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The effect of etching on bacterial microleakage of an adhesive composite restoration

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Abstract

Objectives: The incidence of bacterial microleakage, pulp inflammation and necrosis associated with dentine etching treatments prior to restoration are not known. Consequently, to resolve some of the controversy surrounding the effects and importance of vital dentine etching, the authors investigated these factors.

Methods: 110 standardised class V cavities were cut into buccal dentine, without exposing the pulp of teeth scheduled for extraction for orthodontic reasons. Cavities were either left unetched, or etched with the non-equivalent treatments of phosphoric acid gel for 60 s or Ethylenediaminetetraacetic acid (EDTA) for 30 s, prior to placement of composite resin. Teeth were collected and pulp responses were evaluated according to ISO guidelines, using pathohistomorphometric analysis and ANOVA statistics.

Results: Etching was found to be correlated to bacterial microleakage ($p = 0.0001$) and tertiary dentine formation ($p = 0.0023$). Bacterial microleakage was correlated to inflammatory activity ($p = 0.0001$). The frequency of bacterial microleakage was: no etching (65%), EDTA (51%) and phosphoric acid (PA) (20%).

Significance: Vital dentine etching treatment is of extreme importance for the placement of RC to minimise bacterial microleakage. PA etching proved to be more effective at preventing bacterial microleakage than non-etching, and etching with EDTA.

Keywords: Pulp; Microleakage; Inflammation; Adhesives; Phosphoric acid

1. Introduction

The use of acid etching to condition instrumented cavity surfaces by removing the smear layer has become a standard procedure in restorative dentistry. The smear layer is a 1–5 μm thick layer [1] of denatured cutting debris produced on instrumented cavity surfaces, and is composed of dentine, odontoblastic processes, non-specific inorganic contaminants and microorganisms [2,3]. The smear layer is also forced variable distances into the dentinal tubules beneath the instrumented cavity surfaces [4]. The advantages and disadvantages of the presence of smear layer, and whether

it should be removed or not from the instrumented dentine surfaces, are still controversial [5]. Cavity conditioning requires little time but provides the benefit of better adaption of the restorative or intermediate base to dentine [6]. Hence attention has generally focused on the removal of smear layer to condition cavity preparations for the dentine bonding of restorative materials [7,8]. In the past, there was an initial resistance to the use of acid etching and adhesive systems to place composite restorations following reports of necrosis and inflammation [6]. However, over the past 10 years, the consensus over acid etching of vital dentine has changed, following reports dispelling fears over inflammation [9]. Nevertheless, because of these conflicting data, some questions still remain over the relationships between acid etching, bacterial microleakage, pulp inflammation, and the incidence of necrosis and degree of pulp injuries underlying etched vital dentine.

Table 1
Number of teeth according to etching treatments and postoperative extraction time intervals

Group number	Etching	Postoperative extraction time (days)				
		20–40	41–60	61–100	101–381	Total
1	Phosphoric acid	10	10	6	9	35
2	Ethylenediaminetetraacetic acid	10	13	9	3	35
3	No treatment	11	21	4	4	40

Caries has been identified as the most frequent cause for the failure of restorations [10,11]. Microleakage complications involve inflammation, necrosis, recurrent caries, post-operative sensitivity, periodontal disease, and the eventual need for endodontic therapy [12,13]. Although the relationship between pulp infection, inflammation, and pulp necrosis, has already been established, controversy remains as to the possible differences between etching treatments, and their ability to prevent bacterial microleakage and pulpal necrosis in patients.

Conventionally, the smear layer has been removed with phosphoric acid (PA) or maleic acid, with the objective of enhancing the adhesion of resin composite, or resin modified glass ionomers to enamel and vital dentine surfaces. However, there are other types of etchants in use. Citric acid is the most commonly used etchant in periodontal therapy, and ethylenediaminetetraacetic acid (EDTA) has been used to remove the smear layer in cavity and root canal preparations. Smear layer removal with each of these etchants has been associated with various observations of tissue injury and repair activity [14]. In recent years, the use of a pH neutral etching solution of EDTA, has been found to be as effective as low pH etchants for smear layer removal [15]. Although the bond strength of restorative materials after EDTA application has been examined [16], comparatively little attention has been paid to the pulpal responses following vital dentine etching with EDTA. Of the etchants in clinical use, EDTA is the only one chelating agent at neutral pH, while the low pH of PA acts by dissolving or eroding a mineralised surface [17]. Consequently, there is a need to evaluate the pulp response to vital dentine etching with EDTA, to determine if smear layer removal with EDTA can confer some advantages to restorations, in comparison with PA. Furthermore, the smear layer has come under extensive scrutiny in recent years, and many benefits and liabilities of its retention beneath restorations have been observed. These include, acting as a cavity liner to reduce dentine permeability to the possible cytotoxicity of restorative materials [18], as well as being a liability to restoration longevity, due to the bacteria it can harbour beneath restorations [18]. Characterisation of the effects of etching treatments on vital dentine should help the practitioner to choose to retain the smear layer or remove it with PA or EDTA. Consequently the purpose of this study was to evaluate the incidence of bacterial microleakage, severity of pulp inflammation and

