EVALUATION OF THE ANTIMICROBIAL PROPERTIES OF SELECTED NEW RESIN-MODIFIED MATERIALS USED IN VITAL PULP THERAPY: AN IN VITRO STUDY

Original article

Novotná B.[1, 2, *], Holík P.[1, 2], Morozova Y.[1, 2], Somolová L.[1, 2], Rosa M.[1, 2], Svobodová L.[3, 4]
[1]Institute of Dentistry and Oral Sciences, Faculty of Medicine and Dentistry, Palacký University Olomouc, Czech Republic
[2]Department of Dentistry, Olomouc University Hospital, Czech Republic
[3]Department of Microbiology, Faculty of Medicine and Dentistry, Palacký University Olomouc, Czech Republic
[4]Department of Microbiology, Olomouc University Hospital, Czech Republic
[*]Corresponding author

SUMMARY

Introduction and aim: In the case of deep carious lesions, the preference for the selective excavation method has been increasing. It can be assumed that a small number of microorganisms persist in the soft dentine near the dental pulp. For this reason, it is recommendable to use a filling material with an antimicrobial effect. The aim of this in vitro study is to evaluate the antimicrobial effect of selected resin-modified calcium silicate (TheraCal LC, TheraCal PT) and calcium phosphate (ApaCal ART) cements, and to compare them with the reference material (Biodentine).

Methods: Four groups of microorganisms were selected for this in vitro study: 1. viridans streptococci, 2. *Lactobacillus* spp., 3. *Enterococcus faecalis*, and 4. *Candida albicans*. The antimicrobial effects of the materials were evaluated by measuring the width of the growth inhibition zone using the agar diffusion test. Subsequently, statistical analysis of the obtained data was performed. All these tests were conducted at the 0.05 significance level.

Results: Biodentine was verified to have antimicrobial activity against all selected microorganisms. ApaCal ART showed a low effect only against viridans streptococci, TheraCal PT showed similar activity, but additionally inhibited the growth of *Lactobacillus* spp. However, TheraCal LC showed antimicrobial effects against all tested microorganisms. In addition, for *Enterococcus faecalis*, *Candida albicans*, and *Lactobacillus* spp., the results were not statistically significantly different from those of the reference material.

Conclusion: Resin-modified calcium silicate and calcium phosphate materials generally have lower antimicrobial potential than conventional calcium silicate cements. However, it is important to note that their activity varies among products. Of the materials tested in this in vitro study, TheraCal LC achieved the best results, similar to the reference cement.

Key words: deep carious lesion, indirect pulp capping, calcium silicate cement, calcium phosphate cement

Novotná B, Holík P, Morozova Y, Somolová L, Rosa M, Svobodová L.
Evaluation of the antimicrobial properties of selected new resin-modified materials used in vital pulp therapy: an in vitro study.
Čes. stomatol. Prakt. zub. lék. (Czech Dental Journal). 2026; 126(1): 5–15. doi: 10.51479/cspzl.2025.009

INTRODUCTION AND AIMS

In contemporary dentistry, the emphasis in the treatment of permanent teeth is primarily on preserving the vitality of the dental pulp, and therefore there are several different methods of excavation for the treatment of deep carious lesions that can affect this outcome. The types of excavation can be divided into two main groups: non-selective excavation and selective excavation. Non-selective excavation (formerly complete or total excavation [1]) means the removal of all soft carious dentine from the peripheral and central parts of the cavity until hard, healthy dentine is reached. Hard dentine is characterized by resistance and a creaking sound when using a sharp excavator or probe [2] and does not stain with detection dyes [3]. In recent years, there has been a tendency to move away from this type of excavation in the case of deep carious lesions due to the high risk of opening the pulp cavity and exposing the dental pulp [1, 2, 4]. Selective excavation (formerly partial or incomplete excavation [1]) involves the removal of carious dentine at the periphery of the cavity to hard, healthy dentine, leaving a thin layer of soft or firm dentine in the central part of the cavity. Soft infected dentine can be removed very easily using rotary or hand instruments without much effort. Firm or leathery dentine is demineralized but represents already minimally infected tissue, which can be removed with greater force with a sharp excavator [2]. Both of these tissues (soft and firm dentine) are stained with a detection dye [3]. Currently, there is a growing trend to increasingly recommend this type of excavation for deep carious lesions, either as a definitive one-stage treatment or as part of a two-stage treatment (stepwise excavation, intermittent excavation). The emphasis on this type of excavation is growing in the professional literature, mainly because of the lower risk of exposure and irritation of the dental pulp during preparation [2] and also because a larger amount of hard dental tissues is preserved in this approach [4]. One of the pitfalls of selective excavation is the impossibility of standardizing the degree of excavation. Although the area of softened dentine should be as small as possible, it depends on the experience and preferences of the particular clinician, and in addition, the same dentist can remove more or less carious tissues in different cases when using the same type of excavation. Therefore, it is very difficult to determine the ideal amount of softened dentine that can be left in the cavity [3, 4]. Another pitfall is the impossibility of detecting chronic irreversible pulpitis and the resulting incorrect indication for making a filling or performing indirect pulp capping in a situation that requires a more radical treatment method [3]. In the case of deep caries, studies have shown the presence of bacteria in the dentinal tubules circumpulpally both before treatment and in many cases after selective excavation [5, 6]. Assuming that a small number of microorganisms remain in the tissues, in order to stop the carious process it is necessary to prevent their access to nutrients, limit the space for their multiplication, and the production of toxins. This can be achieved by hermetically sealing the cavity using a suitable agent, which is used as a physical barrier between the dental pulp and the oral cavity. This material should have adhesion to dentine and any other filling materials [7]. Composite materials are the ideal choice for a perfect hermetic closure of the cavity [3]. However, due to the proximity of the dental pulp, it is also appropriate to consider the use of a material that is biocompatible and as minimally cytotoxic as possible to dental pulp cells, stimulates the formation of tertiary dentine, has remineralization potential (release of fluoride or calcium ions), and provides sufficient mechanical and chemical resistance. Last but not least, due to the presence and survival of bacteria in the dentinal tubules, antimicrobial properties are highly desirable [8, 9]. For long-term control, a radiopacity higher than that of dentine is also desirable in order to assess the formation of a dentinal bridge.

