

CHAPTER III

MATERIALS AND METHODS

Materials

- Optibond FL (Kerr, USA) (3 step-etch and rinse adhesive system)
- Clearfil SE Bond (Kuraray, Japan) (2 step self-etching adhesive system)
- Clearfil S³ Bond (Kuraray, Japan) (1 step self-etching adhesive system)
- Composite resin (Filtek Z250 shade A4, 3M ESPE, USA)
- Cylinder diamond bur Ø 1.0 mm (A20, CrossTech Diamond bur # 010, Thailand)
- Fine, flame-shaped diamond bur (B8F, CrossTech Diamond bur # 012, Thailand)
- Sof-Lex[®] disc (3M ESPE, USA.)
- Sticky wax (Kemdent, Associated Dental Products Ltd, UK)
- Nail varnish (Anne & Florio, Thailand)
- Silver nitrate crystals; analytical grade (Merck Chemicals Ltd, Germany)
- Film developer (Kodak GBX Developer and replenisher, Kodak Co., USA.)

Equipments

- Light curing unit (The Elipar[™] FreeLight 2, 3M ESPE, USA)
- Radiometer (Model 100 Optilux, Kerr, USA)
- Mobile unit (Kavo, Heidelberg, Germany)
- Thermo Cycling Unit (King Mongkut's Institute of technology Ladkrabang, Thailand)
- Incubator (Contherm 160M, Contherm Scientific Ltd., New Zealand)
- Slow speed cutting machine (model ISOMET 1000, Buehler, Illinois, USA)
- Stereomicroscope (ML9300 MEIJI, Japan)

Product	Manufacturer	Composition	Application
OptiBond FL	Kerr, USA	Etchant: 37.5% phosphoric acid, silica thickener Primer: HEMA, GPDM, PAMM, ethanol, water, photoinitiator Bond liquid: TEGDMA, UDMA, GPDM, HEMA, bis-GMA, ytterbium trifluoride, fillers, photoinitiators, stabilizers	Apply etchant for 15 sec, rinse for 15 sec; gently air dry, coat with primer with light agitation for 15 sec; gently air dry to remove solvent, apply adhesive in a uniform thin layer and light cure for 30 sec.
Clearfil SE Bond	Kuraray Co Ltd, Osaka, Japan	Primer: MDP, HEMA, hydrophilic dimethacrylate, photoinitiators, water Bond liquid: MDP, bis-GMA, HEMA, hydrophobic dimethacrylate, photoinitiators, filler	Apply primer for 20 sec; gently air blow to dry solvent; apply adhesive; gently blow again and light cure for 10 sec.
Clearfil S ³ Bond	Kuraray Co Ltd, Osaka, Japan	Water, MDP, bis-GMA, HEMA, hydrophobic DMA, CQ, ethyl alcohol, silanated colloidal silica	Apply adhesive for 20 sec; vigorously air blow for 5 sec, and light cure for 10 sec.

Table 1 Chemical composition and manufacturers' instructions of adhesives which were investigated in the present study

Tooth selection

Sixty human premolars, extracted for orthodontic purpose free of cracks, caries and restoration, stored in 0.1% thymol solution at 4°C, were used within 1 month after extraction. The teeth collected after the patient's informed consent, were obtained under a protocol approved by the Ethical Review Board of the Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand. A standard class V cavity was prepared on the buccal surface, locating across the cemento-enamel junction.

Cavity preparation

A class V cavity was prepared on buccal surface, locating across the cemento-enamel junction. Cavity preparation was made by using a cylinder diamond bur at high speed with constant water coolant; so that occlusal cavosurface margin located in enamel with a 45° bevel of 1.0 mm width placed with a fine, flame-shaped diamond bur (CrossTech, Thailand). The gingival margin in dentin/cementum was prepared to a butt joint. All burs were discarded after preparation of two teeth. Cavity dimensions were approximately 1.5 mm deep, 3.0 mm (mesio-distally) wide and 3.0 mm high (occluso-gingivally) as shown in Figure 3.

