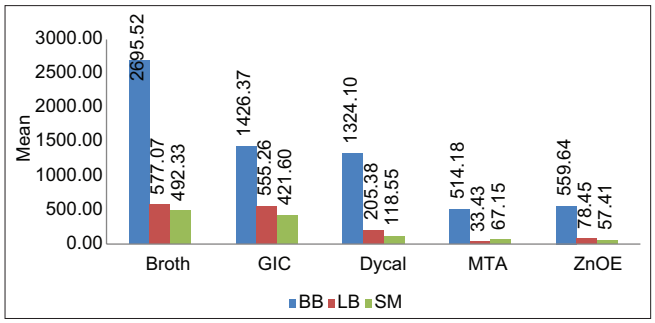
Group	*n*	BB			LB			SM			*P*
		Mean	SD	% reduction	Mean	SD	% reduction	Mean	SD	% reduction	
Broth	100	2695.52	3401.24		577.07	2176.43		492.33	3062.66		<0.001**
GIC	100	1426.37	2765.78	47.08	555.26	2049.75	3.78	421.60	2234.43	14.37	<0.001**
Dycal	100	1324.10	3159.90	50.88	205.38	223.97	64.41	118.55	104.23	75.92	<0.001**
MTA	100	514.18	1705.44	80.92	33.43	200.25	94.21	67.15	64.78	86.36	<0.001**
ZnOE	100	559.64	1923.87	79.24	78.45	445.72	86.41	57.41	160.87	88.34	<0.001**

**-Highly significant ($P<0.001$)

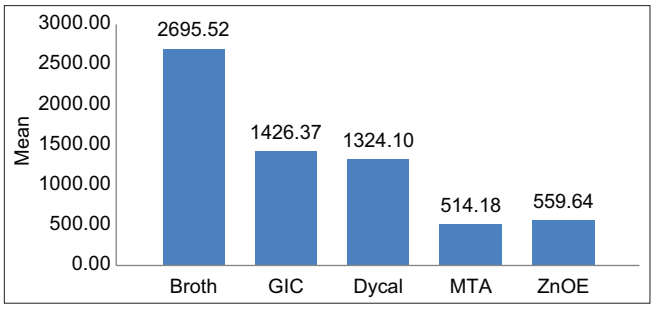
Table 2: Intergroup comparison of Mean CFU of Bifidobacteria

Group	*n*	Minimum	Maximum	Mean	Std. Deviation	*F*	*P*
Broth	100	11.0	20700.0	2695.52	3401.24	10.923	<0.001**
GIC	100	4.0	15240.0	1426.37	2765.78		
Dycal	100	3.0	17970.0	1324.10	3159.90		
MTA	100	1.0	9925.0	514.18	1705.44		
ZnOE	100	0.0	10980.0	559.64	1923.87		

**-Highly significant ($P<0.001$)



Graph 1: Mean Colony forming units (CFUs) of various organisms. BB = Bifidobacterium, LB = Lactobacillus, SM = Streptococcus



Graph 2: Mean CFU of Bifidobacteria

Individual pair-wise comparison did not show statistically significant numbers

Intergroup comparison of mean CFU of Streptococcus mutans showed no statistical significant difference [Table 5 and Graph 4].

On consideration of each microorganism individually, the growth was highly inhibited by MTA as compared to the other cements and with the control group (thyoglycolate broth only).

Streptococcus mutans exhibited least number of CFUs whilst the growth of bifidobacterium was the highest in intergroup comparison.

DISCUSSION

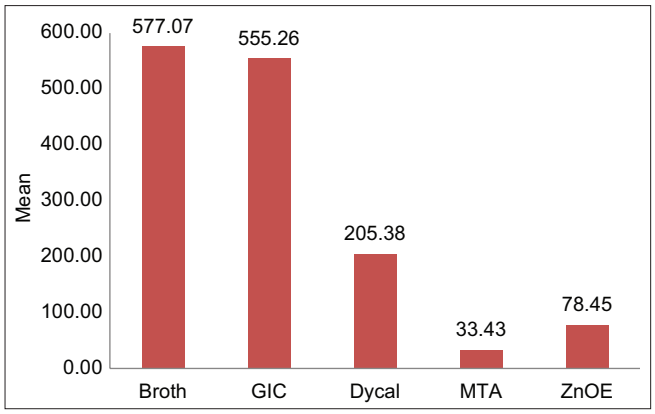
The dental treatment paradigm is shifting towards minimal intervention program that promotes restoration of the teeth in a biological non-operative manner. In this regard, retention of pulp vitality is being given importance in the recent times.

