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Bonding of Self-etch and Total-etch Adhesives to Carious Dentin

INTRODUCTION

In contemporary minimally invasive restorative dentistry, the primary aim in the excavation of carious dentin is to remove only the outer layer of highly infected, denatured caries-infected dentin (Massler, 1967). This facilitates the preservation of the inner layer of intact, bacteria-free remineralizable caries-affected dentin (Wei et al., 1968) and prevents disease progression. Conversely, recent studies showed that the application of adhesive-sealed composite restorations to irreversibly infected dentin did not affect the clinical performance of these restorations (Briley et al., 1997; Ribeiro et al., 1999).

With the advent of contemporary hydrophilic self-etch and total-etch adhesives, it may be possible to bond to and seal vital caries-affected and caries-infected dentin and isolate residual bacteria from any fermentable carbohydrates that are present in the oral fluids or nutrients that are derived from the pulp. This may permit dentinogenesis to isolate residual bacteria even further, causing them to become dormant (Bjørndal and Darvann, 1999). The clinical consequence of leaving residual bacteria underneath bonded restorations is still a subject of considerable debate. Newly developed techniques involving polymerase chain-reaction amplification of bacterial surface protein antigens showed that conventional culture techniques could underestimate the quantity of viable bacteria beneath restorations (Allaker et al., 1998). Remaining viable bacteria may release antigens into the pulp and induce cytokine reactions, evolving to chronic pulpal inflammation (Hahn et al., 2000).

The diagnosis and removal of active caries are therefore crucial (Weerheijm and Groen, 1999), since the inherent subjectivity in detection of the excavation boundary can result in clinically significant differences in the quality and quantity of dentin removed by different operators (Banerjee et al., 2000). Thus, it is possible that clinicians are bonding to a substrate that is composed of sound, caries-affected, and caries-infected dentin in different parts of the same cavity.

The objectives of this study were to examine the microtensile bond strength and interfacial ultrastructure on bonding of a self-etch adhesive and a total-etch adhesive to carious dentin. The hypotheses tested were that: (1) dentin adhesives bond equally well to sound, caries-affected, and caries-infected dentin; and (2) there is no difference between a self-etch and a total-etch adhesive in bonding to these respective dentin substrates.

MATERIALS & METHODS

Selection of Bonding Substrates

Sixteen extracted human molars with coronal dentin caries were used in this study. The teeth were collected after the patients’ informed consent was obtained.
under a protocol reviewed and approved by the institutional review board of the Medical College of Georgia. They were stored in 0.9% NaCl containing 0.05% sodium azide at 4°C, and used within one month following extraction. The occlusal enamel and superficial dentin were removed by means of a slow-speed saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water lubrication, exposing a flat surface of middle to deep dentin where the caries lesion was surrounded by normal dentin (Appendix Fig. A; www.dentalresearch.org). The entire flat surface was flooded with Caries Detector solution to stain the lesion (Kuraray Medical Inc., Tokyo, Japan). Further reduction was performed with 600-grit SiC paper under running water according to the combined criteria of: hardness to a sharp excavator, visual examination, and staining with Caries Detector solution. The relatively soft, dark-stained dentin was classified as caries-infected dentin, while the discolor, harder dentin that stained pink was classified as caries-affected dentin. The surrounding, yellow, hard dentin was classified as normal dentin.

Experimental Design
An experimental self-etch adhesive with antibacterial (Imazato et al., 1998) properties (ABF system, Kuraray) and a commercially available total-etch, moist-bonding adhesive (Single Bond, 3M-ESPE) were used in this study (Appendix Table; www.dentalresearch.org). The experimental self-etching primer system contains an antibacterial monomer, 12-methacryloyloxydodecylpyridinium bromide (MDPB), that is bactericidal before polymerization and bacteriostatic after polymerization (Imazato et al., 1998) and has been suggested to be useful for eliminating residual bacteria in carious dentin.

