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CLINICAL ARTICLES

In Vitro Bacterial Penetration of Endodontically Treated Teeth Coronally Sealed with a Dentin Bonding Agent

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This study evaluated the effectiveness of a dentin bonding agent as a barrier to prevent coronal microleakage and examined the effect of a eugenol-based sealer on the sealing ability of this resin adhesive. Fifty-one extracted human mandibular molars were incorporated in a model system using an oral streptococci as a microbial marker. Group 1 consisted of 15 teeth that were obturated with only gutta-percha and received a coronal barrier of Clearfil Liner Bond 2V. Group 2 was identical to group 1, but included the use of a eugenol-based sealer in the obturation. Group 3 consisted of 15 teeth that were obturated with gutta-percha and sealer, but did not receive a coronal barrier. Six teeth served as controls. Bacterial penetration was monitored for 90 days. Results were analyzed after 30, 60, and 90 days with Fisher's exact test ($p < 0.05$). All controls behaved as expected. Neither group 1 nor group 2 exhibited any bacterial leakage. Eleven of the 15 specimens in group 3 leaked between 15 and 76 days. The coronal barriers in group 1 and group 2 were significantly better in preventing coronal microleakage at 60 days ($p = 0.002$) and 90 days ($p = 0.00005$). The presence of eugenol in the sealer had no significant effect on the sealing ability of Clearfil Liner Bond 2V ($p = 1$).

Clinicians strive to totally seal the root canal system in their attempt to ensure endodontic success (1). Despite these efforts, it has been shown that root canal fillings leak. Leonard et al. (2) stated, "presently there are no available materials or techniques that provide a complete seal of the canal system." When the coronal portion of the root canal is exposed to the oral environment, the obturated canal is a potential route for microorganisms to gain access to the periapical tissues. This situation may lead to endodontic failure. Missing or fractured restorations, restorations with inadequate margins, recurrent decay, or fractured tooth structure are all clinical conditions that can predispose a tooth to coronal microleakage (2).

Numerous studies have demonstrated the ability of microorganisms and saliva to penetrate an obturated canal and reach the apical region. Swanson and Madison (3), in a study using obturated canals exposed to artificial saliva followed by Pelikan ink, found leakage in as little as 3 days. Magura et al. (4) showed that root canals obturated with gutta-percha and Roth sealer, either by lateral or vertical condensation, demonstrated apical contamination within 30 days of coronal exposure to saliva. Trope et al. (5) demonstrated that endotoxin from *Actinobacillus actinomycetemcomitans* was able to pass through obturated root canals within 20 days.

From previous research, a number of conclusions concerning the importance of a coronal seal have been drawn. Saunders and Saunders (6) concluded that coronal microleakage may be the leading cause of nonsurgical endodontic failure and recommended expeditious restoration of the coronal access preparation. Ray and Trope (7) stated that the technical quality of the coronal restoration may be significantly more important than the technical quality of the endodontic treatment for apical periodontal health. Likewise, Klevant and Eggink (8) showed healing in teeth without a canal obturation material, but with a good coronal seal.

A variety of materials have been tested in an attempt to provide a coronal barrier to prevent microleakage. Barrier Dentinal Sealant, temporary endodontic restorative material, amalgam, glass ionomer, mineral trioxide aggregate, Cavit, intermediate restorative material, and Super-EBA have all been tested as intracoronal barriers (9–11).

Dentin bonding agents are widely used in restorative dentistry to improve the bond of materials to teeth and to prevent microleakage under restorations. In endodontics, these agents have been evaluated in root canal obturations, for perforation repairs, and as root-end barriers (2, 12, 13). The sealing ability of dentin bonding agents was demonstrated by the work of Leonard et al. (2). They found that 7 of 20 samples obturated with a dentin bonding agent

and resin displayed a total sealing of the coronal aspect, and they concluded that dentin bonding agents may have the potential to enhance the root canal seal by reducing microleakage. Additionally, the results of Vignaroli et al. (13), using fluid filtration, demonstrated the ability of specific dentin bonding agents to provide an excellent seal when used as root-end sealants. Despite their use in other areas of dentistry, the application of a dentin bonding agent as a coronal barrier is not a common practice. Current research has found conflicting results concerning the ability of dentin bonding agents to prevent coronal microleakage (14, 15), and questions exist concerning their use as endodontic coronal barriers. First, as a coronal barrier, can bacterial penetration be prevented? Next, what effect, if any, does the eugenol of a root canal sealer have on the sealing ability of dentin bonding agents (14, 16, 17)? The purpose of this study was to determine the effectiveness of a light-cured dentin bonding agent, Clearfil Liner Bond 2V (J. Morita USA, Inc., Irvine, CA), in the prevention of bacterial migration in a simulated coronal leakage model and to evaluate the effect of a eugenol-based sealer on the sealing ability of this resin adhesive.

