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Antibacterial action of dental cements: An *in vitro* study

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SUMMARY

The antibacterial activity of seven commercialy available dental cements (Eugespad®, Dentical®, Dycal®, Expaliner®, PR. Scell®, PR. Base Cement®, PR. Lining Cement®) against 1) bacterial species implicated in carious lesions or in dental plaque (*Actinomyces israelii* ATCC 10048, *Actinomyces viscosus* ATCC 19246, *Streptococcus mutans* ATCC 25175, *Streptococcus sanguis* ATCC 10557) and 2) bacterial samples of stimulated saliva was studied, *in vitro,* using a modification of the method of McComb and Ericson (1987).

Dycal® and Expaliner® did not affect bacteria whereas the other dental cements displayed some antibacterial properties. Eugespad® was the most active followed by PR. Base Cement® + PR. Scell® + Dentical® and by PR. Lining Cement®.

Associated with mechanical and biocompatibility properties, these differences could be taken into account when choosing a dental cement for clinical use.

KEY WORDS:

Actinomyces, Dental cements, Microbiology, *Streptococcus.*

RÉSUMÉ

Le but de ce travail a été de comparer, *in vitro,* les éventuelles propriétés antibactériennes de différents ciments: 1 ciment ZOE (Eugespad®), 2 ciments à base d'hydroxyde de calcium (Dentical®, Dycal®), 3 ciments verre-ionomère (PR. glass ionomer LC®, PR. glass ionomer BC®, PR. Scell® glass ionomer) et un ciment adhésif (Expaliner®).

Ces matériaux ont été testés vis-à-vis d'espèces bactériennes retrouvées dans la carie dentaire ou dans la plaque dentaire (*Actinomyces viscosus* ATCC 19246, *Actinomyces israelii* ATCC 10048, *Streptococcus mutans* ATCC 25175, *Streptococcus sanguis* ATCC 10557) et des échantillons de salive totale.

L'activité antibactérienne a été appréciée par une méthode d'inhibition de croissance en milieu solide dérivée de la technique des antibiogrammes (McComb, 1987). Chaque ciment, préparé selon les recommandations des fabricants, était déposé au fond d'un puits creusé dans une gélose (milieu de Schaedler

enrichi avec du sang de mouton) ensemencée avec une préculture de 48 heures de l'une des souches bactériennes retenues. Après incubation à l'étuve à 37°C en anaérobiose, les diamètres des zones d'inhibition de croissance étaient mesurés au temps t = 48 heures. Pour chaque espèce et matériau testés, l'expérimentation a été répétée 10 fois.

Parmi les sept matériaux testés, le Dycal® et l'Expaliner® n'ont entraîné aucune zone d'inhibition de croissance. Les plus fortes zones d'inhibition ont été obtenues avec le ciment ZOE. L'activité des quatre autres ciments a varié en fonction de la nature du ciment et de l'espèce bactérienne.

Ces modifications d'activité pourraient, après confirmation de ces premiers résultats, être prises en compte dans le choix clinique d'un ciment dentaire.

MOTS CLÉS :

Actinomyces, Ciments dentaires, Microbiologie, *Streptococcus.*

INTRODUCTION

According to Browne *et al.* (1983) bacteria which colonize the interface of material and cavity wall are mainly responsible for pulpal inflammation beneath cavities filled with various test materials. They observed a reduction or elimination of pulpal inflammation beneath cavities filled with silicates and composites when bacterial microleakage is prevented or reduced by a surface bactericidal cement.

Their results confirm the observations of numerous workers (Brännström and Nordenvall, 1978; Bergenholtz *et al.*, 1989; Brännström and Nyborg, 1973; Brännström and Vojinovic, 1976; Tobias *et al.*, 1985; Torstenson *et al.*, 1982; Watts and Patterson, 1983).

