

Antibacterial potential of contemporary dental luting cements

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SUMMARY

The aims of this investigation were to evaluate the antibacterial activities of different types of dental luting cements and to compare antibacterial action during and after setting. Agar diffusion testing was used to evaluate the antibacterial properties of seven types of dental luting cements (glass ionomer cements (GICs), resin modified GICs, resin composite, zinc oxide eugenol, zinc oxide non-eugenol, zinc phosphate, zinc polycarboxylate cements) on *Streptococcus mutans* bacteria. Instantly mixed zinc phosphate cements showed the strongest antibacterial activity in contrast to the non-eugenol, eugenol and resin cements that did not show any antibacterial effects. Non-hardened glass ionomer, resin modified and zinc polycarboxylate cements exhibited moderate antibacterial action. Hardened cements showed weaker antibacterial activities, than those ones applied right after mixing.

Key words: *Streptococcus mutans*, luting cements, antibacterial action, secondary caries, agar diffusion test.

INTRODUCTION

In dentistry many different types of luting cements are available. Zinc phosphate, zinc polycarboxylate, GICs, resin modified GICs, resin composite, zinc oxide eugenol, zinc oxide non-eugenol cements are widely used. The following properties of unexceptionable luting cement are desired [1]:

1. low viscosity and film thickness;
2. long working time with rapid set at oral temperatures;
3. low solubility;
4. high compressive and tensile strengths;
5. high proportional limit;
6. adhesion to tooth structure and restorative materials;
7. radiopacity;
8. translucency;
9. biocompatibility;
10. anticariogenic activity.

In spite of an extensive range on the market there is no one ideal dental luting cement. Nevertheless, the effects of luting cements on oral microorganisms and anticariogenic properties have to be considered. *Streptococcus mutans* is one of the bacteria most frequently implicated in dental caries [2-6]. Cariogenic bacteria, such as mutans streptococci, efficiently degrade fermentable carbohydrates to acids, which can demineralise tooth tissue [3-5, 7]. Applying dental crowns, bridges, inlays, onlays or veneers bacteria may be still present under the restoration having not fully removed the tissue affected by caries or if there is microleakage present after cementing. Microleakage is a common clinical phenomenon by which oral fluids, ions, molecules, and bacteria penetrate the tooth-restoration interface and gain access to dentinal tubules and pulp. In the area between the prepared tooth and applied restoration *S. mutans* bacteria are allowed to grow without mechanical disturbance, the shortage of oxygen gradually favours the growth of facultatively anaerobic mutans streptococci at the expense of aerobic bacteria, whose survival depends on an adequate oxygen supply [8]. This may cause an excessive increase of *S. mutans* colonies under the restoration inducing secondary caries and particularly reducing the longevity of the restoration.

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There are no definitive criteria available for the judgement of complete removal of caries during the preparation. It is proved that the residual bacteria of carious lesion may cause increased pulp sensitivity, inflammation and secondary caries as well [9].

Antibacterial activity of dental luting cements, during and after setting, assumes clinical relevance, because this property may help in the elimination or reduction of bacteria that have remained viable on walls of the preparation or bacteria that may gain access to the cavity through microleakage fissures.

The objective of this study was to examine the hypothesis that the effect on the growth of the colonies of *S. mutans* depends on the types of dental luting cements and the respective antibacterial potential varies before and after hardening of the cement.

MATERIALS AND METHODS

Seven types of luting cements were investigated. The brands, types and suppliers of materials used in this study are given in Table 1.

A specimen of bacteria culture was taken from the plaque formed on the patient's teeth. The obtained *S. mutans* bacteria were confirmed by Rapid STR system (Remel Inc.) [10], having previously carried out reactions of catalasis, hemolysis and determined Gram-positive streptococci by Gram paint method.

