Detection of acid diffusion through bovine dentine after adhesive application

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Abstract

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Aim Acidic diffusion through bovine dentine was investigated by measuring pH changes on dentine surfaces after applying three adhesive systems.

Methodology Coronal incisor bovine dentine discs, 0.5 mm thick, were prepared from dentine close to the pulp chamber. A single-bottle adhesive system-Single Bond, a self-etching primer system-Clearfil SE Bond and an 'all-in-one' adhesive system-AQ Bond were used. The labial dentine surfaces were conditioned as follows: Single Bond groups: (SB-1) 35% phosphoric acid etchant was applied and left in place; (SB-2) the etchant was applied for 15 s and rinsed off for 10 s; (SB-3) application of adhesive agent and light curing following step SB-2; Clearfil SE Bond groups: (SE-1) SE primer was applied for 20 s and dried; (SE-2) application of adhesive agent and light curing following step SE-1; AQ Bond groups: (AQ-1) AQ Bond adhesive was applied for 20 s and dried, applied for additional 5 s and dried again; (AQ-2) light curing following step AQ-1. The pH change on the pulpal dentine surface was measured using a pH-imaging microscope.

Results All the Single Bond groups revealed a lower pH on the pulpal surface (pH 6.25, 6.59 and 6.64 for SB-1, SB-2 and SB-3, respectively) compared with intact dentine. Clearfil SE Bond and AQ Bond groups showed no significant deference in pH value from intact dentine.

Conclusions Acid diffusion from phosphoric acid etching was observed when placed on 0.5 mm-thick dentine discs; however, there was only limited evidence of acid diffusion from SE primer and AQ Bond.

Keywords: 'all-in-one' adhesive system, bovine dentine, pH, remaining dentine thickness, self-etching primer system, single-bottle adhesive system.

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Introduction

Dentine bonding systems require the application of acid conditioners to remove the smear layer from the dentine surface and to promote demineralization of the underlying dentine substrate. These products can alter the physical and chemical properties of dentine, facilitating their penetration through the dentinal tubules (Pashley 1992, Hebling *et al.* 1999).

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Etching dentine was believed to increase pulpal irritation through the removal of debris blocking the tubule entrances and facilitating the penetration of acidic irritants into the tubules (Stanley *et al.* 1975). Some researchers have routinely reported that subsequent pulpal inflammation and necrosis was due to acid toxicity (Langeland *et al.* 1970, Stanley *et al.* 1975, Stanley & Parmeijer 1997). In an early study on dental cements, it was suggested that a pH of approximately 2.0 for 5 min would induce a damaging pulpal response (Plant & Tyas 1970).

If acids are thought to irritate the pulp, it is important to demonstrate that they permeate through the dentine to reach the pulpal tissues. A few simple *in vitro* studies have indicated that hydrogen ions did not seem to penetrate through dentine. Lee *et al.* (1973)

placed thin (about 1 mm thick) dentine discs on pH indicator paper and then covered the surface of the dentine with acids of the type used to etch enamel. Little change in the pH of underlying indicator paper was noted. Chan & Jensen (1986) measured how much the pH of phosphoric acids fell across thin dentine discs (about 0.4 mm) using pH electrodes. Their result indicated that brief exposures (1 min) to 37% phosphoric acids led to little penetration of hydrogen ions across dentine of 0.4–0.5 mm thickness. Research on the diffusion of hydrogen and hydroxyl ions through dentine using an electrode, demonstrated that hydrogen ions penetrated more slowly than hydroxyl ions due to the buffering of hydrogen ions by hydroxyapatite and other components of dentine (Wang & Hume 1988).

In the previous study, a pH-imaging microscope (SCHEM-100; Horiba Ltd, Kyoto, Japan) was used to investigate the relationship of remaining dentine thickness (RDT) and acidic diffusion from conventional luting cements by measuring pH value changes beneath cemented dentine (Hiraishi *et al.* 2003a). It was demonstrated that acidic diffusion from glass–ionomer and zinc phosphate cement occurred when placed on 0.25 mm-thick bovine dentine but there was no evidence of acidic diffusion through 0.50 mm-thick dentine.