Antimicrobial action of lining material is clinically important. Therefore, the purpose of this study was to evaluate the antibacterial activity of seven cements commonly used as lining agents on *actinomyces* and *streptococcus* strains and on bacterial samples of saliva. These strains were choosen as test organisms because they are found in deep carious lesions or in dental plaque or are implicated in the aetiology of dental caries (Hardie, 1986; Loesche, 1986; Loesche and Syed, 1973; Schaal, 1986; Shaw, 1987; Van Houte, 1980, 1994).

MATERIAL AND METHODS

Test materials

Brand names, manufacturers, principal constituents of the materials tested have been listed in Table I. Table I also lists the known antimicrobial components of the material as well as the powder-to-liquid ratios used.

Organisms and Culture Conditions

Four reference strains *(Actinomyces viscosus, Actinomyces israelii, Streptococcus mutans, Streptococcus sanguis)* (Table II) and freshly collected stimulated whole saliva were used in this experiment. Cultures of reference strains were reconstituted from lyophilization and grown anaerobically (GasPak System, BBL Microbiology Systems, Cockeysville MD) in Brewer's jar at 37° C onto Schaedler agar (Schaedler broth with 1.8% of agar noble, BBL Microbiology Systems) supplemented with 5% sheep blood (BioMérieux Lyon France).

Antibacterial evaluation method

The testing technique used for this investigation is based on an adaptation of the method of McComb and Ericson (1987).

Under sterile conditions, a suspension (6 according to McFarland scale = 18×10^8 cells/ml, API SYSTEM SA, 1986) was prepared for each reference strain from a 48 h culture. A 0.5 ml aliquot of the suspension containing the appropriate microorganism was poured onto fresh blood Schaedler agar plates ($\varnothing^*$ = 9 cm, t** = 4 mm). Similarly, blood Schaedler agar plates were inoculated with 0.5 ml stimulated whole saliva. Saliva was collected just before use in each experiment. Excess inoculum was removed with a pipette and the inoculated plates were dried for 15 min at 37° C (Tobias *et al.*, 1985). After drying, 6 mm diameter wells were made in the plates and dental materials were placed into them. For each bacterial strain, 20 plates were used and divided into two groups of 10 each. In each plate of one group, 4 wells were realized and filled with Eugespad, PR. Scell, PR. Base Cement and PR. Lining Cement.

* $\varnothing$ = diameter, ** t = thickness

TABLE I: Tested Materials.
TABLEAU I: Matériaux testés.

Brand name Batch	Manufacturer	Principal constituents	Powder/liquid ratio Paste/paste
Eugespad (Eu)	SPAD Quetigny, France	ZnO, Eugenol*	5 - 1
Dentical (DE) 701	SPAD Quetigny, France	$Ca(OH)_2$*, $ZnSO_4$, CaF_2 Diethyleneglycol, Monosalicylate	1 - 1
Dycal (Dy) 860123	CAULK De Trey Dentsply Bois Colombes, France	$Ca(OH)_2$*, ZnO, TiO, ZnO, $CaPO_4$, Ethyltoluolsulfonamid*, Butylglycolsalicylate	1 - 1
PR SCELL (S) 8890	PIERRE ROLLAND Merignac, France	SiO, F*, acrylic and tricarboxilic acids	1 level spoon 2 drops of liquid
PR Base Cement (BC) 8710	PIERRE ROLLAND Merignac, France	Unknown Glass-ionomer cement	1 level spoon 1 drop of liquid
PR Lining Cement (LC) 8712	PIERRE ROLLAND Merignac, France	Unknown Glass-ionomer cement	1 level spoon 1 drop of liquid
Expaliner (Ex) 80621	PIERRE ROLLAND Merignac, France	BisGMA, Triethyleneglycol-dimethacrylate, polycarbonicpolymetacrylic acid	photopolymerization

* = Antibacterially active component.

TABLE II: Reference strains.
TABLEAU II: Souches de référence.