During the preparation of the cavity it often turns out that total removal of decalcified dentin may cause pulp exposure, leading to subsequent root canal treatment. Many studies have proven that although demineralized dentin contains microorganisms, it can be temporarily left intact in order to prevent the pulp from being exposed. Such treatment is acceptable, provided that the materials and medicaments which possess antibacterial activity against cariogenic bacteria are chosen to be placed.[6]

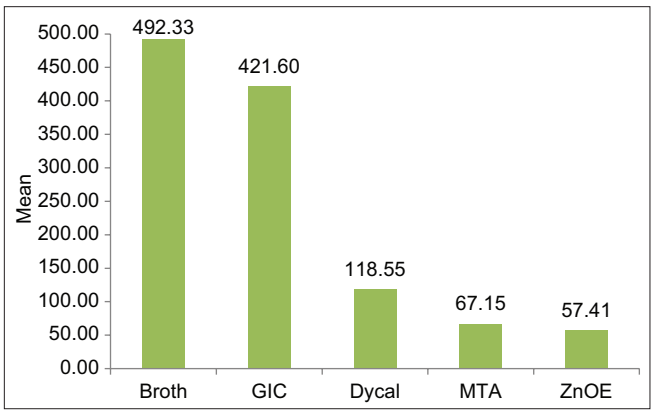
Traditionally, DDC is treated by removal of gross carious debris, followed by the softened dentin at the floor of the cavity. The cavity is then lined by a material for pulp capping procedure.[1] PCC procedures are recommended for those deep carious lesions that are in close proximity to the pulp and have not yet affected the pulp. The objective is to induce the formation of a dentin-bridge formation in order to arrest the caries process, thus protecting the vitality of the pulp.[3]

Golubchin *et al.* in 1967 described two zones in the carious dentin viz., external zone with softened infected dentin which cannot get remineralised and an internal zone comprising of affected dentin which can undergo remineralisation.[2]

Studies on microbiological examination of the deepest layer of carious dentin have exhibited significant bacterial



Graph 3: Mean CFU of Lactobacilli



Graph 4: Mean CFU of Streptococcus mutans

Table 3: Individual pair wise comparison of Mean CFU of bifidobacteria

Comparison between		Mean Difference	Std. Error	Sig.
Broth	GIC	1269.15	378.4850	0.001*
Broth	Dycal	1371.42	378.4850	<0.001**
Broth	MTA	2181.34	378.4850	<0.001**
Broth	ZnOE	2135.88	378.4850	<0.001**
GIC	Dycal	102.2700	378.4850	0.787 NS
GIC	MTA	912.19	378.4850	0.016*
GIC	ZnOE	866.73	378.4850	0.022*
Dycal	MTA	809.92	378.4850	0.033*
Dycal	ZnOE	764.46	378.4850	0.044*
MTA	ZnOE	-45.4600	378.4850	0.904 NS

**-Highly significant ($P<0.001$), *-Significant ($P<0.05$), NS – Not significant ($P>0.05$)

activity, as was quite evident in the present study as well. These microorganisms remaining in dentin following cavity preparation may induce pulp damage, requiring the use of pulp-capping agents with antimicrobial activity underneath permanent restorations.[7-9] Therefore, the anti-bacterial efficacy of the material used for pulp capping at this juncture is of utmost importance.

