

An in vitro study of bacterial inhibition by VLC calcium hydroxide pulp cap compounds

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Abstract

The antimicrobial activity of visible light cure (VLC) calcium hydroxide pulp capping products was compared to self-cure products by an in vitro microbial assay. Individual microbial samples were collected from six deep carious lesions, sonicated, and plated onto duplicate T-soy blood agar. Uniform discs of each capping compound were pressed slightly below the surface of the agar and allowed to incubate at 37° C for 24 hr. The diameter of zones of inhibited bacterial growth were measured to the nearest 0.1 mm and tested for significant differences with ANOVA test. All products tested resulted in similar size zones of inhibition ($P < .10$).

Bacteria play a significant role in the overall status of the dental pulp, not only in causing disease, but also in stimulating the formation of reparative dentin. Although the precise mechanism by which reparative dentin is stimulated to form is not completely understood, it is known that as bacteria multiply and approximate the pulp chamber, odontoblasts produce new dentin effectively walling off the vital pulp tissues from the encroaching bacteria. This response is the underlying principle justifying the indirect pulp cap. Success with this procedure is enhanced when performed in young patients whose teeth have large pulp chambers and a good blood supply. When bacteria are allowed to proliferate to sufficient numbers, decay will advance faster than the formation of reparative dentin and eventually infect the pulp.

In spite of the operator's conscious effort to remove infected dentin during cavity preparation, bacteria are frequently, unintentionally left behind in the dentinal tubules overlying the pulp chamber. Bacteria have been cultured from dentin more than a year after having been sealed under restorations.¹ Fisher (1966) found bacterial activity to be minimal under restorations, so long as the filling "seals" the bacteria in the dentinal tubules and prevents nutrients from reaching them. Should excessive marginal leakage occur, the bacteria can proliferate and eventually infect the pulp.

¹ Besic 1943; Fisher 1966; Schouboe and MacDonald 1962.

Takehashi (1965) demonstrated bacterial contamination to be the major adverse factor influencing the healing of exposed pulps. Attempts by Patterson (1974), Schmidt et al. (1960), and Stark et al. (1976) to disinfect dentin prior to placing the final restoration, have included the use of antibiotics and caustic chemicals. Going (1964) showed copal varnish to effectively inhibit bacterial growth by reducing marginal leakage. Pulp investigators generally agree that decontaminating and sealing the axial walls and pulpal floor of a preparation is the most important factor promoting the health of the pulp. The indirect pulp cap relies mainly on the antimicrobial activity of the pulp capping agent to effectively "hold down" the number of bacteria. Limiting the growth of bacteria allows sufficient time for the pulp tissue to form reparative dentin and to wall itself off from the encroaching bacteria.

The antimicrobial activity of several pulp capping products has been studied in vivo and in vitro. Leung et al. (1980) found a reduction in the number of bacterial colony-forming units (CFU)/mg in 85% of their experimental teeth in which Dycal[†] was placed over remaining carious dentin for a period of four weeks. Sixty per cent of these experimental teeth were found to be "operationally sterile." Fairborn et al. (1980) found similar findings with IRM[†] and Improved Dycal. Lado et al. (1986) studied the in vitro bacterial inhibition of six self-cure pulp capping products on microorganisms cultured from infected dentin. All but one (Pulpdent[™]) were found to be significantly more effective than reagent calcium hydroxide ($P = .05$).

Visible light cure (VLC) pulp-capping products recently have come into use that are much harder than the self-cure materials. A concern has been that the vehicle of these new products may prevent or significantly reduce any antimicrobial effects associated with the self-cure products.

The issues addressed in this study are whether or not the VLC pulp cap products tested inhibit bacterial growth of microorganisms commonly found in dentin,

[†] LD Caulk Co; Milford, DE.

and if they do, how effective they are when compared to commercially available self-cure products.

Materials and Methods

Capping Material

The six products studied included: three unknown VLC calcium hydroxide compositions^a provided by the manufacturer which were labeled BAH-3-155-V, BAH-3-155-F, and BAH-3-155-S; two self-cure calcium hydroxide products, Life^b and Dycal; and IRM. Each product was prepared according to the manufacturer's instructions. Uniform discs (6.5 mm in diameter x 1.5 mm thick) of each were formed using a template and stored in separate, dark, air-tight containers at room temperature for not longer than a week before use.

Bacterial Samples

Individual microbial samples were aseptically collected from the base of deep carious lesions in each of six human teeth. The top material of the lesion (soft decay) was first removed and discarded. The remaining material (hard decay) was then excavated and immediately placed in 10 ml of prerduced anaerobically sterilized Ringer's solution which, while open, was continuously flushed with oxygen-free nitrogen gas to maintain anaerobiosis. Each sample was sonicated for 10 sec to evenly disperse the bacteria before plating onto the assay medium. The microorganisms present in these samples consisted predominantly of *Lactobacillus*, *Actinomyces*, and *Streptococcus* species.

