CHAPTER III

METERIALS AND METHODS

MATERIALS

1. Samples

The samples in this study were extracted maxillary and mandibular premolars from patients (age range 12-19 years) for orthodontic reasons. Tooth collection chose particularly in case of two maxillary and two mandibular premolars that were extracted from each patient. The samples consisted of 128 teeth from 32 patients. All teeth were free from buccal restorations, cracks, carious lesions, and abnormal labial surface anatomy. All teeth were investigated within 6 months after extraction. Each tooth was stored at room temperature in 0.1 % (weight/volume) thymol, an antimicrobial agent to inhibit bacterial growth.

2. Brackets

All brackets used in this study were metal standard edgewise premolar brackets 0.018" X 0.025" slot, minidiamond type. In these foil/mesh backed brackets, there were two components: body and base. Stainless steel bracket bodies were joined with 316 stainless steel foil/mesh bases by brazing with gold alloy. Total area of each bracket was 8.4 square millimeters and the foil mesh had one hundred interlock holes per inch (Ormco, batch No. 350-0506, Ormco Glendora, California).

3. Adhesive systems

3.1. Conventional phosphoric acid etching adhesive system

Conventional 37% phosphoric acid (Ormco, Ormco Corporation, USA) with self-cured composite resin, System 1+ (Ormco, Ormco Corporation, USA)

3.2. Self-etching adhesive systems

Xeno® III (Densply Co, Ltd) self-etching adhesive system with composite resin, System 1+ (Ormco, Ormco Corporation, USA)

ED PRIMER® (J.C.Morita, Kurraray Co, Ltd) self-etching adhesive system with composite resin, System 1+ (Ormco, Ormco Corporation, USA)

AdheSE® (Unity, Vivadent Co, Ltd) self-etching adhesive system with composite resin, System 1+ (Ormco, Ormco Corporation, USA)

Adhesive systems used in this study are shown in figure 3.1-3.4.

Figure 3.1 Conventional phosphoric acid etching adhesive system

Figure 3.2 Xeno® III self-etching adhesive system

Figure 3.3 ED PRIMER® self-etching adhesive system

Figure 3.4 AdheSE® self-etching adhesive system

4. Supplies

4.1. Carborundum disc

4.2. Fluoride free pumice

4.3. Transparent tape in which a hole, 5 millimeters in diameter covering the bonding area was punched.

4.4. 0.018" X 0.025" Stainless steel archwire.

4.5. Cylindrical polyvinylchloride rings, whose diameter, height, and thickness were 25 millimeters, 17 millimeters, and 1 millimeter, respectively, were sealed at the base with green stone.