Most of these required properties are met by the group of calcium silicate cements, which are also recommended for this indication today [1, 2]. These materials have been on the dental market since the 1990s [10] and their properties have already been thoroughly investigated [11]. Although these materials have very good properties, their use sometimes also presents certain disadvantages, including difficult handling, long setting time, or a tendency to discolouration. Manufacturers have tried to eliminate or at least mitigate these negative aspects, and therefore, over time, many different variants of calcium silicates have been developed, which have similar composition and properties, but mainly try to compensate for the aforementioned weaknesses. For better clarity, calcium silicate cements can be divided into four groups:

1. Powder and liquid materials: This group includes all cements supplied in powder and liquid form, e.g., ProRoot® MTA (Dentsply, USA), MTA+ (Cerkamed, Poland), PD™ MTA White (PD dental, Switzerland) and many others, which must be mixed manually to a consistency of “liquid sand” [12]. Setting is ensured by a hydration reaction (mostly tricalcium silicate with water) and the setting time is within a few hours. Due to the difficult handling and long setting time [13], these materials have already been surpassed in vitality-preserving procedures by newer materials, which are preferred for this indication.

2. Powder and liquid materials supplied in capsules: These products are prepared in capsules and mixed by machine just before use. The advantage is a more precise mixing ratio and better mixing of the material (and thus uniform hydration) [14, 15]. This group includes, for example, Biodentine® (Septodont, France), Harvard® MTA-Cap (Harvard, Germany), MTA Biorep (Itena, France) and others. In most cases, a simpler method of application is ensured by the use of special application capsules. Biodentine can be used in the cavity as a "dentine substitute" [16] and its advantage compared to the previous group is the addition of calcium chloride, which ensures setting within 12–15 minutes [17].

3. Premixed materials: These products are available in premixed form, the setting of which is initiated by the presence of moisture. They contain an admixture of calcium phosphate, and therefore they are often referred to as bioceramics. Depending on the different consistencies (sealer, paste, putty), they can be used in many indications. For procedures involving the preservation of vitality (especially for direct contact with the dental pulp), it is ideal to use the paste or putty form, which has good handling properties and can be immediately covered with a permanent filling material [18]. There is also a large number of different products from this group available on the dental market, e.g., NeoPutty™ (Avalon Biomed, USA), Wellroot™ PT (Vericom, Korea), Totalfill® BC RRM Putty (FKG, Switzerland) and others.

4. Hybrid materials – modified with resin: Although the handling properties of the previous two groups have been improved and the setting time has been significantly shortened, they still set within tens of minutes, which extends the total treatment time. The aim of the manufacturers was therefore to innovate the material so that it ideally sets immediately after application. For this reason, hybrids were created that are modified with resin and their setting is ensured either by light polymerization or dually, i.e., both chemically and by light. Recently, a large number of these products have appeared on the market and are stated by the manufacturers as suitable for direct and indirect contact with the dental pulp. They contain various ratios of resin, calcium silicate or calcium phosphate components and radiopaque agents [19–21]. For example, Theracal LC® (Bisco, USA), Harvard BioCal®-Cap (Harvard, Germany), ApaCal ART® (Prevest DenPro, India) and others are available on the market.

It is clear that it can be difficult for a dental practitioner to navigate through this large number of different products and use them in the optimal indication. From the characteristics described above, it can be assumed that for procedures involving the preservation of vitality, it is ideal to use materials from the second, third, or even fourth group. In the case of resin-modified hybrids, which have been on the dental market for a significantly shorter time, the question is how the presence of monomers affects the overall properties of the material (e.g., the cytotoxic effect in direct contact with cell culture is significantly higher than in conventional calcium silicates [20]). Given the above-described issues, the aim of this in vitro study was to evaluate the antibacterial properties of selected resin-modified materials, which we assume will be similar to or better than the reference sample.

Hypotheses

Null hypothesis A_0: Selected calcium silicate or calcium phosphate materials modified with resin have the same or higher antimicrobial effect against selected microorganisms than conventional calcium silicate cements.

Alternative hypothesis A_a: Selected calcium silicate or calcium phosphate materials modified with resin have a lower antimicrobial effect against selected microorganisms than conventional calcium silicate cements.

MATERIALS AND METHODS

Materials

The reference sample for this study was Biodentine™ (Septodont, France), which is considered the gold standard used in vitality-preserving procedures [16, 17].

From a large group of resin-modified hybrid materials, the following were selected based on the type of setting and the particles used: TheraCal LC® (Bisco, USA), as a representative of light-curing materials with a calcium silicate component; TheraCal PT® (Bisco, USA), as a representative of dual curing materials with a calcium silicate component; and ApaCal ART® (Prevest DenPro, India), as a representative of light-curing materials with a calcium phosphate component **(Table 1)**.

Methodology

Four groups of microorganisms were selected and included in the *in vitro* study: 1. viridogenic streptococci (four strains of *Streptococcus mitis*, three strains of *Streptococcus parasanguinis*, and three strains of *Streptococcus vestibularis*), 2. *Lactobacillus* sp. (three strains of *Lactobacillus gasseri*, four strains of *Lactobacillus jensenii*, three strains of *Lactobacillus crispatus*), 3. *Enterococcus faecalis*, 4. *Candida albicans*. The experiment

Tab. 1 *Overview of selected materials.*

Material	Particle type	Curing type	Radiopaque filler
ApaCal ART	Tricalcium phosphate	Light-curing	Barium zirconate
TheraCal LC	Calcium silicate	Light-curing	Barium zirconate
TheraCal PT	Calcium silicate	Dual curing	Barium zirconate Ytterbium fluoride
Biodentine	Tricalcium silicate	Self-curing	Zirconium dioxide

included ten biological replicates for each of the four groups of microorganisms (i.e., 4 × 10 different strains), which came from the collection of microorganisms of the Department of Microbiology, Faculty of Medicine and Dentistry, Palacký University Olomouc, and University Hospital Olomouc. To evaluate the antimicrobial effects of individual materials, an agar diffusion test was performed for these groups, and the experiment was carried out in parallel for all samples.