The major species involved in caries production are Streptococcus mutans followed by Lactobacillus species. The other contributory species include other Streptococcal species and Actinomyces. Newer species which have been found to have an eminent role include Bifidobacteria and Scardovia species associated with adult caries.[10]

However, all the species are not found concomitantly; their presence and colonisation seem to occur in a sequential manner as elucidated by Takahashi *et al*.[11]

Some species thrive in an anaerobic condition whereas others are facultative anaerobes. These species can be further identified by sub-culturing using selective anaerobic media.

S. Saini *et al*.[12] did a study to identify the microbes both aerobes and anaerobes in the deep seated carious lesions and found that most of them are polymicrobial. The most anaerobic species found are Lactobacillus, Veillonella and Actinomyces whereas the most common aerobic organisms found was streptococcus species. An antibiotic sensitivity test using various antibiotics was performed in their study.

Neelakantan *et al*.[1] used swabs from deep dentinal caries to culture the cultivable mixed flora of microorganisms. Their study showed pure culture of lactobacillus before placing the medicament, our study showed more of mixed culture with predominantly bifidobacterium species in control samples not treated with dental cements.

Our results correlate with the studies done by Ledezma-Rasillo *et al*.[13] which also showed the predominant organism in DDC to be Bifidobacteria.

Most researches have employed the use of commercially available bacterial strains in order to test the anti-microbial efficacy of the dental cements or materials used in their experiments,[6,14,15] whereas the novelty in our study was that we used direct samples of deep dentinal caries excavated from the cavities before the restorative procedure.

Three species namely Streptococcus mutans (S. mutans), Lactobacilli and Bifidobacteria which are ubiquitous organisms found in DDC were taken up for the present study. Here the antimicrobial efficacy of cements was tested as against the antibiotics in the study done by S. Saini *et al*.[12]

An ideal PCC cement ought to control infection, maintain dentinal seal (to prevent microleakage), be clinically simple to handle and improve formation of dentin bridge.[14]

A number of materials have been suggested for use in pulp capping. In a survey in which private practitioners were asked about the pulp capping material they use, the

Table 4: Intergroup comparison of Mean CFU of Lactobacilli

Group	*n*	Minimum	Maximum	Mean	Std. Deviation	*F*	*P*
Broth	100	0	20670	577.07	2176.43	3.664	0.006*
GIC	100	0	13635	555.26	2049.75		
Dycal	100	0	1113	205.38	223.97		
MTA	100	0	1428	33.43	200.25		
ZnOE	100	0	3178	78.45	445.72		

*-Significant ($P<0.05$)

Table 5: Intergroup comparison of mean CFU of S mutans

Group	*n*	Minimum	Maximum	Mean	Std. Deviation	*F*	*P*
Broth	100	0	30771	492.33	3062.66	1.468	0.211 NS
GIC	100	0	15810	421.60	2234.43		
Dycal	100	0	392	118.55	104.23		
MTA	100	0	315	67.15	64.78		
ZnOE	100	0	1125	57.41	160.87		

NS – not significant ($P>0.05$)

respondents listed four different materials, with none being preferred by a clear majority of users.[5]

A similar survey was conducted before the commencement of this study amongst the local dental practitioners. As per the results, the most routinely used cements in clinical practice as pulp capping agents were CaOH2 (Dycal) cement, Glass ionomer cement (GIC), Zinc oxide eugenol cement (ZnOE) and mineral trioxide aggregate (MTA).

Therefore, we included these 4 cements – DyCal (CaOH2), ZnOE, GIC and MTA to test the bacterial growth from the mixed culture obtained from DDC samples.

A slightly modified direct contact test was used in this study, wherein the carious sample was kept directly in contact with 1 mm^3 cement blocks of GIC, CaOH2, MTA and ZnoE respectively placed in 1 ml of thioglycollate broth for 24 hours anaerobically. Liquid thioglycolate broth vial which had no cement block was taken as control. Post-incubation, 10 uL from each vial was then streaked on 3 selective media (Bifidobacterium agar, Mitis salivarius agar and Lectobacillus agar) and subject to anareobic environment for a further 24 hours. The CFUs were then counted and statistically analysed.[14]

We found that direct contact test was an extensive procedure as the time required to analyse the growth inhibition by the cements was more than a day. Even then, the method was convenient to assess the antimicrobial efficacy as the colonies could be easily counted and compared to assess the inhibitory effects of the cements.

