Microleakage of Three Cement Bases

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The purpose of this study was to evaluate the ability of a glass-ionomer cement-base material to prevent bacterial penetration along the dentin interface and to compare it with two conventional cement-base materials. A total of 107 Class 5 restorations was placed in Rhesus monkey teeth by means of three test materials [zinc oxide-eugenol (ZOE), copalite varnish + zinc phosphate cement base (V+ZP), and a glass-ionomer lining cement (GI)], with unetched and unbonded resin composite used alone as a control material and as a final restoration over the test base materials. Following disinfection, Class 5 cavities were prepared on the buccal surfaces of the teeth to the inner one-half of dentin. A sterile filter-paper disk was then placed on the axial wall and covered with a Teflon disk. Next, the cavities were based to the dento-enamel junction with one of the test base materials and finally restored with unetched and unbonded resin composite. After five and 16 weeks, the filter-paper disks were retrieved and cultivated for the presence and type of bacteria. The five-week results showed positive growth in two groups: the composite-only controls and the V + ZP group. The 16-week results showed growth in all of the test groups, but only one of nine teeth showed growth in the zinc oxide-eugenol group and one of 16 teeth in the glass-ionomer group. The results of this study indicate that under the conditions tested, a glass-ionomer base was capable of minimizing bacterial penetration along the material-tooth interface.

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Introduction.

Restoration of teeth with deep caries often necessitates the placement of a cement base underneath the final restoration. Historically, the purpose of a cement base was to act as a protective thermal and/or physical barrier between the dentin and the restorative material. Shrinkage of restorative materials leaves gaps between the restoration and the cavity wall that provide an avenue for leakage of oral fluids and bacteria with their by-products along this interface *via* the dentinal tubules to the pulp. This leakage is commonly referred to as microleakage. Articles by Brännström and Nyborg (1973), Skogedal and Eriksen (1976), Bergenholtz *et al.* (1982), Browne *et al.* (1983), and Brännström (1984) indicate that pulpal inflammation in restored teeth is due to the penetration of bacteria and their by-products along the tooth/restoration interface rather than to the direct effects of the restorative material.

Through the use of acid etching and bonding to enamel, the degree of microleakage around resin composites has been reduced significantly (Quist and Quist, 1977; Brännström and Nordenvall, 1978). However, problems still persist in maintenance of a complete seal. This same success has not been obtained at the enamel interface with other restorative materials, such as amalgam or cast restorations. If leakage cannot be controlled at the enamel interface, preventing microleakage at the dentin interface could reduce its potential harmful effects and thus allow for greater freedom in the choice of a final restorative material. Since cement-base materials are frequently used in the restoration of cavities with extensive caries involvement, their ability to prevent microleakage along the cut dentin interface could be significant.

For a cement-base material to be an effective barrier to microleakage, the material should bond to dentin and preferably demonstrate antibacterial or bactericidal properties. The dentin-bonding properties of glass-ionomer materials (Hood *et al.*, 1981; Smith, 1989) would seem ideal for preventing bacterial microleakage along the dentin/restoration interface. However, little is known about their clinical performance and how they compare with other types of base materials beneath a final restorative material. Recent studies by Tobias *et al.* (1985) and McComb and Ericson (1987) have indicated that glass-ionomer cements do inhibit bacterial growth on blood agar plates, and that this inhibition may be due to the low pH of the cement before setting and/or to its high fluoride content. These dentin bonding and bactericidal/static properties of glass-ionomer bases would seem to make them ideal for minimizing the effects of leakage beneath overlying restorations. Thus, it was the purpose of this study to assess the amount of bacterial penetration that occurs clinically along the dentin-restoration interface for three cement-base materials—glass ionomer, zinc phosphate, and zinc oxide-eugenol—placed beneath a leaking restorative material.

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Materials and methods.