A mass spectrometer was used to identify microorganisms. A small amount of the examined culture was transferred to the target of the MALDI plate using a wooden toothpick. In the case of yeast, the test sample was first covered with 1 µl of 70% formic acid solution (Bruker Daltonics, Germany). In the next step, all samples were overlaid with 1 µl of matrix (Bruker Daltonics, Germany). After the reagents had dried, the plate was inserted into the Microflex LT instrument (Bruker Daltonics, Germany) and the protein mass spectrum determination process was started. The MALDI Biotyper program (Bruker Daltonics, Germany) was used to analyze the results.

Before testing, the isolate of each strain was inoculated onto Columbia blood agar in a separate container (plastic Petri dish with a diameter of 90 mm, Trios, Czech Republic) and incubated for 18–24 hours in a large-volume incubator (35 ± 1 °C, 5% CO_2, PHCBi, Japan). The following day, several colonies of the given isolate were transferred to a tube with sterile saline solution and the final density of the suspension was adjusted to 0.5 McFarland on the turbidity scale using a DENSILAMETER-II turbidimeter (EMO, Czech Republic). This corresponds to 1–5 × 10^6 CFU (colony forming units) in 1 ml. Then, the microbial suspension of the given strain was inoculated using a cotton swab onto the entire surface of Mueller Hinton agar with horse blood in a separate container (plastic Petri dish with a diameter of 90 mm, Trios, Czech Republic). Subsequently, four wells with a diameter of 5 mm and a height of 4 mm (depth of the entire agar layer) were created in each of the 40 culture containers with inoculated agar, which were filled with ApaCal ART, TheraCal LC, TheraCal PT, and Biodentine samples under the conditions specified by the manufacturer **(Fig. 1)**.

ApaCal ART, TheraCal LC, and TheraCal PT, materials in pre-mixed form in a syringe with a cannula, were injected into the well and subsequently light-cured from a distance of 2 mm for the time specified by the manufacturer using a Valo™ curing lamp (Ultradent Products, USA) at a standard power of 1000 mW/cm². Biodentine was prepared according to the manufacturer's instructions and applied to the well, where it chemically solidified within 15 min. The inoculated media were incubated for 24–48 hours in a large-volume incubator (35 ± 1 °C, 5% CO_2, PHCBi, Japan). The diameters of the resulting growth inhibition zones were measured using a digital caliper after 24 and 48 hours of incubation **(Fig. 2 and 3)**, and 5 mm (the diameter of the well with the material) was subtracted from the measured values. In the case of *Lactobacillus strains*, inhibition zones were measured only after 48 hours due to the typically slower growth of these microorganisms.

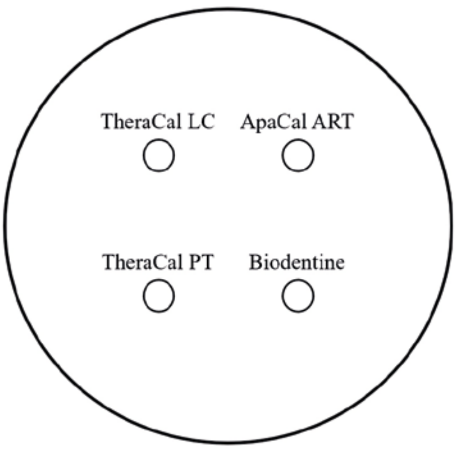

Fig. 1
Diagram showing the localization of samples on the agar surface.

Statistical analysis of data

The obtained data were statistically processed. Shapiro-Wilk normality tests verified that most of the values did not have a normal distribution.

The comparison of the four groups of materials was performed using the Kruskal-Wallis test, followed by post hoc tests with Bonferroni correction to compare the investigated materials with the reference material.

For each material, the sizes of the inhibition zones for all groups of microorganisms were compared using the Kruskal-Wallis test in order to evaluate its antimicrobial effects against specific bacteria or yeasts. If the statistical results differed significantly, post hoc tests with Bonferroni correction followed for pairwise comparisons.

For each material, the sizes of the inhibition zones for each microorganism were compared when measured after 24 and 48 hours using Wilcoxon tests.

All tests were performed at a significance level of 0.05. If the p-value was less than 0.05, differences were considered statistically significant and were marked in bold. Statistical processing was performed using IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

RESULTS

Quantitative variables (means of inhibition zones) are presented using medians, minimum and maximum values, or means and standard deviations (SD).

The sizes of the inhibition zones are shown in the box plot in **Figure 4**. The horizontal line in the box represents the median value, the lower edge of the box represents the 1st quartile value, and the upper edge represents the 3rd quartile value. The brackets indicate the maximum and minimum measured values. Outliers (values that are more than 1.5 times the interquartile range away from the quartiles) are plotted with circles. Extremes (values that are more than three times the interquartile range away from the quartiles) are plotted with asterisks.

A comparison of the four materials is presented in **Table 2**. For all microbial strains at both measured times, the sizes of the inhibition zones within the group of all materials differed statistically significantly ($p < 0.05$).

Table 3 shows the comparison of individual samples with the reference material. In several cases, statistically significant differences were noted between the examined materials and the reference material.

The comparison of the antimicrobial effects of the materials against individual microorganisms is summarized in **Tables 4** and **5**. The inhibition zones formed by ApaCal ART did not differ statistically significantly for individual microorganisms at the time of measurement after 24 or 48 hours. For TheraCal LC, they differed statistically significantly when measured after 24 and 48 hours; the inhibition zones for *Enterococcus faecalis* were significantly smaller than those for *Candida albicans* and *Streptococcus*, other differences were not statistically significant. For TheraCal PT, the size of the inhibition zones differed statistically significantly only when measured after 48 hours, but post hoc tests did not show statistically significant differences; only the differences between *Lactobacillus*, *Enterococcus faecalis*, and *Candida albicans* were at the border of significance, where the antibacterial

Fig. 2
Viridans streptococci culture showing inhibition zones around TheraCal LC, TheraCal PT, and Biodentine.