A pH value analysing technique, using the SCHEM-100, has recently been introduced into dentistry (Hiraishi *et al.* 2003b,c). This method can evaluate various dentine or chemical characteristics of dentine surfaces. Specimen preparation for the SCHEM-100 does not require the samples to be destroyed by dissolution. The pH analysis can be performed by a simplified process of placing flat solid samples on a semiconductor silicon sensor with photocurrent characteristics (Nomura *et al.* 1997). This method is more sensitive and accurate than pH indicator papers or pH electrodes (Nomura *et al.* 1997).

In the present study using the SCHEM-100, the pH value analysing technique was employed to examine the presence of acidity on the pulpal surface under conditioned labial bovine dentine after applying different adhesive systems.

Materials and methods

Sample preparation

Bovine mandibular incisors were extracted from cattle between 20 and 24 months of age and stored frozen for no longer than 1 month. Teeth with evidence of fractures or other defects were rejected and a total of 120 extracted intact teeth retained for the study. Roots were sectioned at the cement–enamel junction with a high-speed diamond bur and the crown segments sliced using a diamond saw (Leitz Instruments, Heidelberg, Germany) under water coolant (Hiraishi *et al.* 2003a). The first section was through the labial dentine and the second made parallel to the first section through the pulp chamber, keeping the pulpal wall intact (Fig. 1). The pulpal surfaces of the sliced discs were ground with 600-grit silicon carbide paper under running water until the pulpal walls were removed. The labial dentine surfaces were ground in the same manner until discs approximately 0.55 mm-thick were obtained. The dentine discs were treated with 0.5 mol L^{-1} EDTA (pH 7.4) for 2 min to remove the smear layers on the both surfaces, which promoted permeability through the dentinal tubules. Following this, the labial surfaces were ground again with 600-grit silicon carbide paper under running water to reduce the thickness to 0.50 mm and create smear layers only on the labial dentine surfaces as in the clinical situation. Thus, dentine discs were produced with a smear layer on the labial surface and no smear layer on the pulpal surface. The discs were washed for 30 s in distilled water to remove chemical contaminants from the 0.5 mol L^{-1} EDTA. The tested area of the labial surface was demarcated by attaching a piece of vinyl masking tape in which a 3 mm diameter hole was made.

Three dentine bonding systems were used: a single-bottle adhesive system, Single Bond (3M-ESPE, St Paul, MN, USA); a self-etching primer adhesive system, Clearfil SE Bond (Kuraray Medical Inc., Tokyo, Japan); an ‘all-in-one’ adhesive system (a single-step adhesive system), AQ bond (Sun Medical Co., Kyoto, Japan) (Table 1). The discs were randomly divided into seven groups of 15 discs each and prepared as follows (Table 2):

Single Bond groups: (i) 35% phosphoric acid etchant was placed on the demarcated area of the labial surface and left in place for the duration of the pH-measurement (SB-1); (ii) etchant was applied for 15 s to the tested area, rinsed with water for 10 s, then carefully blot-dried but remaining visibly moist (SB-2); (iii) an adhesive agent was applied according to the manufacturer’s instructions and light-cured for 10 s following step 2 (SB-3).

Clearfil SE Bond groups: (i) SE primer was applied to the demarcated area of the labial surface (SE-1); (ii) SE primer was applied for 20 s on the demarcated area of

