In vitro dentine permeability: the relative effect of a dentine bonding agent on crown preparations


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KEYWORDS
Fluid filtration; Dentine permeability; Dentine bonding; Microleakage; Crown preparation; Provisional crowns; Dentinal tubules

Summary Objective. The aim of this study was to determine whether a dentine bonding agent (DBA) had an effect in reducing fluid filtration under cemented provisional crowns.

Methods. Crown preparations on 34 premolars, randomly allocated to two equal-sized test and control groups, were assessed for dentine permeability by a fluid filtration technique. In the test group, prepared crowns were acid-etched and then treated with a DBA (Prime & Bond NT). In the control group, no such DBA was applied. Provisional crowns were cemented on both groups of teeth using non-eugenol zinc oxide cement. Fluid filtration rates were measured repeatedly after crown preparation, at cementation, 1 h, 1 day, 1 week and 3 weeks following crown cementation for both groups. Filtration rates were also measured after acid-etching and dentine bonding for the test group.

Results. There was insufficient evidence to suggest any statistically significant differences either between the two groups or, on average, across the five time points in each group in terms of the relative percentage of fluid filtration.

Conclusion. The DBA used did not seal dentinal tubules any more than did the smear layer and provisional crowns luted with a non-eugenol ZnO cement.

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Introduction

Crown preparations are often required to restore teeth. A full coverage restoration requires removal of most of the enamel resulting in an exposed dentine surface. Dentine is a tissue traversed by tubules 0.6–2.0 μm in diameter. Crown preparation of a molar tooth can expose up to 2 million dentinal tubules. These tubules provide routes for the passage of substances to and from the dental pulp. In the case of crown preparations, these tubules will eventually be sealed by the luting cement used to lute the crown. However, microleakage has been found to occur with all luting cements to different extents for both permanent and provisional crowns. Provisional crowns

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and the way they are made, makes them more likely to have ill-fitting margins. Polymerisation shrinkage of some provisional crown materials was shown to create a gap between the crown margin and the tooth. The positioning of crown margins in the cervical area of the tooth makes this part of the tooth the first area to be affected by the leaking irritants from the oral environment. Interestingly, dentine in this area was shown to have a higher permeability than occlusal dentine. There are several ways of sealing dentinal tubules.

The smear layer

This can be defined as an amorphous coating of loosely bound debris present on the dentine surface after hand or rotary instrumentation and is about 1–5 μm thick. Pashley reported that the smear layer created by some low and high speed burs could reduce dentine permeability by 98% (i.e. to 2% of etched values) compared to etched dentine, which was considered as 100% permeable. However, if microleakage is a common occurrence, the smear layer would only last for a few days due to acidity from the oral environment.

Oxalate

When potassium oxalate is applied to a dentine surface, it reacts with the calcium in the dentine to form insoluble crystals of calcium oxalate that block the dentinal tubules. Using scanning electron microscopy (SEM) and hydraulic conductance, Pashley and Galloway found that the treatment of a dentine surface with oxalate solutions significantly reduced hydraulic conductance and also provided a significant protection against acid etching when compared to potassium chloride treated smear layers.

Dentine bonding agents

Over the last few years, dentine bonding agents (DBA) have been introduced as a new way of sealing dentinal tubules. Their advantages over the smear layer are the mechanical retention (hybrid layer) and the ability to withstand moisture and acid, however, it is not clear how effective this barrier is or how long it lasts.

This study aimed to determine whether application of a DBA to teeth prepared for full veneer ceramic crowns affected fluid filtration over a three week period when provisional crowns were cemented, compared with a control group of teeth with provisional crowns where no DBA was applied.

Materials and methods

Thirty-four intact premolars were used in this study. Teeth had been extracted for orthodontic reasons and were stored in saline with added thymol until they were used. A pressurized-fluid apparatus using the same principles described by Pashley et al. was used. This apparatus was constructed and previously used by Youngson et al. (Fig. 1).

