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Effect of different methods for creating smear layer on fluid movement across resin/dentin interfaces created by a current self-etching adhesive

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Objective: The purpose of this study was to evaluate fluid movement across resin/dentin interfaces created by a current self-etching adhesive applied onto dentin with different prepared smear layer.

Materials and Methods: Dentin discs were prepared from extracted third molar and divided into 2 groups of smear layer-cover dentin discs created with either 360-grit silicon carbide paper or superfine grit diamond bur. For SEM investigation, smear layer free specimens by treating with 17% EDTA for 5 min and smear layer covered specimens were observed. The hydraulic conductance model was set and delivers the pressure of 20 cmH_2O for all measurement. For measurement of maximum permeability, fluid filtration rate of smear layer free specimens were measured in the hydraulic conductance model. Then, smear layer was created with either silicon carbide paper or diamond bur and the fluid filtration rate was measured. The relative smear layer coverd dentin permeability (%P) was calculated. The smear layer covered specimens were further treated with a universal adhesive. The fluid infiltration rate and the relative bonded dentin permeability of bonded dentin were also measured and calculated.

Results: The results of SEM observation demonstrated 1-2 microns thick of smear layer covered both dentin prepared with diamond bur and silicon carbide paper. There was no significant difference between specimens prepared with silicon carbide paper and diamond bur in the aspect of fluid infiltration rate of smear layer covered dentin and fluid filtration rate of bonded dentin. The difference of %P of smear layer coverd dentin and %P of bonded dentin between dentin prepared with diamond bur and silicon carbide paper could not found.

Conclusions: In conclusion, the fluid movement across resin/dentin interfaces created by application of the universal adhesive was not interfered by different methods for creating smear layer.

Keywords: dentin permeability, fluid infiltration rate, resin/dentin interface, smear layer, universal adhesive

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Smear layer created by cavity preparation consists of a mixture of enamel, dentin or cementum, contaminating blood, saliva and microorganism. [1] The presence of smear layer on a dentin surface occludes the underlying dentinal tubules and reduces dentinal permeability. [2] According to previous studies, the presence of the smear layer provided the adverse effect to the bond strength. Therefore, the smear layer should be modified, removed or impregnated to optimize the resin-dentin bond interface. [3-5]

Surface preparation methods create different characteristics of smear layer. [6] In laboratory testing, SiC papers produce thick smear layer with irregular surfaces, while clinically, dental burs produce thin and dense smear layer. [5-7] The different of smear layers has been reported affecting the bond strength of self-etching adhesives. [5-10]

Currently, the most simplified system, a one-step self-etch adhesive has been developed by incorporating priming and bonding resin into a single solution to reduce the step of application

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and technical sensitivity. [11-12] Despite the benefit of the less application's time, these one-step self-etch adhesives demonstrate highly hydrophilicity even after polymerization. [13] Additionally, the cured surface of one-step self-etch adhesives contain a high concentration of ionic monomer, water, and dissolved mineral. [13] Thus, these simplified adhesives exhibit high water sorption and permeability. [13, 14]

The nanoleakage caused by the hydrophilicity of adhesive has been demonstrated. It presented of water-filled channel within the hybrid and adhesive layers. These channels allow water movement from the underlying hydrated dentin through hybrid and adhesive layer that cause degradation of resin/dentin interface. [15-16]

There was a limited data regarding the effect of the different smear layer preparation on the water movement across resin/dentin interfaces especially when current self-etching adhesives were applied. [17,18] The knowledge regarding to this effect might have a benefit to predict the sustainability of resin/dentin interface via the study of resin/dentin permeability with different smear layer creation.

The objective of this study was to evaluate the fluid movement across resin/dentin interfaces created by a current self-etching adhesive applied onto dentin with different smear layer preparation.

Materials and Methods

Eighteen caries-free human third molars were collected under a protocol approved by the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University Institutional Review Board. The teeth were stored in 0.1% thymol solution at 4°C for less than one month. Eighteen dentin discs from middle 1/3 of coronal dentin were prepared by two slices cutting perpendicular to the crown, with approximately 1 mm thickness. Each disc was polished with 360-grit silicon carbide papers (Buehler, Illinois, USA) to produce a dentin disc that has a thickness of 0.7-0.8 mm, which were calibrated by a pincer-type caliper (Mitutoyo, Tokyo, Japan).