A popular method used in this study to evaluate antibacterial properties was the agar diffusion test, which is based on placing samples on agar plates seeded with microorganisms and then evaluating antibacterial activity by taking gauge of inhibition zone around the discs [11-13]. The Petri dishes containing Columbian agar (Liofilchem S.r.l) and 5% sheep

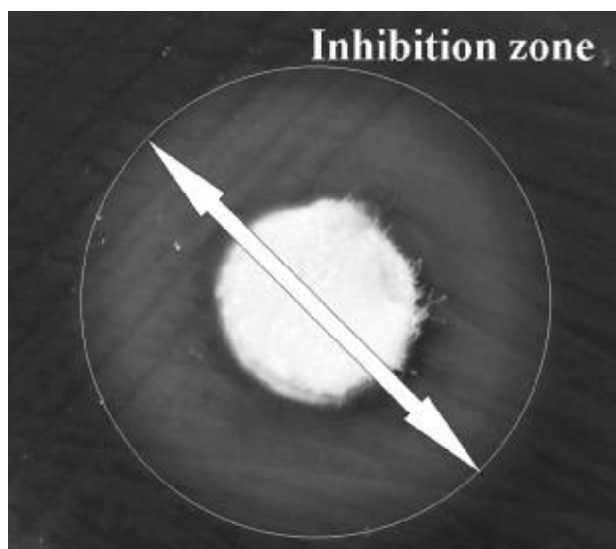


Fig. 1. Evaluation of inhibition zones using agar disc diffusion method

blood as nutritional medium were seeded with *S. mutans* bacteria. Then sterile discs, 7 mm in diameter, were soaked with the cements mixed according to the instruction and placed onto seeded nutritional medium. The Petri dishes were then incubated aerobically for 24 hours at the temperature of 37°C.

The respective antibacterial activities of luting cements were investigated during and after setting. In the first case cements were applied the first minute after mixing (not hardened), in the other case they were applied hardened, that is 24 hours after mixing. Light cured resinous Bifix QM and Variolink II cements were polymerized for 60 s on both sides with a light-curing unit XL2500 (3M ESPE) i.e. 'cured materials'.

Antibacterial effect of either cement was evaluated by blindly measuring mean diameter (mm) of complete inhibition zones of bacterial growth around

Table 1. Materials used in the study

Brand	Type	Manufacturer
Meron	Glass ionomer	Voco
Ketac Cem	Glass ionomer	3M ESPE
Meron Plus	Resin modified GIC	Voco
Fuji Plus	Resin modified GIC	GC Europe
Bifix QM	Resin-based	Voco
Variolink II	Resin-based	Ivoclar
Repin	ZnO eugenol	Spofa Dental
Provicol	ZnO non-eugenol	Voco
Temp Bond NE	ZnO non-eugenol	Kerr
Unifas-2	Zinc phosphate	Medpolimer
Hoffmann's Cement	Zinc phosphate	Hoffmann Manufaktur
Adhesor Carbofine	Zinc polycarboxylate	Spofa Dental
Carboco	Zinc polycarboxylate	Voco

Table 2. Inhibition zone diameters of hardened and non-hardened cements

Material	n	Non-hardened	n	Hardened
Unifas-2	12	25.27 ± 0.63 ¹	10	8.57 ± 0.32
Hoffmann's	16	20.47 ± 0.88	10	8.82 ± 0.23
Fuji Plus	16	13.82 ± 0.86	12	12.42 ± 0.50
Adhesor Carbofine	16	11.82 ± 0.78	10	9.60 ± 0.34
Meron	12	10.78 ± 0.51	10	8.60 ± 0.34
Meron Plus	12	10.30 ± 0.38	12	8.24 ± 0.55
Carboco	12	9.63 ± 0.54	10	7.37 ± 0.21
Ketac Cem	12	9.51 ± 0.49	10	8.62 ± 0.37
Bifix QM	9	7.10 ± 0.11	6	7 ± 0
Provicol	6	7 ± 0	6	7 ± 0
Temp Bond NE	6	7 ± 0	6	7 ± 0
Repin	6	7 ± 0	6	7.07 ± 0.08
Variolink II	6	7 ± 0	6	7 ± 0
Penicillin	(n=10)	28.97 ± 0.90		

¹All values are the mean of n experimentations (mm) ± standard deviation.

