

# Antibacterial effect of two luting cements on prepared dentin *in vitro* and *in vivo*

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The antibacterial effect of a polycarboxylate cement (Durelon<sup>®</sup>) and a zinc phosphate cement (De Trey's Zink Zement Improved<sup>®</sup>) was studied *in vitro* and *in vivo*.

For *in vitro* experiments a streptomycin resistant strain of *Streptococcus mutans* (*Strep. mutans* PRSR) was used. In one experimental series 1 ml of an overnight bacterial culture was suspended in 20 ml of nutrient agar, and poured to set in Petri dishes. Holes 5 mm in diameter were punched in the gel and filled with freshly mixed cement. The antibacterial effect was estimated by measuring the diameter of the zone of bacterial growth inhibition surrounding the holes after 24 h. In another experimental series two cavities were cut into the dentin of newly extracted human third molars. On the floor of both cavities a circular disc of filter paper saturated with *Strep. mutans* PRSR was placed. A sterile brass cylinder with freshly mixed cement covered one of the paper discs for 15 min. Both discs were then transferred to a streptomycin-containing growth medium of trypticase soy broth (TSB) and incubated at 37 °C for 6 days. Scrapings from the cavity floor subjacent to the paper discs were also incubated. Bacterial growth was recorded as positive, no growth as negative.

*In vivo* vital crown and bridge abutments with preparations into the dentin were used from several patients. Discs of filter paper saturated with the patient's own saliva were placed on sprayed and dried dentin and covered with freshly mixed cement as described above. Again, after 15 min the paper disc was liberated from the cement, placed in TSB and incubated for 6 days, as were scrapings from the dentin subjacent to the disc.

In the gel diffusion test the zinc phosphate cement exhibited the strongest antibacterial properties, while in the other *in vitro* test the polycarboxylate cement appeared to be most strongly antibacterial against *Strep. mutans* PRSR. *In vivo* positive cultures were obtained with both cements, indicating that the saliva always contains some bacteria resistant to the antibacterial effect of the cements studied.

**Key-words:** Dental materials; antibacterial effect; crown and bridge prosthetics

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Adverse pulp reactions to various dental materials have long been recognized (6). However, several authors have implicated bacteria as the actual source of pulp damage (3, 9, 16). It has been claimed that bacterial contamination of cavity walls may precede

the insertion of restorations, and to prevent this, thorough cavity cleansing has been advocated (4, 5, 10, 11). However, *in vitro* studies (21, 22) have demonstrated that several dental materials are bactericidal and it is therefore conceivable that the materials

themselves may eliminate any bacteria left on the cavity walls (14).

In this context, relevant materials in crown and bridge prosthetics are the luting cements. Accordingly, a study of their antibacterial properties might be of interest.

The purpose of the present investigation was to assess *in vitro* and *in vivo* the antibacterial activities of freshly mixed samples of polycarboxylate and zinc phosphate cements.

#### MATERIALS AND METHODS

##### *Cements*

The cements studied were a polycarboxylate cement (Durelon<sup>®</sup>, ESPE GmbH, Seefeld/Oberbay, W. Germany), and a zinc phosphate cement (De Trey Zink Cement Improved<sup>®</sup>, De Trey Frères S.A., Zürich, Switzerland). Both cements were mixed to a consistency suitable for luting cast crown and bridge restorations.

##### *Test bacteria*

For *in vitro* tests *Streptococcus mutans* PRSR was used. The strain is a human plaque isolate, made streptomycin resistant (17). It belongs to biotype I of Shklair & Keene (18), and is agglutinated to high titres by an antiserum to *Strep. mutans* IB, serotype c (2).

For the experiments the bacteria were cultivated in Trypticase Soy Broth (TSB, B.B.L., Cockeysville, Md., U.S.A.) containing 1000 µg/ml Streptomycin (Glaxo, London, U.K.). The streptomycin containing medium will be referred to as TSB/S in the text. Following incubation for 18 h the bacteria were washed three times by centrifugation (10.000 x g, 15 min., 4 °C) in reduced transport fluid (RTF, (13)), and resuspended to some 4 x 10<sup>10</sup> colony-forming units/ml in RTF.

In the *in vivo* tests a mixed, undefined bacterial culture from the patients own saliva was used.

