CHAPTER III

MATERIALS AND METHODS

I. Materials

The following materials were used in the experiments.

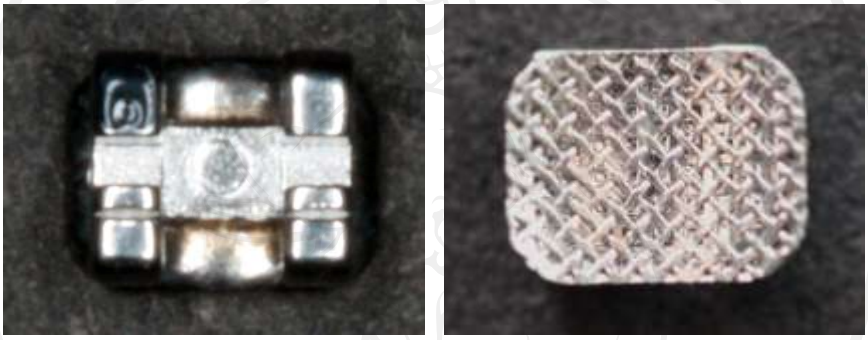
1. Samples

One hundred and sixty maxillary first premolar teeth (Figure 11), extracted for orthodontic reasons, were included in this study. The inclusion criteria were that the buccal surface of all teeth had sound enamel, with an absence of caries, restorations, flourosis (Tooth Surface Index of Fluorosis/TSIF score is '0')[58] or other enamel defects. The extracted teeth were stored in 0.1% (weight/volume) thymol solution for one to six months prior to the bonding process. Random number tables were used to randomly categorize all teeth into eight groups of 20 premolar teeth each.

Figure 11 An extracted maxillary first premolar**.**

2. Brackets

The Mini Masters Series brackets (American Orthodontics, Sheboygan, Wisconsin, USA) (Figure 12) had the following characteristics: a) 17-4 stainless steel bracket with 0.022 x 0.028 inch-slot, b) Roth prescription, c) the bracket base had a mono-layered mesh pattern with diagonal metal wires size 110 μm. The total projected surface area of the bracket base was 8.82 square millimeters.[59]



a. **b.**

Figure 12 a. Metal bracket, **b.** Base of bracket.

3. Adhesive systems

Four types of adhesives systems were used in the present study.

3.1 Transbond™ XT total-etching system

- 35% Phosphoric acid (Scotchbond™, 3M Unitek®, Monrovia, California, USA) (Figure 13)
- Transbond™ XT primer (3M Unitek®) (Figure 14)
- Transbond™ XT Light Cure Adhesive Paste (3M Unitek®) (Figure 14)

Figure 13 35% Phosphoric acid.

Figure 14 Transbond™ XT primer and Transbond™ XT Light Cure Adhesive Paste.

3.2 Transbond™ PLUS self-etching system

- Transbond™ Plus Self Etching Primer (3M Unitek®) (Figure 15)
- Transbond™ PLUS Color Change Adhesive (3M Unitek®) (Figure 16)

Figure 15 Transbond™ Plus Self Etching Primer.

Figure 16 Transbond™ PLUS Color Change Adhesive.

3.3 Beauty Ortho Bond® self-etching system (Shofu®, Kyoto, Japan) (Figure 17)

- Primer A and B
- Beauty Ortho Bond® Paste
- Salivatect

Figure 17 Beauty Ortho Bond®.

3.4 Assure® total-etching system (Reliance® Orthodontic Products, Inc., Itasca, Illinois, USA) (Figure 18)

- 37% Phosphoric acid
- Assure® Bonding Resin
- Assure® Light Cure Adhesive

Figure 18 Assure® total-etching system.

4. Artificial saliva

An artificial saliva was produced by the Department of Pharmacy, Faculty of Medicine, Chiang Mai University. The composition of the artificial saliva[60] is shown in Table 3.

Table 3 The composition of artificial saliva 1,000 ml.

