Effects of Acetone Evaporation Duration of Dentin Bonding Agent on Microleakage

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Abstract

The purpose of this study was to investigate the effect of acetone evaporation duration of dentin bonding agent on microleakage. Class V cavities (3x4x1.5 mm) were prepared on buccal surfaces of 40 human premolars, 1.5 mm above and below the cemento-enamel junction. Specimens were randomly divided into 4 groups (n=10 in each group), which were applied with dentin bonding agent at 0, 10, 20, and 30 min after dispension before being restored with resin-matrix composite. Specimens were stored in distilled water for 24 h at 37^{o}C before and after being thermocycled for 500 times (5^{o}C-55^{o}C). After being immersed in 0.5% basic fuchsin for 24 h at 37^{o}C, teeth were cut bucco-lingually and evaluated for dye penetration using a light microscope. The results from this study showed that the duration of acetone evaporation did not significantly affect the microleakage level ($p=0.720$), however cervical margins leaked significantly more than occlusal margins ($p<0.05$).

Keywords: Dentin bonding agent, Acetone, Microleakage

Introduction

Adhesive techniques combined with the use of tooth-colored restorative materials are frequently utilized to restore teeth that give good results in anatomy, function, esthetics and invisibleness (Van Meerbeek et al., 2003). Previous studies have shown that acids could be used as a surface treatment before application of acrylic resin (Buonocore, 1955) and resin-matrix composite (Swift et al., 1995). However, the major shortcoming of tooth-colored restoration is polymerization shrinkage. This phenomenon leads to microleakage, which is the clinically undetectable passage of bacteria, fluid, molecules or ions at the tooth/restoration interface (Ferrari & Garcia-Godoy, 2002). Microleakage may lead to secondary caries, postoperative hypersensitivity, and pulpal inflammation (Gallo et al., 2001; Piemjai et al., 2002).

Acetone is used as one of the solvents of dentin bonding systems since it can efficiently remove water from the tooth substrate. One variable which has not been investigated is the microleakage occurred when using the adhesive dispensed after exposure to the environment for a certain period of time. The purpose of this study was to investigate the microleakages affected by application dentin bonding agent after being dispensed for 0, 10, 20, and 30 min.

Materials and Methods

The research protocol was approved by the Ethics Committee for Human Research of Naresuan University. Forty extracted caries-free human premolars were collected and used in this study. They were immersed in 0.1% thymol solution which was used within 3-month storage. Class V cavities (3 mm height x 4 mm width x 1.5 mm depth) were prepared on the buccal surfaces 1.5 mm above and below the Cemento-Enamel Junction (CEJ). The surrounding walls of the cavities were prepared as butt joints using cylinder diamond burs with a high speed hand-piece under air-water spray. The cavities were etched with 37% phosphoric acid for 15 s, rinsed thoroughly with air-water spray for 20 s and gently air-dried for 5 s.

Subsequently, 40 teeth were randomly divided into 4 groups (n=10) according to different durations of dispensation of the dentin bonding agent. For the control group (group A), the bonding agent (Prime & Bond NT, Dentsply, Konstanz, Germany) was immediately applied to the tooth surface. For the experimental groups (group B, C and D), the adhesive was dispensed into a covered plastic dish for 10, 20, and 30 min prior to application, respectively. The bonding

agent was applied in two thin layers, lightly dried for 2 s and light-cured for 20 s. Then, a light-cured resin-matrix composite (Ceram X, Dentsply, Konstanz, Germany) was placed in the cavities incrementally. Each 1.5-mm increment was cured for 40 s. All restorations were polished with the coarse, medium, fine, and ultrafine discs (Soflex, 3M ESPE, St.Paul, MN, USA).

Following the restoration process, the teeth were stored in distilled water for 24 h at 37°C before they were thermocycled 500 times between 5°C and 55°C for 20 s at each temperature. After that, nail vanish was applied to the entire surface of the teeth except for the restorations and approximately 1 mm around them. Teeth were immersed in 0.5% basic fuchsin dye solution at 37°C for 24 h after they were stored in distilled water at 37°C for 24 hours. The teeth were rinsed, cleaned, gently dried, mounted in acrylic epoxy resin, and sectioned in a bucco-lingual direction across the center of the restorations using a slow-speed saw (Isomet 1000, Buehler, USA). The sectioned teeth were examined by two calibrated inspectors for dye penetration levels along the occlusal and cervical margins under a light microscope (Olympus, Model BX50, Tokyo, Japan) at 40x magnification. Criteria for analysis of microleakage are presented in Table 1.

Table 1 Criteria for microleakage analysis.