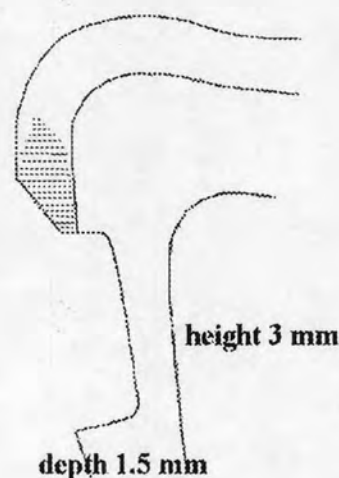


Figure 3 Design of class V cavity preparation.

Restorative procedure

The sample teeth were randomly divided into 6 groups with 10 samples each

Group 1 Optibond FL (used following manufacture's instruction)

A cavity was etched by 37.5% phosphoric acid for 15 seconds, rinsed with water for 15 seconds, air dried readily leaving moisture on tooth surface, and then cavity was coated with primer using light agitating action for 15 seconds, gently air dried for at least 5 seconds to remove solvent. Adhesive was then applied in a uniform thin layer and exposed with a curing light for 30 seconds.

Group 2 Clearfil SE Bond (used following manufacture's instruction)

Cavity was coated with Clearfil SE Primer for 20 seconds, gently air dried to remove solvent, and then coated with Clearfil SE Bond, gently air dried again and exposed with a curing light for 10 seconds.

Group 3 Clearfil S³Bond (used following manufacture's instruction)

Cavity was coated with Clearfil S³Bond for 20 seconds, vigorously air dried for 5 seconds, and exposed with a curing light for 10 seconds.

Group 4 Optibond FL (modified application by selective enamel acid etching)

A cavity was etched by 37.5% phosphoric acid for 15 seconds, rinsed with water for 15 seconds, air dried readily leaving moisture on tooth surface, and then the entire cavity was coated with primer using light agitating action for 15 seconds, gently air dried for at least 5 seconds to remove solvent. Adhesive is then applied in a uniform thin layer to the entire cavity and exposed with a curing light for 30 seconds.

Group 5 Clearfil SE Bond (modified application by selective enamel acid etching)

A cavity was etched by 37.5% phosphoric acid for 15 seconds, rinsed with water for 15 seconds, air dried, and then both enamel and dentin were coated with Clearfil SE Primer for 20 seconds, gently air dried, and then coat with Clearfil SE Bond to the entire cavity, gently air dried and exposed with a curing light for 10 seconds.

Group 6 Clearfil S³Bond (modified application by selective enamel acid etching)

37.5% phosphoric acid was applied on enamel only for 15 second, rinsed with water for 15 seconds, air dried. Clearfil S³Bond was then applied to the entire cavity for 20 seconds, vigorously air blown for 5 seconds, and exposed with a curing light for 10 seconds.

The placement of the restorative resin composite Z250 (3M ESPE, USA) was performed incrementally as shown in Figure 4. The first layer was inserted occlusally and obliquely, with the second layer against gingival wall of the cavity. Each increment was light activated for 40 seconds. Excess of resin composite in all the filled specimens was removed with a number 12 blade, and then polished with a series of abrasive discs (Sof-Lex[®], 3M ESPE, USA).

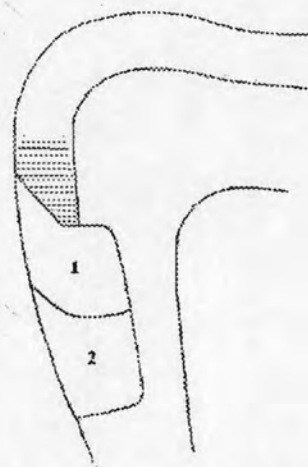


Figure 4 Incremental placement of resin composite

Thermocycling and microleakage preparation

The teeth were kept for 24 hours in 37 °C distilled water, followed by 5,000 thermal cycles between 5±1 and 55±1 °C with a dwell time of 30 seconds. All remaining surfaces were coated with 2 coated of nail varnish, except for 1 mm around the restoration. The root apices were sealed with sticky wax.