injury and repair responses, following the placement of resin composite in unexposed cavities, following smear layer removal with EDTA and PA, as well as with not etched smear layer.

2. Materials and methods

One hundred and ten teeth, scheduled for extraction for orthodontic reasons from healthy patients (63.4% female) aged between 9 and 25 years of age, had class V cavities prepared in non-carious intact first or second maxillary or mandibular premolars. Teeth were extracted between 20 and 381 days following cavity preparation, etching and extraction, using a local anaesthetic with patient and parental informed consent in Marseille Hospital dental care centres. Our standardised methods and procedures used in this study have been described elsewhere [19–22]. Briefly, class V cavity preparations were placed in the buccal surface of teeth, 1 mm above the level of the cemento-enamel junction.

Preparation forms were cut into the tooth dentine using the least possible pressure at a drill speed of 400,000 revolutions per minute with water spray coolant. Cavity dimensions were estimated during cutting, however a histometric analysis of the histological sections found the cavities were cut into dentine to a range of cavity remaining dentine thicknesses (RDT) between 0.008 and 1.610 mm, and axial floor widths between 1.01 and 3.39 mm.

Teeth were assigned to three experimental groups (1–3) for restoration (Table 1). All the products were used strictly according to their manufacturers instructions. *Group 1*: Enamel and dentine cavity walls were etched with 37% PA gel. The gel was left in place for 60 s and then rinsed for 30 s with water. This was followed by air-drying for 20 s. Adhesive (Scotchbond dual cure, 3M Dental, St. Paul, MN, USA) was applied to the cavity walls using the brush supplied for the purpose. The cavity was then dried for 30 s to remove alcohol from the resin. This procedure was repeated for a second coating of adhesive. The RC system (Silux, 3M Dental, St. Paul, MN, USA) was then used to fill the cavity and was polymerised with a curing light for 40 s. *Group 2*: Following cavity preparation, 17% EDTA (dentine conditioner, 3M Dental Products, St. Paul, MN, USA) was applied to cavity walls for 30 s to remove smear layer, and then rinsed for 30 s with water. This was followed by air-drying for 20 s. These teeth were restored

Table 2

Frequency bacterial microleakage, inflammation and necrosis following cavity etching (n = number of teeth)

Etching Treatment	Total		Inflammation category					
			Absent/slight		Moderate		Necrosis	
	+ Bact	– Bact	+ Bact	– Bact	+ Bact	– Bact	+ Bact	– Bact
No etching	65% % (n 26)	35% (n 14)	50% (n 8)	50% (n 8)	75% (n 18)	25% (n 6)	0% (n 0)	0% (n 0)
EDTA	51% (n 18)	49% (n 17)	38% (n 3)	62% (n 5)	57% (n 13)	43% (n 10)	50% (n 2)	50% (n 2)
Phosphoric acid	20% (n 7)	80% (n 28)	0% (n 0)	100% (n 14)	19% (n 3)	81% (n 13)	80% (n 4)	20% (n 1)

with Scotchbond/Silux as described above. *Group 3*: The cavities in this category were left unetched and were restored with Scotchbond/Silux as described above. Extracted teeth were prepared for light microscopy and a histomorphometric analysis was conducted, as described previously [19–22]. Briefly, 5 μ m tooth sections were stained with haematoxylin and eosin, the area of tertiary dentine was estimated histomorphometrically at 10 $\times$ magnification using a grid eyepiece graticule, and the RDT was also measured. Odontoblasts with a distinct nuclei and intact cytoplasm were counted beneath the cavity preparations per mm² unit of pulp area, as well as odontoblast numbers per mm² opposite and independent of the cavity preparation.