Actinomyces israelii:	ATCC 10048
Actinomyces viscosus:	ATCC 19246
Streptococcus mutans (serotype c):	ATCC 25175
Streptococcus sanguis (type II):	ATCC 10557

ATCC = American Type Culture Collection.

3 wells were made in each plate of the other group and Dentical, Dycal and Expaliner were placed into them. Each of the 20 plates was poured with the same bacterial suspension. All cements were mixed according to the manufacturer's instructions just before use. The Expaliner cement was polymerized with a Visilux 2 light Unit (Dental Products Division/3 M, St-Paul, USA) for 40 secondes both above and below the appropriate well. Similarly, plates without bacterial inoculation were realized. The blood agar Petri dishes were incubated anaerobically at 37° C and examined after 48 h for 1) the presence of bacterial growth inhibition (clear, circular halo without bacteria surrounding the sample of material) and for 2) the change of agar translucency surrounding each well. The diameter of each inhibition zone was measured by 2 different examiners with calipers.

From the values obtained at 48 h, the average number and standard deviation were evaluated. These results were analyzed by analysis of variance and t test ($p<0.05$) so that significant differences between test materials could be determined (Schwartz, 1980).

RESULTS

Inhibition of growth

The mean diameter of zone of complete inhibition of bacterial growth at 48 h is shown in Fig. 5. Fig. 1 and Fig. 2 show typical zones of bacterial inhibition.

Analyses of variance demonstrated a significant overall difference between means at the 5% level: $F>F_S$ ($2.17 < F_S < 2.25$) (with *S. mutans* F=28,91; with *S. sanguis* F = 457,89; with *A. viscosus* F=70,87; with *A. israelii* F=18,18; with microbial saliva strains F=31,41).

Detailed analyses using t tests showed that all cements (except Dycal and Expaliner) displayed some antibacterial activity against the four reference strains and the saliva microorganisms. Table III gives us the statistical interpretation of the antibacterial activity of the different cements for each test organism.

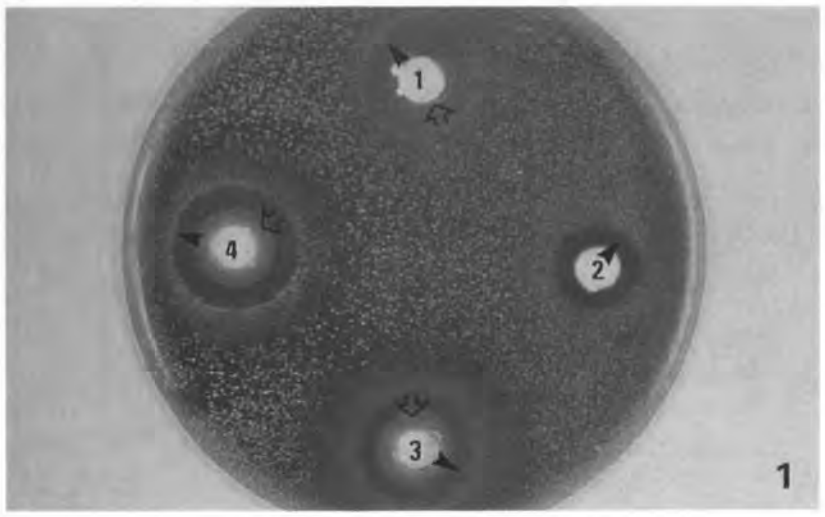

Fig. 1: Typical zones of bacterial inhibition with *Streptococcus sanguis.*
1=Eugespad, 2=PR Lining Cement, 3=PR Scell, 4=PR Base Cement
◊ indicates agar color change
◗ indicates limit of zone of bacterial inhibition
Fig. 1: Zones d'inhibition de croissance avec Streptococcus sanguis.
1=Eugespad, 2=PR Lining Cement, 3=PR Scell, 4=PR Base Cement
◊ *variations de la couleur de la gélose*
◗ *limites des zones d'inhibition de croissance*