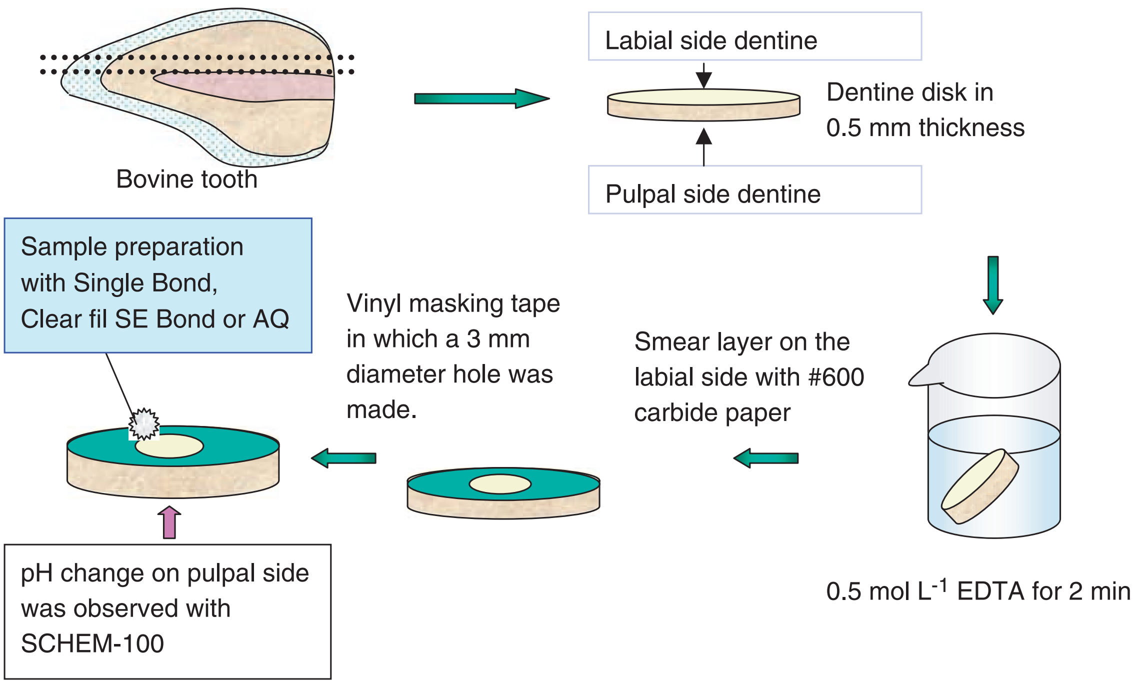

Figure 1 Bovine dentine sample preparation.

Table 1 Information on the materials used

Products (manufacturer)	Material (Batch no.)	Ingredients	Manufacturers' instructions
Single Bond (3M-ESPE, St Paul, MN, USA)	Etchant (7423) Adhesive (3411)	35% phosphoric acid Polyalkenoic acid-copolymer, HEMA, Bis-GMA, ethanol, water, photo initiator	Apply 15 s and rinse 10 s Light-cure 10 s
Clearfil SE Bond (Kuraray Medical Inc., Tokyo, Japan)	Primer (00047A) Adhesive (00111A)	MDP, HEMA, water, initiator MDP, HEMA, dimethacrylates, initiator, microfiller	Apply 20 s and dry Light-cure 10 s
AQ Bond (Sun Medical Co., Kyoto, Japan)	Base (7423) AQ Sponge (contains P-toluenesulfinate-salt)	4-META urethandimeth-acylate (Meth)acrylates, photo initiators, water and acetone stabilizers	First coat: apply 20 s and dry Second coat: apply 3–5 s and dry light-cure 10 s

HEMA, 2-hydroxy-ethylmethacrylate; Bis-GMA, bisphenyl-glycidyl-methacrylate; MDP, 10-methacrloyloxydecyl dihydrogen phosphate; 4-META, 4-methacryloxyethyl trimellitate anhydride.

the labial surface, dried with a mild stream of oil-free air, followed by the application of the adhesive agent and light-curing for 10 s according to the manufacturer's instructions (SE-2).