Score	Criteria
0	No leakage
1	Dye penetration confined to half the depth of cavity wall
2	Dye penetration extending to but not including the axial or pulpal wall
3	Dye penetration extending onto one-fourth of the axial or pulpal wall
4	Dye penetration extending onto half of the axial or pulpal wall

To determine the significant differences in microleakage at different durations after the dispense of dentin bonding agent, the data was analyzed using the Kruskal-Wallis test, corrected for ties. The comparison of microleakage between occlusal surface and cervical surface were assessed using the Mann-Whitney U test. The p-value of less than 0.05 was considered as significant.

Results

The mean and standard deviation (S.D.) of microleakage score in each group are presented in Table 2 and 3. The Kruskal-Wallis test showed that there was no significant difference in leakage between immediate and delayed application of the bonding agents ($p=0.720$). The Mann-Whitney U test showed that the leakage observed on the cervical margins was statistically greater ($p<0.05$) than the leakage found on the occlusal margins.

Table 2 Microleakage scores at different durations after the dispense of dentin bonding agent.

Time (minutes)	Microleakage score		Number of surfaces analyzed	p value
	Mean	S.D.		
0	0.5750	0.6360	40	0.720
10	0.7750	0.8912	40	
20	0.5750	0.6360	40	
30	0.5500	0.6385	40	

Table 3 Microleakage scores on occlusal and cervical surface at different durations after the dispense of dentin bonding agent.

Time (minutes)	Mean ± S.D. of Microleakage score		p value
	Occlusal surface	Cervical surface	
0	0.2000± 0.4104	0.9500± 0.6048	0.000
10	0.2000± 0.5231	1.3500± 0.8127	0.000
20	0.1000± 0.3078	1.0500± 0.5104	0.000
30	0.1000± 0.3078	1.0000± 0.5619	0.000

Discussion

This study examined microleakage affected by delayed application of the acetone-based dentin bonding agent (being dispensed for 0, 10, 20 and 30 min prior to application). Acetone has a relatively high vapor-pressure value (184 mmHg at 20°C) compared to ethanol (43.9 mmHg at 20°C) or water (17.5 mmHg at 20°C). A higher vapor pressure will allow the solvent to evaporate more easily. In support of the previous study, after the solvent evaporation, there was a decrease in the ability of the bonding system to penetrate around the exposed collagen fibers and the opened dentin tubules producing poor and incomplete hybrid layers (Gallo et al., 2001). The penetration of resin into partially demineralized dentin relies on the micromechanical retention between resin and hydroxyapatite crystals, being the key to a successful bonding to dentin. We speculate that the solvent evaporation could affect the effectiveness of the dentin bonding agents.

The present study shows that there was no significant difference in leakage between 0, 10, 20, and 30 min of delayed application. The solvent may be less evaporated due to covered plastic dish. A recent study suggested the importance of adequate care during clinical usage of adhesives (Abate et al., 2000). Containers such as bottles should prevent evaporation of solvents and should be kept tightly closed. Dispensing should be carried out immediately before use and it should be exposed to room environment as little as possible. These are the important clinical steps to avoid changes in the relative proportions of their components.

One of the goals of an ideal restoration is to prevent microleakage. Different methods have been employed to evaluate microleakage around restorations. Dye penetration is frequently used because of its low cost and ease of application. The disadvantages are that it is subjective and that the dye penetration is not indicative of bacterial penetration. The microleakage test using dyes could detect leakage where bacteria could not penetrate.

According to the results, microleakage observed in cervical surfaces was statistically greater than that observed in occlusal surfaces, which is in agreement with the findings in previous studies (Santini et al., 2004; Tredwin et al., 2005). The quality of bonding to dentin surfaces and the microleakage depends on a number of variables. The direction of dentin tubules is important to obtain a hybrid layer, which is considered mandatory in obtaining a lasting marginal seal. At approximately 1 mm from the CEJ, Scanning Electron Microscope (SEM) studies have shown that tubular direction is almost parallel to the cavity floor, therefore the classical hybrid layer is virtually absent. A bevel at this cavo-surface angle could expose dentin tubules leading to a stronger hybrid layer formation (Santini et al., 2004).

Moreover, the difference between leakage in enamel and cementum/dentin margins could be attributed to the fact that cementum and dentin are less mineralized than enamel. Dentin is moist tubular tissue, making adhesion less stable than in enamel. This was expected, as bond strength to enamel is usually higher than bond strength to dentin. Dentin is a less favorable bonding substrate than enamel and the enamel margins of composite restoration are reported to have less leakage than cementum/dentin margins (Tredwin et al., 2005). In addition, the greater permeability of dentin to dyes has been reported as a confusing factor in microleakage tests at the cementum/dentin margin (Cenci et al., 2004).

Conclusions

There was no significant difference in leakage between immediate (0 min) and delayed (10, 20, and 30 min) application of the bonding agents. However, the leakage observed on the cervical margins was greater than the leakage found on the occlusal margins.

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