Pulpal inflammatory responses were categorised as either absent/slight, moderate or severe, on the basis of standards set by the Federation Dentaire Internationale and International Organisation for Standardisation, and previously published criteria [23,24]. Essentially, in addition to other distinguishable pathological features, ‘necrosis’ was observed as disintegration of pulp tissue, particularly a complete disintegration of odontoblasts beneath the cavity floor. A moderate response indicated a reduction of the odontoblasts, localised inflammatory lesions, as well as tissue infiltration of polynuclear lymphocytes or polynuclear leukocytes. An absence or slight inflammatory response indicated that the odontoblast layer appeared normal, and few inflammatory cells were observed. Bacterial contamination of the restorations, or the underlying dentinal tubules, was assessed using the Brown–Brenn procedure [25] for the presence of gram positive and negative bacteria. The raw numerical data was examined using multivariate Analysis of Variance (ANOVA) (StatView software, SAS). These procedures are reportedly among the most versatile and most conservative of the multiple comparison tests [26].

3. Results

3.1. Bacterial microleakage

The Brown–Brenn procedure identified bacteria in 43.6% of all the restored cavities. The ability of cavity etching to prevent the microleakage of bacteria varied, the rank order

of materials was; PA (80%), EDTA (49%) non-etched (35%) (Table 2). Overall, the use of cavity etching was found to be very important to prevent bacterial microleakage of the cavity preparations (ANOVA p = 0.0001.) Furthermore, the quantity of bacteria in non-etched cavities, was found to be much greater than following EDTA or PA etching (Fig. 1).

3.2. Pulp inflammatory activity

Pulpal inflammatory activity was not found to be increased by etching treatments (ANOVA p = 0.0936) rather inflammatory activity was found to be correlated with bacterial microleakage (ANOVA p = 0.0001.) Necrosis was observed primarily in the presence of bacterial microleakage with PA etching, and also following EDTA etching (Table 2). Higher grades of inflammation were observed following bacterial microleakage into cavities, which were etched with PA (Fig. 2). The lowest levels of inflammatory activity were observed in cavities, which were non-etched (Fig. 2). Moreover, no pulp necrosis was observed in any cavities, which were non-etched, although these cavities appeared to contain the highest levels of bacterial contamination (Table 2). This is presumably because the bacteria in non-etched cavities did not invade tubules and migrate closer to the pulp than in etched cavities.

3.3. Odontoblast density

Odontoblast density was reduced beneath cavities, in comparison with odontoblast density independent of the cavity preparation (ANOVA p = 0.0001). However, these reductions in odontoblast numbers may be attributed to cavity preparation trauma, rather than an effect of etching, as odontoblast density was not reduced significantly beneath cavities with a RDT below 0.5 mm, in comparison with those with a RDT above 0.5 mm (p = 0.8238) (Fig. 3).

3.4. Tertiary dentine area

A histomorphological evaluation of the extracted premolar specimens revealed the presence of a tertiary dentine matrix beneath 66% of the restorations. In all cases, a tubular continuity was maintained between the secondary dentine matrix and the preexisting odontoblasts. These

Fig. 1. Quantity of bacterial microleakage into cavities.

observations classify the secreted tertiary dentine matrix as reactionary in origin [27,28], and consequently our observations of tertiary dentine activity should not be confused with dentine bridge formation, which is an entirely different form of dentine repair activity.

Etching treatments of vital dentine appeared to be correlated with increases in tertiary dentine activity (ANOVA $p = 0.0023$). EDTA etching of vital dentine, appeared to increase the area of tertiary dentine beneath cavities with a RDT less than 0.5 mm, while PA etching

did not appear to appreciably influence the tertiary dentine activity (Fig. 4).

3.5. Analysis of the postoperative extraction time within groups to ensure the test comparisons were valid

Bacterial microleakage, pulpal response to bacterial leakage and the area of tertiary dentine might be expected to be dependent on the postoperative extraction time. However in this study, conducted between 20 and 381 days, no

Fig. 2. Pulp inflammatory activity following vital dentine etching.

Fig. 3. Odontoblast density following vital dentine etching.

Fig. 4. Tertiary dentine area following vital dentine etching.

significant differences were observed at the $p = 0.05$ level using multivariate ANOVA statistics.