AQ Bond groups: (i) 'all-in-one' adhesive was applied to the demarcated area of the labial surface for 20 s and dried with a mild stream of oil-free air (first coat), and then applied for an additional 5 s and air-dried (second coat) (AQ-1), (ii) light-curing for 10 s following step 1 according to the manufacturer's instructions (AQ-2).

pH measurement of adhesive systems and pulpal dentine surfaces after adhesive system application

A glass electrode pH meter (TWIN pH; Horiba Ltd) was used to measure the pH of 35% phosphoric acid etchant, SE primer and a mixture of AQ Bond base and the AQ sponge. pH analysis using the SCHEM-100 was performed by placing flat solid samples on a semiconductor silicon sensor with photocurrent characteristics (Nomura *et al.* 1997, Hiraishi *et al.* 2003b). The pH-imaging sensor is based on a light addressable

Table 2 Dentine conditioning and bonding procedure

Adhesive systems	Application steps[a]
Single Bond	
SB-1	a
SB-2	a (15 s), b (10 s), c1
SB-3	a (15 s), b (10 s), c1, e, f
Clearfil SE Bond	
SE-1	d
SE-2	d (20 s), c2, e, f
AQ Bond	
AQ-1	e (20 s), c2, e (5 s), c2
AQ-2	e (20 s), c2, e (5 s), c2, f

[a]Procedure: (a) etch dentine; (b) rinse etchant; (c1) blot dry; (c2) dry with mild air; (d) apply primer; (e) apply adhesive agent; (f) light-cure for 10 s.

potentiometric sensor (LAPS) made of Si_3N_4/SiO_2 and Silicon (Si) (Hafeman *et al.* 1988). The pH measurement was conducted at multiple points, and the pH distribution displayed as pH images. The spatial resolution and the pH resolution of the sensor were 300 μm and 0.1 pH units, respectively.

Uncured or cured adhesive agents of Single Bond and Clearfil SE Bond, and cured adhesive agent of AQ Bond were placed on the sensor of the SCHEM-100 and their pH measured. Immediately after sample preparation each dentine specimen was placed on the sensor, pulpal surface down, on the sensor of the SCHEM-100. The lowest pH value on the pulpal dentine surface under the tested areas was recorded 3 min after preparation.

Statistical analysis

The means and standard deviations were calculated for the pH values of the materials and the tested pulpal surfaces. For statistical analysis, one-way ANOVA and Fisher's PLSD test were performed to determine if there was a statistically significant difference ($P < 0.05$) among the pH values and groups.

Results

The pH values of the three dentine bonding systems are shown in Table 3. For Single Bond, the pH of 35% phosphoric acid etchant, uncured and cured adhesive agent were less than 0.2, 4.60 and 4.70, respectively. For Clearfil SE Bond, the pH of SE primer, uncured and cured adhesive agents were 2.0, 4.67 and 4.97, respectively. For AQ Bond, the pH of uncured and cured AQ Bond adhesives with the AQ sponge were 2.5 and 4.02.

Table 3 pH values of materials, mean (SD)

Single Bond	
Etchant	<0.2[a]
Adhesive	
Uncured	4.60[b] (0.03)
Cured	4.70[b] (0.03)
Clearfil SE Bond	
Primer	2.00[a]
Adhesive	
Uncured	4.67[b] (0.02)
Cured	4.97[b] (0.02)
AQ Bond	
Base with AQ Sponge	
Uncured	2.50[a]
Cured	4.02[b] (0.03)

[a]pH value was measured with a glass electrode pH meter.
[b]pH value was measured with a pH-imaging microscope ($n = 5$).

Table 4 pH value on the pulpal side 3 min after adhesive systems application, mean (SD)

Single Bond	
SB-1	6.25[b] (0.24)
SB-2	6.57[c] (0.17)
SB-3	6.64[c] (0.06)
Clearfil SE Bond	
SE-1	6.82[a] (0.10)
SE-2	6.86[a] (0.10)
AQ Bond	
AQ-1	6.82[a] (0.09)
AQ-2	6.84[a] (0.09)
Intact dentine (control)	6.92[a] (0.08)

Means with the same letters are not significantly different at $P > 0.05$, $n = 15$.

