CHAPTER III

EXPERIMENTAL DESIGN

Materials

1. 18 non-carious, non-defect human premolar (extracted for orthodontics treatment)
2. 600 grit Silicon carbide (SiC) abrasive papers, Buehler, Lake Bluff, IL, USA
3. Topical anesthetic gel, Benzo-jel, Henry Schein Inc., USA (details in Table 1)
4. Adhesives (details in Table 1)
    a. Two- step self-etch adhesive system, Clearfil Protect Bond, Kuraray Med. Inc., Japan
    b. One-step self-etch adhesive system, Clearfil Tri-S Bond, Kuraray Med. Inc., Japan
    c. Two-step total-etch adhesive system, Single Bond Plus, 3M, ESPE, USA
5. Resin composite, Clearfil AP-X , Kuraray Med Inc., Japan (details in Table 1)
6. Distilled water
7. Stop watch
8. Cyanoacrylate adhesive, Zapit, Dental Ventures of America, CA, USA

Table 1 The Details of Materials Used in this Study

Materials/Companies	Lot. No.	Compositions
Benzo-jel Henry Schein Inc., USA	24205	polyethelylene glycol water base, 20% benzocaine, flavoring, sodium saccharin
Clearfil Protect Bond Kuraray Med. Inc., Japan	Primer: 000010 (pH=2) Bonding:000017	Primer: MDP, MDPB, HEMA, hydrophilic dimethacrylate, water Bonding agent: MDP, HEMA, Bis-GMA, hydrophobic dimethacrylate, silanated colloidal silica, N,N-diethanol-P-toluidine, d,l-camphorquinone, sodium fluoride
Clearfil Tri-S Bond Kuraray Med. Inc., Japan	040219 (pH=2)	MDP, HEMA, Bis-GMA, silinated colloidal silica, d, l-camphorquinone, ethyl alcohol, water
Single Bond Plus 3M, ESPE, USA	Etchant: 5CL (pH=1) Bonding: 5CJ	Etchant: 35% phosphoric acid gel Bonding agent: HEMA, Bis-GMA, water, copolymer of acrylic & itaconic acid, ethyl alcohol, UDMA, silica nanofiller, glycerol 1,3-dimethacrylate
Clearfil AP-X Kuraray Med. Inc., Japan	00800A	Bis-GMA, TEGDMA, d,l-camphorquinone, silanated silica

MDP= 10-methacryloyloxydecyl dihydrogen phosphate, MDPB= 12-methacryloyloxydodecylpyridinium bromide,
HEMA= 2-hydroxyethyl methacrylate, Bis-GMA= bisphenol A diglycidylmethacrylate,
TEGDMA= triethyleneglycol dimethacrylate UDMA= urethane dimethacrylate

Instruments

1. Ultrasonic and hand scaler
2. Low speed cutting machine, Isomet Buehler, Lake Bluff, IL, USA (Figure 1)

Figure 1 Isomet Cutting Machine

3. Light curing unit (Optilux 501, Kerr, USA)
4. Digital micrometer (Mitutoyo, Japan)
5. Universal testing machine, EZ-Test, Shimadzu Corporation, Kyoto, Japan (Figure 2)

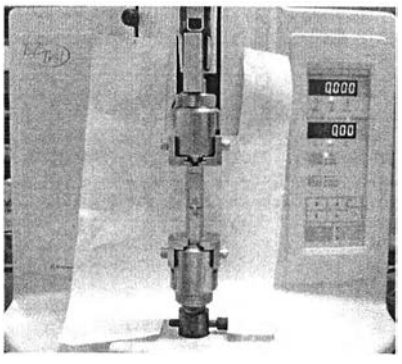

Figure 2 EZ-Test Testing Machine

6. Desiccator (Drykeeper, Sanplatec Corp., Japan)
7. Scanning electron microscope (JSM-5410LV, JEOL, Japan)

Methodology

1. Preparation for the micro-tensile testing

1.1 Eighteen non-carious extracted human premolars were collected in 0.1% thymol solution at room temperature. The teeth were cleaned by ultrasonic and hand scaler to remove soft tissue and debris, then stored in distilled water at 4 ±1 °C until used.

1.2 The teeth were randomly assigned into three groups according to the adhesives.

1.3 The teeth were cut perpendicular to the long axis approximately 1.0 mm above the CEJ (Tjan *et al.*, 1996) by a low speed cutting machine (Figure 1) under running water to expose the dentin surfaces (Figure 3a). Because the temperature in the refrigerator was different from the room temperature, the teeth were left for 10 minutes to equilibrate to the environment before cutting.

1.4 The superficial dentin surface was polished by silicon carbide abrasive paper (grit #600) under running water (Burrow *et al.*, 1994; Frankenberger *et al.*, 2001; Say *et al.*, 2005).

1.5 For control groups, after drying, adhesives were applied onto the dentin surfaces using an applicator following the manufacturers' instructions. The bonding procedures are shown in Table 2.

