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Antibacterial Actions of Dental Cements

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Summary

We examined antibacteriral activities of ten clinically used dental cements on the oral indigenous bacteria. The order of the intensiveness of antibacterial activity in wideness of spectrum of various bacterial species was eugenol group, carboxylate group, zinc phosphate group, copper group=silicate phosahate group, silicate group. The two cements in eugenol group were active on all the indicator strains. Carboxylate group inhibited preferably anaerobes. The others were active on several strains. The action of cements on the susceptible cells was found to be both bactericidal and bacteriostatic, in which eugenol group acted mainly bactericidally. This bactericidal effects were demonstrated also in the teeth experimentally infected. Silicate group exhibited no measurable antibacterial activity. The inhibitory activities were rather stable, they did not lose at least 6 weeks in a solution except for one cement sample. The inhibitory activities were detected in the both of powder components and liquid components of the cements and the activities in the former components were stronger than those of the latter components. Of the authentic reagents consists of cements, eugenol, rosin, zinc oxide, and zinc phosphate exhibited inhibitory activity against various bactrial species.

INTRODUCTION

Dental cements are required to have technologically the most appropriate properties. However, since they contact always with oral fluids and oral microbes on the mouth, antibacterial[1) 4) 5) 9) 10) 12) 13)] activity is desirable in the cements. From this point of view, many reports concerning to action of dental restoractive materials have been published. This paper was undertaken to assess the antimicrobial actions of commercially available dental cements specially against the indigenous bacteria of the human mouth.

MATERIALS AND METHODS

Cements : Ten commercially available cements including eugenol group(3), zinc phosphate group(2), carboxylate group(2), silcate phosphate group(1), copper group(1), and silicate group(1), listed in Table 1, were used throughout the experiments (Table 1).

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Table 1 : List of cements

Cement (trade name)	
1. Eugedain	Eugenol group
2. Neodyne	Eugenol group
3. E. B. A.	Eugenol group
4. Micro Cement	Zinc phosphate group
5. Crown Bridge & Inlay Cement	Zinc phosphate group
6. Durelon	Carboxylate group
7. Carbo Cement	Carboxylate group
8. Posterit Cement	Silicate group
9. Copr-Seal Cement	Copper group
10. Luxsilit	Silicate group

In the subsequent tables, the cement sample was expressed by the enumerated number of the begining of the trade name

Bacterial strains: Caries-or periodontal disease-associated bacteria and bacteria found in the normal oral flora[2] were used as indicator strains for assay of antibacterial activites. They were *Streptococcus mutans* Ingbritt, *Streptococcus sanguis* ATCC 10557, *Streptococcus mitis* ATCC 9895, *Streptococcus salivarius* ATCC 9759, *Actinomyces viscosus* ATCC 19246, *Actinomyces naeslundii* ATCC 12104, *Propionibacterium acnes* ATCC 6919, *Fusobactreium nucleatum* ATCC 25286, *Bacteroides ochraceus*[7] #85*(Capnocytophaga)*, and *Bacteroides melaninogenicus* NM-3.[6] Cultivation for preparation of indicator cells of the above strains except for *Bacteroides* species were carried out in GAM-semisolid medium (Nissui). The *Bacteroides* strains were cultured in a medium consisting of heart infusion (Difco, 2.5%), hemin (5 μg/ml), menadione (0.5 μg/ml), horse defibrinated blood (10%v/v), and agar (1.2%) in anaerobic glove box.

Detection of antibacterial activity: The cements were solidified aseptically in plastic templates sizing 2mm in thickness and 6 mm in diameter by mixing the powder components and liquid components which have been sterilized with ethylene oxide and millipore filter membrane, respectively, prior to subject to solidification. The tablets finished to solidify were placed onto plates of brain heart infusion (Difco) or trypticase peptone[8] (BBL), and soft agar previous been seeded with about 1×10^5 cells of each indicator strain was overlaid. The plates were cultured for 3 to 5 days anaerobically and the diameters of the resultant clear inhibitory zones were measured. To evaluate the antibacterial activities of powder-and liquid-components, the former (about 2 mg) was adhered to sterile disk (6 mm in diameter) and placed onto agar plates containing indicator strains and the inhibitory was measured accordingly to the way described above (disk-method). On the other hand, liquid component was poured into well (8 mm in diameter) in indicator cells-

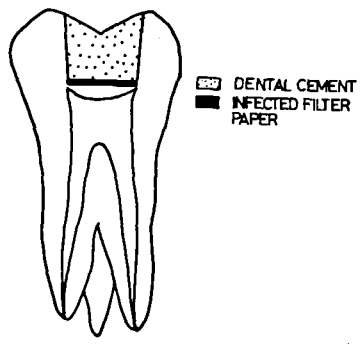

Fig. 1 Schematic representation of infected teeth for antibacterial action

containing agar plate and the inhibitory activity was measured after incubation (well-method).[3)]

Stability of the antibacterial activities: Each component was immersed in 0.1 M phosphate buffer (pH 7.0) and kept at 37°C for 1 to 6 weeks. The residual activities were assayed.