Evaluation of Silver Uptake

The teeth were immersed in a 50 wt% silver nitrate solution for 24 hrs. The silver-stained specimens were then rinsed with distilled water and placed in photodeveloping solution for 8 hrs under a fluorescent light to reduce the diamine silver ions into metallic silver grains.

Microleakage evaluation

Samples were sectioned longitudinally across the resin-dentin interface from facial to lingual surface into 0.8 mm-thick serial slabs using a slow speed cutting machine (model ISOMET 1000, Buehler, USA). Three longitudinal sections were performed: one in the middle and the other close to the mesial and distal margins of the restoration as shown in Figure 5. Three sections from each tooth were used for the experiments and evaluated for microleakage at the enamel and dentin margins under a stereomicroscope (ML9300 MEIJI, Japan) at 15x magnification.

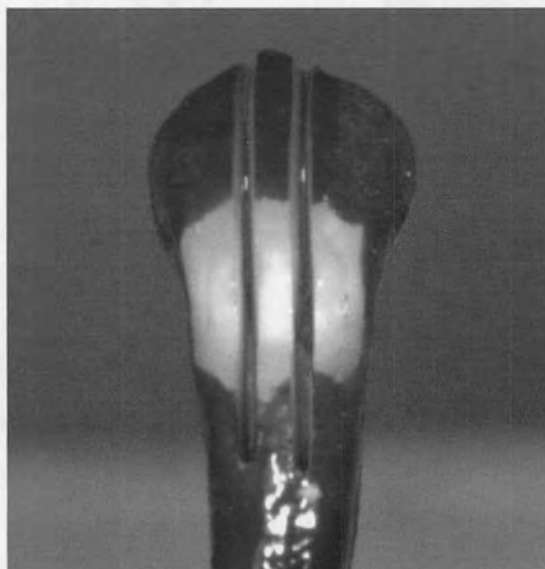


Figure 5 Three longitudinal sections were performed for microleakage evaluation.

Tracer penetration was evaluated by two calibrated examiners. When disagreement occurred, consensus was obtained. Scores applied for qualifying microleakage were recorded as distance of penetration along resin bonded interface. Enamel and dentin margins were scored separately for microleakage using 0-4 ordinal ranking systems. (Degree of penetration score are shown in Figure 6)

- 0 = No dye penetration
- 1 = Dye penetration no more than 1/3 of the cavity depth
- 2 = Dye penetration no more than 2/3 of the cavity depth
- 3 = Dye penetration more than 2/3 of the cavity depth
- 4 = Dye penetration along the axial wall

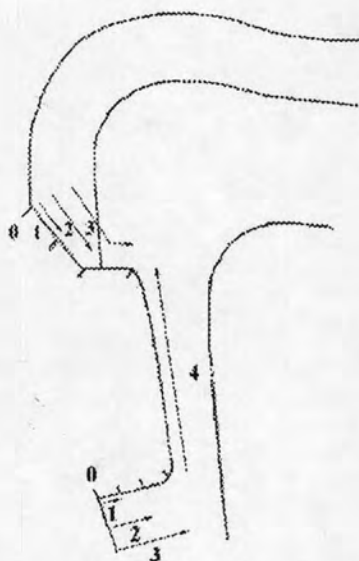


Figure 6 Score of microleakage

Data analysis

The differences in a frequency distribution of microleakage scores at enamel and dentin margins among the adhesive groups were analyzed using Chi-Square test. An inter-group comparison was performed using Wilcoxon Signed Rank test. All tests were conducted with statistical significance set at $\alpha = 0.05$ using SPSS version 13.0 (Chicago, IL, USA).