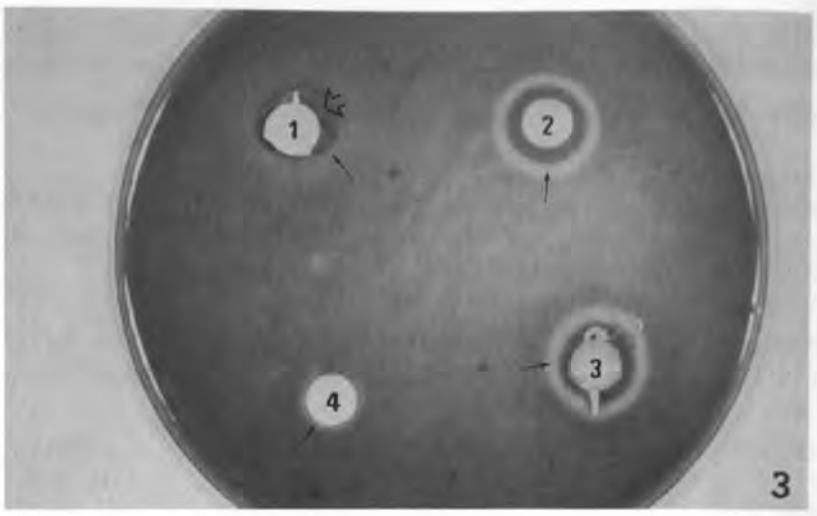

Fig. 3: Change of agar translucency.
1=Eugespad, 2=PR Base Cement, 3=PR Scell, 4=PR Lining Cement
◊ indicates agar color change
→ indicates zone of hemolysis-like agar change
Fig. 3: Changement de couleur de la gélose.
1=Eugespad, 2=PR Base Cement, 3=PR Scell, 4=PR Lining Cement
◊ *changement de couleur de la gélose*
→ *zones d'hémolyse*

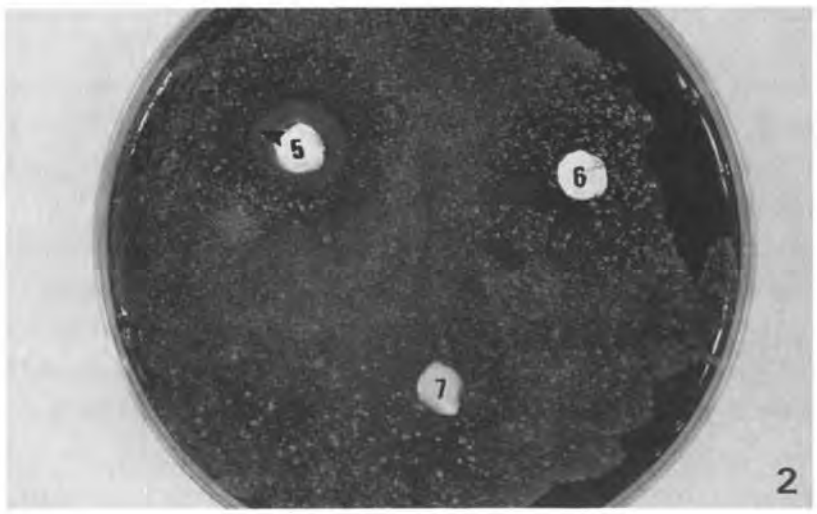

Fig. 2: Typical zones of bacterial inhibition with saliva samples.
5=Dentical, 6=Dycal, 7=Expaliner
◊ indicates agar color change
◗ indicates limit of zone of bacterial inhibition
Fig. 2: Zones d'inhibition de croissance avec les échantillons de salive.
5=Dentical, 6=Dycal, 7=Expaliner
◊ *variations de couleur de la gélose*
◗ *limites des zones d'inhibition de croissance*