Table 4 shows the pH value of the pulpal side on tested areas of each sample. The intact pulpal dentine exhibited a pH value of 6.92. All Single Bond groups indicated significantly lower pH on the pulpal surface than the intact dentine ($P < 0.0001$). When intact dentine was etched, the pH value significantly decreased to 6.25 (SB-1) ($P < 0.0001$). After rinsing the etched surface, the pH value increased to 6.59 (SB-2). Following light-curing, the pH was found to be 6.64 (SB-3), similar to SB-2. However, the pH values of SB-2 and SB-3 were significantly lower compared to intact dentine ($P < 0.0001$). The Clearfil SE Bond and AQ Bond groups showed no significant difference in pH value from intact dentine (pH 6.92), indicating no chemical effect on pulpal surfaces with regards to acidity. Figure 2 shows representative images of the pH change of the pulpal surface beneath conditioned dentine 3 min after each preparation. The images revealed a decrease in pH on the pulpal side in all Single

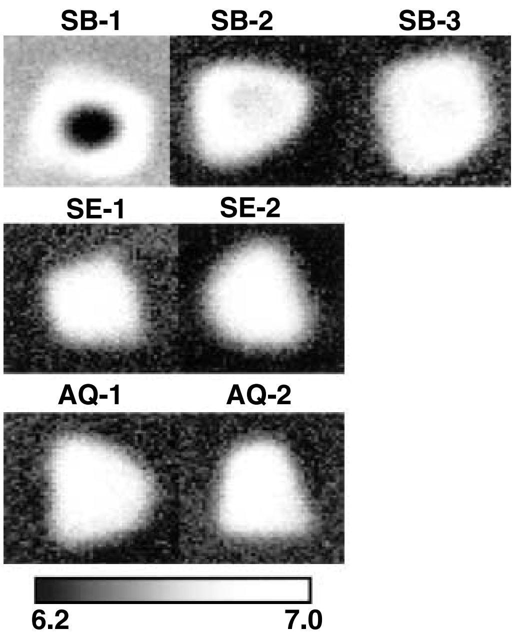

Figure 2 Representative pH images of the pulpal side beneath conditioned dentine with adhesive systems application. Note: the horizontal bar shows the grey scale for pH value.

Bond groups, indicating acidic diffusion through the 0.5 mm-thick dentine discs to the pulpal dentine surface. With Clearfil SE Bond and AQ Bond, there were no remarkable differences between their pH images.

Discussion

Successful adhesion of a bonding resin to dental hard tissues is a fundamental requirement of adhesive materials. Micromechanical retention can occur when the resin completely infiltrates the dentinal surface and creates a hybrid layer (Nakabayashi *et al.* 1982). A hybrid layer can be produced by the procedure of etching, priming and bonding to the dentine surface. Dentine etching involves the application of acid conditioners to remove the smear layer from the dentine surface and demineralize the superficial dentine matrix enabling resin infiltration into the dentine surface, resulting in exposure of the collagen fibrils of the dentinal matrix (Nakabayashi *et al.* 1982, Pashley 1992). During priming, hydrophilic monomers diffuse across the demineralized dentine, stabilize the hydrated collagen network and displace water with polymerizable monomers. Finally, the adhesive resins are applied to the primed dentine and polymerized.

Depending on their composition, current conventional adhesive systems can be classified as either a self-priming bonding system (single-bottle adhesive system) or a self-etching primer system. Single-bottle adhesive systems, based on acid etching, are characterized by the combination of the priming and bonding steps into a 'one-bottle' liquid containing primer and adhesive resin. Self-etching primer systems are characterized by the combination of the etching and priming steps into a self-etching primer followed by an adhesive resin (Chigira *et al.* 1994, Watanabe *et al.* 1994, Harada *et al.* 2000, Nakaoki *et al.* 2002). The self-etching primer, which contains an acidic adhesive resin monomer, simultaneously modifies or removes the smear layer and decalcifies both enamel and dentine surfaces. The acidity of the primer is weaker than an acid enchant such as phosphoric acid (Harada *et al.* 2000, Nikaido *et al.* 2002). 'All-in-one' adhesive systems are characterized by the combination of the etching, priming and bonding steps, and were introduced as a further development of the self-etching primer systems to simplify the clinical procedures. 'All-in-one' adhesive systems are claimed to reduce the critical procedures into one step using one material, which simultaneously etches and primes dentine, and consequently infiltrates the smear-covered dentine with acidic resins (Frankenberger *et al.* 2001, Tay *et al.* 2002).