Smear layer-covered dentin discs were created using 2 different surface preparations (N=9): 360-grit silicon carbide paper and flame-shaped superfine grit diamond burs (Diamond Point FG, Shofu, Kyoto, Japan). For silicon carbide prepared surface, dentin discs were manually polished with 360-grit SiC paper (25.8 microns abrasive) at a constant weight for 30 sec under running water. For diamond bur prepared surfaces, dentin discs were grinded with flame-shaped superfine grit diamond burs (25 microns abrasive) utilized with a high-speed handpiece (Twinpower Turbine, J Morita, Kyoto, Japan) including copious water spray for 5 light pressure strokes in order to create a uniform surface.

Four smear layer covered specimens from either dentin prepared with diamond bur or dentin prepared with silicon carbide paper were randomly taken and treated with 17% EDTA (ethylenediaminetetraacetic acid) for 5 min. A transverse groove with 0.3 mm in depth was created on the pulpal side of every specimen using a flame-shaped superfine grit diamond burs (Diamond Point FG, Shofu, Kyoto, Japan). The specimens were further divided into 2 subgroups. Two specimens from the first subgroup were re-created smear layer with either diamond bur or silicon carbide paper and used as smear layer covered dentin observation. Two remaining specimens of the second subgroup were used as smear layer free dentin observation. The dentin discs were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 at 4°C for 12 h, then rinsed with 20 ml of 0.2 M sodium cacodylate buffer at pH 7.4 for 1 h. Then, all dentin discs were rinsed with distilled water for 1 min, dehydrated in ascending grades of ethanol: 25% for 20 min; 50% for 20 min; 75% for 20 min; 95% for 30 min; and 100% for 60 min. After that, the discs were immersed in hexamethyldisilazane (HMDS, Sigma Chemical Co., St Louis, MO, USA) for 10 min and then left drying in desiccator for 24 h. Ultimately, the discs were fractured into 2 halves and sputter coated with gold. The surface topography and thickness of the smear layer on dentin surfaces were observed under a scanning electron microscope (SEM, JSM-6610LV, JEOL LTD, Tokyo, Japan).

Five specimens from either dentin prepared with diamond bur or dentin prepared with silicon carbide paper were used for the permeability measurement. The smear layer was first removed by rinsing with 17% EDTA for 5 min. The fluid filtration rate of each EDTA-treated specimen was calculated and designated as a value of 100% or initial maximum permeability. Then the smear layer on the dentin surfaces was re-created with the methods as described above. The permeability of smear layer coverd dentin discs were then measured. Furthermore, the smear layer coverd dentin disc was treated with a universal adhesive resin (Single Bond Universal, 3M ESPE, St.Paul, MN, USA) with self-etch mode and the permeability was also measured. The compositions and pH values of this adhesive are shown in Table 1. The adhesive was applied and rubbed for 20 sec according to the manufacturer's protocol onto smear layer covered dentin under simulated pulpal pressure. Gently air dry for 5 sec and light-activated for 10 sec by using an LED light-curing unit with a power intensity of 1,200 mW/cm^2 (LED Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein) at a distance of 2 mm from the bonding surface. All measurments were performed under simulated pulpal pressure of 20 cmH_2O. The method for simulating pulpal pressure was described as following.

Each specimen was attached to a custom-made artificial pulp chamber with the infusion area of 0.196 cm^2. The artificial pulp chamber was connected to a hydraulic conductance device (tubing pump, ISMATEC®, Wertheim, Germany) that delivers the pressure of 20 cmH_2O during the measurement of the dentin permeability (Figure 1).

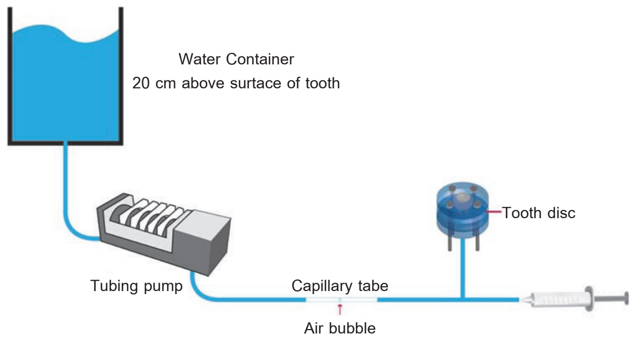

Figure 1 Schematic showing the hydraulic conductance model use in this study.