Eight teeth (4 with caries-affected dentin and 4 with caries-infected dentin) were treated with the ABF primer for 20 sec under agitation and gently air-dried. The ABF adhesive was then applied, gently air-thinned, and light-cured for 20 sec. Another 8 teeth (4 with caries-affected dentin and 4 with caries-infected dentin) were etched with 35% phosphoric acid gel for 15 sec and rinsed for 15 sec, leaving a visibly moist surface. Two consecutive coats of Single Bond adhesive were applied and light-cured for 10 sec. Composite build-up was performed with Clearfil AP-X (Kuraray) in 3 1.5-mm-thick adhesive were applied and light-cured for 10 sec. Composite build-up was performed with Clearfil AP-X (Kuraray) in 3 1.5-mm-thick slabs of resin-bonded dentin from each group. The remaining 3 to 5 slabs of resin-bonded dentin from each group were cut into 1 x 0.8-mm sticks and prepared according to the transmission electron microscopy protocol described by Tay et al. (1999). Undemineralized, 90-nm-thick ultrathin sections of the epoxy-resin-embedded bonded specimens containing the bonded dentin substrate of interest were examined either unstained, or double-stained with uranyl acetate and Reynold’s lead citrate, with the use of a transmission electron microscope (Philips EM208S, Eindhoven, The Netherlands) operating at 80 kV.

RESULTS
Microtensile bond strength results are shown in the Table. Analysis of variance indicated that there were significant differences (p < 0.001) among dentin types, but not between adhesives (p = 0.35). There was no significant interaction between the two factors (p = 0.29). Bond strengths to sound dentin were significantly higher than those to caries-affected dentin, which, in turn, were significantly higher than those to caries-infected dentin (p < 0.05).

Transmission electron microscopy of resin-dentin interfaces in sound dentin showed that 0.5- to 1-μm-thick hybrid layers were produced by the self-etch ABF system (Fig. 1A) and 5-μm-thick hybrid layers were created when the total-etch Single Bond adhesive was used (Fig. 1B). For both adhesives, hybrid layers in caries-affected dentin were much thicker than those observed in sound dentin, and their dentinal tubules were often obliterated with heavy mineral deposits.
to 8 μm thick for the self-etch system (Fig. 2A), and between 15 and 19 μm thick for the total-etch system (Fig. 2B), porous zones of carious-affected dentin could be seen either beneath the hybrid layer in the self-etch adhesive (Fig. 2A) or along the base of the hybrid layer in the total-etch adhesive (Fig. 2C).

When thin layers of caries-infected dentin were encountered, the self-etch adhesive could form hybrid layers that incorporated the superficial caries-infected dentin and part of the underlying caries-affected dentin (Fig. 3A). However, when thick layers of caries-infected dentin were present, the self-etch adhesive was unable to etch and infiltrate beyond this infected, grossly denatured layer. The dentinal tubules remained incompletely sealed with resin (Fig. 3B). In areas that were about 100 μm beneath the bonded interface, we observed isolated regions containing large bacteria-infected zones with complete destruction of both intertubular and peritubular dentin (Frank et al., 1989). The bacteria in these zones exhibited intact cell walls and contained electron-lucent, glycogen-like intracellular polysaccharide granules (Hamilton, 1976) in their cytoplasm (Fig. 3C).

For the total-etch adhesive, loose dentin chips were often trapped within the adhesive. Numerous bacteria could be seen in the tubules (Fig. 3D). Bacteria along the surface of the caries-infected dentin were often trapped within the polyalkenoic acid copolymer component of the adhesive. Most of them exhibited intact cell walls and contained carbohydrate granules in their cytoplasm. Some of these bacteria were in the process of cell division before laboratory fixation (Fig. 3C).

**DISCUSSION**

Since both adhesives exhibited higher tensile bond strengths to sound dentin than to caries-affected or caries-infected dentin, we must reject the first null hypothesis. However, there was no difference between the self-etch and the total-etch adhesive in bonding to these respective dentin substrates. Thus, we must accept the second null hypothesis.