Chemical agent	Weight
Carboxymethylcellulose Sodium	1.0000 g
Glycerin	5.0000 g
Sodium Chloride	0.0812 g
Potassium Chloride	0.1490 g
Magnesium Chloride.6H_2O	0.01255 g
Calcium Chloride	0.01662 g
Sodium Monohydrogen Phosphate.7H_2O	0.12372 g
Sodium Dihydrogen Phosphate.2H_2O	0.03737 g
Sodium Fluoride	0.01105 g

5. Supplies

5.1 Fluoride-free pumice.

5.2 0.020 inch stainless steel wires.

5.3 0.016 x 0.022 inch stainless steel wires.

5.4 Cylindrical polyvinylchloride rings, 26 mm in outside diameter, 16 mm in height and 2 mm thickness (Figure 19).

Figure 19 Cylindrical polyvinylchloride ring.

5.5 Elastic ligatures.

5.6 2.0 mm thickness of clear plastic plate.

5.7 Self-cured acrylic resin and monomer.

6. Distilled water

Distilled water was prepared by the Dental Materials Laboratory Unit, Faculty of Dentistry, Chiang Mai University.

7. Thymol solution

A 0.1% (weight/volume) thymol solution was prepared by dissolving 10 g of thymol in 100 ml of 95% ethyl alcohol.

II. Instruments

1. Sectioning saw

An IsoMet® 1000 sectioning saw (BUEHLER®, Lake Bluff, Illinois, USA) (Figure 20) was set to section the teeth at a speed of 100 rpm**.**

Figure 20 IsoMet® 1000.

2. Force gauge

A Tension and Compression Gauge (DENTAURUM GmbH & Co. KG, Ispringen, Germany) (Figure 21) was used to calibrate force for bracket placement.

Figure 21 Tension and Compression Gauge.

3. Pipette

A Proline® mechanical pipette (Biohit®, Helsinki, Finland) covering the volume range 20-200 µl (Figure 22) was utilized to fix the volume at 20 µl.

Figure 22 Proline® mechanical pipette.

4. Light curing unit

A Mini LED™ light curing unit (Satelec®, Acteon, Merignac Cedex, France) (Figure 23) provided light intensity at 1,250 mW/cm^2.

Figure 23 Mini LED ™ light curing unit.

5. Incubator

An incubator Model 200 (Memmert Corporation, Bayern, Germany) (Figure 24) was set to maintain the temperature at 37±1 °C.

Figure 24 Incubator.

6. Thermocycling machine

The thermocycling equipment (Medical and Environment Equipment Research Laboratory, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand) consisted of three parts (Figure 25):

6.1 Thermocycling machine (model TC 301) was set for 2,000 thermal cycles.

6.2 Hot-water bath (model HWB332R) was set at 55^{o}C.

6.3 Cold-water bath (model CWB332R) was set at 5^{o}C.

Figure 25 Thermocycling equipment.

7. Instron® Universal Testing Machine

An Instron® Universal Testing Machine (model number 5566**,** Instron Ltd., High Wycombe, England**)** was used for measuring shear bond strength with a load cell of 500 Newtons, and the data was analyzed with the Bluehill software CAT No. 2603-080 (Instron, Norwood, MA, USA) (Figure 26).

8. De-bonding plate

A de-bonding plate was designed to apply vertical force under the bracket wings (Figure 27).

9. Mounting jig

A mounting jig was designed to hold the cylindrical polyvinylchloride ring in position with the bracket base parallel to the direction of force (Figure 28).

Figure 26 Instron® Universal Testing Machine.

Figure 27 De-bonding plate.

Figure 28 Mounting jig**.**

III. Methods

The study was divided into three parts.

1. Shear bond strength testing
2. Adhesive remnant evaluation
3. Statistical analysis

1. Shear bond strength testing

1.1 One hundred and sixty orthodontic upper first premolar teeth were sectioned using an IsoMet® 1000 sectioning saw at 2 to 3 mm below the cemento-enamel junction before being embedded. All buccal tooth surfaces were gently polished with fluoride-free pumice and a rubber cup for 10 seconds, rinsed with water for 10 seconds, and dried with an oil-free air source. The lingual half of each sample was embedded in a clay block, which was used as a stabilizer (Figure 29). The long axis of the samples were laid as parallel as possible to the base of the block.