This hydraulic conductance model imitated the protocol proposed by Pashley and Stewart. [19] Furthermore, this model was modified by adding the infusion pump to mimic the movement of the blood from human blood flow. The procedures start with creating an air bubble in the capillary tube, which was positioned between the water reservoir and the dentin disc in the artificial pulp chamber. The infusion pump that was set the fluid flow rate at 0.36 microlitre /min was activated. The picture of air bubble was recorded by a digital camera at 0 minute and 10 minutes. Then the distance of air bubble movement in the capillary tube (volume: 50 μL, total length: 125 mm) from 2 pictures was analyzed and measured with ImageJ software (NIH Image, Bethesda, Maryland, USA). The fluid filtration rate of each specimen

Table 1 Chemical composition, pH and source of adhesives were used in this test

Adhesive	Chemical composition	pH value
Single Bond Universal (3M ESPE, St.Paul, MN, USA)	MDP Phosphate Monomer, Dimethacrylate resins, HEMA, Vitrebond™ Copolymer, Filler, Ethanol, Water, Initiators, Silane	2.7

after treated with EDTA, created smear layer with either diamond bur or silicon carbide paper and treated with the adhesive were calculated using the following equation:

$$\text{The fluid filtration rate} = \frac{\text{The distance of air bubble (mm) x 50 (μL)}}{\text{125 (mm) x 10 (min)}}$$

In adddition, the permeability as the relative smear layer coverd dentin permeability (%SMP) of specimen was calculated using the following equation:

$$\%SMP = \frac{\text{Fluid filtration rate of smear layer coverd dentin}}{\text{Fluid filtration rate of EDTA-treated dentin}} \times 100$$

The permeability from this equation means the smear layer coverd dentin relative to its maximum EDTA-treated permeability, with each tooth acting as its own control.

The relative bonded dentin permeability (%BP) was also calculated using the following equation.

$$\%BP = \frac{\text{Fluid filtration rate of bonded dentin}}{\text{Fluid filtration rate of EDTA-treated dentin}} \times 100$$

The means and standard deviations of fluid infiltration rate and relative permeability (%SMP and %BP) were analyzed using Kolmogorov Smirnov test (K-S test) to determine the distribution of the data and Levene's test to test the homogeneity of variance. The difference of means between groups were further analyzed using independent T test with a significance level of $\alpha = 0.05$.

Results

SEM observation showed that dentin prepared with either diamond bur or 360-grit silicon carbide paper demonstrated plenty of grooves, scratches, and was covered with a visible smear layer with some evidence of dentinal tubules occluded by smear plugs (Figure 2 and Figure 3). The thickness of smear layer was approximately 1-2 microns for both dentin prepared with diamond bur and silicon carbide paper. Dentinal tubules, which were apparently occluded by smear plugs, were relatively more evident in the silicon carbide paper than in the diamond group. On the other hand, dentin treated with the EDTA had no evidence of smear layer or smear plug.