A nitrogen gas cylinder (B.O.C., Leeds, UK) fitted with a Saffire N-3.5 multi-stage pressure regulator (B.O.C) was used to pressurise a sealed chamber made from 8-mm thick Perspex. The chamber contained a beaker of phosphate buffered saline (PBS) that was connected to the system by a tube through an outlet. A tap in the outlet tube was used...
to disconnect the system from the chamber while changing specimens. A small air bubble was introduced into the fluid-filled tubing from a 1-ml Plastipak syringe (Bectone-Dickinson, Madrid, Spain) via a 1.2-mm diameter cannula (Venflon 2, viggo-Spectrameter, Helsingborg, Sweden) set in a T-junction. The tubing was connected to a modified 10-µl microsyringe calibrated in 0.1 µl (S.G.E., Deutschland, Germany) which led into a modified 1-ml syringe. A second syringe fitted with a microscrew was then connected with a cannula to allow adjustment of the position of the air bubble within the syringe.

Teeth were decoronated 2 mm below the cemento-toenamel junction (CEJ) using a high speed diamond annular saw with water coolant (Meadside Eng. & Co. Ltd, Waltham Abbey, Essex). The cut surface was tried on a piece of Perspex to make sure that the surface was flat. If not, it was lapped on 600 grit Silicon Carbide paper until a flat surface was obtained. The residual pulpal tissue was removed using barbed broaches taking care not to touch the pulpal wall to avoid creating smear layer. The pulp chamber was then irrigated with PBS to remove any remnants from the lapping process. Perspex base plates (5 × 5 × 0.37 cm³) were prepared and perforated in the centre using a 0.5 mm cylindrical steel drill through which a cannula (Etching gel dispensing tip, No 7523T, 3M Dental Products) was inserted. Cyanoacrylate and then epoxy resin (Araldite Resin, Bostik Ltd, Leicester, UK) cements were used to secure the cannula.

The projecting cannula was cut short using a tungsten carbide high speed bur so that the coronal tooth segment could fit on the perspex with the cannula’s tip projecting into the pulp chamber. The patency was checked after cutting by inserting an endodontic finger spreader into the cannula and then by air. The coronal tooth segments were then cemented onto the Perspex by cyanocrylate, which was applied on the Perspex around the cannula. Specimens were then left in 100% humidity at 37 °C for 24 h, for the cement to set. Epoxy resin was then used to seal the root dentine and to give some support (Fig. 2). Specimens were subsequently left in the same incubator for another 24 h before they were stored in PBS plus thymol at room temperature.

A silicone putty index was taken for every tooth using polyvinylsiloxane impression material (President, Coltene, Switzerland) with marks made on the Perspex base and the index for orientation. This index was used at a later date for fabrication of the provisional crown.

There is increased clinical usage of dentine-bonded luting agents for all forms of crown restorations where a perceived benefit is the reduction of marginal leakage that may lead to crown failure. To assess the effect on dentine permeability on a relatively minimal preparation, a full veneer crown preparation (Fig. 3) was completed on each tooth using a medium grit diamond bur in a high-speed handpiece with copious water-cooling. All preparations were performed by the same operator (TE). The preparations, including depth of reduction, followed the principles outlined by Shillingburg et al. A 0.5 mm chamfer was created cervically and all enamel was removed from the axial walls coronal to the finishing line. No effort was made to remove all occlusal enamel to gain results close to those expected in the clinical environment. The remaining dentine thickness was not examined but was considered within the normal clinical range for a ceramic crown preparation.
Specimens were then numbered and randomly divided into equal-sized test and control groups.

**Test group**

After crown preparation, the cannula and tooth specimen were connected to the apparatus and the fluid filtration was measured with the smear layer still present. The next step was to etch all the prepared surfaces with 36% orthophosphoric acid (DeTrey, Conditioner 36) for 15 s using a hypodermic needle. The etchant was then rinsed for 15 s and air dried for 3 s to remove excess water and leave a moist surface. The fluid filtration was then measured once more (Fig. 4).