4.6. Elastic ligatures

4.7. 1.5 Millimeters thickness of hardened plastic sheath

4.8. Acrylic resin and monomer

4.9. 2 % Methylene blue

5. Instruments

5.1. A universal testing machine (Instron®) was used for measuring shear bond strengths (Figure 3.5).

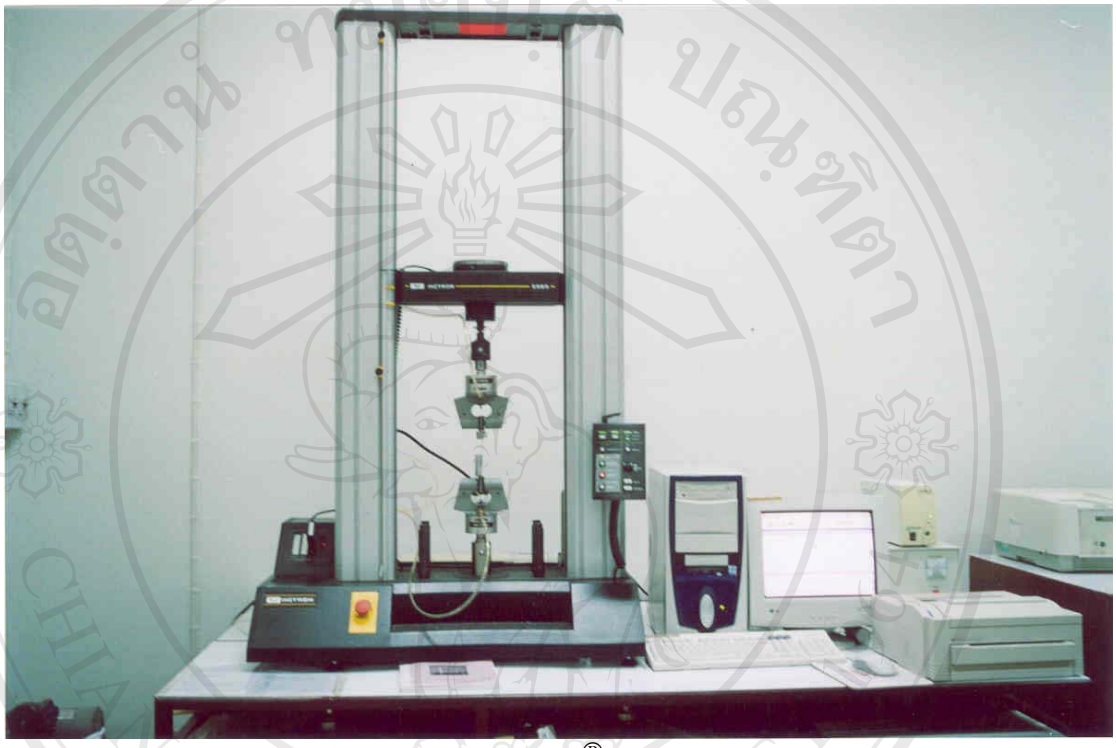

Figure 3.5 Universal testing machine (Instron®)

5.2. A debonding plate was designed to fit under the bracket wing to ensure vertical force application between the bracket base and the enamel surface (Figure 3.6).

Figure 3.6 Debonding plate

5.3. A mounting jig was designed to hold the tooth in a vertical position with the bracket base parallel to the direction of force (Figure 3.7).

Figure 3.7 Mounting jig

5.4. Water bath was maintained at 37°C for this study (Figure 3.8).

Figure 3.8 Water batch

5.5. Nikon stereozoom microscope X15 magnification that was connected to a computer for determining the failure modes (failure sites and amount of residual adhesives on debonded enamel surfaces) (Figure 3.9).

Figure 3.9 Nikon stereozoom microscope

5.6. A computerized transparent grid was made from transparent paper and used for determining the amount of residual adhesive on the debonded tooth in each photograph (Figure 3.10).