Figure 29 Tooth sample embedded in clay block.

1.2 The 160 orthodontic upper first premolar metal brackets were used. All teeth were bonded to the specimens according to one of the following eight protocols:

Group I (Control): The enamel surfaces were treated with 35% phosphoric acid etching gel for 15 seconds, rinsed for 20 seconds, and dried with an oil-free air stream. Transbond™ XT Light Cure Adhesive Primer was applied on the etched surfaces with a microbrush, and the brackets were bonded to the tooth surface using Transbond™ XT Light Cure Adhesive Paste.

Group II: The enamel surfaces were treated with 35% phosphoric acid etching gel for 15 seconds, rinsed for 20 seconds, and dried with an oil-free air stream. Transbond™ XT Light Cure Adhesive Primer was applied with a microbrush on the etched surfaces. Artificial saliva 0.02 ml was dropped onto the primed surface with a micropipette, left for 10 seconds and then blown off with an air syringe for five seconds. After that, the brackets were immediately bonded using Transbond™ XT Light Cure Adhesive Paste.

Group III: Transbond™ Plus Self Etching Primer was applied and rubbed with a microbrush on the enamel surfaces for approximately three seconds. An oil-free air stream was gently applied to the enamel, and the brackets were bonded using Transbond™ PLUS Color Change Adhesive.

Group IV: Transbond™ Plus Self Etching Primer was applied and rubbed with a microbrush on the enamel surfaces for approximately three seconds. An oil-free air stream was gently applied to the enamel. Artificial saliva 0.02 ml was dropped onto the primed surface with a micropipette, left for 10 seconds and then blown off with an air syringe for five seconds and the brackets were immediately bonded using Transbond™ PLUS Color Change Adhesive.

Group V: Beauty Ortho Bond® Primers A and B were mixed. The enamel surfaces were rubbed with the mixture for approximately three seconds. An oil-free air stream was gently applied to the enamel, and the brackets were bonded using Beauty Ortho Bond® Paste.

Group VI: Beauty Ortho Bond® Primers A and B were mixed. The mixed primers were then rubbed onto the enamel for approximately three seconds. An oil-free air stream was gently applied to the enamel. Then SALIVATECT was applied on the primed tooth surface according to the manufacturer's directions. Artificial saliva 0.02 ml was dropped onto the surface with a micropipette, left for 10 seconds and then blown off with an air syringe for five seconds.

The brackets were immediately bonded using Beauty Ortho Bond® Paste.

Group VII: The enamel surfaces were treated with 37% phosphoric acid etching gel for 15 seconds, rinsed for 20 seconds, and dried with an oil-free air stream. Assure® Bonding Resin was applied with a microbrush on the etched surface, and the brackets were bonded using Assure® Light Cure Adhesive.

Group VIII: The enamel surfaces were treated with 37% phosphoric acid etching gel for 15 seconds, rinsed for 20 seconds, and dried with an oil-free air stream. Assure® Bonding Resin was applied with a microbrush on the etched surface. Artificial saliva 0.02 ml was dropped onto the primed surface with a micropipette, left for 10 seconds and then blown off with an air syringe for five seconds. The brackets were then immediately bonded using Assure® Light Cure Adhesive.

The study design is shown in Table 4.

Table 4 The study design.

Group	Etching and priming	Conditions	Adhesives
I	35% Phosphoric acid + Transbond™ XT Light Cure Adhesive Primer	Non-contaminated	Transbond™ XT Light Cure Adhesive Paste
II	35% Phosphoric acid + Transbond™ XT Light Cure Adhesive Primer	Saliva-contaminated	Transbond™ XT Light Cure Adhesive Paste
III	Transbond™ Plus Self Etching Primer	Non-contaminated	Transbond™ PLUS Color Change Adhesive
IV	Transbond™ Plus Self Etching Primer	Saliva-contaminated	Transbond™ PLUS Color Change Adhesive
V	Beauty Ortho Bond® Primers A and B	Non-contaminated	Beauty Ortho Bond® Paste
VI	Beauty Ortho Bond® Primers A and B	Saliva-contaminated	Salivatect + Beauty Ortho Bond® Paste
VII	37% Phosphoric acid + Assure® Bonding Resin	Non-contaminated	Assure® Light Cure Adhesive
VIII	37% Phosphoric acid + Assure® Bonding Resin	Saliva-contaminated	Assure® Light Cure Adhesive