##### *Experimental design*

##### *In vitro* growth inhibition by agar diffusion

One millilitre of an overnight culture of *Strep. mutans* PRSR in TSB was added to 20 ml of TSB containing 1.5 % Noble agar (Difco, Detroit, Mi., U.S.A.) kept molten at 50 °C. The bacteria were evenly suspended in the nutrient agar which was then poured into Petri dishes to set. Holes 5 mm in diameter were punched in the gel, and subsequently filled with freshly mixed cement. Four dishes were poured with two samples of either cement in each. Control wells contained distilled water and 0.05 % chlorhexidine digluconate (ICI, Macclesfield, U.K.). After incubation for 24 h at 37 °C in an atmosphere of 95 % N<sub>2</sub> and 5 % CO<sub>2</sub> the antibacterial effect of the cements was estimated, measuring the diameter of zones of growth inhibition in a stereo microscope.

##### *Survival of bacteria underneath luting cements in vitro*

Twenty-four newly removed impacted human third molars were used, twelve with either cement. Two cavities, approx. 3 mm in diameter, were cut in the dentin with a tungsten carbide bur in an air turbine. The cavities were sprayed with water and dried with compressed air.

Sterile paper discs (1.5 mm diameter, grade 3 MM chroma, W. & R. Balston Ltd., Kent, U.K.) were saturated with *Strep. mutans* PRSR in RTF and placed on the cavity floors. Freshly mixed cement was then applied to one cavity covering the paper disc by means of a sterile brass cylinder and left in place for 15 min (Fig. 1). The brass cylinder was then removed, the filter paper liberated from the cement and placed

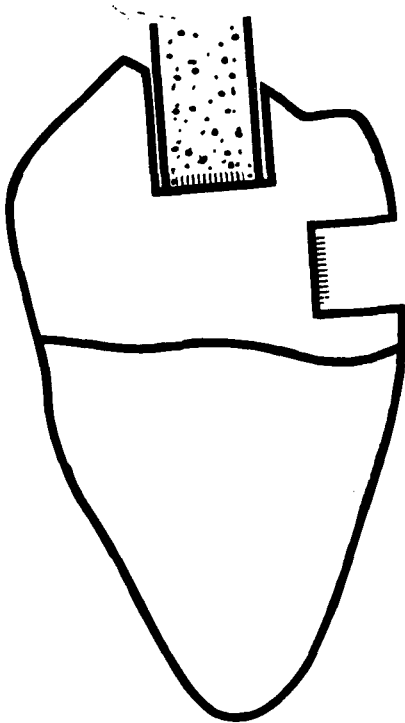


Fig. 1. Schematic illustration of newly extracted human third molar with two cavities. On the floor of both cavities a disc of filter paper saturated with *Strep. mutans* PRSR in TSB/S is found. In the test cavity the disc of filter paper is covered with a sterile brass cylinder containing freshly mixed cement.

in a tube containing 2 ml TSB/S. The filter paper from the control cavity was placed in another tube.

Prior to cementation bacterial sampling was made from both cavities by means of the pointed end of sterile toothpicks moistened in TSB and TSB/S and placed in 2 ml of the same growth media respectively. After removal of the paper discs at the end of the 15 min period, the same procedure of bacterial sampling was made from the floor of both cavities placing the toothpicks in 2 ml TSB/S. In addition, for control purposes, a test for the presence of streptomycin resistant bacteria on the outside of the brass ring and the adjacent cavity wall was made using the above described technique. All tubes containing paper discs and

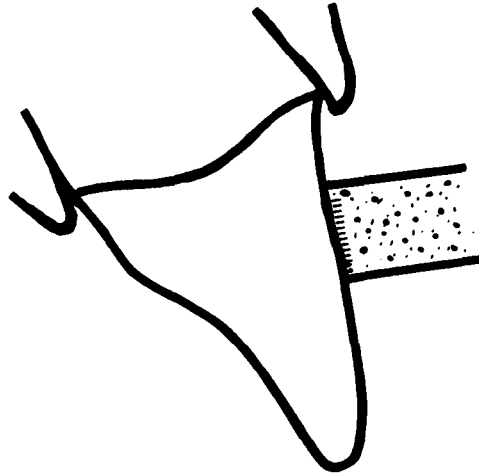


Fig. 2. Prepared abutment with saliva-contaminated disc of filter paper covered with a sterile brass cylinder containing freshly mixed cement.

toothpicks were incubated at 37 °C for 6 days. Bacterial growth, represented by turbidity of the growth solution, was recorded as positive, no growth as negative.