A total of 107 Class 5 restorations was placed in four adult Rhesus monkey teeth with use of three test cement base materials—Zinc Oxide-Eugenol (ZOE) (IRM, L.D. Caulk Co., Div. of Dentsply International, Inc., Lake View Ave., Milford, DE 19963), Copalite Varnish (Harry J. Bosworth Co., 7227 North Hamlin Ave., Skokie, IL 60076) plus Zinc Phosphate Cement Base [V+ZP] (Modern Tenacin, L.D. Caulk Co., Div. of Dentsply International, Inc., Lake View Ave., Milford, DE 19963), and GC Glass Ionomer Lining Cement [GI] (Fuji, Type I, G-C International, Inc., 8096 North 85th Way, Ste. 100, Scottsdale, AZ 85228)—and a control material, composite-only (Heliosit, Vivadent [USA] Inc., 182 Wales Ave., Tonawanda, NY 14150). There were two evaluation periods: five weeks and 16 weeks following preparation and restoration. The final restorative material used to cover the test materials was a light-cured microfilled resin composite placed without enamel etching or bonding. Both the test and control materials were randomly distributed, as listed in Table 1. The specific numbers of teeth for each test group differ due to variations resulting from the random placement.

A protocol similar to that used by Bergenholtz *et al.* (1982) was used for pre-treatment preparation of the animals and for materials' placement and retrieval procedures. Three weeks prior to cavity preparation and material placement, the dentition of each animal was scaled and polished, followed by toothbrushing with a 0.2% chlorhexidine-digluconate solution (Stuart Pharmaceuticals, Division of I.C.I., United States Inc., Wilmington, DE) three times/week. Immediately prior to cav-

TABLE 1
NUMBER OF TEST SAMPLES AND NUMBER WITH BACTERIAL GROWTH AT FIVE AND 16 WEEKS

Test Material	Number of Five-week Samples	Number with Growth	Number of 16-week Samples	Number with Growth
Composite only	11	6	10	6
ZOE	13	0	11	1
V + ZP	15	2	15	3
Glass Ionomer	16	0	16	1
Total	55	8	52	11

ity preparation and material placement, the teeth were polished, and groups of three or four teeth were isolated with rubber dams. The rubber dam was held in place with rubber-dam clamps and a metal frame, the individual teeth ligated with dental-tape ligatures, and the resultant isolated field of teeth and surrounding rubber-dam material disinfected with two consecutive 15-second swabs of 30% H_2O_2 followed by another two consecutive 15-second swabs of 5% tincture of iodine. Following rubber-dam isolation and disinfection of the operative site, Class 5 cavities located 1 mm occlusal to the free gingival margin and extending into the inner one-half of dentin were prepared on the buccal surfaces of the teeth with an ultraspeed air turbine with a sterile saline coolant and a sterile #34 carbide bur. The cavities were cleaned with sterile saline and dried with sterile cotton pellets. A sterile filter-disk 0.3 mm thick and 1.5 mm in diameter was placed on the pulpal floor of the preparation and covered with a sterile Teflon disk 0.1 mm thick and 1.5 mm in diameter. This Teflon disk was used to separate the filter-paper disk from the restorative material. The cavities were then based to the dento-enamel junction with one of the three test base materials. In the test cavities restored with varnish plus zinc phosphate (V + ZP), two layers of varnish were placed prior to the filter paper and the Teflon disk. The varnish on the prepared enamel cavity walls was removed with a #34 carbide bur in the ultraspeed handpiece. In the test cavities restored with the ZOE, an additional Teflon disk was placed between the ZOE and the resin composite to prevent the eugenol from interacting with the composite and preventing its set. The final restoration of these cavities was completed with unetched and unbonded resin composite. Twenty-one cavities were restored with microfilled resin composite, unetched and unbonded, to serve as controls. The teeth were not brushed after cavity preparation and restoration.

After periods of five and 16 weeks, the teeth were isolated, polished, and disinfected as described previously. The three experimental restorative materials were removed, and the filter paper was recovered and placed in a vial containing 5 mL of RTF transport medium (Syed and Loesche, 1972).

All bacteriological sampling and processing were completed in the following way. Prior to disinfection, immediately after disinfection, and after placement of the final restorative material, control bacteriological samplings of the operative field were taken. Control samples, to test for the presence of bacteria in the preparation at the time of restoration, were also taken for each cavity preparation just prior to material placement. All of the control samples were taken by sterile endodontic paper points to absorb sterile RTF transport medium that had been placed onto the surfaces of selected teeth or into the prepared cavities. Following sampling, these paper points, as well as samples of unused portions of each of the restorative materials, filter-paper disks, and Teflon disks left over from the surgical session, were incubated anaerobically for seven days in an enriched nutrient broth and evaluated for the presence or absence of bacterial growth.