3.6. Analysis of the cavity remaining dentine thickness within groups to ensure that the test comparisons were valid

To remove the possibility of a mismatch of RDT's between each of the restoration variables, the RDTs were measured and compared with each other using multivariate ANOVA statistics. No differences were observed at the $p = 0.05$ significance level.

4. Discussion

According to many longitudinal investigations, there remains a high number of primarily vital teeth which exhibit symptoms requiring endodontal treatment, following restorative treatments [29]. Consequently, there is a need to examine all aspects of cavity preparation and restoration, to identify possible sources of pulp injury, which may mediate these post-operative complications. This information may prove useful to guide clinicians as to where to concentrate their efforts in order to reduce the frequency of postoperative complications in patients. Often pulp responses have been ascribed to the activity of particular restorative materials, with seemingly little regard given to the possible effects of vital dentine etching treatments, and other factors such as the dimensions of the cavity preparation forms. Recent studies have indicated that pulp responses are the summation of all the cavity preparation and restoration events, in addition to patient factors such as age and history [19–22]. However, evidence is lacking to explain the relationships between vital dentine etching, bacterial microleakage and pulp inflammation, which cause pulp pathology. This justified our attempt to evaluate the variables of vital dentine etching with PA and EDTA, as well as to evaluate the effects of non-etching of vital dentine prior to the placement of resin composite, using morphometric and analytical evaluation. Preparation of cavities in caries-free teeth, avoided the possibility of including residual caries effects within the data, and pulp responses were examined according to ISO guidelines.

Vital dentine etching and smear layer removal appears to have been best studied at the electron microscopical level to evaluate the hybridisation interface between adhesive bonded materials and tooth structure. Nevertheless, little previous efforts has been made to examine the effects of vital dentine etching treatments, or the presence of smear layer on underlying pulp tissue in patients. However, this study has highlighted the importance of vital dentine etching in terms of underlying pulpal responses. Vital dentine etching can affect the incidence of bacterial microleakage, increase the severity of pulp inflammatory activity, increase tertiary dentine secretion, and decrease odontoblast cell density beneath restorations.

In the clinical situation, the adequate removal of smear

layer is difficult to achieve. This explains attempts to develop adhesive products which did not require smear layer removal prior to filling [30]. However, these early products proved unsuccessful and low restoration bond strengths were reported [31,32]. In accord with these findings, our observations show that a failure to etch the smear layer permitted bacterial microleakage into 65% of cavity restorations (Table 2), and that relatively high quantities of bacteria were present in these cavities (Fig. 1). Moreover, in accord with previous observations [17,18], our findings indicate that the presence of smear layer also appeared to prevent the further microleakage of bacteria through cut dentinal tubules in a pulpward direction as no necrotic pulps were observed (Table 2), and this explains why the level of inflammatory activity was generally less than that observed with PA or EDTA etching (Fig. 2). Furthermore, it has been suggested that the presence of smear layer, containing trapped bacteria [17,18] and denatured smear layer debris [33] could diffuse through the dentine floor of the cavity preparations, and increase the level of pulp injury in comparison with etched dentine cavity restorations. Our observations show that the density of odontoblasts beneath unetched vital dentine was maintained at higher levels than following EDTA or PA etching (Fig. 3). These observations suggest that non-etching of vital dentine can be beneficial for pulp responses in some respects. However, the length of this study was limited to 380 days, and our observations of high-levels of bacterial colonisation of these non-etched cavity restorations, would almost certainly result in a high frequency of pulp complications with time. The onset of recurrent caries beneath posterior composites can take place within 6 months of a cavosurface margin defect [34]. In the clinical situation, the presence of a smear layer has been observed to prevent the formation of an adequate seal between the cavity surface and the restoration, resulting in microleakage and eventual pulpal inflammation [35,36]. The effect of this, is that the cavity restoration will fail more quickly, than it would if the smear layer was removed [6]. On the basis of this evidence and the results presented in this study, non-removal of smear layer prior to filling with resin composite cannot be recommended for work on patients.