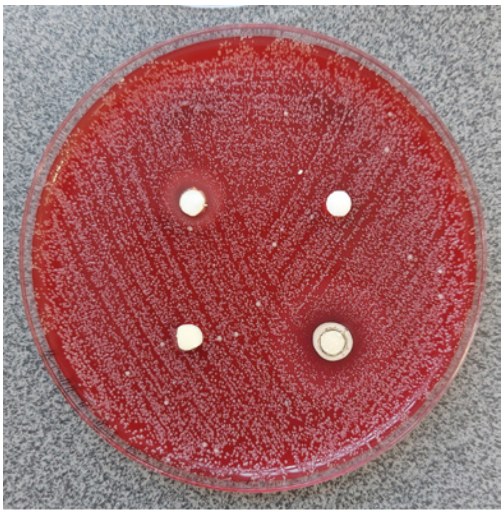

Fig. 3
Candida albicans culture, inhibition zones around TheraCal LC, and Biodentine.

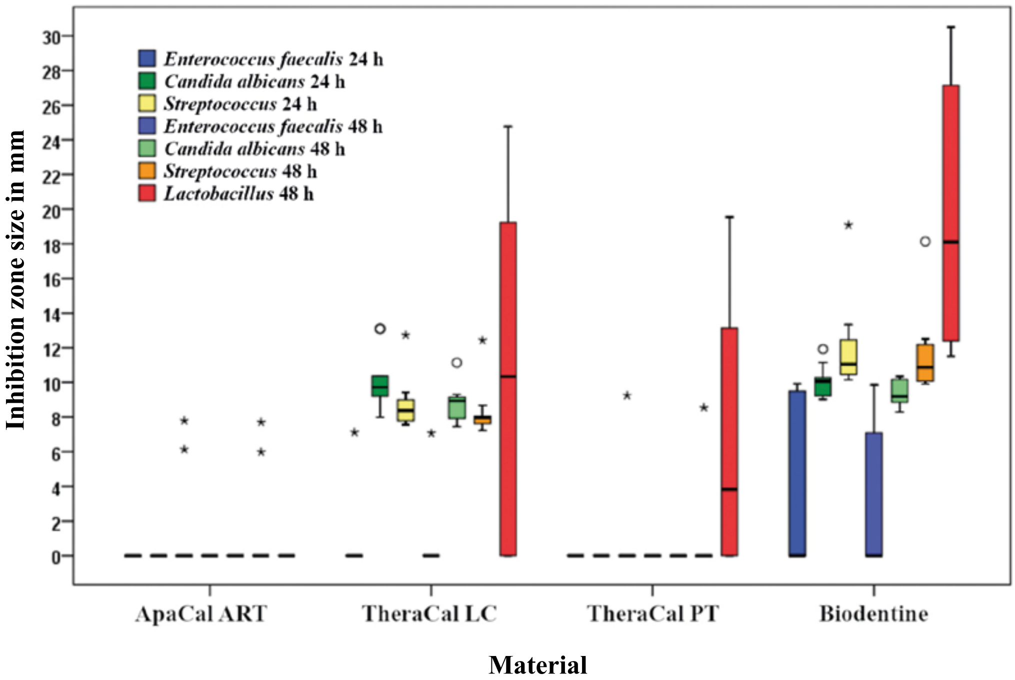

Fig. 4
Distribution of measured values of inhibition zone sizes (in mm) for all microorganisms after 24 and 48 hours for all tested materials: horizontal line in the box – median value; lower edge of the box – 1st quartile value (25th percentile); upper edge – 3rd quartile value (75th percentile); whiskers – maximum and minimum measured values;
o – outliers (more than 1.5 times the interquartile range from the quartiles);
** – extreme values (more than three times the interquartile range from the quartiles).*

effect against the *Lactobacillus* group was higher. The size of the inhibition zones for Biodentine differed statistically significantly when measured after 24 and 48 hours; the differences between all microorganisms were statistically significant, the highest effect was for *Lactobacillus*, followed by *Streptococcus*, *Candida albicans*, and the lowest effect was for *Enterococcus faecalis*.

A comparison of the size of the inhibition zones over time is shown in **Table 6**. The difference in the size of the inhibition zones after 24 and 48 hours for the ApaCal ART and TheraCal PT samples was not statistically significant for any microorganisms. For TheraCal LC and Biodentine materials, significantly lower values were found after 48 hours compared to the measurement after 24 hours for the *Candida albicans* and *Streptococcus* groups.

DISCUSSION

Since selective excavation leaves softened dentine in the cavity, which may contain a small amount of bacteria, it is desirable that the filling material has antibacterial and possibly antifungal properties [8]. The aim of this study was to evaluate the antimicrobial effect of selected calcium silicate and calcium phosphate cements modified with resins using an agar diffusion test against 1. viridizing streptococci, 2. *Lactobacillus* species, 3. *Enterococcus faecalis*, 4. *Candida albicans*.

Tab. 2 *Kruskal-Wallis test for comparing the four groups of examined materials. Values are given in mm, with p < 0.05 indicated in bold.*

		Material												p
		ApaCal ART			TheraCal LC			TheraCal PT			Biodentine			
Time	Microorganism	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	
24 h	*Enterococcus faecalis*	0.00	0.00	0.00	0.00	0.00	7.12	0.00	0.00	0.00	0.00	0.00	9.92	**0.018**
	Candida albicans	0.00	0.00	0.00	9.72	7.98	13.10	0.00	0.00	0.00	10.07	9.03	11.92	**<0.0001**
	Streptococcus	0.00	0.00	7.79	8.38	7.56	12.73	0.00	0.00	9.25	11.06	10.15	19.08	**<0.0001**
	Lactobacillus	-	-	-	-	-	-	-	-	-	-	-	-	**-**
48 h	*Enterococcus faecalis*	0.00	0.00	0.00	0.00	0.00	7.07	0.00	0.00	0.00	0.00	0.00	9.86	**0.020**
	Candida albicans	0.00	0.00	0.00	8.94	7.45	11.14	0.00	0.00	0.00	9.19	8.29	10.35	**<0.0001**
	Streptococcus	0.00	0.00	7.70	7.98	7.23	12.43	0.00	0.00	8.54	10.8	9.91	18.13	**<0.0001**
	Lactobacillus	0.00	0.00	0.00	10.34	0.00	24.77	3.83	0.00	19.53	18.10	11.52	30.51	**0.0002**