The five- and 16-week filter-disk samples were removed from the pulpal floor of the preparations using sterile forceps and placed into 5 mL of RTF transport medium. Each sample was dispersed by sonication with an ultrasonic disrupter followed by Vortex mixing. The dispersed sample was plated automatically on enriched trypticase soy agar (ETSA) plates by means of a spiral plater. After seven days of anaerobic incubation, the plates were read, the number of colony-forming units (CFU) of bacteria was determined, and identifications of bacteria according to colony morphology and Gram stain were made.

Fisher's exact probabilities with a Bonferroni Correction factor were used to compare the numbers of positive growth samples at five and 16 weeks, both among various time periods for similar treatment materials and among treatment materials within the same time periods. ANOVA tests were used to compare the CFU numbers of those cases with positive growth. All tests were conducted at the 0.05 confidence level.

Results.

The results of the control bacteriological samples of the general operative field were as follows: (1) All samples taken prior to disinfection were positive; (2) all samples [a] taken immediately after disinfection and [b] of the restorative materials, filter-paper disks, and Teflon disks were negative; and (3) all samples taken after the five- and 16-week samplings were negative. All bacteriological samplings of the cavity preparation taken just prior to material placement were negative, except where noted, in the five- and 16-week results.

The results of the five- and 16-week bacteriological test samplings are summarized in Tables 1 and 2.

At the five-week test period, 55 test cavities were evaluated. Of the bacteriological control samples taken of the cavity prep-

TABLE 2
BACTERIA CULTIVATED AT FIVE AND 16 WEEKS

	Control Sample Prior to Disinfection	Five Weeks				16 Weeks			
		Composite only	V + ZP	Glass Ionomer	ZOE	Composite only	V + ZP	Glass Ionomer	ZOE
Bacteroides sp.	x	x							
F. nucleatum		x					x		
Fusobacterium sp.	x	x	x			x			
Capnocytophaga sp.			x				x		
Selenomonas sp.	x								
S. sanguis	x					x			
Gram+ rods	x	x	x			x			x
Gram− rods	x					x			
Gram− cocci							x		
Gram+ cocci								x	

x = Positive growth in at least one sample.

aration prior to placement of the restorative materials, four test cavities had positive growth, but none of these four cavities were positive for growth at the end of the five-week interval. Of the 55 test teeth, one tooth in the composite-only control group lost its composite restoration after placement during this five-week period and was eliminated from the study. Eight of the 55 teeth had cultivable bacteria after five weeks, of which six teeth were in the composite-only control group and two teeth were in the V + ZP group. No positive growth was observed in the glass-ionomer test group or in the ZOE test group in this time period.

In the 16-week test period, 52 test cavities were evaluated. Two teeth were eliminated from the study because they showed positive growth both at the pre-restorative control sampling of the cavity preparation and at the 16-week sampling. An additional two teeth were eliminated from the V + ZP group because one filter-paper disk was lost during sample retrieval and one other tooth had lost a restoration prior to the time of the final sampling. Eleven of 48 teeth evaluated during the 16-week test period had cultivable bacteria that consisted of six teeth in the composite-only control group, one tooth in the ZOE group, three teeth in the V + ZP group, and one tooth in the glass-ionomer group.

All samples showing positive growth in the five- and 16-week test periods were cultivated on agar plates to determine the specific bacterial types present. The types of bacteria present were similar to those which normally constitute the oral flora. The specific types of bacteria present are listed in Table 2.

Discussion.