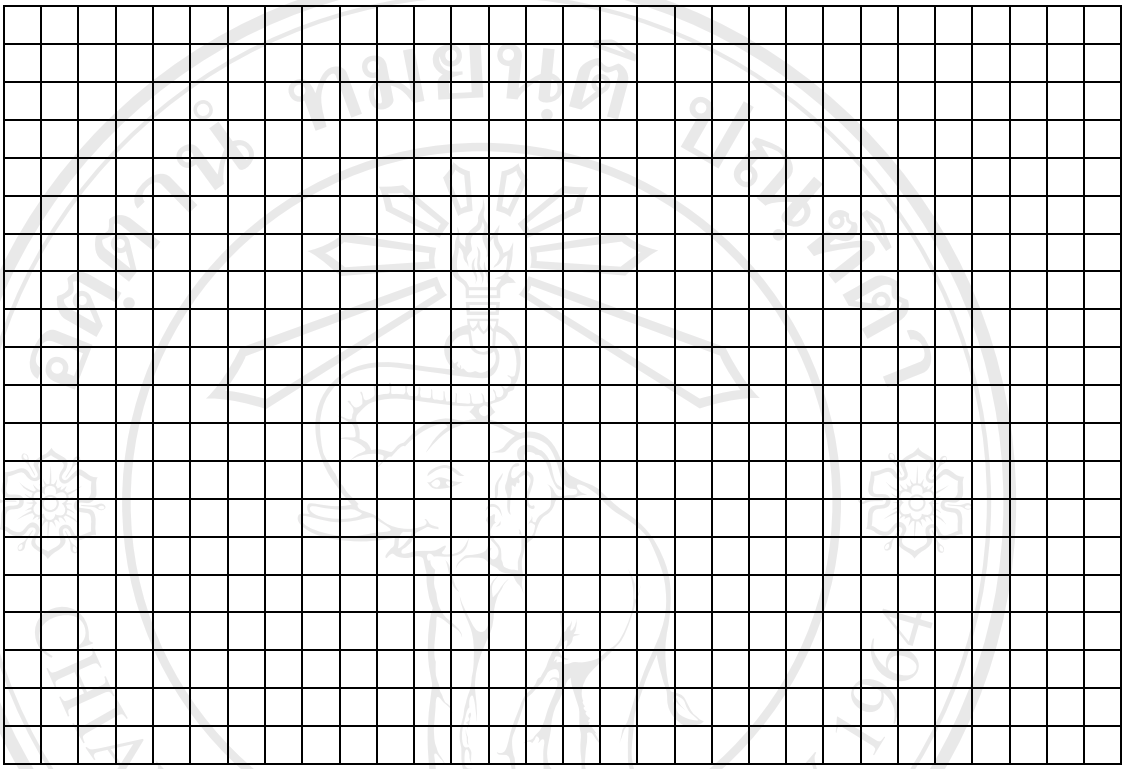

Figure 3.10 Computerized transparent grid

Methods

The experimental process was divided into four parts as follows:

I. Tooth allocation for experimental groups

II. Tooth preparation

III. Shear bond strength testing

IV. Examination of the failure modes

I. Tooth allocation for experimental groups

Four groups of adhesive systems were used in these investigations: conventional phosphoric acid etching and three self-etching adhesive systems (Xeno® III, ED PRIMER® and AdheSE®).

Four extracted premolar teeth from each patient, consisting of 14 or 15, 24 or 25, 34 or 35, 44 or 45, were randomly allocated for each experimental group to avoid a

selection bias. Therefore, a completely randomized block design was the choice for the allocations. The arrangements of these four teeth were 24 possible blocks that could be allocated for the four groups, as shown in Table 3.1.

Table 3.1 Twenty-four possible blocks that used for tooth allocation.

Order of Block design	Phosphoric acid etching adhesive system	Self-etching adhesive system		
		Xeno® III	ED PRIMER®	AdheSE®
1	14 / 15	24 / 25	34 / 35	44 / 45
2	14 / 15	24 / 25	44 / 45	34 / 35
3	14 / 15	34 / 35	44 / 45	24 / 25
4	14 / 15	34 / 35	24 / 25	44 / 45
5	14 / 15	44 / 45	24 / 25	34 / 35
6	14 / 15	44 / 45	34 / 35	24 / 25
7	24 / 25	14 / 15	34 / 35	44 / 45
8	24 / 25	14 / 15	44 / 45	34 / 35
9	24 / 25	34 / 35	44 / 45	14 / 15
10	24 / 25	34 / 35	14 / 15	44 / 45
11	24 / 25	44 / 45	14 / 15	34 / 35
12	24 / 25	44 / 45	34 / 35	14 / 15
13	34 / 35	14 / 15	24 / 25	44 / 45
14	34 / 35	14 / 15	44 / 45	24 / 25
15	34 / 35	24 / 25	44 / 45	14 / 15
16	34 / 35	24 / 25	14 / 15	44 / 45
17	34 / 35	44 / 45	14 / 15	24 / 25
18	34 / 35	44 / 45	24 / 25	14 / 15
19	44 / 45	14 / 15	24 / 25	34 / 35
20	44 / 45	14 / 15	34 / 35	24 / 25
21	44 / 45	24 / 25	34 / 35	14 / 15
22	44 / 45	24 / 25	14 / 15	34 / 35
23	44 / 45	34 / 35	14 / 15	24 / 25
24	44 / 45	34 / 35	24 / 25	14 / 15

When the four teeth were extracted from each patient, a block were simple randomly selected from all possible blocks. Then, these teeth were allocated to the experimental groups according to the sequence of teeth in the chosen block.

II. Tooth preparation

1. All teeth were prepared by sectioning of the roots with carborundum discs. The buccal surfaces were cleaned with fluoride-free pumice and water for 15 seconds, rinsed with distilled water and then dried with a stream of oil-free compressed air. A standardized area was obtained by masking off the enamel surface with transparent tape in which a hole, 5 millimeters in diameter covering the bonding area, was punched.

2. Each tooth was bonded with one adhesive system, as suggested by the manufacturer. Standard brackets were used for bonding each tooth. The long axis of the bracket was paralleled to the long axis of the tooth and the position of the bracket was in the center of the clinical crown.

Each sample was bonded by one of the following procedures:

2.1 Control group: Conventional phosphoric acid etching adhesive system with composite resin, System+1 (Ormco, Ormco Corporation, USA)

Each buccal surface was etched with 37% phosphoric acid solution (Ormco, Ormco Corporation, USA) for 30 seconds, rinsed with distilled water for 10 seconds and dried thoroughly with a stream oil-free compressed air. The liquid activator was applied on the etched enamel surface and the bracket base. Then, the composite resin was applied to the bracket base. After that the bracket was firmly placed on the enamel surface and excessive resin was removed from the enamel surface with an explorer.