Neelakantan *et al*.[1] studied the antibacterial effects of calcium hydroxide, polyantibiotic paste, novel light cured fluoride-releasing hydroxyapatite-based liner and MTA invivo under amalgam restorations and found that hydroxyapatite-based liner and MTA gave significant antibacterial effects**.** We had concurring results wherein MTA had the highest anti-bacterial action as compared to GIC, ZnOE and Dycal against Bifidobacterium and lactobacilli with 94.21% and 80.92% reduction respectively.

Yalcin *et al*.[14] studied the antimicrobial efficacy of CaOH2, Calcimol LC and BioAggregate on 10 microliter lactobacillus commercial strain suspension using the technique of direct contact test where a 96-well microliter plate coated with the 3 cements was used. The results showed that none of the pulp capping agents had antimicrobial effect as a logarithmic growth increase of the test strain was observed as against the control**.**

In one of the clinical studies, when various cements were used to assess the antimicrobial efficacy, sterility was 61.4% of all the tooth cavities filled with calcium hydroxide as against 81.8% of cavities filled with zinc oxide-eugenol cement**.**[12] In the present study, the carious broth samples exposed to CaOH2 cement showed approximately 63.7% growth reduction whereas ZnOE showed an overall reduction of 84.6%.

ZnOE showed comparable results to MTA with 79% reduction against bifidobacterium, 86.4% against Lactobacilli and 88.34% against S mutans, which was surprisingly higher than even MTA in the current study.

It is known that ZnOE releases eugenol in concentrations that are cytotoxic. It also demonstrates high interfacial leakage**.**[16]

In the study by Saini *et al*.,[12] Fuji Triage cement inhibited the growth of all bacterial strains. Fuji IX cement demonstrated the most potent antibacterial activity against *S. sanguis*. Ketac Molar showed antibacterial activity against

S. sanguis and *S. salivarius*, whereas Ketac Silver was efficient against *S. mutans* as well. Neither of the Ketac cements inhibited growth of the standard *L. casei* strain.

But our findings were contrary, where GIC exhibited the least anti-microbial properties of all the cements with only about 47% reduction against bifidobacteria, 14% reduction against S mutans. A very negligible activity of 3.78% of the cements against Lactobacilli in the present study was comparable to the study by Saini *et al.*

Nowadays, bioactive cements on a calcium silicate basis such as MTA are the preferred pulp capping agents. Studies advocate the use of calcium silicate cements like Portland cement or Mineral Trioxide Aggregate (MTA), as they yield better results than the traditional and established techniques which used CaOH2. The calcium silicate cements possess the capacity to disinfect, form a bacteria-tight seal and also are capable of inducing calcium-phosphate precipitation in their vicinity. It could be suggested that the antimicrobial activity of MTA results from its high pH and the substances released to the environment.[15]

MTA, after hardening, leads to calcium oxide formation that reacts with tissue fluids to form calcium hydroxide. Fibronectin is secret by pulp cells adjacent to the necrotic layer under the capping material. The secreted layer further forms collagen fibrils which undergo reorganization forming reparative dentin tissue. Thus, MTA can stimulate reparative dentin formation.[3]

Accordingly, we found that MTA possesses better anti-microbial activity with statistically significant *P* value <0.001 and overall 87.2% reduction against the bacterial species as against Ca0H2 which showed only about 63% efficacy; which correlate with Hashem *et al.*[17] and Hilton *et al.* who advocate that MTA yields better results than other traditionally used pulp-capping materials like CaOH2.[4,18]

CONCLUSION

In this experiment, a modification of the direct contact test was employed as the bacterial source was carious material directly obtained from the deep dentinal cavities. The broth culture with carious samples was exposed to 1 mm3 cement cubes as direct contact and further incubated anerobically on selective culture media.

We found that Bifidobacteria was the prevalent bacteria in samples of deep dentinal caries as compared to S mutans and lactobacilli.