Survival of bacteria underneath luting cements in vivo Vital human crown and bridge abutments with full crown preparations into the dentin were used. After preparation the teeth were sprayed with water, dried with compressed air, and kept free from moisture by means of cotton rolls. A sterile disc of filter paper, as described above, was moistened in the patient's saliva and placed on the labial surface of the preparation. Covering the disc, a sterile brass cylinder was cemented (Fig. 2). Ten cylinders were cemented with polycarboxylate cement and 11 with zinc phosphate cement. After 15 min the cylinder was removed and the filter paper liberated from the cement and placed in 2 ml TSB. Using the technique with sterile toothpicks outlined above, surface scrapings were obtained from the prepared dentin before and after application of the saliva-contaminated disc covered by cement. The

toothpicks were also placed in 2 ml TSB. All tubes containing paper discs or toothpicks were incubated at 37 °C for 6 days. Saliva-contaminated discs of filter paper (n = 10) left uncovered on sprayed and dried, prepared dentin for 15 min were also incubated in 2 ml of TSB at 37 °C and served as controls.

## RESULTS

### *Bacterial inhibition in agar diffusion tests*

Both cements displayed antibacterial activity in this system (Fig. 3). For polycarboxylate cement the mean diameter of the zone of inhibition was 0.82 cm (SD = 0.03 cm, range: 0.80–0.85 cm), while for zinc phosphate cement the corresponding figures were 1.15 cm (SD = 0.12 cm, range: 0.90–1.30 cm). For comparison the mean diameter of the zone created by 0.05 % chlorhexidine was 1.64 cm (SD = 0.07 cm, range: 1.55–1.90 cm) whereas no inhibition zone could be detected around wells filled with distilled water.

### *Bacterial inhibition on prepared dentin in vitro*

Growth tests showed that bacteria were always present on the cavity floor after preparation and before cementation. In two cavities streptomycin resistant bacteria appeared to be present before application of the test bacteria, but in no case were streptomycin resistant bacteria present on the outside of the brass ring or the surrounding cavity wall immediately before removal of the ring. Growth did not take place from any of the paper discs having been covered with polycarboxylate cement (Table 1). For zinc phosphate cement growth occurred in 8 out of 12 tubes, but signs of turbidity were not visible till after 2–3 days of incubation. Scrapings from the dentin subjacent to the cement covered discs did not give rise to bacterial growth for either cement.

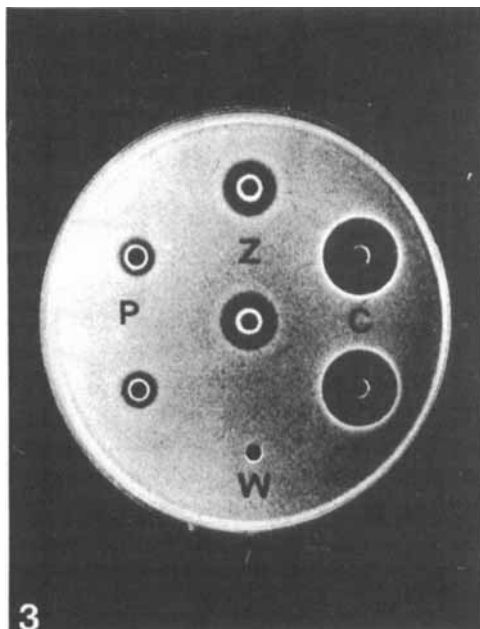


Fig. 3. Petri dish with nutrient agar containing *Strep. mutans* PRSR. Wells with surrounding zones of bacterial growth inhibition are seen. The wells contain from left to right: polycarboxylate cement (P), zinc phosphate cement (Z), chlorhexidine digluconate (C), and distilled water (W).

The discs from the control cavities gave rise to growth in all instances after one day of incubation. Scrapings from the subjacent dentin revealed growth in a total of 14 out of 24 instances.

### *Bacterial inhibition on prepared dentin in vivo*

In Table 2 the bacterial growth in relation to the two cements *in vivo* is shown. Scrapings from the prepared abutments obtained before cementation gave rise to bacterial growth in all cases. Bacterial growth took place from 4 out of 10 discs covered with polycarboxylate cement and from 4 out of 10 scrapings from the underlying dentin. In one case only did growth occur both from the disc and from the subjacent dentin surface. With zinc phosphate cement the corresponding numbers were 7

Table 1. Effect of luting cements on bacteria on prepared dentin surfaces *in vitro* expressed as proportions of positive cultures of *Strep. mutans* PRSR in TSB/S

	Test cavity		Control cavity	
	Paper disc	Dentin subjacent to disc	Paper disc	Dentin subjacent to disc
Polycarboxylate cement	0/12	0/12	12/12	4/12
Zinc phosphate cement	8/12	0/12	12/12	10/12

Table 2. Effect of luting cements on bacteria on prepared dentin surfaces *in vivo* expressed as proportions of positive cultures of *Strep. mutans* PRSR in TSB

	Prepared dentin	Paper disc	Dentin subjacent to disc
Polycarboxylate cement	10/10	4/10	4/10
Zinc phosphate cement	11/11	7/11	2/11

out of 11 discs and 2 out of 11 scrapings. Again, growth from both disc and subjacent dentin was recorded in one case only. All the controls resulted in growth after one day of incubation.