2.2 Experimental groups: Three self-etching adhesive systems

Experimental group 1: Xeno® III (Densply, Germany CO, LTD) with composite resin, System+1 (Ormco, Ormco Corporation, USA)

Equal amounts of liquid Xeno® III A and B were dispensed into a clean mixing well and mixed for 5 seconds with the applicator tip supplied. The Xeno® III was applied to the buccal surface and left undisturbed for at least 20 seconds. The adhesive was dispersed by using a gentle stream of oil-free air for at least 2 seconds until there was no more flow of the adhesive to ensure proper removal of solvent. Excessive air-drying was avoided to prevent thinning of the adhesive. The adhesive was cured with a light-curing unit for at least 10 seconds. The liquid activator was applied on the bracket base. Then, the composite resin was applied to the bracket base. After that the bracket was firmly placed on the enamel surfaces. Excess resin was removed from the enamel surface with an explorer.

Experimental group 2: ED PRIMER® (J.C.Morita, Kurraray Co, Ltd) with composite resin, System1+ (Ormco, Ormco Corporation, USA)

Equal amounts of liquid ED PRIMER® A and B were dispensed into a clean mixing well and mixed for 5 seconds with the applicator tip supplied. The ED PRIMER® was applied to the buccal surface and left undisturbed for at least 60 seconds. The adhesive was dispersed by using a gentle stream of oil-free air for at least 2 seconds until there was no more flow of the adhesive to ensure proper removal of solvent. Excessive air-drying was avoided to prevent thinning of the adhesive. The liquid activator was applied on the bracket base. Then, the composite resin was applied to the bracket base. After that the bracket was firmly placed on the enamel surfaces. Excess resin was removed from enamel surface with an explorer.

Experimental group 3: AdheSE® (Unity Co, Ltd) with composite resin, System1+ (Ormco, Ormco Corporation, USA)

The AdheSE Primer was applied to the buccal surface and left undisturbed for at least 30 seconds and dispersed by using a very weak stream of oil- free air. Then, The AdheSE Bond was applied on the buccal surface and dispersed by using a very weak stream of oil-free air. The adhesive was cured with a light-curing unit for at least

10 seconds. The liquid activator was applied on the bracket base. Then, the composite resin was applied to the bracket base. After that the bracket was firmly placed on the enamel surfaces. Excess resin was removed from enamel surface with an explorer.

All experimental samples were allowed to cure at least 10 minutes and then the adhesive tape was removed from each tooth.

Each sample was embedded in cylindrical polyvinylchloride rings, a 0.018" X 0.025" stainless steel wire was placed on the bracket slot and elastic was used to ligate the body of the bracket perpendicular to the shear force. This wire was attached to the hardened plastic sheath by transparent tape (Figure 3.11).

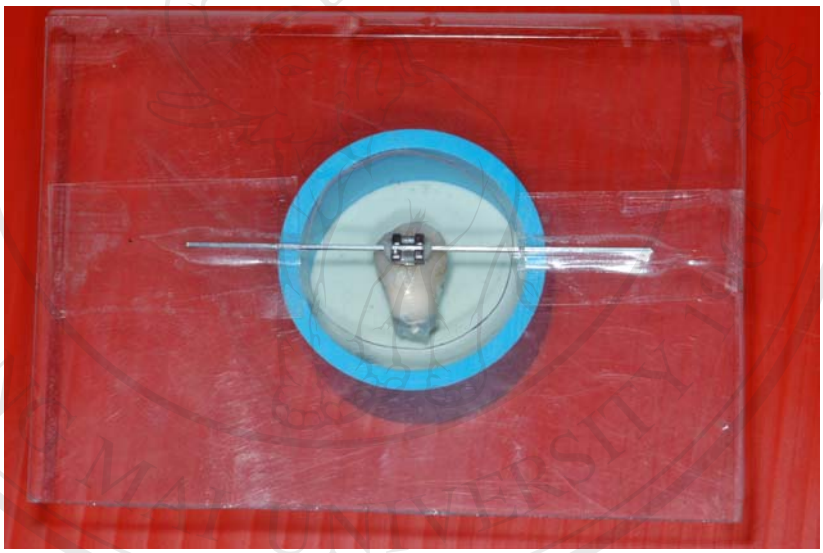

Figure 3.11 A 0.018" X 0.025" stainless steel wire was placed on the bracket slot by ligating the elastic ligature to the body of the bracket.