1.3 All brackets were firmly placed on the teeth at the middle part of the buccal surface by one experienced operator using the Tension and Compression Gauge to define 300 *g* of force for three seconds in order to achieve a comparable resin layer thickness.[20] An orthodontic sickle was used to remove any excess adhesive. A 0.020 inch stainless steel wire was put in the bracket slot to determine the space between the bracket and the light guide tip before light curing. The wire was black-colored marked with a 5-mm distance at both ends. The light tip was set at

the end of the wire, away from the interproximal edges of the bracket base and parallel to the lateral edge of the bracket (Figure 30).

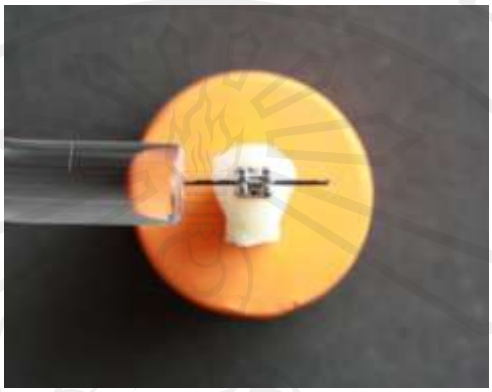


Figure 30 The placement of light tip 5-mm away from the interproximal edges of the bracket base.

1.4 Prior to activation with the Mini LED^{TM} curing unit, the built-in radiometer was used to check light intensity. The curing unit was set in the fast mode that emitted light at an intensity of 1,250 Mw/cm^2 instantly. The light guide tip was placed in the aperture at the front part of the base. The curing unit handpiece was turned on, and when the green light in the base illuminated, indicating adequate light intensity, the handpiece was used to cure the adhesive (Figure 31).

Figure 31 Light intensity check for Mini LED^{TM} curing unit.

All samples were light cured for 10 seconds equally on both mesial and distal aspects of the teeth. The flow chart of the bracket bonding procedure according to the manufacturers's instruction is shown in Figure 32.