Before applying dentine adhesive, the tubing was disconnected from the pressure chamber and the system depressurised through the first syringe. This step was taken to mimic the clinical situation when the pulp is under the effect of the vasoconstrictors in the local anaesthetic and pulpal pressure is essentially zero,22 and also because the pressure used was much higher than the physiological pulpal pressure. Prepared surfaces were subsequently rewetted and gently dried to leave a moist surface. DBA (Prime & Bond NT (Quix-Unit Dose) Trial Kit, Dentsply, DeTrey GmbH, Germany) was used according to the manufacturer’s instructions to seal the dentinal tubules. Using its new pre-dosed, single use, bottles and the applicator tips provided, the adhesive was applied to the dentine surface and left undisturbed for 20 s for the solvent to evaporate, then air-thinned with the 3-in-1 syringe. The adhesive was light cured for 20 s using an Optilux light unit (Demetron Research Corporation, Danbury, USA). The fluid filtration was measured again at this stage (Fig. 4).

Yellow Soft Paraffin BP (Adams Health Care, England) was applied to the bonded crown preparation to prevent adhesion between the provisional crown material and the DBA. The corresponding silicone putty index was filled with a temporary crown material (Iso-Temp, 3M Dental Products, St Paul, USA) before it was placed onto the crown preparation using the orientation marks on the index and the perspex. An atomising gun was used to inject the temporary crown material (Iso-Temp) into the silicone putty index. This material was left for 5–6 min to achieve an initial set then the index was removed and the crown was removed carefully and trimmed with a pair of scissors. The crown was then refitted and carefully removed once or twice to prevent it from sticking due to polymerisation shrinkage before light curing for 30 s to complete the setting reaction. Final marginal finishing was performed using a sand paper disc with a low-speed handpiece. The Yellow Soft Paraffin was removed from the tooth with a piece of gauze and then the crown was cemented with Temp-Bond NE (Kerr, Non-Eugenol Temporary Cement, Italy). This cement was used to avoid possible disruption of the polymerisation of the DBA by eugenol, especially the oxygen-inhibited superficial layer of the DBA, although this was possibly removed with gauze. After the cement was set, the excess was removed with a sharp dental probe. The fluid filtration was again measured at this stage and subsequently after 1 h, 1 day, 1 week and 3 weeks (Fig. 4).

Measurement of the fluid filtration was performed by pressurizing the apparatus described previously with PBS to 70 kPa (i.e. 714 cm H₂O). The PBS was previously degassed for 30 min in a vacuum oven (-1 bar/40 °C) in order to reduce the compressibility of the fluid therefore minimising any false movement of the air bubble. At every stage, the fluid filtration was measured by recording, three times, the movement of the air bubble over 2 min periods. The means were obtained

![Figure 4](image-url)  
*Figure 4*  
Diagram showing the different stages of the experiment in both control and test group.
and the fluid flow rates in $\mu$l/min were calculated by dividing them by two. During the experiment, teeth were stored in PBS and thymol at room temperature.

Control group

In this group, there was no etching or DBA application. The fluid filtration was measured after crown preparation, provisional crown cementation, 1 h, 1 day, 1 week and 3 weeks. As in the test group, Yellow Soft Paraffin applied to teeth was then removed with a piece of gauze before making the provisional crown. Fig. 4 shows the different stages of the experiment in both groups.

Collection of data

In order to make each tooth serve as its own control, the relative percentage of fluid filtration was calculated using the following equation:

$$\left(\frac{V_2 - V_1}{V_1}\right) \times 100$$

where

- $V_1 = \text{volume of fluid flow after crown preparation with smear layer (}$\mu$l/min); $V_2 = \text{volume of fluid flow at any assessment stage (}$\mu$l/min).

Statistical analyses were used to compare the relative percentage of fluid filtration, both within each of the two groups, and between the two groups. The relative percentage of fluid filtration at each pair of successive time points were compared using paired t-tests, incorporating multiple comparisons, for each group separately. To examine differences between the two groups, repeated measures analysis of variance was used, incorporating possible effects of group, time and tooth, together with the two-way interaction of group and time; tooth was modelled as a random effect, group and time as fixed effects. Finally, to examine whether the first two stages of the test group resulted in a significant change from zero in the relative percentage of fluid filtration, one sample t-tests were used.