On the other hand, the application of etchants to remove smear layer has long been an issue of concern for pulp viability [36,37]. Despite reports that EDTA can be less harmful to oral tissues when removing smear layer [15], EDTA has also been reported to be cytotoxic to tissues [38] and EDTA treatment of a pulp exposure or a very deep cavity preparation is not recommended [6]. These observations highlight the difficulty in assessing the possible effects of vital dentine etching. Although lower quantities of bacteria were detected in cavities following EDTA dentine etching, in comparison with cavities which were non-etched (Fig. 1), more than 50% of EDTA etched cavity restorations were found to be infected with bacteria (Table 2). This would suggest that EDTA etching marginally improves

the bacteriometric sealing of adhesive bonded composite to teeth, but is not as effective as PA (Table 2). Odontoblast density beneath EDTA etched vital dentine was found to decrease to similar levels as unetched and PA treated cavities (Fig. 3) suggesting that reductions in odontoblast cell survival beneath vital dentine, was more the result of cavity preparation trauma, than the primary effects of vital dentine etching treatments.

One effect of vital dentine etching with EDTA, which as not observed with PA etching or non-etching treatments, was a large increase in tertiary dentine deposition beneath cavities, with a RDT below 0.5 mm (Fig. 4). The stimulatory effect of EDTA on tertiary dentine area may be a result of solubilisation of endogeneous growth factors from the dentine matrix [39,40]. Growth factors such as Transforming Growth Factor Beta (TGF β) and bone morphogenic proteins, may play a critical role in dentinogenic events [41,42]. EDTA can solubilise a number of dentine matrix components [40] including growth factors, and it's chelating action at neutral pH [15] may contribute to this action. Thus smear layer removal with EDTA in accordance with the manufacturers recommended treatment, may be beneficial to the patient in terms of pulpal repair response, and contribute to greater bond strength of restorations [32]. However, it is unlikely to be effective if the RDT is above 0.5 mm.

In terms of bond strengths, the presence of smear layer in non-etched cavities (Group 3) probably produced low adhesive bond strengths. Although evidence is generally lacking to explain the relationship between bond strength and bacteriometric sealing, the observations of a high level of microleakage in these restorations (Table 2), suggest that low bond strengths may be related to a reduction in the quality of bacteriometric sealing. The etching treatments evaluated in this present study are not equivalent. The 30 s of etching with 17% EDTA is a less active etching treatment than 60 s of etching with 37% PA. To achieve the same degree of etching with PA, the EDTA would probably have to be applied for 5–10 min. The EDTA etching (Group 2) of cavity walls probably increased the bond strength of adhesive to enamel, in comparison with non-etched cavities. However EDTA etching has been observed to reduce the already low bond strength of the original dual-cure Scotchbond to dentine [43]. Thus, the EDTA etched cavities were probably only adequately bonded at the acid-etched enamel interface. This relatively small area of bonding could reduce the quality of bacteriometric sealing, culminating in over 50% of restorations becoming contaminated with bacteria (Table 2). In Group 1, the PA etching removed the enamel and dentine smear layers, thereby increasing the resin–enamel bonds to 20 MPa, but lowering the resin–dentine bonds [43]. Presumably, the same result occurred with EDTA etching, but the enamel bonds were not as high, because EDTA probably did not remove the entire enamel smear layer. The greater enamel bond strength associated with PA etching [43] may be related to superior bacteriometric sealing, explaining the low numbers of

restorations contaminated with bacteria (Table 2). These observations, together with continual improvements in the enamel and dentine bonding performance of adhesive systems [44], explain the need to periodically examine these adhesive systems clinically.

There has been a noticeable lack of consensus about the effects of smear layer and etching treatments to remove smear layer [4,18]. After considering all the effects of EDTA and PA etching, as well as the effects of non-etching vital dentine, seemingly the most important factor to prevent pulp complications, and maintain restoration longevity [13,38,45] is postulated to be the ability of the restoration to prevent bacterial microleakage. Consequently as more than 50% of non-etched and EDTA etched cavity restorations became infected, compared with only 20% of PA etched cavity restorations (Table 2), smear layer removal with PA must be recommended in preference to EDTA etching and non-etching of vital dentine. However, this does represent a compromise since there will be minimal tertiary dentine deposited beneath PA etched restorations. Furthermore, our quantitation of bacteria represents only total cell numbers and does not consider their relative metabolic activity.

We have shown that the incidence, and quantity of bacterial contamination, is correlated to the presence of smear layer and vital dentine etching treatments. The presence of bacteria increases the severity of pulp inflammation. Etching treatments can also influence underlying odontoblast density and tertiary dentine repair activity. It is now clear that the use of the most appropriate vital dentine etching treatment, can provide clinicians with the ability to minimise the incidence of bacterial microleakage, inflammation and pulp necrosis, although this does require a compromise in terms of tertiary dentine repair activity.

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