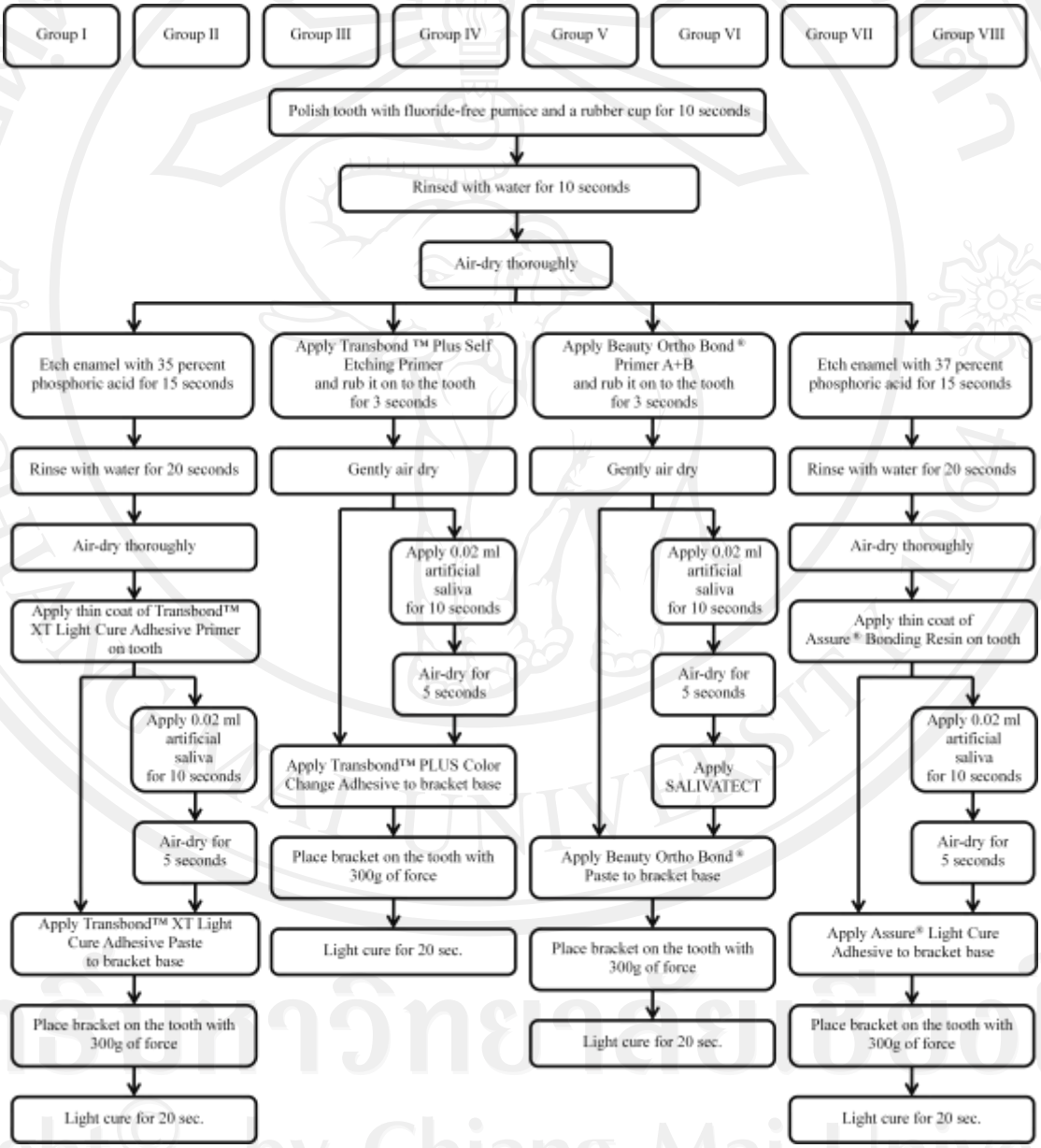


Figure 32 Flow chart of bracket bonding procedure.

(Modified from Yussa, *et al*.,2009)

1.5 After the bonding procedure, each tooth was prepared for embedding in a cylindrical polyvinylchloride ring block. The clay block and the 0.020 inch wire were removed from the teeth. A 0.016 x 0.022 inch straight stainless steel wire was placed in the bracket slot and an elastomeric ligature was used to tie each bracket. In order to control the angulation of the mounted tooth and direction of the force applied, the 0.022 inch side of the wire was fully seated in the bracket slot.

Each wired sample was installed in the center hole of a 2-mm thick flat plastic plate. The 0.022 inch side of the wire was placed parallel on the surface of the plate. The tooth was hung in the middle of the hole (Figure 33). The wire was fixed with adhesive tape to control the angulation of the wire on the plate. The whole set of tooth with wire and plastic plate was placed on the top of a polyvinylchloride ring. The lingual part of the tooth was embedded in the center of the ring. Self-cured acrylic resin was used to fill the space in the polyvinylchloride ring to allow exposure of only the tooth-bracket assembly surface. All samples were left until the acrylic resin was fully cured. The elastomeric ligatures, wires and plastic plates were then removed (Figure 34).

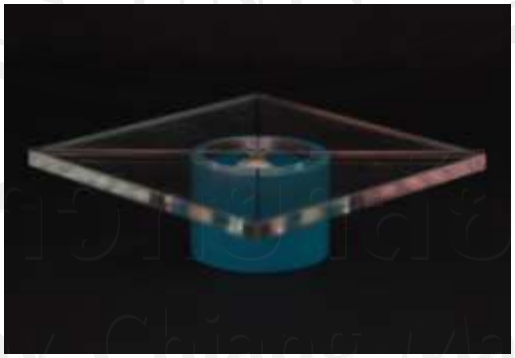


Figure 33 Sample in a polyvinylchloride ring held with a 0.016 x 0.022 inch stainless steel wire, tied with an elastomeric ligature on a 2-mm plastic plate.