Results

The means and standard deviations of the fluid flow rates in both groups were calculated (Table 1). Means and standard deviations of the percentage of fluid filtration were also calculated (Table 2).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Test group ($n = 17$)</th>
<th>Control group ($n = 17$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>After preparation</td>
<td>0.44 (0.37)</td>
<td>0.71 (1.12)</td>
</tr>
<tr>
<td>After etching</td>
<td>1.05 (1.16)</td>
<td>-</td>
</tr>
<tr>
<td>After DBA</td>
<td>0.41 (0.47)</td>
<td>-</td>
</tr>
<tr>
<td>After cementation</td>
<td>0.21 (0.40)</td>
<td>0.09 (0.03)</td>
</tr>
<tr>
<td>After 1 h</td>
<td>0.14 (0.13)</td>
<td>0.10 (0.05)</td>
</tr>
<tr>
<td>After 1 day</td>
<td>0.11 (0.07)</td>
<td>0.08 (0.04)</td>
</tr>
<tr>
<td>After 1 week</td>
<td>0.09 (0.08)</td>
<td>0.07 (0.04)</td>
</tr>
<tr>
<td>After 3 weeks</td>
<td>0.12 (0.07)</td>
<td>0.09 (0.02)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage</th>
<th>Test group ($n = 17$)</th>
<th>Control group ($n = 17$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>After etching</td>
<td>179.4 (338.7)</td>
<td>-</td>
</tr>
<tr>
<td>After DBA</td>
<td>37.0 (183.7)</td>
<td>-</td>
</tr>
<tr>
<td>After cementation</td>
<td>-43.9 (74.3)</td>
<td>-57.87 (33.06)</td>
</tr>
<tr>
<td>After 1 h</td>
<td>-58.1 (39.6)</td>
<td>-58.54 (39.41)</td>
</tr>
<tr>
<td>After 1 day</td>
<td>-61.9 (25.5)</td>
<td>-64.14 (33.94)</td>
</tr>
<tr>
<td>After 1 week</td>
<td>-67.3 (29.7)</td>
<td>-63.65 (35.34)</td>
</tr>
<tr>
<td>After 3 weeks</td>
<td>-63.9 (20.8)</td>
<td>-55.24 (34.95)</td>
</tr>
</tbody>
</table>
being 0.94, 0.21 and 0.49, respectively. When the outliers were excluded, the conclusions did not change with p-values of 0.76, 0.54 and 0.78, respectively. In both cases, the random tooth effect was highly significant ($p < 0.01$). Therefore, there is insufficient evidence to suggest any significant differences either between the two groups or, on average, across the five time points in terms of the percentage of fluid filtration.

The p-values from the one-sample t-tests examining whether the first two stages of the Test group (Acid-etching and DBA) resulted in a significant change in the percentage of fluid filtration were 0.04 and 0.42 for After Etching and After DBA, respectively. Thus, there was evidence to suggest that the mean percentage of fluid filtration after acid-etching was significantly different from the baseline (after preparation). The 95% confidence interval for this was (5.3, 353.6), so whilst on average there was a significant increase of 179.4%, this increase could be anywhere between 5.3 and 353.6%. After DBA application, the fluid filtration was not significantly different from the smear layer-covered dentine baseline (after preparation).

**Discussion**

The hydrodynamic theory of dentine sensitivity states that movement of fluid in the dentinal tubules is the mechanism by which pain is expressed when exposed dentine is stimulated. Fluid filtration testing works on this principle and has become increasingly popular because of its non-destructive nature and the ability to provide quantitative measurements.