Antibacterial activities of cements in extracted teeth: After cavities were prepared in extracted molars, as illustrated in Fig. 1, they were sterilized with autoclave and infected in cavity areas with *Strep. sanguis* and *P. acnes,* separately by contact of the cavity areas with each bacteria on the filter paper containing about 1×10^7cells. After incubation, cavities were filled with EBA or Luxsilit cement followed by embedding of the teeth in brain heart infusion agar and kept at 37°C for 2 days. After which, cements were removed carefully using dental engine and viable cells remained in the cavity areas were counted.

RESULTS

Antibacterial activities of the cements against various oral indigenous bacteria are summarized in Table 2. Neodyne, one of the eugenol group was active on the all indicator strains and the strongest activity. EBA followed Neodyne in spectrum and intensivity, but it did not inhibit *A. naeslundii.* Eugedain inhibited only five species, its spectrum was narrower than the former two. In zinc phosphate group, Micro and Crown bridge & Inlay cement were active on three species, Posterit and Copr-Seal were on two species. Luxsilit, a silicate group was ineffective on all the strains tested (Fig. 2).

The stability of antibacterial activities of the six out of ten cements was examined on *Strep. mutans, Strep. sanguis,* and *P. acnes.* As shown in Table 3, five cement samples maintained almost the same level of activities measured at the initial time, but one cement, Posterit cement showed lowering of the activity on *Strep. sanguis* during 1 to 6 weeks.

Investigation was made to examine the antibacterial action is whether bactericidal or bacteriostatic. The tablets were adhered with about 1×10^7 cells of each testing bacterial strain and then they were placed onto the plates of brain heart infusion agar or trypticase peptone agar and incubated at 37°C anaerobically. After 2 to 4 days incubation, sterile loop was streaked across the tablet areas and the plates were incubated for a further 2 to 4 days. If growth was seen where the cells had been translocated away from the tablet areas,

Table 2 : Inhibitory action of various dental cements

indicators	dental cements									
	1	2	3	4	5	6	7	8	9	10
Strep. mutans Ingbritt	0	16	10	0	0	0	0	0	0	0
Strep. sanguis ATCC 10557	8	18	14	10	8	13	8	10	8	0
Strep. mitis ATCC 9895	0	16	14	0	0	0	0	0	0	0
Strep. salivarius ATCC 9759	0	20	8	0	0	0	0	12	0	0
A. viscosus ATCC 19246	0	26	10	0	0	8	8	0	0	0
A. naeslundii ATCC 12104	0	16	0	0	0	0	0	0	0	0
P. acnes ATCC 6919	16	18	16	8	10	10	14	0	0	0
F. nucleatum ATCC 25286	25	26	23	23	12	16	18	0	16	0
B. ochraceus #85 (*Capnocytophaga*)	15	22	15	0	0	10	11	0	9	0
B. melaninogenicus NM-3	20	14	16	0	0	8	9	0	0	0

Values : inhibitory diameter (mm)

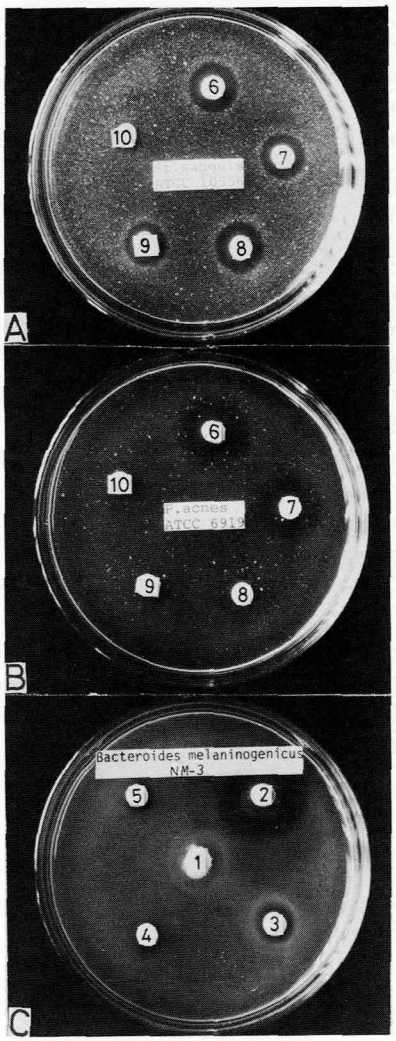

Fig. 2 Antibacterial activity of various dental cements
indicator strain:
A : *Strep. sanguis* ATCC 10557
B : *P. acnes* ATCC 6919
C : *B. melaninogenicus* NM-3
dental cement:
1 : Eugedain
2 : Neodyne
3 : E. B. A
4 : Micro Cement
5 : Crown Bridge & Inlay Cement
6 : Durelon
7 : Carbo Cement
8 : Posterit Cement
9 : Copr-Seal Cement
10 : Luxsilit