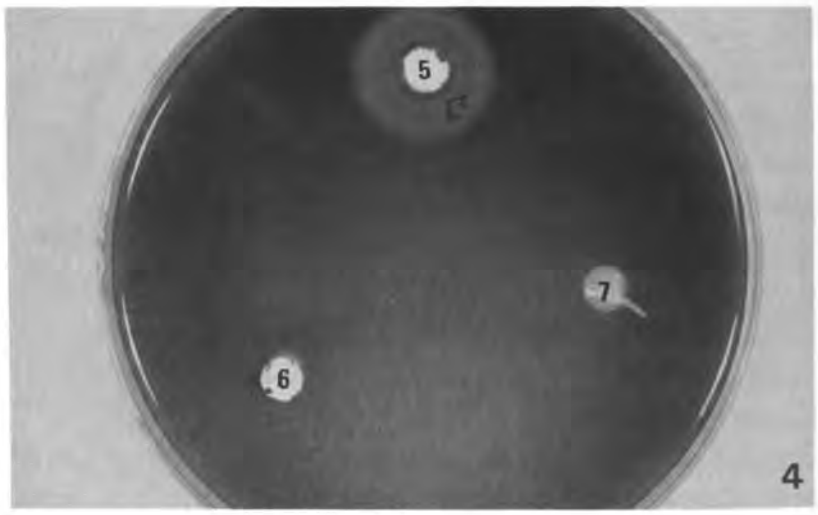

Fig. 4: Change of agar translucency.
5=Dentical, 6=Dycal, 7=Expaliner
◊ indicates agar color change
→ indicates zone of hemolysis-like agar change
Fig. 4: Changement de couleur de la gélose.
5=Dentical, 6=Dycal, 7=Expaliner
◊ *changement de couleur de la gélose*
→ *zones d'hémolyse*

The Eugespad cement has the most pronounced effect on bacterial growth followed by BC+DE+S and by LC.

Change of agar translucency

Dycal and Expaliner do not affect agar gel whereas hemolysis-like agar change or agar color change are noted with Eugespad, Dentical and the glass-ionomer cements (Figs 1, 2, 3, 4).