The interstitial fluid pressure (IFP) of the pulp is estimated to be about 15-20 cm H$_2$O in normal pulps (1.47-1.96 kPa). However, pressures higher than 15-20 cm H$_2$O have been used in different investigations. The pressure of 70 kPa was selected because the apparatus used in the current investigation had been used in a previous experiment that had used a pressure of 69 kPa. The slight increase was made because it was easier for the operator to adjust the pressure meter every time. However, it could be argued that the DBA tested might behave differently at pressure equal to the physiological pulpal pressure. In other words, the presence of fluid flow across dentine at this high pressure does not necessarily mean that such fluid flow will occur at a lower pressure, and therefore the DBA should be tested at a lower pressure. On the contrary, the absence of fluid flow makes us more certain that it is much less likely to occur at a lower pressure.

Due to the difficulty in obtaining sound premolars, some teeth had to be stored in saline saturated with thymol for few months before they were used. However, previously it has been found that from the second day post-extraction, dentine permeability did not change over the following 3-4 weeks.
With regard to whether resin-bonding to dentine was affected by post extraction time, it has been reported that there were no significant differences in resin adhesion between the first hour up to 26 days post-extraction.\textsuperscript{26} Movement of fluid through tubules is controlled by several factors as stated in the Poiseuille-Hagen’s equation:\textsuperscript{12}

\[ Q = \frac{\pi \Delta P r^4}{8 \eta l} \]

where

- \( Q \) = rate of fluid flow
- \( \Delta P \) = pulpal pressure
- \( r^4 \) = tubule radius
- \( \eta \) = viscosity of the dentinal fluid
- \( l \) = length of tubule

In this experiment, the variables of ‘pulpal pressure’ and fluid viscosity were all constant. Differences in dentine thickness and surface area were overcome by using the relative percentage of fluid filtration, i.e. the tooth acted as its own reference point. Therefore, only two variables were left, the volume of fluid flow as a dependent variable related directly to changes in tubular radius which was the independent variable.

The age and gender of the people from which these teeth were obtained was not known; therefore, the quality of dentine could have been different from one tooth to another. This was also compensated by using the relative percentage of fluid filtration so each tooth served as its own control.\textsuperscript{12,19,20,27} Pashley et al.\textsuperscript{28} used the fluid filtration technique to compare third generation DBAs in their ability to seal the dentine surface after crown preparation. These authors used the actual filtration rate instead of the percentage because there was no statistically significant difference between the groups either before or after crown preparation. In the current experiment, it can easily be seen that there is a large variation within and between groups which is a problem found commonly in biological systems. For that reason, the relative percentage of fluid filtration was used to allow every tooth to act as its own control. Pashley et al.\textsuperscript{29} had the baseline dentine permeability for one of their groups significantly lower than the other two and for that reason they used the relative percentage of dentine permeability along with the absolute values. The use of crown preparations or cavities makes it more likely for the baseline dentine permeability to vary. Therefore, it might be beneficial to balance groups by permeability prior to protocol. On a few occasions after etching, the air bubble moved rapidly through the microsyringe and then suddenly stopped and started to move slowly again. The explanation for this could be that loose fibrils and collagen present within the tubules were originally situated near the pulpal surface (wide dentinal tubules) and were jammed in the periphery as the tubule radius decreased.

It was also noticed in a pilot study that, in most cases after fitting the specimen onto the apparatus, the air bubble moved faster during the first reading, less in the second and least in the third occasion. The first reading tended to be considerably greater than any subsequent one, therefore, it was decided that, for the main study, 1 min would elapse before adjusting the air bubble for the first reading. The reason for this is likely to be the initial movement of fluid into the dentinal tubules or/and an air bubble in the pulp chamber. A recent article regarding the effect of pressure and measuring time on fluid filtration in endodontics has been published.\textsuperscript{30} These authors reported a significant difference in filtration rate between measuring time (3 min, 1 and 4 h) and also the pressure (15 cm \( H_2O \), 150 cm \( H_2O \)), i.e. the longer the time, the less the air bubble moved. Conversely, higher pressure caused more movement. This article finally suggested waiting 1 h before taking the first reading. This latter paper was, however, published following commencement of the current investigation.