The microfilled resin composite was chosen to provide a final restorative material that would leak. To ensure this, we did not etch or use a bonding agent to enhance the marginal seal. It has been well-documented that resin composites shrink when set (Craig, 1985). This results in a space between the restoration and the cavity wall, thus opening the way for microleakage of bacteria and their by-products. Indeed, a reproducible percentage of leaking restorations (60%), as indicated by the presence of cultivable bacteria, was achieved in the composite-only control group teeth at both the five- and 16-week test periods. However, if bacterial penetration along the tooth/restoration interface is a continuous process, then one would anticipate an increase in cultivable bacteria along the axial walls of the composite-only control teeth as post-operative time increases. The absence of this trend may be due to the properties of the resin composite used in this study. This resin composite absorbs moisture with exposure to water and, as post-operative time increases, may improve its marginal adaptation and limit bacterial penetration.

At 16 weeks, the V + ZP group lost some of its capacity to prevent bacterial penetration. These results were not unexpected, since zinc phosphate cement shrinks when set, does not bond to dentin, and has not been considered to have any bactericidal effect after it has set. The capacity of this cement to inhibit bacterial penetration seems limited. The use of a cavity varnish in this study did not reduce bacterial penetration along the tooth/material interface, because the varnish was applied prior to the filter-disk placement and did not influence the degree of bacterial penetration past the zinc phosphate cement base along the restoration-cavity preparation interface. The cavity varnish may be important in reducing penetration of bacteria along with their by-products into dentin tubules; however, assessment of this possibility was outside the scope of the present study.

The results of the ZOE test group showed no bacterial penetration at the five-week time period, and at 16 weeks, only one of nine teeth in this group was positive for bacterial growth. These results are consistent with the pharmacological and toxicological properties of ZOE. When placed in contact with dentin, ZOE seals and excludes substrate for micro-organisms (thus reducing their metabolism), decreases their tendency to spread, and prevents inward diffusion of irritant end-products (Hume, 1986). More importantly, this material is bacteriostatic and has been used as a control standard to evaluate antimicrobial properties and to prevent microleakage (Brännström and Nyborg, 1973; Cox *et al.*, 1987). Tobias *et al.* (1981, 1982) state that bacteria are very seldom observed at the material interface or on the cavity walls in preparations filled with ZOE-containing materials, even after time periods extending to six months.

The glass-ionomer group had none of the 16 teeth in the five-week test period and only one of 16 teeth in the 16-week test period with positive bacterial growth. These results are comparable with those in the ZOE test group. In a study evaluating various luting cements, Fitzgerald *et al.* (1988) found that crowns cemented with V + ZP showed significant increases in the number of cultivable bacteria as the post-operative time increased from ten to 56 days, while crowns cemented with a glass-ionomer luting cement had no difference in cultivable bacteria as the post-operative time increased from ten to 56 days. The results of that study parallel the findings in this study and support the fact that as the post-operative time increases, the capacity of zinc phosphate cement to inhibit bacterial penetration is minimal, and that the glass-ionomer material maintains its capacity to inhibit bacterial penetration. What this present study cannot determine is how this material limits such penetration.

Two possible mechanisms of bacterial exclusion exist. One is *via* physical exclusion due to a bond to dentin so intimate that no bacteria and/or fluids can pass through the interface. The other mechanism would be through antimicrobial activity of the material preventing penetration of viable bacteria. One or both may play a role in this material's ability to limit bacterial penetration. The literature is inconclusive in determining the relative importance of each. Findings of Graver *et al.* (1990) suggest that glass-ionomer materials, when used as luting agents, are quite capable of preventing dye penetration along the tooth/material interface, and that ZOE used in a similar fashion allows substantial dye penetration to occur. These findings would indicate that the bond between glass-ionomer materials and dentin is tight enough to exclude the penetration of either bacteria or fluids, and that the lack of cultivable bacteria under the ZOE bases in the present study was due to its bacteriostatic and bactericidal properties. However, Gordon *et al.* (1985) and Crim and Shay (1987) found that a glass-ionomer material was ineffective in preventing dye penetration at the restoration/tooth interface. These findings would indicate that either the gap between the glass-ionomer material and dentin was too small to allow the passage of bacteria but large enough to permit dye penetration, or the bacteriostatic/bactericidal properties of the material were sufficient to prevent penetration of viable bacteria. Findings in this area are seemingly in conflict and, thus, further study is needed to determine the relative contributions of the two possible exclusion mechanisms.

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