MATERIALS AND METHODS

The Microorganisms

Human supragingival plaque was harvested and placed in a medium of Todd Hewitt Broth (30 g/L) (Difco, Detroit, MI) and lactalbumin hydrolysate (5 g/L) (Difco), with a pH of 7.4 and incubated at 37°C in the presence of CO_2 for 24 h. The culture underwent 1:10 serial dilutions. Each dilution was streaked on Mitis-Salivarius agar plates (Difco) and incubated in the same manner overnight. An isolated, gumdrop-like colony, characteristic of *Streptococcus mutans*, was selected from the agar plates and was used to inoculate a fresh medium. After incubation, this culture was plated to ensure colony uniformity. Gram stains revealed a Gram-positive cocci with visible chaining. Sequential additions of streptomycin sulfate (Sigma Chemical Co., St. Louis, MO) were made to the culture until a resistance level of 500 μg/ml was achieved. This streptomycin-resistant oral streptococci culture (7.9×10^8 colony-forming units/ml) was used to inoculate the upper chamber of the model system.

The Dentin Bonding Agent

This study evaluated Clearfil Liner Bond 2V as a coronal barrier to microleakage. This material is a light- and dual-curing bonding system and consists of a self-etching primer and a bonding agent. The primer and bonding agent contain the well-known adhesion monomer (10-methacryloyloxydecyl dihydrogen phosphate) and HEMA (2-hydroxyethyl methacrylate). The primer offers simultaneous treatment of both dentin and enamel.

The dentin bonding agent was applied according to the manufacturer's instructions (18) by combining equal mounts of primer liquid A and B into a well of a mixing dish immediately before application. The mixture was applied to the entire pulp chamber with a disposable brush, left in place for 30 s, and then dried with a mild air stream. One drop of Bond liquid A was dispensed into the well of a mixing dish and applied to the entire pulp chamber with a disposable brush. The Bond liquid A was light-cured for 30 s using a Demetron Optilux curing unit (Demetron Kerr, Danbury, CT) with an output of 800 mw/cm^2. A second layer of Bond liquid A was applied and cured in an identical manner to the first layer.

The Model

Fifty-one extracted human mandibular molars were selected for this study. All specimens were free of restorations or had only minimal occlusal caries. The apical half of the roots were removed with a diamond bur on a high-speed handpiece. The occlusal surfaces and an adjacent band of 4 mm of enamel were abraded with a model trimmer. Standard endodontic access preparations were made through the occlusal surface of each tooth, incorporating any occlusal caries, if present. The canals in the remaining coronal half of the roots were enlarged with a .12 ProFile GT rotary file (Dentsply, Tulsa Dental, Tulsa, OK). Adjustment of the remaining root lengths with a model trimmer produced 4-mm-long standardized canals, when measured from the pulpal floor to the apical extent of each sectioned root. The chamber and the canals were irrigated with 5.25% NaOCl (Clorox, Clorox Co., Oakland, CA) and stored in sterile saline until obturation. Twenty-one teeth were obturated with gutta-percha without sealer, using the Obtura II injectable system (Texceed Corp., Costa Mesa, CA). Vertical pressure was applied with standard endodontic pluggers. The remaining 30 teeth were obturated in a similar manner except with the addition of Kerr Root Canal Sealer EWT (Kerr USA, Romulus, MI). The sealer was applied to the canal walls with an endodontic explorer before injection and compaction of the gutta-percha. All pulp chambers were cleaned of excess gutta-percha and/or sealer with a chloroform-moistened cotton pellet, rinsed with sterile saline, and dried with an air/water syringe. The teeth were divided into three groups. Group 1 consisted of 15 teeth, obturated without sealer, which received a coronal barrier of light-cured Clearfil Liner Bond 2V. Group 2 consisted of 15 teeth obturated with gutta-percha and sealer and received a coronal barrier identical to group 1. Group 3 consisted of 15 teeth obturated with gutta-percha and sealer that did not receive a coronal barrier. The positive control group consisted of three teeth obturated without sealer and without a coronal barrier. The negative control group consisted of three teeth obturated without sealer, but with a barrier of two layers of nail polish (Maybelline, Inc., New York, NY) in the pulp chamber.