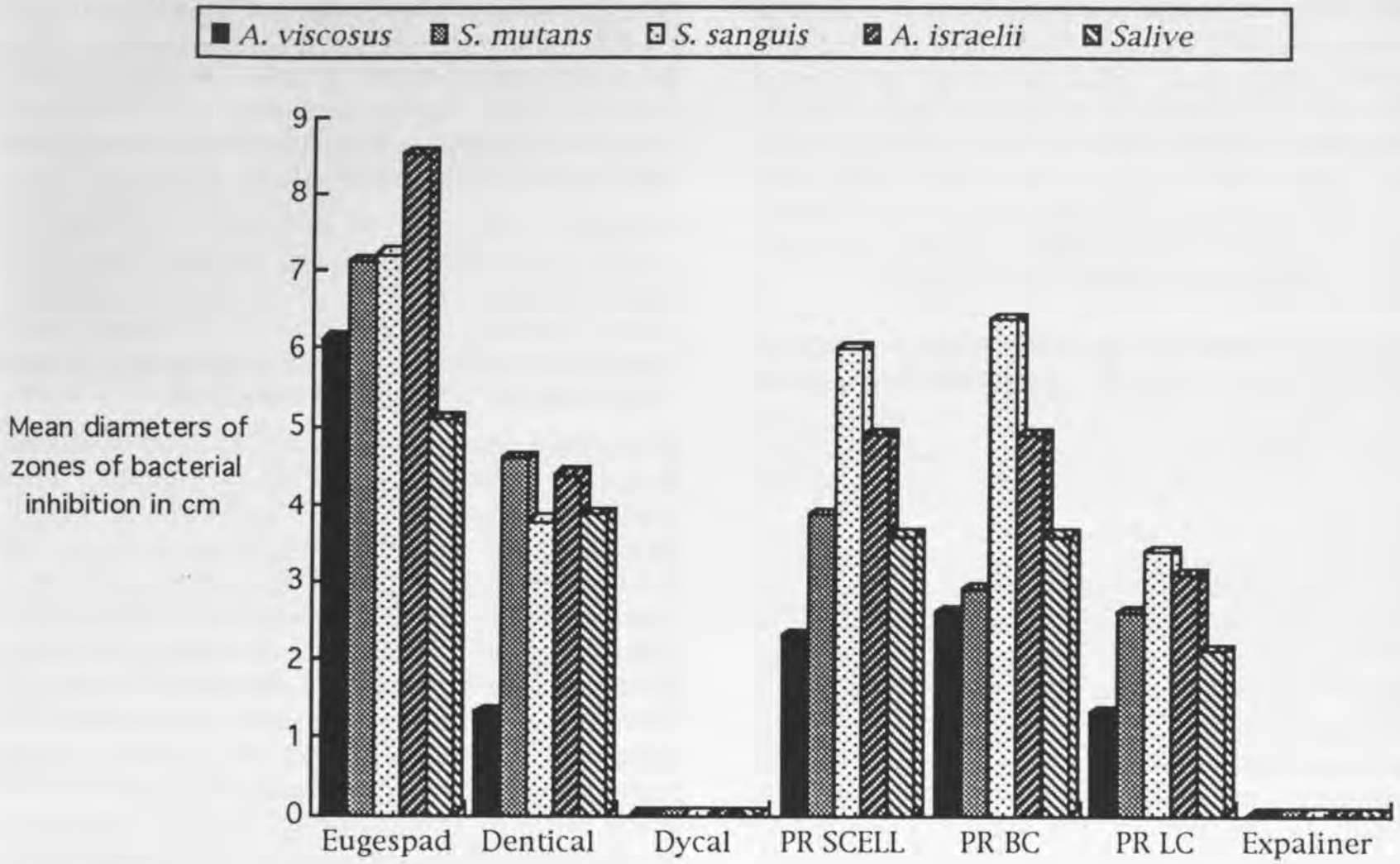

Fig. 5: Mean diameter of zones of bacterial activity at 48 hours.
Fig. 5: Diamètres moyens des zones d'inhibition de croissance à 48 heures.

TABLE III: Statistical interpretation of antibacterial activity of each material.
TABLEAU III: Interprétation statistique de l'activité antibactérienne de chaque matériau.

– With *A. israelii:*	Eu > BC = S = De = LC
– With *A. viscosus:*	Eu > BC = S > De = LC
– With *S. mutans:*	Eu > De = S > BC = LC
– With *S. sanguis:*	Eu > BC > S > De > LC
– With saliva:	Eu > BC = S = De > LC

= t-tests do not show a significant difference between means at the 5 % level: $t < 1,96$.

> t-tests show a significant difference between means at the 5 % level: $t > 1,96$.

DISCUSSION

Under the conditions of this study, only five cements (Eugespad, Dentical and the three glass-ionomer cements) inhibited growth of test microorganisms.

This antimicrobial action was cement and bacterial dependant. Eugespad was the most inhibitory on all test organisms whereas Dycal and Expaliner did not exibit any bacterial inhibition. PR. Lining Cement was less inhibitory. Dentical, PR. Base Cement and PR. Scell showed intermediate activity.

The demonstration of antibacterial properties in the method used depends 1) on the contact between dental materials and agar gel and 2) on the diffusability of antimicrobial agents in the agar. Therefore, according to Tobias *et al.* (1985) the agents playing a role in antibacterial action are F^+, H^+, OH^- ions and eugenol which diffusion occurs readily through agar gel. Since bacteria are present in dentinal tubuli (Adriaens *et al.*, 1988; Mjor, 1977; Oguntebi, 1994), the demonstration of diffusion of an antimicrobial agent is of clinical interest. It can be supposed that if a diffusion occurs in agar gel, such situation could be possible in dentinal tubuli.

This inhibition of growth is presumably due to the release of fluorides from glass ionomer cements and/or the low pH of these cements whereas release of hydroxyl (OH^-) ions and the high pH probably are responsible of the antibacterial effect of Dentical.