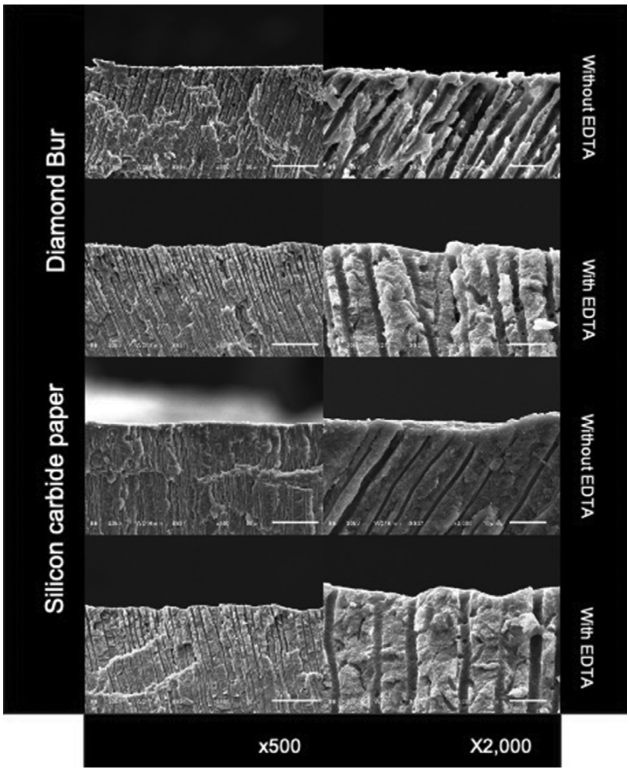

Figure 2 SEM images of dentin surfaces after prepared with either diamond bur or silicon carbide paper with or without EDTA treatment at x500 and x2,000 magnification

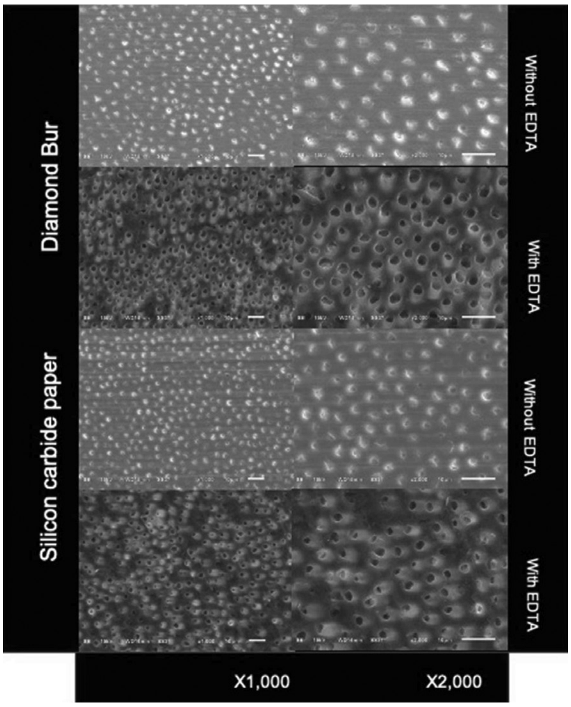

Figure 3 SEM images of dentin top surfaces after prepared with either diamond bur or silicon carbide paper with or without EDTA treatment at x1,000 and x2,000 magnification

The means and standard deviations of fluid infiltration rate of dentin disc after treated with EDTA, created smear layer with either diamond bur or silicon carbide paper and treated with the adhesive are demonstrated in Table 2.

From statistical analysis of the data within the same column, no statistically significant differences were found. The maximum permeability was found for the smear layer removal group by treating with 17% EDTA for 5 min groups. The maximum fluid infiltration rate between diamond bur group and silicon carbide group were not significant different (p=0.57) with the rate of 0.610 ± 0.196 microlitre/min and 0.680 ± 0.173 microlitre/min, respectively. The reduction of fluid infiltration rates was found after smear layer creating. The fluid infiltration rates of smear layer covered dentin created by diamond bur and silicon carbide were 0.060 ± 0.007 microlitre/min and 0.081 ± 0.032 microlitre/min, respectively. Therefore, no statistical significance was found between both groups (p=0.22). After treatment with a universal adhesive (Single Bond Universal), dentin discs showed reduction of fluid infiltration rate comparing with smear layer covered dentin. No statistical significance of infiltration rate was found (p=0.10) between after application of adhesive onto smear layer covered dentin created by diamond bur (0.039 ± 0.010 microlitre/min) and silicon carbide paper (0.051 ± 0.011 microlitre/min).

The means and standard deviations of %SMP (smear layer-coverd dentin permeability compared with EDTA treated dentin permeabilty) and %BP (bonded dentin permeability compared with EDTA treated dentin permeabilty) were calculated and showed in Table 2. No statistical differences were found between group prepared with diamond bur and with silicon carbide paper for both %SMP (p=0.48) and %BP (p=0.67). Thus, %BP was less than %SMP.

Table 2 Means and standard deviations of fluid infiltration rate of dentin disc (μL/min) after treated with EDTA, created smear layer with either diamond bur or silicon carbide paper and treated with the adhesive.