***Tab. 3** Post hoc test with Bonferroni correction comparing the investigated materials with the reference material for all groups of microorganisms at 24 and 48 h. Results with p < 0.05 are indicated in bold.*

	Inhibition zone after 24 hours				Inhibition zone after 48 hours			
	Enterococcus faecalis	*Candida albicans*	*Streptococcus*	*Lactobacillus*	*Enterococcus faecalis*	*Candida albicans*	*Streptococcus*	*Lactobacillus*
ApaCal ART vs. Biodentine	**0.092**	**0.0002**	**0.0003**	-	0.092	**0.0002**	**0.0003**	**0.0002**
TheraCal LC vs. Biodentine	0.274	1.000	**0.004**	-	0.336	0.596	**0.004**	0.121
TheraCal PT vs. Biodentine	0.092	**0.0002**	**0.0002**	-	0.092	**0.0002**	**0.0002**	**0.023**

***Tab. 4** Comparison of inhibition zone sizes for all microorganisms for each material using the Kruskal-Wallis test. Values with p < 0.05 are indicated in bold.*

Material	Measurement time	p
ApaCal ART	24 h	0.126
	48 h	0.104
TheraCal LC	24 h	**<0.0001**
	48 h	**0.001**
TheraCal PT	24 h	0.368
	48 h	**0.004**
Biodentine	24 h	**<0.0001**
	48 h	**<0.0001**

***Tab. 5** Post hoc tests with Bonferroni correction for pairwise comparisons (E.F. – Enterococcus faecalis, C.A. – Candida albicans, S. – Streptococcus, L. – Lactobacillus). Results with p < 0.05 are indicated in bold.*

	TheraCal LC		TheraCal PT		Biodentine	
	24 h	**48 h**	**24 h**	**48 h**	**24 h**	**48 h**
E.F. vs. C.A.	**0.0004**	**0.0004**		1.000	**0.022**	**0.006**
E.F. vs. S.	**0.0004**	**0.0004**		1.000	**0.001**	**0.001**
E.F. vs. L.		0.078		0.078		**0.001**
C.A. vs. S.	1.68	0.786		1.000	**0.114**	**0.012**
C.A. vs. L.		1		0.078		**0.001**
S. vs. L.		1		0.270		**0.012**

Viridizing streptococci are known as the main cariogenic pathogens [22], especially *Streptococcus mutans*, against which antibacterial activity has already been investigated [23–25]. However, in deep carious lesions, other genera of bacteria come to the fore; the species *Streptococcus* is present here to a lesser extent and there is greater variability of species, which can often be difficult to specify [26–28]. The aim of our *in vitro* study was to evaluate the antimicrobial activity against other selected species of oral streptococci (apart from *S. mutans*) that occur in deep caries, specifically the available strains of the species *Streptococcus mitis* (four strains), *Streptococcus parasanguinis* (three strains), and *Streptococcus vestibularis* (three strains). In this work, the species described above are included in one group of viridizing streptococci, since the aim was to evaluate the antibacterial properties of materials against these streptococci in general. The species *Lactobacillus* belongs to the dominant group in deep carious lesions, the main species being *Lactobacillus acidophilus* [29], however, according to the available literature, it is clear

***Tab. 6** Comparison of the inhibition zone sizes depending on the measurement time (after 24 and 48 h). Values with p < 0.05 are indicated in bold.*

Material	Microorganism	p
ApaCal ART	*Enterococcus faecalis*	1.000
	Candida albicans	1.000
	Streptococcus	0.180
	Lactobacillus	
TheraCal LC	*Enterococcus faecalis*	0.317
	Candida albicans	**0.005**
	Streptococcus	**0.005**
	Lactobacillus	
TheraCal PT	*Enterococcus faecalis*	1.000
	Candida albicans	1.000
	Streptococcus	0.317
	Lactobacillus	
Biodentine	*Enterococcus faecalis*	0.068
	Candida albicans	**0.005**
	Streptococcus	**0.005**
	Lactobacillus	

that there is a great variability of many other species [22, 26–28]. For this reason, the available strains of the species *Lactobacillus gasseri* (three strains), *Lactobacillus jensenii* (four strains), and *Lactobacillus crispatus* (three strains) were selected for our *in vitro* study. In the text, these strains are included in one investigated group of *Lactobacillus* for simplification and better clarity, as the aim was to evaluate whether the materials have an antibacterial effect in general against the species *Lactobacillus*. *Enterococcus faecalis* was chosen because this pathogen is often resistant to dental agents and, if present in a carious lesion, often persists there even after treatment [30]. *Candida albicans* is also currently referred to as one of many potential cariogenic pathogens, and was therefore also included in this study [31].

Given that the available literature is mostly focused on antibacterial activity against one or two types of bacteria, the intention of this *in vitro* study was to combine in one research design both the main cariogenic pathogens [22] and also yeasts and bacteria, which are very resistant and can be problematic to remove [30, 31]. Another aim was to compare the effect of the investigated samples with a reference material, which has been in clinical practice for a long time, and its properties have been thoroughly investigated [32, 33].

In evaluating the obtained results, the main problem for comparison with other publications was the lack of standardization of the methodologies. Many studies used completely different types of testing [29, 34], different incubation environments [25], in the case of the agar diffusion test, different culture media were used [24], or different times for measuring the inhibition zones were set [35]. Another difficulty may be the different virulence of the available bacterial strains, which were obtained from different sources, which is why the sizes of the inhibition zones were different when using similar methodologies [35].