The strong activity of Eugespad could be related to the release of Eugenol. According to Orstavik (1981), Tobias *et al.* (1985), the overall antibacterial effect of zinc oxide-eugenol based materials was substantially greater than that of the other materials. This study confirms theirs observations and agrees with the findings of Barkhordar *et al.* (1989), McComb and Ericson (1987) regarding the antibacterial activity of glass ionomer cements.

No specific observations on the measure of surface pH of freshly set cement specimens were made in this study: according to McComb and Ericson (1987), simple analysis of cement surface pH is not a good indicator of antimicrobial activity of the material.

The two calcium hydroxide cements (Dentical and Dycal) have very different bacterial activity. The non bacterial effect of Dycal is puzzling regarding earlier findings (Fisher and McCabe, 1978, McComb and Ericson, 1987). Dycal did not affect agar gel and bacteria whereas with Dentical change of agar translucency and inhibition of growth were observed (Figs 2, 4). This data could be explained by a lack of water solubility of Dycal samples used in this study. McComb and Ericson (1987) have showed that antibacterial activity was dependant of the formulation of the dental cement: Prisma VLC Dycal did not affect bacteria and agar whereas Advanced Formula II Dycal showed antibacterial activity. In our investigation, the formulation of Dycal was different of the Fisher's one and could explain this lack of activity. According to Schröder (1985), the release of calcium hydroxide in a particular environment will depend on the composition of the material and its solubility. These conclusions could explain the difference between Dentical and Dycal.

It is not surprising to observe a relationship between inhibition of bacterial growth and change of agar translucency (=agar color change or hemolysis-like agar zone). These changes are probably due to the diffusion of the same components of the cement and particularly diffusion of the antimicrobial agents. Dycal and Expaliner did not affect bacteria and did not induce agar change: these two materials could be considered to be inert. These results agree with the findings of McComb and Ericson (1987): antibacterial activity is associated with agar color change.

However antibacterial effect is not the only base for choosing a lining cement. An antibacterial effect is not always beneficial and may be toxic for the pulp in a deep cavity. This is the case with Eugenol or glass ionomer cements when dentin thickness between pulp and cement is about 0.5 mm or less (Stanley, 1990). Biocompatibility and mechanical properties are also essential. All these factors must be taken into consideration in clinical practice.

Attemps to release this *in vitro* antibacterial activity to the *in vitro* clinical activity meet with some difficulties. *In vivo* antibacterial effect may also depends upon 1 bacterial concentration in the dentin and 2 the presence at the interface between cavity wall and dental material of a complex microflora.

All cements except Dycal and Expaliner displayed some *in vitro* antibacterial properties. This inhibitory effect was cement and bacterial dependent. Although significant differences between the antibacterial properties of dental cements were observed in the present experiment, more studies will be required to determine the antibacterial properties of these materials and particularly by testing them against more bacterial species incriminated in dental decay. Combined with the mechanical and biocompatibility tests, the bacteriological study of dental materials against microbial strains frequently isolated from dental supragingival plaque and caries could be taken into account by clinicians when choosing a dental cement.

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REFERENCES

Adriaens, P.A., Edwards, C.A., De Boever, J.A., Loesche, W.J. — Ultrastructural observations on the bacterial invasion of cementum and radicular dentin of periodontally diseased teeth. *J. Periodontol., 59:* 493-503, 1988.

API System S.A. — Préparation de l'échelle étalon de densité optique de McFerland. API ZYM. Système de recherche d'activités enzymatiques. Notice technique 2520 Version C: 1, 1986.

Bergenholtz, G., Cox, C.F., Loesche, W.J., Syed, S.A. — Bacterial leakage around dental restorations: its effect on the dental pulp. *J. Oral Pathol., 11:* 439-450, 1989.