#### DISCUSSION

The role of particular microbial species in recurrent caries and pulpal inflammation in connection with cast restorations is not known. *Strep. mutans*, a common inhabitant of the oral cavity, has been implicated as an etiological factor in primary dental caries (8). Moreover, the activities of this species in relation to dental filling materials have been given some consideration (15, 19). Therefore, a strain of *Strep. mutans* was chosen for the *in vitro* tests of the antibacterial properties of the luting cements used in the present study. As demonstrated in the present *in vivo* series, conventional preparation procedures regularly leave viable bacteria on the prepared dentin surface. The use of a streptomycin resistant strain of *Strep. mutans* permitted specific tests to be made against this organism without inter-

ference of other organisms that might have been present in the test cavities.

The gel diffusion tests provided a baseline for comparison of the two cements in regard to their activity towards *Strep. mutans* PRSR. However, it was noticed in preliminary studies that the width of the inhibition zone was obviously influenced by the consistency of the cement mixes. Yet, the low range of individual width recordings might imply that achieving what is considered a clinically suitable cement mix, is a fairly reproducible procedure with regard to antibacterial activity, and should not influence the findings to any extent.

From the results of the present study it is apparent that after *in vitro* preparation all cavities contained viable bacteria. In two cases these bacteria were resistant to streptomycin. However, no contamination of the cavities with *Strep. mutans* PRSR from the outside had taken place during the actual cementation procedure, since all controls for this purpose were negative.

The polycarboxylate cement prevented growth of *Strep. mutans* PRSR from the paper discs whereas the zinc phosphate cement prevented growth in 1/3 of the cases. It should be noted, however, that

when growth did occur, it started only after 2–3 days of incubation. This could mean that the number of surviving bacteria was either very much reduced or the capacity for growth of the surviving bacteria was somehow affected by the zinc phosphate cement. However, both cements seemed equally effective in preventing bacterial growth from the surface of the underlying dentin. The observation that growth of *Strep. mutans* PRSR took place in only 14 out of 24 scrapings from the dentin subjacent to the paper discs in the control cavities may be due to methodological shortcomings. Still, antibacterial effects of the dentinal fluid towards this bacterium should not be ruled out, considering the presence of immunoglobulins in dentin (20).

The last series of tests was performed to study the antibacterial activity of the luting cements *in vivo*, and it was obvious from the results that vital bacteria will always be present on prepared dentin after spraying with water and drying with compressed air. Apparently, there was some difference in the antibacterial activity of the two cements towards a mixed salivary flora. Again the polycarboxylate cement appeared to be most effective but this time growth occurred in 40% of the cases. Since very dense suspensions of *Strep. mutans* PRSR were killed under similar conditions *in vitro*, it seems reasonable to assume that the mixed salivary flora contains bacteria with greater resistance to the antibacterial action of the polycarboxylate cement. A rather wide variation among species in their susceptibility to this type of cement has indeed been demonstrated (23).

Pulpal damage depending on the activities of living bacteria on cavity floors may be prevented by the bactericidal action of liners and dental cements. However, it is conceivable that even products of dead bacteria may cause pulpal inflammation (1). Therefore, mere killing of the bacteria in the dentin smear may not suffice to eliminate

bacteria dependent pulp inflammation. Rather, it might be speculated that mechanical removal of living and dead bacteria as well as their toxic and immunogenic products would be desirable. Moreover, mechanical cleansing of the prepared surface has been found recommendable also for the purpose of improving the retentive ability of luting cements (7).

It may be concluded that polycarboxylate and zinc phosphate cements possess certain antibacterial properties. Apparently the composition of the bacterial flora in question influences the degree of antibacterial activity registered. The necessity for removal of all bacteria and their products to preserve pulp health is a question yet to be solved. Even after infectious insults the dental pulp exhibits marked healing potentialities after the use of zinc phosphate and polycarboxylate cements (12).

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