Biodentine was found to have antimicrobial activity against all groups tested. This finding is consistent with other studies [23, 35–37]. The largest inhibition zones in the range of 11.52–30.51 mm were formed in the *Lactobacillus* group, followed by *Streptococcus* with values ranging from 9.91–19.8 mm, *Candida albicans* with values ranging from 8.29–11.92 mm, and *Enterococcus faecalis* with values ranging from 0–9.92 mm. When compared over time, the zones decreased in the *Streptococcus* and *Candida albicans* groups after 24 hours from the first measurement. The sizes of the inhibition zones in all groups of microorganisms compared with each other and also after 24 and 48 hours in the case of ApaCal ART were not statistically significantly different, so it can be assumed that this material has similar antimicrobial activity against all microorganisms tested. However, these were mostly zero values, only *Streptococcus* had zones of 0–7.79 mm. When compared with the other samples examined, and especially with the reference material, it is clear that the antimicrobial potential of the ApaCal ART material is the lowest of all, more precisely, it is limited only to the *Streptococcus* group. Given that this is a relatively new product, about which only a limited number of studies have been published in the last five years, mainly evaluating physical properties [38, 39], bond strength to adhesive systems [40, 41], radiopacity [21], and possibly cytotoxicity [20], but not antimicrobial properties - it was not possible to compare the results of this *in vitro* study with another publication focusing on the same topic.

TheraCal PT showed the formation of an inhibition zone in the range of 0–19.53 mm for the *Lactobacillus* group, and 0–9.25 mm for *Streptococcus*, and no inhibition zones were formed in the case of *Candida albicans* and *Enterococcus faecalis*. No statistically significant differences were demonstrated over time for any group. However, in comparison with the reference material, there were also statistically significantly different (lower) values for most of the inhibition zones. Non-significant differences were only found for *Enterococcus faecalis*, where TheraCal PT did not form inhibition zones and growth restriction was also insignificant for the other materials. Although this is not a new product, publications investigating this material mostly focus on bioactivity or cytocompatibility [19, 42, 43]. Only one publication also focused on the inhibition of *Enterococcus faecalis* growth, where TheraCal PT showed significantly higher antimicrobial activity than Biodentine, which does not match the result of this *in vitro* study [34]. The reason for the different results may be the choice of a different methodology and the way of using the sample, since in the aforementioned publication the material was used to create an extract in an unsolidified form.

The antimicrobial properties against the other microorganisms studied could not be compared with the literature. However, according to this study, TheraCal PT can be described as a material with better antimicrobial properties than ApaCal ART, but still not quite reaching the potential of the reference material.

TheraCal LC has been available on the dental market for over a decade, and therefore its properties have been much more thoroughly investigated and described [44]. The antimicrobial effect has been compared in some publications with conventional base materials, mainly with calcium salicylate or glass ionomer cements, using different methodologies. For example, Moradi *et al.* reported similar activity against *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Streptococcus mutans* for TheraCal LC as for light-curing calcium salicylate or glass ionomer cements, but lower activity than for self-curing calcium salicylate materials [29]. Given that the number of publications focusing on the antimicrobial effect of TheraCal LC has increased in recent years, for the comparison of this in *vitro study*, mainly publications focusing on the corresponding spectrum of microorganisms and comparing TheraCal LC with pure calcium silicate cements were selected. In our *in vitro* study, it was demonstrated that TheraCal LC exhibits antimicrobial activity against all groups examined, which is in accordance with other studies in which activity against the groups *Lactobacillus* [29], *Streptococcus* [24, 25, 45], and *Enterococcus faecalis* [24, 25]. Only in the case of activity against *Candida albicans* are no other publications available yet. When comparing TheraCal LC with the reference material, the differences in values are statistically significantly different only for the *Streptococcus* group; the results are similar in the other groups, although the inhibition zones for TheraCal LC were slightly smaller. This finding for activity against *Lactobacillus*, *Enterococcus faecalis*, and *Streptococcus* correlates with other studies [24, 35, 45]. In the case of the research by Niranjan *et al*., the sizes of the inhibition zones for the *Enterococcus* and *Streptococcus* groups were similar, while in our study they were significantly smaller for the *Enterococcus* group. The reason is probably the difference in the agar test methodology, since in our study blood agar was used for all the groups studied, while in the mentioned study Mueller Hinton agar was used for the incubation of the *Enterococcus* group [24]. Although in our study the sizes of the inhibition zones of the individual microorganisms were statistically significantly different, the values were higher than for the other materials studied and similar to the reference sample. It is therefore clear that TheraCal LC has the greatest antimicrobial potential of the products studied.

After evaluating all the results, the null hypothesis A_0 is rejected, since all the materials studied do not have the same or higher antimicrobial effect against the selected microorganisms than conventional calcium silicate cements. Since they have it to varying degrees lower than the reference material, the alternative hypothesis A_a is accepted.

CONCLUSION

Calcium silicate and calcium phosphate resin-modified hybrid materials show a lower antimicrobial effect against selected microorganisms than conventional calcium silicate cements. For this reason, it is still more appropriate to prefer the use of unmodified calcium silicate materials in clinical practice. However, due to the significant difference in results between the individual samples examined, the statement about low antimicrobial capabilities cannot be generalized for all resin-modified hybrid materials. ApaCal ART shows almost no antimicrobial activity against selected microorganisms, TheraCal PT has better results, whereas TheraCal LC achieves the most pronounced antimicrobial effect. Based on this *in vitro* study, TheraCal LC can therefore be chosen as an alternative to conventional calcium silicate cements, however, further *in vitro* and *in vivo* studies are needed to more thoroughly investigate other properties of these materials.

Acknowledgements

The authors thank Mgr. Kateřina Langová, Ph.D. (Department of Medical Biophysics, Faculty of Medicine and Dentistry, Palacký University Olomouc) for statistical processing of the obtained data.

Conflict of interest declaration

The authors declare that they have no conflict of interest regarding the issues investigated in this original article.

Authors' contribution to the publication

B. N., P. H. and Y. M. participated in the designing of the experiment. B. N. and L. Sm. participated in the preparation of samples. B. N. and L. Sv. performed their own testing and subsequent measurements. L. Sm. and M. R. evaluated the obtained data. All authors contributed to writing the manuscript.

Data availability

The data are available from the corresponding author upon justified request.