Many studies have examined through *in vivo* histological observation, the cytotoxicity of adhesive systems against pulpal tissue; however, the results have been contradictory (Inokoshi *et al.* 1982, Cox 1987, Elbaum *et al.* 1992, Pashley 1992, Akimoto *et al.* 1998, Cox *et al.* 1998, Kitasako *et al.* 1998, 1999, Hebling *et al.* 1999, Costa *et al.* 2002). The effects of acid pretreatment of dentine, prior to restoration with resin composite, have been evaluated to determine if the pulpal responses to composite resins would intensify if dentine permeability was increased with acid pretreatment (Stanley *et al.* 1975, Fujitani *et al.* 1987, Tagami *et al.* 1990, Pashley 1992, 1996). Most dentine acid conditioners are hypertonic, and their application on dentine removes the smear layer and decalcifies the peritubular dentine, causing increased dentinal tubule diameter (Pashley 1996). This procedure drastically increases dentine permeability (Pashley 1996), and consequently causes pulpal irritation by facilitating the penetration of acid into the tubules (Stanley *et al.* 1975). *In vivo* studies on human teeth have shown that a diffusion of bonding agent particles across dentine to the pulp tissue causes a pulpal response (Tay *et al.* 1994, Gwinnett & Tay 1998).

However, the RDT between the cavity floor and pulp tissue may prevent the penetration and diffusion of toxic ingredients released from resin materials into pulp tissue (Gerzina & Hume 1995). In the present study, the pH value analysing technique was performed on extracted bovine dentine with a 0.5 mm thickness. Today, all animal or human usage tests are done at an RDT of about 0.5 mm or less (Hanks *et al.* 1988). Clinically, in a deep cavity, dentine is often reduced to within 0.5 mm of the pulp, therefore it seems appropriate to use dentine discs with a 0.5 mm thickness to observe the possible effects of acidic diffusion (Hanks *et al.* 1988).

In the present study, when the dentine was etched with phosphoric acid (etchant group), a pH value of 6.25 was recorded on the pulpal surface, indicating acidic diffusion through to the pulpal dentine surface. Pashley (1992) reported that the factors that could lead to pulpal response following acid etching include the type of acid, its pK_a and pH values, its molecular weight, hydrogen ion concentration and time of etching. Phosphoric acid has strong acidity and a low molecular weight (formula weight: 98), which probably contributed to its diffusion through dentine. Regarding the etching time, the etchant remained on the labial surface for approximately 3 min because it took that time to scan a sample with the SCHEM-100. This prolonged exposure time at a low pH was possibly another reason for the acidic diffusion found on the pulpal surface.

When the etched surfaces were rinsed and dried after etchant application for 15 s, there was a significant increase in pH value from the etchant group. This was probably due to the short etching of 15 s compared with 3 min. When compared with intact dentine, although the etched surfaces were rinsed, the pulpal dentinal surfaces presented a significantly lower pH value. This finding indicated that phosphoric acid was so strong that its effect contributed to acidic diffusion through dentine even though the etching time was only 15 s. Furthermore, when the adhesive agent was applied and light-cured, there was no significant change in pH value from that of the prepared surface by etching and rinsing. This indicates that there remained evidence of acidic diffusion on the pulpal surface even after light curing. However, it was not possible to evaluate whether this acid diffusion was due to remaining etchant after rinsing or the adhesive agent of Single Bond, which has a low uncured pH of 4.60 and a cured pH of 4.70.

For the Clearfil SE Bond group, no significant change in pH was observed on the pulpal surface after application of SE primer, whether an adhesive agent was applied or not. As the acidity of SE primer (pH 2.0) is weaker than that of phosphoric acid (pH < 0.2), the dentine surface was slightly etched (Harada *et al.* 2000). Many primers on the market contain polyacrylic acids, which have molecular weights that vary from 5000 to 25 000 or higher (Pashley 1992). This may be another contributing factor to SE primer's reduced diffusion coefficient. SEM observation of the primer-treated dentine surface demonstrated that SE primer removed the smear layer; however, smear plugs were observed within dentinal tubules (Harada *et al.* 2000). This was attributed to the small demineralization effect of SE primer. In addition, no acidic effect of adhesive agent was noted under slightly etched dentine with SE primer.