A 4-dram scintillation vial (Fisher Scientific, Pittsburgh, PA) with a rubber-lined plastic top was modified to create each model. A ½-inch drill bit on a drill press was used to create a hole in the center of each top. A 1000-μl polyethylene pipette (Fisherbrand Redi-Tip, Fisher Scientific, Pittsburgh, PA) was placed through the hole in the vial top. From the external surface, the junction of the pipette and the top was secured with cyanoacrylate (Goop, Eclectic Products, Inc., Pineville, LA) and sealed with nail polish. The tip of each pipette was abraded with sandpaper and modified with scissors until it fit securely into the occlusal access of a prepared molar. The assembly was labeled with a paint pen on the vial top. The tooth/pipette interface was secured with a thin layer of sticky wax, followed by a layer of cyanoacrylate. After drying for 24 h, the tooth/pipette assembly was immersed in a mixture of epoxy resin (Tabletop & Finish Resin, Dynatron/Bondo Corporation, Atlanta, GA) to a level approximately 15 mm above the occlusal surface. After another 24-h dry-time, the apical end of the roots were exposed with the use of a model trimmer. Inspection at $\times$20 magnification (Global Microscope, St. Louis, MO) ensured a sur-

TABLE 1. No. of teeth exhibiting leakage over time ($n = 15$)

Group	Days			Total
	1–30	31–60	61–90	
1	0	0	0	0
2	0	0	0	0
3	4	4*	3*	11*

* Statistically significant with Fisher's exact test at $p < 0.05$.

face of dentin and gutta-percha without residual epoxy resin. Each tooth assembly was paired with a vial, packaged individually, and sterilized by a 12-h cycle in an ethylene oxide gas sterilizer.

After sterilization, a sterile medium composed of Todd Hewitt (30 g/L), lactalbumin hydrosalate (5 g/L), and streptomycin sulfate (500 μg/ml) with a pH of 7.4 was aseptically placed into the vials to a level of approximately 2 mm above the root ends. The vials were placed in trays and incubated at 37°C for 24 h. Lack of turbidity ensured sterility of the models. A 0.5-ml sample of an overnight culture of the 500 μg/ml streptomycin-resistant oral streptococci was used to inoculate the pulp chamber of each tooth. The pipette openings were covered with parafilm (American Can Co., Greenwich, CT), and the models were incubated at 37°C. The lower chamber of each model was monitored daily for signs of turbidity, which would indicate that microleakage had occurred from the coronal area to the apical area. Based on a pilot study, replacement of the culture in the upper chamber was accomplished aseptically with the use of disposable Pasteur pipettes (Fisher Scientific) every 6 days to ensure viability. The broth in the lower chambers was replaced aseptically after 45 days. Results for each model were recorded as either positive or negative for leakage, with no attempt to quantify the leakage. Cultures of the lower chambers of the models that demonstrated leakage were examined by Gram stains to confirm Gram-positive cocci. In addition, Gram crystal violet (Difco) was added to the upper chamber of these models to demonstrate the path of leakage. For the models that did not exhibit leakage, the Gram crystal violet was added at 90 days. At the conclusion of the study, all samples were sectioned with the use of a model trimmer to enable evaluation of the pulp chamber. Results were analyzed using Fisher's exact test after 30, 60, and 90 days. A value of $p < 0.05$ was considered statistically significant.

RESULTS

All of the positive controls leaked within 9 days. Throughout the duration of the experiment, no leakage was recorded for the negative control group, group 1, or group 2. In group 3, 11 of 15 samples without the coronal barrier displayed turbidity within a range of 15 to 76 days. Table 1 shows the leakage of each group in 30-day intervals. The analysis of the results at the 30-day interval was not statistically significant ($p = 0.1$). However, Fisher's exact test showed statistical significance for the comparison of both group 1 and group 2 to group 3 at 60 days ($p = 0.002$) and 90 days ($p = 0.00005$). There was no difference between group 1 and group 2 with regard to the effect of eugenol on the sealing ability of the Clearfil Liner Bond 2V ($p = 1$).