Corresponding author

MDDr. Barbora Novotná
Institute of Dentistry and Oral Sciences
Faculty of Medicine and Dentistry,
Palacký University Olomouc
University Hospital Olomouc
Palackého 12
779 00 Olomouc
e-mail: barbora.novotna@upol.cz

REFERENCES

1. Innes NPT, Frencken JE, Bjørndal L, Maltz M, Manton DJ, Ricketts D, et al. Managing carious lesions: consensus recommendations on terminology. Adv Dent Res. 2016; 28(2): 49–57. doi: 10.1177/0022034516639276

2. Duncan HF, Galler KM, Tomson PL, Simon S, El-Karim I, Kundzina R, et al. European Society of Endodontology position statement: Management of deep caries and the exposed pulp. Int Endod J. 2019; 52(7): 923–934. doi: 10.1111/iej.13080

3. Alleman DS, Magne P. A systematic approach to deep caries removal end points: the peripheral seal concept in adhesive dentistry. Quintessence Int. 2012; 43(3): 197–208.

4. Barros MMAF, De Queiroz Rodrigues MI, Muniz FWMG, Rodrigues LKA. Selective, stepwise, or nonselective removal of carious tissue: which technique offers lower risk for the treatment of dental caries in permanent teeth? A systematic review and meta-analysis. Clin Oral Investig. 2020; 24(2): 521–532. doi: 10.1007/s00784-019-03114-5

5. Ricucci D, Siqueira JF, Li Y, Tay FR. Vital pulp therapy: histopathology and histobacteriology-based guidelines to treat teeth with deep caries and pulp exposure. J Dent. 2019; 86: 41–52. doi: 10.1016/j.jdent.2019.05.022

6. Iwami Y, Hayashi N, Takeshige F, Ebisu S. Relationship between the color of carious dentin with varying lesion activity, and bacterial detection. J Dent. 2008; 36(2): 143–151. doi: 10.1016/j.jdent.2007.11.012

7. Wells C, Dulong C. Vital pulp therapy for endodontic treatment of mature teeth: a review of clinical effectiveness, cost-effectiveness, and guidelines. Canadian Agency for Drugs and Technologies in Health; 2019: 1–31.

8. Hanna SN, Alfayate RP, Prichard J. Vital pulp therapy an insight over the available literature and future expectations. Eur Endod J. 2020; 5(3): 46–53. doi: 10.14744/eej.2019.44154

9. Novotná B, Harvan Ľ, Somolová L, Morozova Y, Voborná I. Treatment of deep caries lesion and stepwise excavation technique. Čes. stomatol. Prakt. zub. lék. (Czech Dental Journal). 2021; 121(3): 83–89. doi: 10.51479/cspzl.2021.011

10. Lee SJ, Monsef M, Torabinejad M. Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. J Endod. 1993; 19(11): 541–544. doi: 10.1016/S0099-2399(06)81282-3

11. Cervino G, Laino L, D'Amico C, Russo D, Nucci L, Amoroso G, et al. Mineral trioxide aggregate applications in endodontics: A review. Eur J Dent. 2020; 14(4): 683–691. doi: 10.1055/s-0040-1713073

12. Žižka R, Šedý J, Voborná I. Příprava kalciumsilikátových cementů. LKS. 2020; 30(1): 8–11.

13. Kadali N, Krishna Alla R, Guduri V, Av R, Sajjan Mc S, Venkateswara Raju R, Lecturer R. Mineral trioxide aggregate: an overview of composition, properties and clinical applications. Int J Dent Mater. 2020; 2(1): 11–18. doi: 10.37983/IJDM.2020.2103