Of the cements used for testing the anti-microbial properties, the most effective inhibition of bacterial growth was by MTA. A close second was ZnOE interestingly, followed by CaOH2. GIC showed the least antimicrobial activity. Long term studies will reveal the effectiveness of these pulp capping agents and their antimicrobial action in vivo.

Acknowledgements

We are grateful to Rajiv Gandhi University of Health Sciences, Bangalore, Karnataka for providing grant for this Original research work.

Financial support and sponsorship

This original research work has been funded by Rajiv Gandhi University of Health Sciences, Bangalore.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Neelakantan P, Rao CVS, Indramohan J. Bacteriology of deep carious lesions underneath amalgam restorations with different pulp-capping materials – An *in vivo* analysis. J Appl Oral Sci 2012;20:139-45.
2. Golubchin D. Current therapeutic options for treating deep carious lesions: A review. Odontoestomatología 2017;19:4-17.
3. Mathur VP, Dhillon JK, Logani A, Kalra G. Evaluation of indirect pulp capping using three different materials: A randomized control trial using cone-beam computed tomography. Indian J Dent Res 2016;27:623-9.
4. Tanner CRA. Anaerobic culture to detect periodontal and caries pathogens. J Oral Biosci 2015;57:18–26.
5. Northwest PRECEDENT. A survey of practitioner preference in direct pulp capping materials. 2007 Internal data, available upon request.
6. Łuczaj-Cepowicz E, Marczuk-Kolada G, Zalewska A, Pawińskam M, Leszczyńska K. Antibacterial activity of selected glass-ionomer cements. Postepy Hig Med Dosw 2014;68:23-8.
7. Byun R, Nadkarni MA, Chhour KL, Martin FE, Jacques NA, Hunter N. Quantitative analysis of diverse *Lactobacillus* species present in advanced dental caries. J Clin Microbiol 2004;42:3128-36.
8. Davies EE, Nemes JL. Anaerobic bacteria in carious dentine. Oral Surg Oral Med Oral Pathol 1955;8:526-9.
9. Martin FE, Nadkarni MA, Jacques NA, Hunter N. Quantitative microbiological study of human carious dentine by culture and real-time PCR: Association of anaerobes with histopathological changes in chronic pulpitis. J Clin Microbiol 2002;40:1698-704.
10. Beighton D, Al-Haboubi M, Mantzourani M, Gilbert SC, Clark D, Zoitopoulos L, *et al.* Oral Bifidobacteria: Caries-associated bacteria in older adults. J Dent Res 2010;89:970–4.
11. Takahashi N, Nyvad B. The role of bacteria in the caries process: Ecological perspectives. J Dent Res 2011;90:294-303.
12. Saini S, Mahajan A, Sharma JK, Arora, Saini OP. Polymicrobial etiology of dental caries. Indian J Pathol Microbiol 1999;42:25-9.
13. Ledezma-Rasillo G, Flores-Reyes H, Gonzalez-Amaro AM, Garrocho-Rangel A, Ruiz-Rodriguez Mdel S, Pozos-Guillen AJ. Identification of cultivable micro- organisms from primary teeth with

necrotic pulps. J Clin Pediatr Dent 2010;34:329–33.
14. Yalcin M, Arslan U, Dundar A. Evaluation of antibacterial effects of pulp capping agents with direct contact test method. Eur J Dent 2014;8:95-9.
15. Özyürek T, Demiryürek E. Comparison of the antimicrobial activity of direct pulp-capping materials: Mineral trioxide aggregate-Angelus and Biodentine. J Conserv Dent 2016;19:569-72.
16. Hilton TJ. Keys to clinical success with pulp capping: A review of the literature. Oper Dent 2009;34:615–25.
17. Hashem D, Mannocci F, Patel S, Manoharan A, Brown JE, Watson TF, *et al*. Clinical and radiographic assessment of the efficacy of calcium silicate indirect pulp capping: A randomized controlled clinical trial. J Dent Res 2015;94:562–8.
18. Hilton TJ, Ferracane JL, Mancl L. Comparison of CaOH with MTA for direct pulp capping: A PBRN randomized clinical trial. J Dent Res 2013;92:16–22.