Microbial Assay

An in vitro microbial assay was used to evaluate the growth inhibitory effects of the pulp-capping products. Trypticase-soy agar supplemented with 5% whole defibrinated sheep blood and adjusted to pH 7.9-7.2 was used as the assay medium. Twelve milliliters of molten medium at ~55°C was dispensed into sterile Petri plates (100 mm x 15 mm). Discs of the six products were then placed into the molten medium with sterile forceps so that the discs were just beneath the surface of the agar medium. After the medium was allowed to solidify on a level surface, 0.1 ml of sonicated microbial sample was poured and spread over it. Duplicate plates were used for each sample; thus, there were a total of 12 plates (two for each of the six microbial samples). The inoculated assay plates were incubated at 37°C for 24 hr in an anaerobic chamber containing an atmosphere of 10% H₂, 5% CO₂, and 85% N₂. After incubation, the circular zones of inhibition that had formed around each disc were measured with calipers to the nearest 0.1 mm.

ANOVA tests for significant differences in zone diameters were performed with the component of vari-

ance (random effects) model described by Ostle (1969).

Results

The means, standard deviations, and the ranges of zones of bacterial inhibition obtained from six duplicate determinations (N = 12) of the tested products are given in Table 1.

TABLE 1. Means, Standard Deviations, Medians and Ranges of the Diameters of Zones of Inhibition for the Six Capping Products

	Mean ± SD (mm)	Median (mm)	Range (mm)
Dycal	10.88 ± 1.65	11.15	8.90-12.90
IRM	9.72 1.65	9.55	7.90-12.30
Life	10.07 1.35	9.75	8.80-12.40
BAH-3-155-F	10.07 1.26	10.40	8.10-11.60
BAH-3-155-V	9.65 1.52	9.35	7.90-11.80
BAH-3-155-S	9.58 ± 1.48	9.45	7.80-11.90

A comparison of the antibacterial activities of the six products, based on the average diameter of the zones of inhibition, resulted in no significant differences at $P < .10$. The mean diameter of the zones for all products ranged from 9.58 to 10.88 mm.

Discussion

The three VLC calcium hydroxide products provided by the manufacturer were coded and their compositions were identified at the conclusion of the study. BAH-3-155-V was Prisma VLC™ pulp cap material presently commercially available. BAH-3-155-F was Prisma VLC with 0.75 mg calcium fluoride, and BAH-3-155-S was Prisma VLC with 0.5 mg Santicizer 8® (a sulfonamide plasticizer used in regular Dycal). Calcium fluoride and Santicizer 8 were added to the commercially available VLC product to evaluate any potential effect they may have had on bacterial growth. The results indicate that the two additives had little if any effect on bacterial growth.

Conclusions

Since this was an in vitro study of the antimicrobial effects of various pulp-capping products, conclusions cannot be drawn as to the effectiveness of these products in stimulating reparative dentin. However, it can be concluded that the VLC products are equally effective as standard self-curing, pulp-capping products in inhibiting the growth of organisms commonly found at the base of a cavity preparation. The ease of manipulation and strength of the VLC calcium hydroxide products is certainly an advantage over the self-cure products, thus justifying their acceptability for use in indirect capping procedures.

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^a Kerr-Sybron; Romulus, MI.

^b BBL Microbiology Systems; Cockeysville, MD.

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Tooth fairy rewards average nearly a dollar

In 1900 the tooth fairy deposited an average of 12¢ under the pillows of young sleepers. But, by the mid-1980s that amount averaged close to \$1, according to one researcher.

Each decade since the turn of the century has seen an increase in the amount left by the tooth fairy. During the Depression, if a child received a coin from the tooth fairy it was very special because money was so scarce at that time.

Although the average payout now is close to a dollar, most children still receive a quarter. If they do receive a dollar, it's more significant if it's a silver dollar because silver coins are the tooth fairy's favorite exchange for a child's tooth, according to the researcher.

Commemorating the loss of a primary tooth is a tradition that goes back before Christianity. One theory relates teeth to man's desire for immortality. Some civilizations found the presence of teeth after death despite the disintegration of the rest of the body and, as a result, the tooth became a symbol of immortality.

Each culture has its own way of commemorating the loss of the first tooth. One culture will have the tooth buried under a tree; one will give the tooth to the mother to keep; and yet in another culture if a primary tooth falls out the child is supposed to throw it away over his shoulder backward, or over a roof, or into a mouse hole and then ask a mouse, rat, squirrel, or fox to take the tooth and give the child a better one instead.

Though the evolution of the tooth fairy is unresolved, the tradition has become an established part of American culture and will probably be around for many years to come.