	EDTA	Smear layer	Adhesive
Diamond bur	0.610 ± 0.196	0.060 ± 0.007	0.039 ± 0.010
Silicon carbide paper	0.680 ± 0.173	0.081 ± 0.032	0.051 ± 0.011

Table 2 Means and standard deviations of relative permeability of dentin disc.

	%SMP (Smear layer / EDTA)	%BP (Bonded dentin / EDTA)
Diamond bur	10.40 ± 2.90	6.76 ± 2.52
Silicon carbide paper	12.46 ± 5.44	7.51 ± 2.79

Discussion

From previous studies, the higher cutting speed of diamond burs produced dense and thin layer whereas the smear layers created by SiC paper were thicker but loosely organized that affected the bond strength. [5, 7, 20] Additionally, the effect of the residual of smear layer after application of self-etching adhesive adhesives especially from the surfaces with thick smear layer has been reported. [21] However, the smear layer created by both 360-grit SiC paper with 25.8 microns abrasive and superfine grit diamond burs with 25 microns abrasive produced the same characteristic of smear layer with well organize thin layer. This result was different from previous finding that showed the difference of smear layer characteristic between the preparation with diamond bur and silicon carbide paper. [5, 7, 18] The difference of testing conditions, burs, silicon carbide papers and force might affect to the difference of results. However, the effect of smear layer to bond strength has been reported which to be adhesive dependence. [22, 23]

The maximum permeability of dentin was observed when the dentin was treated with EDTA. From this study, after EDTA treatment, SEM images showed clear tubular structures of dentin without any smear layer or smear plug and the maximum fluid infiltration rate was about 0.610 - 0.680 microlitre/min. The reduction of fluid infiltration through dentin disc was observed after creating of smear layer with both diamond bur and silicon carbide paper. The fluid infiltration rate of smear layer covered dentin was reduced to the rate of about 0.060 – 0.081 microlitre/min. This was corresponding to the %SMP (smear layer/EDTA) that showed the reduction of permeability to 10.40-12.46% comparing to EDTA treated dentin. The reduction of permeability regarding to smear layer coverage are in the line with the study by Yiu and co-worker reported that the permeability of smear layer covered dentin varied from 9-11% of acid etched dentin. [24]

When the smear layer covered specimens were bonded with Single Bond Universal under a pulpal pressure of 20 cmH_2O, the fluid movement of the specimens measured following bonding was 0.039 ± 0.01 microlitre/min for the group that smear layer was created by diamond bur and 0.051 ± 0.01 microlitre/min for the group that smear layer was created by silicon carbide paper without any significant difference. These fluid movement of specimens after adhesive application was less than that of smear layer covered specimens. Although, the adhesive resins apparently bonded to dentin, fluid flow was still recorded through the bonded interfaces. These fluid movements might be large enough to contribute to degradation of resin/dentin interfaces. [25] Therefore, the application of this universal adhesive could reduce the permeability with maximal permeability of EDTA treated dentin about 92.49-93.24% as demonstrated in Table 2. This result might provide higher leakproof than in a previous study reported by Suaro S and co worker. [18] They reported the 73.6-88.8% reduction of dentin permeability when the previous generation one-step self-etch adhesives was applied onto EDTA treated dentin under 20 cmH_2O of pulpal pressure. The reducing tendency of dentin permeability after adhesive application was observed. This result might be caused by the improvement of current universal adhesives.

The imperfect sealing ability of current adhesive resins still be found in this study that confirmed results of several previous studies. Our results confirmed those studies with the additional demonstrating the influence of pulpal pressure on resin/dentin adhesion. [27,28]

The result from this study revealed that the different method for creating smear layer did not affect the fluid movement across resin/dentin interfaces created by a universal adhesive. Thus, the researcher can choose the preferred method to produce the smear layer in the permeability testing. For future study, other parameters like different application modes of dental adhesive or different type of universal adhesives could be investigated that might have an affect on the resin/dentin permeability.

Conclusions

Different methods for creating smear layer did not affect the fluid movement across resin/dentin interfaces created by application of a universal adhesive. In addition, the application of the adhesive could reduce the dentin permeability when comparing with the smear layer covered dentin.

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