Table 2 Bonding Procedures

Material	Etching	Priming	Bonding
Clearfil Protect Bond	-	Apply 20sec, air dry	Apply with brush, air thin, light cure 10sec
Clearfil Tri-S Bond	-	-	Apply 20sec, air with high pressure 5sec, light cure 10sec
Single Bond Plus	Etch15sec, rinse 10sec, blot dry	-	Apply 3 coats 15sec, air 5sec, light cure 10sec

1.6 For experimental groups, a topical anesthetic gel (Benzo-jel, Henry Schein Inc., USA) was applied using a brush on exposed dentin, left for 5 minutes then rinsed with water for 30 seconds and gently air dried before an application of the adhesives and restoration with resin composite.

1.7 The resin composite was placed on the tooth surface approximately 1.5 mm thick in 3 incremental layers to ensure adequate height of the specimens for the microtensile testing (Figure 3b). Each layer was light cured (Optilux 501, Kerr, USA) for 40 seconds at 600 mW/cm^2 output.

1.8 The bonded specimens were stored in distilled water at 37 ±1 ^{o}C for 24 hours (Frankenberger *et al.*, 2001; Say *et al.*, 2005).

1.9 The bonded specimens were sectioned by a low speed saw (Figure 1) perpendicular to the bonded surfaces to obtain stick-shape specimens with a square cross-sectional bonded surface area of 1.0 mm^2 (Sonoda et al., 2005) (Figure 3c). Twenty specimens were obtained for each subgroup.

1.10 The thickness and width of a stick were determined by a digital micrometer (Mitutoyo, Japan) (Figure 3d).

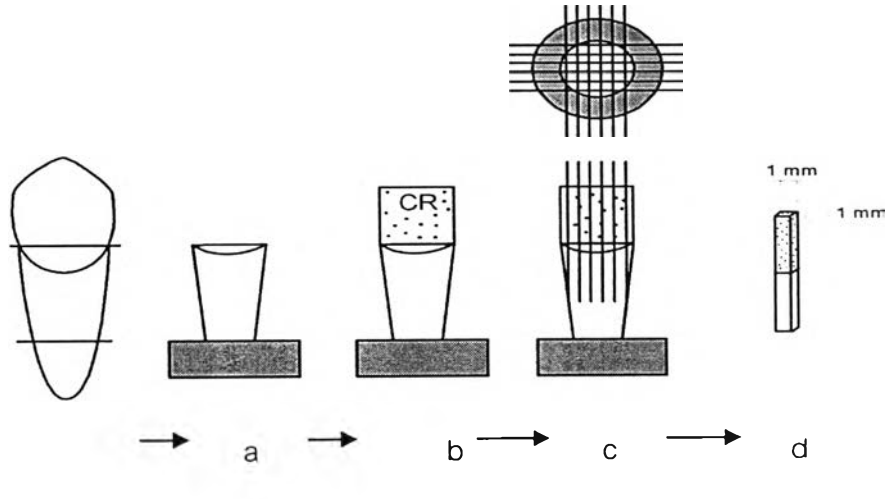

Figure 3 Specimen Preparation

a: a tooth will be cut perpendicular to the long axis at 1mm above cemento-enamel junction

b: resin composite will be built-up on dentin surface

c: serial sectioning will be conducted on the specimen

d: specimen with cross-sectional area of 1 mm^2 for the micro-tensile testing

1.11 The dentin-composite sticks were cemented to the testing device with cyanoacrylate adhesive. For each group, twenty specimens were subjected to micro-tensile bond test using a universal testing machine (Figure 2) at a testing speed of 1.0 mm/min (Say *et al.*, 2005) until fracture occurred.

1.12 The maximum loads at break (KgF) were recorded and converted to the bond strength values (MPa).

After fracture, all fractured surfaces will be observed by a scanning electron microscope to identify the mode of failure.

2. Preparation for SEM analysis

2.1 The fractured surfaces of dentin and composite were adhered to aluminum stubs with a carbon tape.

2.2 Stored in a desiccator for 24 hours.

2.3 The surfaces were sputter-coated with gold for 2 minutes.

2.4 All fractured surfaces were examined using SEM and allocated to one of five failure types;

Type 1: adhesive failure at dentin-resin interface, if the majority part of the bonded interface failed between dentin and the bonding resin

Type 2: cohesive failure in dentin, if the majority of the bonded interface failed in dentin

Type 3: cohesive failure in adhesive resin, if the majority part of the bonded interface covered with adhesive resin

Type 4: mixed, if the failures were partially adhesive and partially cohesive in resin and/or dentin

Type 5: cohesive failure in resin composite, if the majority part of the bonded interface failed in resin composite

Analyses of Data

Because data distribution is normal, it was analyzed by independent t-test and multiple comparisons Bonferroni's test. Failure modes were analyzed by Chi-Square Tests.

All data was analyzed at 95% significant levels (a p-value of 0.05) using a computer statistics package SPSS for Windows Version 11.5 (SPSS Inc., Illinois, USA).