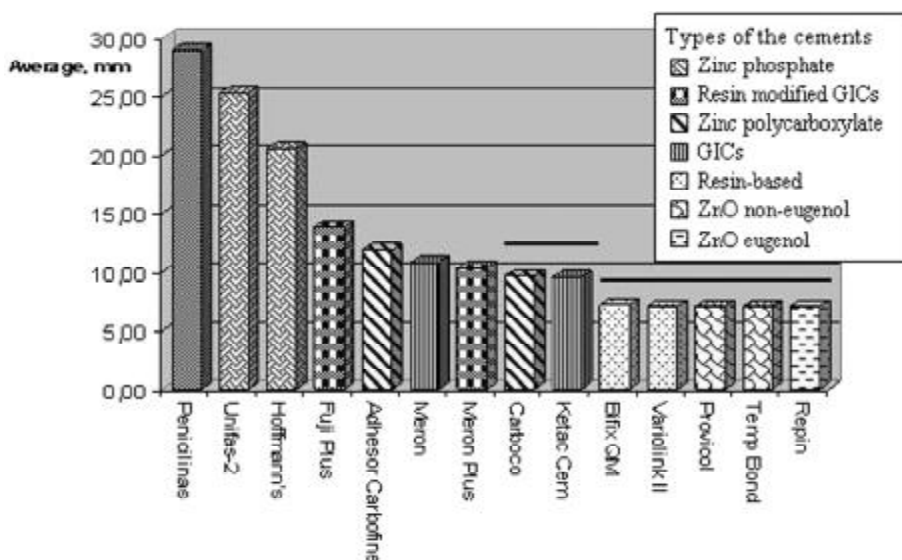


Fig. 2. Antibacterial effect of non-hardened cements (average of the diameter of the bacterial growth inhibition zone). No significant difference ($P \leq 0.05$) among lined materials was observed.

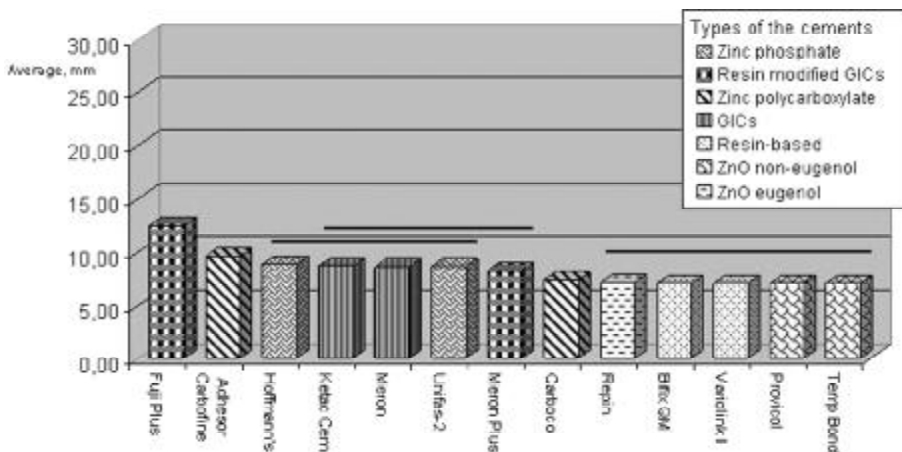


Fig. 3. Antibacterial effect of hardened cements. No significant difference ($P \leq 0.05$) among lined materials was observed.