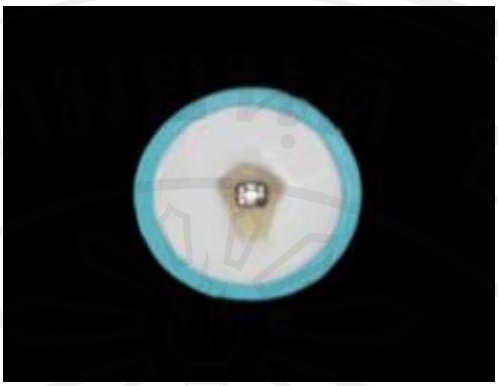


Figure 34 Sample embedded in polyvinylchloride ring with self cured acrylic resin.

1.6 All samples were incubated in distilled water at 37°C for 24 hours. Then a thermocycling procedure was performed using a thermocycling machine in cold-water bath at 5°C and hot-water bath at 55°C for 30 seconds per bath and with a transfer time of 10 seconds for 2,000 cycles (Figure 35 a, b, and c).

a.

b.

c.

Figure 35 a. Thermocycling machine set at 2,000 cycles, **b.** Cold-water bath set at 5° C, and **c.** Hot-water bath set at 55° C.

1.7 After completion of the thermocycling process, the brackets were de-bonded with a Universal Testing Machine (Instron® testing machine). The de-bonding plate was fixed into the upper pneumatic grip, while the mounting jig was attached to the lower pneumatic grip (Figure 36). The polyvinylchloride ring was attached to the mounting jig. The de-bonding plate was vertically adjusted and force applied to the ligature groove between the bracket base and wings at the time of testing (Figure 37). Brackets were de-bonded from the tooth surfaces using the Instron® testing machine at a cross head speed of 0.5 mm per minute and a load cell of 500 newtons. The occluso-gingival force was provided parallel to the buccal tooth surface, until the bracket was dislodged from the tooth surface. The force values in Newtons were divided by the area of the bracket base, which is 8.82 square millimeters.[59] The bond strength for removing brackets was recorded in units of Megapascals (MPa). All bonding and de-bonding procedures were performed by one operator.