DISCUSSION

Methods to measure coronal microleakage have included the use of dyes, radioisotopes, fluid filtration, and microorganisms. For each of these methods, the inadequacies have been highlighted and clinical significance questioned (1, 4, 14). The model used in this study was patterned after an in vitro model system designed by Mortensen et al. (19) to study the resistance of restorations to bacterial leakage. This model is more closely related to the clinical situation, and has been modified and used by several other researchers to study coronal microleakage (5, 11). Our study included additional modifications. Streptomycin-resistant bacteria and a medium containing streptomycin sulfate were used to help eliminate false-positive results. The apical half of the roots were removed in an attempt to standardize canal length that would hasten the appearance of turbidity, if leakage did occur. A pilot study for the model design encountered leakage problems at the tooth/pipette interface. Layering sticky wax, cyanoacrylate, and epoxy resin in conjunction with abraded enamel proved successful in eliminating model leakage at this junction.

Gram stains of the lower chambers with turbidity confirmed the presence of Gram-positive cocci that matched that of the initial inoculum. Application of the Gram crystal violet to the upper chambers of these teeth demonstrated that the paths of leakage occurred through the canal system, with no failures of the model system. Sectioning group 1 and group 2 revealed the average thickness of Clearfil Liner Bond 2V was 3.18 mm. The Gram violet stain did not penetrate past the dentin bonding agent in any case. The average length of gutta-percha for all groups was 3.63 mm.

The clinical significance of in vitro leakage studies is questionable (1), and the amount of leakage that is clinically significant is not known. Nevertheless, the development and maintenance of a sealed root system are considered to be important for successful endodontic treatment. Hence, evaluation of the quality of the seal using leakage tests is still a relevant concept, even though a universally accepted model is nonexistent (2).

Research has shown that coronal microleakage is an important factor in endodontic failure (6–8). Gutta-percha and sealer provide minimal resistance to bacterial microleakage (3–5). A variety of materials have been tested as coronal barriers (9–11), but all have demonstrated leakage. A dentin bonding agent has the potential for providing a long-term seal.

The results of this study agree with those of Ferraz et al. (15), who found dentin bonding agents capable of preventing microleakage. However, this study contradicts the results of Richie-Gillespie et al. (14), who found a dentin bonding agent, All Bond 2, to be inferior to gutta-percha and sealer, alone or in conjunction with Barrier Dentinal Sealant and Tublitec. They concluded that the effect of eugenol from the sealer and/or shrinkage contraction of the bonding agent actually accelerated microleakage. On the contrary, our study showed that the use of a eugenol-containing sealer had no effect on the sealing ability of Clearfil Liner Bond 2V. If, in fact, eugenol does diminish the adhesiveness of a dentin bonding agent, our results suggest cleaning the pulp chamber with chloro-

form-moistened cotton pellets is sufficient in neutralizing this effect.

Dentin bonding agents like Clearfil Liner Bond 2V possess many characteristics that may be desirable for a coronal barrier. First, the material has demonstrated excellent sealing capabilities when placed on exposed dentin (20). Next, placement of the material is quick, easy, inexpensive, and requires minimal armamentarium. Finally, the dentin bonding agents are translucent, which allows for visualization of the gutta-percha. This property may decrease the risk of perforating the chamber floor in cases of post space preparations or retreatment, if necessary. In cases with sufficient coronal tooth structure, amalgam, or resin build-ups could be bonded directly to the coronal barrier.

For over a decade, research (2–12, 14, 15) has been presented that emphasizes the important role coronal microleakage plays in endodontics. However, a standard regimen incorporating some form of coronal barrier after obturation is nonexistent. The placement of a barrier to prevent coronal microleakage may be one simple, additional step that may improve the success rate of endodontic treatment.

This study found that Clearfil Liner Bond 2V as a coronal barrier provided an adequate seal against the migration of oral streptococci in an in vitro model, and that the use of a eugenol-containing sealer had no effect on the sealing ability of this dentin bonding agent.

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