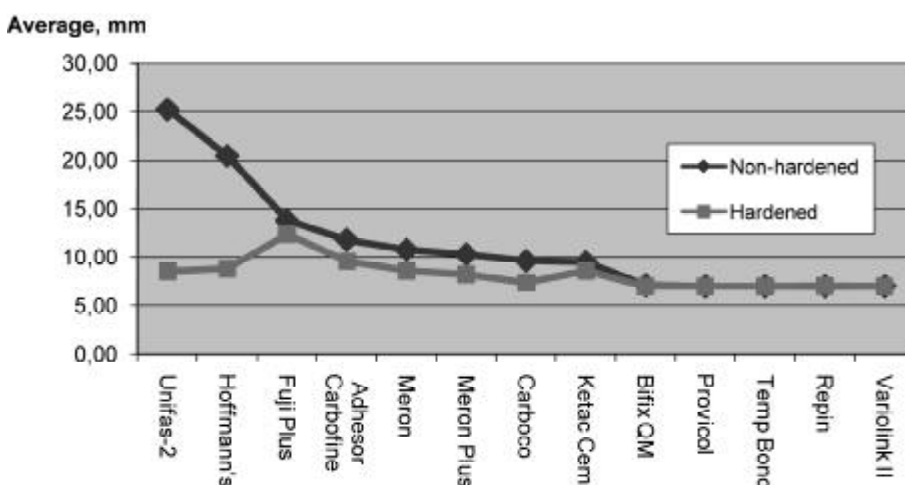


Fig. 4. Comparison of antibacterial properties of non-hardened and hardened cements

the discs (Fig. 1). Statistical analysis ($P \leq 0.05$, based on χ^2 criterion and t-test) was carried to determine the different activity significantly. Penicillin (10 µg discs) was used as a control.

RESULTS

Table 2 shows the antibacterial effect of non-hardened and hardened cements (average of the diameter of the bacterial growth inhibition zone). Control samples (penicillin) showed 28.97 ± 0.90 mm effect on bacterial growth.

The strongest antibacterial characteristics were exhibited by freshly mixed zinc phosphate cements (25.27 ± 0.63 mm). Instantly mixed Unifas-2 and Hoffman's cements have as nearly strong antibacterial action as penicillin (Fig. 2).

Five non-hardened cements (Provilol, Temp Bond NE, Repin, Bifix QM and Variolink II) have not shown significant antibacterial properties, it did not matter if they were applied right after mixing or cured. Thereby investigated zinc oxide non-eugenol, eugenol and resin-based cements don't inhibit the growth of bacteria. Although hardened Repin opposite to non-hardened showed very little antibacterial effect, no significant difference between these results could be observed ($P \leq 0.05$).

There is significant difference between hardened and non-hardened cements' antibacterial effect ($P \leq 0.05$). Hardened cements have weaker antibacterial effect than those applied right after mixing (Fig. 3). Only Fuji Plus and Ketac Cem showed quite stable results. Fuji Plus exhibited 13.82 ± 0.86 mm (non-hardened) and 12.42 ± 0.50 mm (hardened) activity on bacterial growth. Otherwise zinc phos-

phate cements' antibacterial potential particularly varies while they are non-hardened and hardened (especially Unifas-2, Fig. 4).

DISCUSSION

Sufficient control of dental plaque is the most important factor in caries prevention. However, not all patients take care of their oral hygiene perfectly. If an ideal oral hygiene is not maintained constantly, antibacterial properties of luting cements are desirable. Ideally, cements should possess antibacterial properties that will prevent bacteria-induced pulpal irritation, tooth sensitivity, and recurrent caries. *S. mutans* is the most common caries-associated bacteria.

The antibacterial properties of luting cements may be attributed to:

- the low pH level while setting and after maturation [14];
- fluoride and zinc releasing properties [15-21].

Uncured cements may have antibacterial effect on bacteria remained after cementing under the restoration, otherwise, in the case of microleakage, antibacterial properties of hardened cements are essential.