The present study did not simulate pulpal pressure and outflowing fluid. In the case of studying dentine permeability when pulpal fluid was considered *in vitro*, the permeability of dentine treated with primers has been shown to decrease (Nikaido *et al.* 1995, Tagami *et al.* 1995). Serum proteins were precipitated by the primers in the dentinal tubules and blocked the dentinal tubules (Tagami *et al.* 1995).

A similar phenomenon was observed in the AQ Bond group. Application of self-conditioning adhesive presented no significant change in pH on the pulpal surface compared with intact dentine, whether light cured or not. As the acidity of self-conditioning adhesive is weak, pH 2.5, it was unable to penetrate bovine dentine of a 0.5 mm thickness.

The present study demonstrated the possibility of acidic diffusion after dentine conditioning using bovine dentine to speculate the effect on pulpal tissue. Another possible factor influencing the pulpal response is the cytotoxicity of dental restorative materials. Most reagents used in dentine bonding are hypertonic (Pashley *et al.* 1992). These bonding agents present an osmotic challenge to the pulpodentine complex. It was reported that HEMA was very cytotoxic to cultured cells even after diffusing across dentine (Hanks *et al.* 1988). In addition, unpolymerized resin may leach resin monomers over several days close to pulpal cells, which was probably a potential for pulp irritation (Pashley 1992). If the dentine surface is etched sufficiently, the acid will dissolve both the smear layer and the entire length of the smear plugs, permitting penetration of the cytotoxic components into the tubules close to the pulp tissue (Hamlin *et al.* 1990).

However, in other studies, the most important factor that determined whether a pulpal reaction occurred following acid etching was the adequacy of the subsequently placed restorative materials to seal the cavity margins, preventing microleakage and blocking bacterial substances from penetrating through dentinal tubules to the pulp (Inokoshi *et al.* 1982, Cox 1987, Hume 1988, Shimada *et al.* 1995, Akimoto *et al.* 1998, Cox *et al.* 1998). It is believed that many pulpal reactions were due to bacteria and their products leaking into the depths of the cavity rather than a direct effect of acids or dental materials (Brännström & Nyborg 1972, Inokoshi *et al.* 1982). This concept is supported by the results of recent studies showing pulp healing in resin-capped teeth when bacteria and microleakage are eliminated (Otsuki *et al.* 1997, Cox *et al.* 1998, Kitasako *et al.* 1998, 1999, Medina *et al.* 2002).

The pH value analysing method using the SCHEM-100 was performed on bovine dentine as a substitute for human dentine, The dentine permeability of bovine dentine differs from that of human dentine depending on tubule densities and diameters (Tagami *et al.* 1989). Tagami *et al.* (1989) reported that the permeability of coronal incisor bovine dentine is six to eight times less than that of unerupted coronal human third molar dentine but similar to that of human root dentine. Moreover, in the present study on extracted bovine teeth, dynamic physiological effect was not considered. *In vivo*, outflowing tissue fluid due to physiological intrapulpal pressure reduces the effects of etching or priming (Zheng *et al.* 2000). Much research remains to be done to determine whether the pulpal reactions are dependent on the acid leached from dentine conditioning or not. With respect to chemical diffusion through dentine following acidic conditioning, it was possible to evaluate the effect on dentine permeability as demonstrated in this study. This technique was advantageous for observation of dentine permeability and chemical diffusion through dentine.

Conclusions

This study using the SCHEM-100 demonstrated that 35% phosphoric acid etchant increased bovine dentine permeability even after the etched dentine surface was rinsed. However, the acid from Clearfil SE bond primer and AQ bond conditioning adhesive failed to penetrate through bovine dentine of 0.5 mm thickness. The techniques developed for measuring chemical diffusion will be useful in testing whether restorative materials have chemical effects on tooth structure and pulp tissues.

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