There are several potential problems that may affect bonding and sealing efficacy when hydrophilic self-etch and
total-etch adhesives are used on caries-affected and caries-infected dentin. Caries-affected dentin is softer than normal dentin (Fusayama et al., 1966; Ogawa et al., 1983; Marshall et al., 2001a) because it is partially demineralized. Carious intertubular dentin exhibits a higher degree of porosity than sound intertubular dentin, due to the loss of mineral. Our ultrastructural results agreed with previous studies that hybrid layers in caries-affected dentin were thicker than those in sound dentin (Nakajima et al., 1995), suggesting easier diffusion of acidic conditioners and adhesive monomers, due to increased porosity in the intertubular dentin. Conversely, resin infiltration into dentinal tubules was severely hampered by the presence of porosity in the intertubular dentin. Acid-resistant intratubular mineral deposits; and decreases in the modulus of elasticity (Marshall et al., 2001a,b) and the cohesive strength of such dentin (Appendix Fig. B, www.dentalresearch.org). We speculate that the unmeasurable Knoop hardness of caries-affected dentin is due to the near-complete loss of the mineral phase of dentin and to denaturation of its collagen matrix. The low Knoop hardness values in caries-infected dentin may reflect a smaller number of larger apatitic crystals that no longer fit properly into inter- and intrafibrillar spaces in a normal collagen matrix. To the extent that there is any chemical bonding between carboxylic or phosphate derivatives of methacrylates with the mineral phase, then fewer, larger crystals would offer less surface area for interaction. Hydrogen bonding between resins and collagen may contribute to bond strength in normal dentin and perhaps to caries-affected dentin if it has normal collagen, but it could not occur with the denatured matrix of caries-infected dentin.

The intrinsic weakness of caries-affected and caries-infected dentin may not be a clinical problem if there is normal dentin and/or enamel surrounding the excavated lesion that can provide high bond strengths with resin adhesives. This was probably responsible for the excellent 10-year results of clinical trials of resin-bonded caries-affected dentin (Marshall et al., 1995). This can lower resin retention, particularly when the relatively mild-acting self-etching primers are used. In parallel experiments, we measured the Knoop hardness and ultimate tensile strength of normal and caries-affected dentin. Caries-affected dentin was softer and weaker than normal dentin (Appendix Fig. A; www.dentalresearch.org). Many specimens of resin-bonded caries-affected dentin failed cohesively in dentin, presumably because it was weaker than the bonding resin. This did not occur in normal dentin, where the bonds failed adhesively. Thus, the lower tensile bond strength of the two tested adhesives to caries-affected and infected dentin compared with normal dentin is probably due to several factors: the lack of resin tag formation due to the presence of acid-resistant intratubular mineral deposits; and decreases in the modulus of elasticity (Marshall et al., 2001a,b) and the cohesive strength of such dentin (Appendix Fig. B, www.dentalresearch.org). We speculate that the unmeasurable Knoop hardness of caries-affected dentin is due to the near-complete loss of the mineral phase of dentin and to denaturation of its collagen matrix. The low Knoop hardness values in caries-infected dentin may reflect a smaller number of larger apatitic crystals that no longer fit properly into inter- and intrafibrillar spaces in a normal collagen matrix. To the extent that there is any chemical bonding between carboxylic or phosphate derivatives of methacrylates with the mineral phase, then fewer, larger crystals would offer less surface area for interaction. Hydrogen bonding between resins and collagen may contribute to bond strength in normal dentin and perhaps to caries-affected dentin if it has normal collagen, but it could not occur with the denatured matrix of caries-infected dentin.

In conclusion, we do not advocate that these adhesives be bonded to clinically detectable soft, wet, carious dentin. However, the boundary between caries-affected and caries-infected dentin is often not clear. Our results suggest that the resins can infiltrate into porous caries-affected dentin matrices and into thin zones of caries-infected dentin. Much more research is needed to determine the effectiveness of phosphoric acid gel (Jensen and Handelman, 1980) compared with antibacterial self-etching adhesive monomers in killing bacteria in dentin, the permeability of polymerized resins to water and fermentable sugars, whether monomers penetrate the cytoplasm of bacteria, and whether bacteria can degrade the resin. Until more information is available on these questions, clinicians are advised to remove as much caries-infected dentin as possible. Any thin region of residual caries-infected dentin may be sequestered by adhesive resins. The long-term benefits/risks of this remain to be determined.

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REFERENCES