The strongest antibacterial activity was exhibited by instantly mixed zinc phosphate Unifas-2 and Hoffmann's cements. Freshly prepared phosphate cements are characterized as having a very low pH (under 2), which rises promptly while curing and slowly increases after maturation (up to 5.4 pH level in 24 hours) [1, 22]. Current studies show, that the growth of *S. mutans* colonies significantly decrease at pH 5.1 and completely appears at pH 4.8 or lower level [2, 23]. Accordingly remarkable strong antibacterial effect exhibited by newly mixed Unifas-2 and Hoffmann's may be due to their low pH in the first minute after mixing. The pH rise to higher level in 24 hours after mixing could explain the decreased antibacterial action of phosphate cements placed after hardening similarly (Fig. 4).

GICs, resin modified GICs and zinc polycarboxylate cements are described as having low initial pH level after mixing as well, but opposite to zinc phosphate cements, their instant pH after preparation is slightly higher and reaches non-destructive for *S. mutans* level more rapidly [24, 25]. The longer lasting low pH phase and remarkable low initial pH may be a ground of significantly stronger antibacterial action of zinc phosphate than GICs, resin modified GICs and polycarboxylate cements. Notwithstanding that the last three may release fluoride.

It must be noted that the low pH of the luting cement may result in a strong irritation of the dental pulp after application [26-30]. Therefore it requires clinical consideration of choosing zinc phosphate luting cement and also promotes the research to further look into the pulp non-irritant luting materials having antibacterial properties.

Fluoride is widely used as an anticariogenic material in many dental products [31-33]. GICs, resin modified GICs and polycarboxylate cements investigated in this study manage to leach fluoride as well [1, 34]. A variety of mechanisms are involved in the anticariogenic effects of fluoride on the teeth, including the reduction of demineralization, the enhancement of remineralization, the interference of pellicle and plaque formation, and the inhibition of microbial growth and metabolism. The most important anticariogenic property of fluoride in luting cements is the effect on cariogenic oral bacteria, especially on *S. mutans*. Fluoride can inhibit many enzymes involved in bacteria metabolism (the inhibition of the glycolytic enzyme enolase and the proton-extruding ATP-ase; acid phosphatase, pyrophosphatase, peroxidase and catalase may be affected by fluoride ions also [32]). In such a way fluoride inhibit production of bacterial acids and glucans, especially insoluble glucan produced by *Streptococcus mutans*. As insoluble glucans are important for virulence of mutans streptococci, the inhibitory actions of fluoride could significantly affect cariogenicity [35].

The other action of fluoride ions leading to inhibition of glucans and acid production by cariogenic bacteria at low pH values involves its capacity to induce acidification and starvation stresses on the cell [35]. Fluoride is acting in the form of protonated fluoride (HF) as a transmembrane proton carrier. It enhances proton permeability of cell membranes (to HF the cell is some 10^7 times more permeable than to F^-). Proton-extruding ATP-ases are overloaded and disturbed to extrude proton because excreted proton back into the cell due to movements of HF. It causes absence of ATP and starvation of bacterial cell. Moreover HF dissociates to the F^- (enzyme poison) and H^+ , which acts to acidify the cytoplasm and inhibit glycolytic enzymes. Eventually lowering pH compromises the energetic status of the cell by increasing re-entry of protons across the cell membrane. It increases the demand on ATP for acid-base regulation [35, 36].

In spite of widely described antibacterial properties of fluoride, its activity still remains in question [34]. Current studies show that fluoride can inhibit the growth of oral streptococci *in vitro* at concentra-

tions in the order of 0.16–0.31 mmol/l [37]. GICs and resin modified GICs are described as the materials leaching the highest amount of fluoride among luting cements, but even those products severely reach the inhibitory release of fluoride [38].