Barkhordar, R.A., Kempler, D., Pelzner, R.R.B., Stark, M.M. — Technical note: Antimicrobial action of glass-ionomer lining cement on. *S. sanguis* and *S. mutans. Dent. Mater., 5:* 281-282, 1989.

Brännström, M., Nyborg, H. — Cavity treatment with a microbicidal fluoride solution: growth of bacteria and effect on the pulp. *J. Prosthet. Dent., 30:* 303-310, 1973.

Brännström, M. and Nordenwall, K.J. — Bacterial penetration, pulpal reaction and the inner surface of Concise enamel bond. Composite fillings in etched and unetched cavities. *J. Dent. Res., 57:* 3-10, 1978.

Brännström, M. and Vojinovic, O. — Response of the dental pulp to invasion of bacteria around three filling materials. *J. Dent. Children, 43:* 83-89, 1976.

Browne, R.M., Tobias, R.S., Crombie, I.K., Plant, C.G. — Bacterial microleakage and pulpal inflammation in experimental cavities. *Int. J. End., 16:* 147-155, 1983.

Fisher, F.D. and McCabe, J.F. — Calcium hydroxide base materials. *Brit. Dent. J., 144:* 341-344, 1978.

Hardie, J.M. — Genus *Streptococcus.* In Sneath P.H.H., Mair N.S., Sharp M.E. and Holt J.G. (ed.), Bergey's manual of systematic bacteriology. Vol. 2 Williams and Wilkins Baltimore, pp. 1043-1071, 1986.

Loesche, W.J. — Role of *Streptococcus mutans* in human dental decay. *Microbiol. Reviews, 50:* 353-380, 1986.

Loesche, W.J. and Syed, S.A. — The predominant cultivable flora of carious plaque and carious dentin. *Caries. Res., 7:* 201-216, 1973.

McComb, D. and Ericson, D. — Antimicrobial action of new, proprietary lining cements. *J. Dent. Res., 66:* 1025-1027, 1987.

Mjor, I.A. — Histologic demonstrations of bacteria subjacent to dental restorations. *Scand. J. Dent. Res., 85:* 169-174, 1977.

Oguntebi, B.R. — Dentine tubule infection and endodontic therapy implications. *Int. End. J., 27:* 218-222, 1994.

Orstavik, D. — Antibacterial properties of root canal sealers, cements and pastes. *Int. End. J., 14:* 125-133, 1981.

Schaal, K.P. — Genus *Actinomyces* — In: Sneath P.H.H., Nair N.S., Sharpe M.E. and Holt J.G. (ed.), Bergey's manual of systematic bacteriology. Vol. 2 Williams and Wilkins Baltimore, pp. 1383-1434, 1986.

Schwartz, D. — Comparaison de plusieurs moyennes. Analyse de la variance. Dans Flammarion et Cie (ed.), Méthodes statistiques à l'usage des médecins et des biologistes. 3e ed., pp. 173-186. Paris, 1980.

Shaw, J.H. — Causes and control of dental caries. *New England J. Medicine, 317:* 996-1004, 1987.

Schröder, U. — Effects of calcium hydroxyde containing pulp-capping agents on pulp-cell migration, proliferation and differentiation. *J. dent. Res., 64:* 541-548, 1985.

Stanley, H.R. — Pulpal responses to ionomer cements - biological characteristics. *J. Amer. Dent. Assoc., 120:* 25-29, 1990.

Tobias, R.S., Browne, R.M., Wilson, C.A. — Antibacterial activity of dental restoration materials. *Int. End. J., 18:* 161-171, 1985.

Torstenson, B., Nordenvall, K.J., Brännström, M. — Pulpal reaction and microorganisms under Clearfil composite resin in deep cavities with acid etched dentin. *Swedish Dental J., 6:* 167-176, 1982.

Van Houte, J. — Bacterial specificity in the etiology of dental caries. *Int. Dent. J., 30:* 305-326, 1980.

Van Houte, J. — Role of micro-organisms in caries etiology. *J. Dent. Res., 73:* 672-681, 1994.

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