14. Žižka R, Šedý J, Škrdlant J, Kučera P, Čtvrtlík R, Tomášťík J.
Kalciumsilikátové cementy. 1. část: Vlastnosti a rozdělení. LKS. 2018; 28(2): 37–43.
15. Domingos Pires M, Cordeiro J, Vasconcelos I, Alves M, Quaresma SA, Ginjeira A, et al.
Effect of different manipulations on the physical, chemical and microstructural characteristics of Biodentine.
Dent Mater. 2021; 37(7): e399–406.
doi: 10.1016/j.dental.2021.03.021
16. Awawdeh L, Al-Qudah A, Hamouri H, Chakra RJ.
Outcomes of vital pulp therapy using mineral trioxide aggregate or Biodentine: a prospective randomized clinical trial. J Endod. 2018; 44(11): 1603–1609. doi: 10.1016/j.joen.2018.08.004
17. Kadali NS, Alla RK, Av R, Mc SS, Raju Mantena S, Raju RV.
An overview of composition, properties, and applications of Biodentine. Int J Dent Mater. 2021; 03(04): 120–126.
doi: 10.37983/ijdm.2021.3404
18. Lozano-Guillén A, López-García S, Rodríguez-Lozano FJ, Sanz JL, Lozano A, et al.
Comparative cytocompatibility of the new calcium silicate-based cement NeoPutty versus NeoMTA Plus and MTA on human dental pulp cells: an in vitro study. Clin Oral Investig. 2022; 26: 7219–7228.
doi: 10.1007/s00784-022-04682-9
19. Rodríguez-Lozano FJ, López-García S, García-Bernal D, Sanz JL, Lozano A, Pecci-Lloret MP, et al.
Cytocompatibility and bioactive properties of the new dual-curing resin-modified calcium silicate-based material for vital pulp therapy. Clin Oral Investig. 2021; 25: 5009–5024.
doi: 10.1007/s00784-021-03811-0/Published
20. Novotná B, Holík P, Morozova Y, Rosa M, Galandáková A, Langová K.
Evaluation of cytotoxicity of the dental materials TheraCal LC, TheraCal PT, ApaCal ART and Biodentine used in vital pulp therapy: in vitro study. Dent J. 2024; 12(8): 249.
doi: 10.3390/dj12080249
21. Karadas M, Köse TE, Atıcı MG.
Comparison of radiopacity of dentin replacement materials. 2020; 9(4): 195–202.
doi: 10.22038/jdmt.2020.48196.1366
22. Tanzer J. Microbiology of dental caries. J Biol Earth Sci. 1992; 1(1): 377–424.
23. Esteki P, Jahromi M, Tahmourespour A.
In vitro antimicrobial activity of mineral trioxide aggregate, Biodentine, and calcium-enriched mixture cement against *Enterococcus faecalis*, *Streptococcus mutans*, and *Candida albicans* using the agar diffusion technique. Dent Res J (Isfahan). 2021; 18(1).
doi: 10.4103/1735-3327.310032
24. Niranjana M, Sunil J, Thomas G, Shyam A, Aparna M.
Antimicrobial properties of newer calcium silicate-based pulp-capping agents against *Enterococcus faecalis* and *Streptococcus mutans*: an in-vitro evaluation. Cureus J Med Sci. 2024; 16(9): e70459.
doi: 10.7759/cureus.70459
25. Morita M, Kitagawa H, Nakayama K, Kitagawa R, Yamaguchi S, Imazato S.
Antibacterial activities and mineral induction abilities of proprietary MTA cements.
Dent Mater J. 2021; 40(2): 297–303.
doi: 10.4012/DMJ.2019-351
26. Liu G, Wu C, Abrams WR, Li Y. Structural and functional characteristics of the microbiome in deep-dentin caries. J Dent Res. 2020; 99(6): 713–720.
doi: 10.1177/0022034520913248
27. Spatafora G, Li Y, He X, Cowan A, Tanner ACR.
The evolving microbiome of dental caries. microorganisms. 2024; 12(1): 121.
doi: 10.3390/MICROORGANISMS12010121
28. Rôças IN, Alves FRF, Rachid CTCC, Lima KC, Assunção I V., Gomes PN, et al.
Microbiome of deep dentinal caries lesions in teeth with symptomatic irreversible pulpitis. PLoS One. 2016; 11(5): e0154653.
doi: 10.1371/JOURNAL.PONE.0154653
29. Moradi Z, Abbasi M, Bahador A, Yekaninejad MS, Khanghah XM, Hoseini AP, et al.
Evaluation of cytotoxicity and antibacterial activity of different pulp capping liners.
Biomater Investig Dent. 2023; 10(1): 2287019.
doi: 10.1080/26415275.2023.2287019
30. Kuzmanović Radman I, Djeri A, Arbutina A, Milašin J.
Microbiological findings in deep caries lesions.
Stomatol Glas Srb. 2016; 63(1): 7–14.
doi: 10.1515/sdj-2016-0001
31. Pereira DFA, Seneviratne CJ, Koga-Ito CY, Samaranayake LP. Is the oral fungal pathogen *Candida albicans* a cariogen? Oral Dis. 2018; 24(4): 518–526. doi: 10.1111/odi.12691
32. Davaie S, Hooshmand T, Ansarifard S.
Different types of bioceramics as dental pulp capping materials: A systematic review. Ceram Int. 2021; 47(15): 20781–20792.
doi: 10.1016/j.ceramint.2021.04.193
33. Docimo R, Carrante VF, Costacurta M.
The physical-mechanical properties and biocompatibility of Biodentine: A review. J Osseointegration. 2021; 13(1): 47–50.
doi: 10.23805/JO.2021.13.01.8
34. Kim M, Lee SH, Shin DH.
In vitro study of the biological and physical properties of dual-cure resin-modified calcium silicate-based cement. Dent J. 2023; 11(5): 120.
doi: 10.3390/DJ11050120
35. Eissa M, Ibrahim S, Abdelfattah M, Elawady B.
Antimicrobial activity of different bioactive therapeutic pulp capping materials with relevance to their biological and clinical consideration: A comparative in vitro study.
Egypt Dent J. 2024; 70(2): 2107–2016.
doi: 10.21608/edj.2024.267931.2925
36. Bhavana V, Chaitanya KP, Gandi P, Patil J, Dola B, Reddy RB.
Evaluation of antibacterial and antifungal activity of new calcium-based cement (Biodentine) compared to MTA and glass ionomer cement. J Conserv Dent. 2015; 18(1): 44.
doi: 10.4103/0972-0707.148892
37. Subhi H, Subhi N, Alhaidary S, Azeez MS, Tabnjh AK.
Antibacterial activity of biodentine against *Enterococcus faecalis*: a systematic review.
Front Dent Med. 2024; 5(1498353): 1–19.
doi: 10.3389/fdmed.2024.1498353
38. Swathi Priyadharshini S, Ragavendran C, Anand Sherwood I, Jeyapalan R.
Comparative evaluation of compressive strength and morphological interface of carbonated hydroxyapatite with other pulp capping materials: An in vitro analysis.
Endodontology. 2025; 37(1): 90–95.
doi: 10.4103/ENDO.ENDO_92_24
39. Anthrayose P, Aggarwal A, Yadav S, Nawal RR, Talwar S.
Microscopic and elemental characterization of hydrated dental pulp capping agents.
J Conserv Dent Endod. 2021; 24(5): 496–501.
doi: 10.4103/jcd.jcd_460_21
40. Bakir Ş, Bakir EP, Akbiyik SY.
Evaluation of the bond strength of resin-modified glass ionomer enhanced with bioactive glass to composite resin with different dental adhesive systems.
Anal Quant Cytopathol Histopathol. 2021; 43(4): 235–241.
41. Akbiyik SY, Bakir EP, Bakir Ş.
Evaluation of the bond strength of different pulp capping materials to dental adhesive systems: an in vitro study.
J Adv Oral Res. 2021; 12(2): 286–295.
doi: 10.1177/2320206821997983
42. Park SH, Ye JR, Asiri NM, Chae YK, Choi SC, Nam OH.
Biocompatibility and bioactivity of a dual-cured resin-based calcium silicate cement: in vitro and in vivo evaluation.
J Endod. 2024; 50(2): 235–242.
doi: 10.1016/J.JOEN.2023.11.009
43. Sanz JL, Soler-Doria A, López-García S, García-Bernal D, Rodríguez-Lozano FJ, Lozano A, et al.
Comparative biological properties and mineralization potential of 3 endodontic materials for vital pulp therapy: Theracal PT, Theracal LC, and Biodentine on human dental pulp stem cells.
J Endod. 2021; 47(12): 1896–906.
doi: 10.1016/j.joen.2021.08.001
44. Arandi NZ, Rabi T. TheraCal LC.
From biochemical and bioactive properties to clinical applications.
Int J Dent. 2018; 2018: 6 pages.
doi: 10.1155/2018/3484653
45. Poggio C, Beltrami R, Colombo M, Ceci M, Dagna A, Chiesa M.
In vitro antibacterial activity of different pulp capping materials. J Clin Exp Dent. 2015; 7(5): e584-588.
doi: 10.4317/JCED.52401