Soft paraffin jelly was used as a separating medium to prevent bonding between the DBA and the temporary crown material, and it was also placed on the control group crown preparations to ensure variables were applied equally to control and test specimens. As expected, the use of the soft paraffin jelly on the control group, in many specimens, slowed down the movement of the air bubble. Therefore, this step could be used clinically to minimise bacterial leakage during impression taking and provisional crown fabrication.

A further advantage of this in vitro investigation was the ability to move the teeth around and view them from a close distance. This could have made it easier to make better crowns with close fitting margins than in-vivo, especially with no marginal gingiva to disturb. Monday and Blais,\textsuperscript{31} found that provisional crown margins made with the indirect technique were more accurate than those made with the direct technique. This means that well fitting provisional crowns were produced, which might have reduced dentine permeability beyond the ability of the DBA. This may explain the close similarity of hydraulic conductance of both groups after crown cementation.
In a recent study, Gregoire et al. found that applying DBAs on dentine discs led to a significant decrease in the permeability when compared to the initial penetration values. The reason for that could be the fact that their baseline was taken after 'conventionally' etching both sides of the dentine discs which meant higher permeability values and more likelihood for finding a significant difference. In fact, one of the bonding agents they used was Prime & Bond NT and it did as well as the smear layer in reducing dentine permeability. In the current study, on average, this bonding agent did not reduce dentine permeability beyond or to the level of the smear layer. In other words, dentine permeability after the application of the DBA was higher than that of the smear layer, however, this was not statistically significant.

Bouillaguet et al. found that the DBAs did not always seal dentine any better than did the smear layer. However, dentine adhesives have an advantage over the smear layer as Pashley et al. found that some lining agents (light-cured Scotchbond, Hyroxyline, or DDS 1 and 2) did significantly well in keeping dentine permeability down after an acid challenge.

In an attempt to seal dentinal tubules and minimise microleakage Pashley et al. reported that the smear layer reduced the permeability to 9.7% of the acid-etched control value. When this smear layer was treated with ferric oxalate the permeability dropped to 35% (smear layer permeability was considered 100%). However, the effect of ferric oxalate alone gradually deteriorated under water, from the first week up to 3 months due to dissolution. In fact, the permeability increased more than the control smear layer indicating the later was also dissolved to a certain extent. They also used the Bowen bonding system on top of the ferric oxalate by treating it with N-tolyl glycine-glycidylmethacrylate (NTG-GMA) and pyromellitic dianhydride + 2-hydroxyethylmethacrylate (PMDM) which further reduced the permeability to 25.6%. Despite the significant increase in permeability (68.4%) after bonding composite resin to the bonding agent and after the bond was broken and specimens were stored in water, the permeability was 34.8, 33.2 and 41.3% when measured at 1 week, 1 month and 3 months, respectively. They stated that all tensile strength tests resulted in adhesive failure implying the DBA was removed with the composite. This would further imply that the Multi-step Bowen bonding agent used was not tested under water. This is important because any material used to seal dentinal tubules should be able to withstand moisture, especially if it is going to be used under provisional crowns. In the current investigation, the smear layer in the control group seemed to have been kept away from moisture by the sealing crown and cement. This might have lead to the small and non-significant fluctuation within the control group, even after three weeks storage under water.

The presence of a few outliers could not be explained, and nothing distinctive about these teeth could be observed. However, intratubular contents could have been flushed out by the high pressure used causing large increase in the fluid filtration. Statistical analysis that assessed the effect of excluding these 'outliers' still failed to demonstrate statistically significant difference.

**Conclusion**

1. Treating an acid-etched crown preparations with a DBA (Prime & Bond NT) before provisional crown cementation did not lower in vitro permeability of dentine over that of smear-layer covered controls during the time of the study.
2. Pressure, measuring time and the size of the air bubble should be standardised for better comparison between different experiments.
3. Etched dentine should be well isolated from contamination due to the increase in dentine permeability after etching.
4. Teeth should be divided into groups after measuring dentine permeability in such a way that their means are comparable.

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**References**