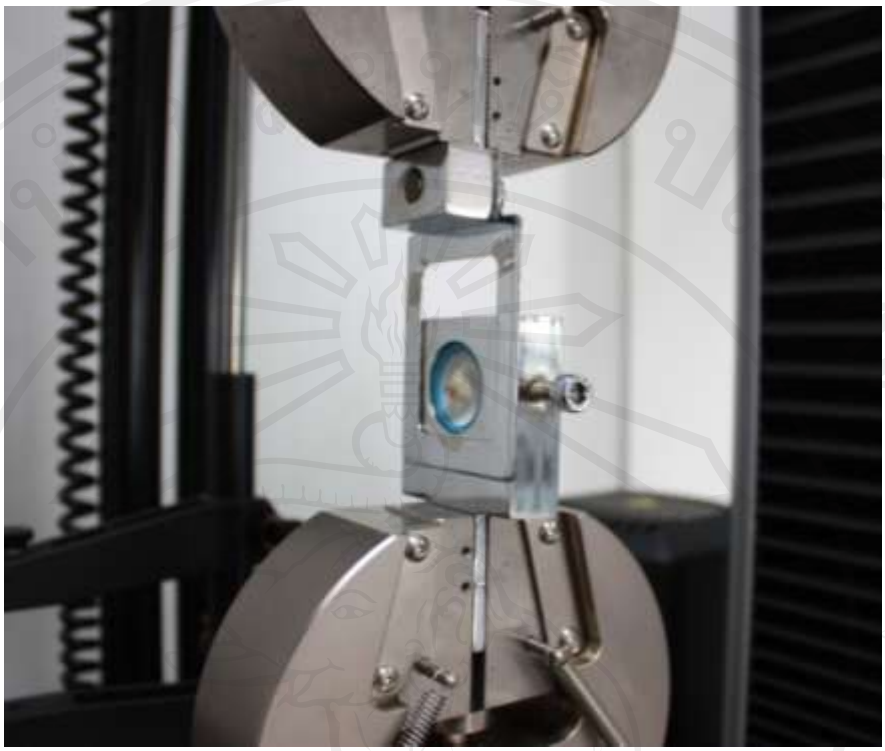


Figure 36 Polyvinylchloride ring mounted into the mounting jig attached to the lower pneumatic grip with the de-bonding plate attached to the upper pneumatic grip.

Figure 37 The apparatus assembled for testing shear bond strength.

2. Adhesive remnant evaluation

Adhesive remnants on the enamel surfaces were evaluated by converting the reciprocal of the bracket base. A digital single-lens reflex camera (Canon 450D, Canon Inc., Tokyo, Japan) and a Canon macro lens at 1x magnification was used to take a photograph of the adhesive remnant on each bracket base. The amounts of adhesive left on the bracket base were measured by superimposing the pictures of the bracket base on a line grid using Adobe Photoshop CS3 version 10.0 software (Adobe Systems Incorporated, San Jose, California, USA) (Figure 38). The area of the adhesive left on the bracket base and the adhesive-free area were calculated. Each specimen was assigned an Adhesive Remnant Index (ARI) value according to the following criteria:[61]

• Score "0" = no adhesive retained on the specimen (tooth) (Figure 39 a).

• Score "1"= less than or equal to 50 percent of the adhesive retained on the specimen (Figure 39 b).

• Score "2"= greater than 50 percent, but less than 100 percent of the adhesive retained on the specimen (Figure 39 c).

• Score "3" = all adhesive retained on the specimen, with a distinct imprint of the bracket base. (Figure 39 d).

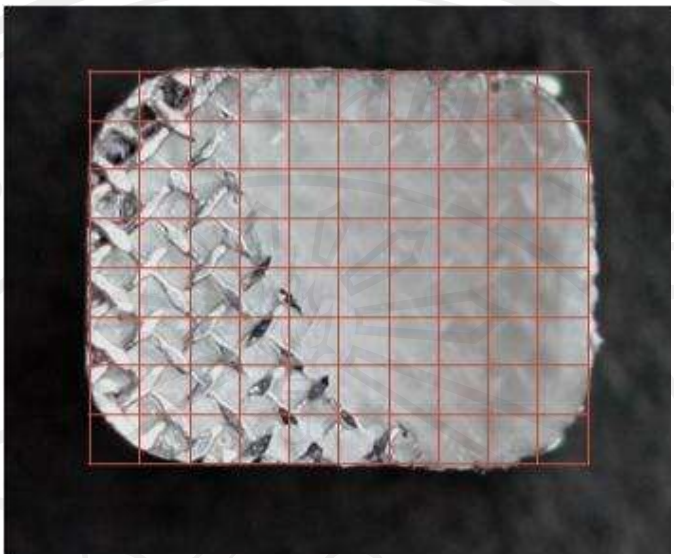


Figure 38 Bracket base superimposed on a line grid.


a.

b.

c.

d.

Figure 39 ARI **a.** Score “0”, **b.** Score “1”, **c.** Score “2”, **d.** Score “3”.

3. Statistical analysis

The SPSS for Windows Release 17.0 program (IBM Corporation, Armonk, New York, USA) was used to calculate the following analysis:

3.1 The shear bond strength values in each sample group were described by means, standard deviations, and ranges.

3.2 Analysis of variance (ANOVA) was used to compare the mean shear bond strength values among eight groups followed by multiple comparisons (Tukey's test).

3.3 Descriptive statistics were used to determine the frequency of ARI scores. Chi-square was used to evaluate if there was any differences of ARI scores distribution among the experimental group.