Uncured polycarboxylate Adhesor Carbofine and Carboco cements, which in theory leach less fluoride than GICs [1], had very similar antibacterial properties like conventional and resin modified GICs. Consequently the meaning of fluoride release in antibacterial action of investigated cements is probably less than the considerably low pH produced by freshly mixed cements. The only exception is uncured Fuji Plus cement, which has shown slightly higher antibacterial properties than other fluoride releasing materials investigated in this study. Furthermore, Fuji Plus had the strongest inhibitory effect on growth of bacteria among the cured materials.

Zinc-containing materials such as zinc phosphate, zinc polycarboxylate, zinc oxide eugenol and zinc oxide non-eugenol cements have been utilised for a number of years in clinical dentistry, due to their ability to release zinc ions that inhibit the growth of caries-related bacteria [9, 39]. Zinc acts as an inhibitor of multiple activities in the bacterial cell, such as glycolysis, transmembrane proton translocation and acid tolerance [19].

Antibacterial action of zinc is similar to fluoride, but it works better in neutral pH (while the inhibitory potency of fluoride for glycolysis is very much greater at acid pH values) [19, 35, 40].

Zinc also can enhance proton permeability of bacterial cells membrane [19]. It reduces proton-extruding ATP-ase activity. Moreover zinc acts to diminish ATP synthesis in glycolyzing cells because it can inhibit the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenases and pyruvate kinase, as well as the metabolism of phosphoenolpyruvate [21, 35].

The high proportion of zinc in powder compound [1] may contribute to antibacterial properties of investigated phosphate (Unifas-2, Hoffmann's) and polycarboxylate (Adhesor Carbofine, Carboco) cements. On the other hand less zinc containing zinc oxide eugenol and zinc oxide non-eugenol cements haven't shown any significant growth inhibitory effect on *S. mutans*. Consequently zinc is normally described as acting in high concentrations, bacteriostatic rather than bactericidal agent [19, 35].

Resin based cements (Bifix QM, Variolink II) as well as zinc oxide eugenol and non-eugenol materials haven't exhibited significant antibacterial action neither right after mixing, nor after maturation. The lack of antibacterial agents and relatively high pH may

cause the poor antibacterial properties of those cements. Otherwise the avoidance of low pH during and after setting minimizes irritation of the pulp and ensures better features of biocompatibility.

Comparing antibacterial properties of cured and uncured materials, significant drop of inhibitory effect on *S. mutans* after maturation of the cements was observed. As setting materials are much more soluble, and more able to diffuse in agar gel than the set ones. The decreased diffusion of ions also reduces the ability to inhibit the growth of bacterial colonies. It must be noted, that due to remarkable decrease of antibacterial effect after setting cements will be less effective in the case of microleakage, when bacteria are able to penetrate for a long time after cementation.

The considerably decrease of antibacterial action of phosphate Unifas-2 and Hoffmann's cements after setting indicates, that the low pH of those materials rises after maturation and has less inhibitory effect on *S. mutans*. The least difference of antibacterial effect before and after maturation was exhibited by resin modified Fuji Plus and glass ionomer Ketac Cem cements. It indicates the stable diffusion of antibacterial agents in 24 hours after mixing. Fuji Plus exhibited the strongest antibacterial properties between cured materials. However Ketac Cem had weak antibacterial effect no matter it was applied uncured or matured.

CONCLUSIONS

Instantly mixed zinc phosphate cements showed the strongest antibacterial characteristics that considerably decreased after setting. Comparing to uncured phosphate cements, fluoride or high percentage of zinc containing materials (GICs, resin modified GICs, polycarboxylate cements) exhibited weaker inhibitory effect on the growth of *S. mutans*. Hardened cements have weaker antibacterial effect than their cured homologues due to decreased diffusion of antibacterial agents after setting. Zinc oxide non-eugenol, eugenol and resin cements (Provicol, Temp Bond NE, Repin, Bifix QM, Variolink II) have not shown significant antibacterial properties, due to the lack of acidity and release of antibacterial agents.

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