Table 3 : Stability of inhibitory activity of dental cements

days	indicators	dental cements 2	3	4	6	7	8
Initial	*Strep. mutans* Ingbritt	16	10	0	0	0	0
1 week		16	10	0	0	0	0
6 weeks		16	10	0	0	0	0
Initial	*Strep. sanguis* ATCC 10557	18	14	10	13	8	8
1 week		18	14	10	13	8	8
6 weeks		16	12	9	12	7	0
Initial	*P. acnes* ATCC 6919	18	16	8	10	14	0
1 week		18	16	8	10	13	0
6 weeks		16	16	8	8	13	0

Values : inhibitory diameter (mm)

action of cement is referred to bacteriostatic, but if growth was never seen over the whole plates, it is obvious that its action is bactericidal. The results are presented in Table 4, in eugenol group, Neodyne did not permit growth of all indicator strains over the whole plates, indicating its action was bactericidal. Similarly, Eugedaine and EBA acted on *P. acnes*, *A. viscosus*, and *Bacteroides* as a bactericidal agent. In carboxylate group, Durelon killed *A. viscosus* and *Bacteroides*. Carbocement killed *B. melaninogenicus*. They acted on the other species bacteriostatically.

The results of antibacterial effect in the infected teeth, EBA reduced viable cells of *Strep. sanguis* slightly, whereas remarkable lowering of viable cells was seen in *P. acnes*. As a control experiment, Copr-Seal which is not potent inhibitor did not show the lowering viable cells, rather increasing of slight amount of them was observed (Table 5).

Antibacterial activities of powder components and liquid components of cements were tested. In eugenol group and Durelon, obviously inhibitory activity was observed in all indicator strains except for *Strep. mutans*. The significant activities were also demonstrated in powder components of the other kinds of cements against *P. acnes* and Gram-negative anaerobes. It was failed to detect the activity in powder component of Luxsilit (Table 6). In liquid components, all of them showed antibacterial activity, even in ten-fold diluted samples with 0.1 M phosphate buffer (pH 7.0), which made free from the effect of low pH on growth of indicator strains of the stock solution of liquids (Table 7).

Antibacterial activities of four authentic reagents, rosin, zinc oxide, zinc phosphate, alminum oxide, and eugenol, contained in powder components of cements as in the case of test of powder cement effect. As shown in Table 8, high activity in eugenol, especially on Gram-negative anaerobes, was evident. Rosin, zinc phosphate also effective on all the indicator strains. Zinc oxide was active on *Strep. sanguis* and anaerobes but aluminum oxide was essentially harmless on all the strains.

Table 4 : Bactericidal or bacteriostatic action of various dental cements

indicators	dental cement									
	1	2	3	4	5	6	7	8	9	10
Strep. mutans Ingbritt	—	BC	BS	—	—	—	—	—	—	—
Strep. sanguis ATCC 10557	BS	BC	BS	BS	BS	BS	BS	BS	BS	—
Strep. mitis ATCC 9895	—	BC	BS	—	—	—	—	—	—	—
Strep. salivarius ATCC 9759	—	BC	BS	—	—	—	—	BS	—	—
A. viscosus ATCC 19246	—	BC	BC	—	—	BC	BS	—	—	—
A. naeslundii ATCC 12104	—	BC	—	—	—	—	—	—	—	—
P. acnes ATCC 6919	BC	BC	BC	BS	BS	BS	BS	—	—	—
F. nucleatum ATCC 25286	BS	BC	BS	BS	BS	BS	BS	—	BS	—
B. ochraceus #85 *(Capnocytophaga)*	BC	BC	BC	—	—	BC	BS	—	BS	—
B. melaninogenicus NM-3	BC	BC	BC	—	—	BC	BC	—	—	—

BC : bactericidal
BS : bacteriostatic
— : not effect

Table 5 : Antibacterial effect of cement in the experimental infected teeth

indicators / cement	*Strep. sanguis* ATCC 10557		*P. acnes* ATCC 6919	
	3	9	3	9
starting time	3.9×10^7	3.9×10^7	2.3×10^7	2.3×10^7
after 2 days	2.0×10^7	4.8×10^7	1.6×10^2	2.6×10^8

Values : viable cells

Table 6 : Inhibitory action of the powder component

indicators	powder of cements									
	1	2	3	4	5	6	7	8	9	10
Strep. mutans Ingbritt	10	0	0	0	0	12	0	0	0	0
Strep. sanguis ATCC10557	12	10	10	8	0	10	0	0	10	0
Strep. mitis ATCC 9895	8	10	8	0	0	12	0	0	0	0
Strep. salivarius ATCC 9759	8	10	10	0	0	16	0	0	0	0
A. viscosus ATCC 19246	10	14	12	0	0	18	18	0	0	0
A. naeslundii ATCC 12104	14	12	14	8	0	10	0	0	0	0
P. acnes ATCC 6919	12	12	14	14	12	18	14	16	16	0
F. nucleatum ATCC 25286	17	12	20	18	15	23	14	10	12	0
B. ochraceus #85 (*Capnocytophaga*)	22	17	15	12	10	13	12	13	8	0
B. melaninogenicus NM-3	12	14	15	11	9	8	13	8	8	0

Values : inhibitory diameter (mm) in disk method

Table 7 : Inhibitory action of various the liquid component

indicators	liquid (10 fold diluted concentration)									
	1	2	3	4	5	6	7	8	9	10
Strep. mutans Ingbritt	18	22	22	20	22	12	14	22	14	16
Strep. sanguis ATCC 10557	14	22	28	26	34	14	16	34	16	30
P. acnes ATCC 6919	34	24	14	12	24	20	16	26	14	18

Values : inhibitory diameter (mm) in well-method

Table 8 : Inhibitory action of some reagents contained in dental cements

indicators	※				eugenol (dilution) ※※							
	Rosin	ZnO	Zn_3PO_4	Al_2O_3	×2	×4	×8	×16	×32	×64	×128	×256
Strep. mutans Ingbritt	14	0	10	0	22	19	18	17	16	0	0	0
Strep. sanguis ATCC 10557	12	9	10	0	15	14	13	12	11	0	0	0
Strep. mitis ATCC 9895	12	0	8	0	18	16	14	12	11	0	0	0
Strep. salivarius ATCC 9759	12	0	8	0	15	15	14	13	12	0	0	0
A. viscosus ATCC 19246	14	0	10	0	30	28	26	25	20	12	0	0
A. naeslundii ATCC 12104	12	10	11	0	19	18	17	15	12	0	0	0
P. acnes ATCC 6919	12	14	9	0	23	21	20	19	17	0	0	0
F. nucleatum ATCC 25286	10	14	12	0	32	29	27	26	24	14	12	0
B. ochraceus #85 *(Capnocytophaga)*	13	12	12	0	33	30	26	23	19	15	12	10
B. melaninogenicus NM-3	14	14	11	0	36	32	28	24	20	12	11	10

Values : inhibitory diameter (mm)
※ : disk method
※※ : well-method

DISCUSSION

Antibacterial activities of dental cements demonstrated in the present paper may be, although minor effect from an original purpose, valuable for treatment of oral infectious diseases or their prevention. Of the ten commercially available cements which are commonly used, no antibacterial activity was detected in silicate group only, but all others, more or less, were active on the various bacterial species. Action of eugenol group is characterized by its effect on wide spectrum of bacterial species and its bactericidal effect on almost all the indicator strains. These tendency of effects coincided nearly with the results of the preceding reports,[1) 4) 10)] but minor inconsistents were still observed. Carboxylate group cements showed intermediately wide spectrum and they were characterized by preferable inhibition of anaerobic species. Unfortunately, antibacterial activities of cements against anaerobes have not enoughly been carried out, therefore, it is almost impossible to compare the activities of cements against anaerobes.

A order of the wideness of effective spectrum of bacterial species of the cements tested was eugenol group, carboxylate group, zinc phosphate group, silicate phosphate group=copper group, silicate group. The activities of solidified cements were maintained at least 6 weeks in a solution, expected clinical values for control of oral bacterial flora. Furthermore, the bactericidal effects of cements in infected teeth provide a better expectation in this sense.

Even the solidified cement which did not exhibit antibacterial activity, that was detected in its liquid component (Luxsilit). As for the powder components, all of them except for silicate group had activities and their active spectrum of bacterial species were wider than solidified state of cements in general. In the case of the reversal correlation, certain mechanisms might exist to decline the antibaterial activity by mixing of the powder and liquid and/or cementing process. It was reported that if liquid and powder was mixed, the antibacterial activity was lost in amalgam.[11) 14)]

From the results of effects of the chemical reagents, alminum oxide had individually no activity, but rosin, zinc phosphate, and eugenol were active on all the indicator strains. Zinc oxide was also considerably effective. Therefore, it is conclusive that the antibacterial activities found in the cements may be sole or synergistical actions of the chemical compunds contained cements.

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