Mixed acrylic monomer was poured into the ring. The tooth-bracket was placed at the center of the ring and above the rim of the ring by the 1.5 millimeters thickness of hardened plastic sheath. Only the buccal tooth surface and attached orthodontic bracket were exposed (Figure 3.12). After the acrylic resin was cured, the elastic ligature, the wire, and the plastic sheath were removed.

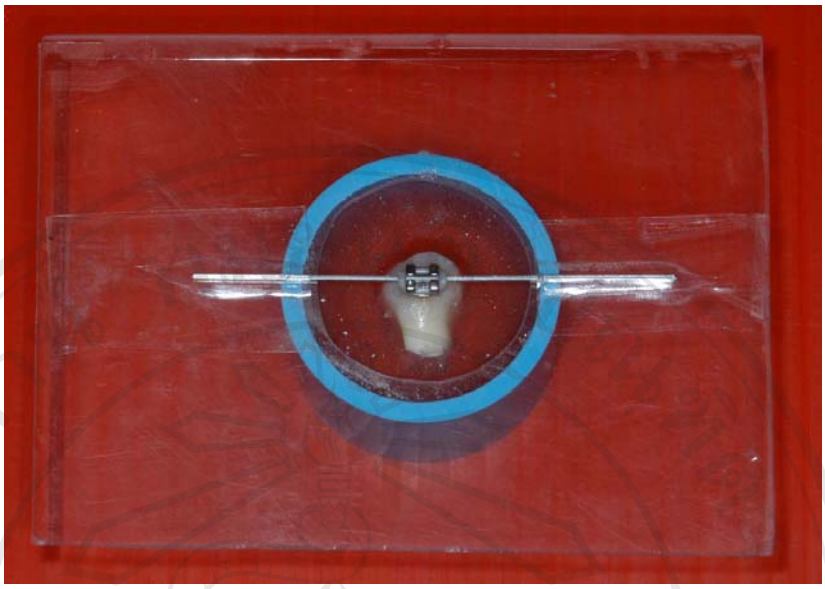

Figure 3.12 Tooth embedded in polyvinylchloride ring

The specimens were immersed in distilled water at 37°C for 24 hours in a water bath to achieve maximum bond strength prior to testing.

III. Shear bond strength testing

Shear bond strength was determined by using a universal testing machine (Instron®) at 0.1 millimeter per minute in crosshead speed and five kilonewtons load cell. Each ring was mounted into the jig which was fixed into the lower pneumatic grip. The debonding plate was fixed into the upper pneumatic grip of the machine. When the universal testing machine (Instron®) worked, the shear bond strength at the bond failure was recorded (Figure 3.13 and 3.14).

Figure 3.13 The debonding plate was fixed into the upper pneumatic grip and the mounting jig was fixed into the lower pneumatic grip.

Figure 3.14 An apparatus assembled for testing shear bond strength

IV. Examination of the failure modes

Failure modes were examined from the failure sites and the amount of residual adhesives on debonded enamel surfaces.

Examination of the failure sites

After debonding, failure sites were determined by examination of both the debonded enamel surfaces and the bracket bases under a Nikon stereozoom microscope at x15 magnification. The failure sites were divided into five locations according to Alexander (1993) and Jou, *et al*. (1995) as follows:

1: within the enamel

2: adhesive/enamel interface

(0-25% of the residual adhesives left on the debonded enamel surfaces)

3: within the adhesive

(25-75% of the residual adhesives left on the debonded enamel surfaces)

4: adhesive/bracket interface

(75-100% of the residual adhesives left on the debonded enamel surfaces)

5: within the bracket

Examination of the amount of residual adhesives on debonded enamel surface

Debonded teeth were immersed in 2% methylene blue for 3 days and then they were washed with distilled water. The amounts of residual adhesives on the debonded enamel surfaces were determined from photographs taken from the Nikon stereozoom microscope at X15 magnification (Figure 3.15). Transparent paper was placed on the photograph. The border of the debonded enamel surface area was drawn on the transparent paper (Figure 3.16). The percentages of residual adhesives per total debonded enamel surface area were carried out by placing the computerized transparent grid on the photograph. Each dot of computerized transparent grid was subjectively observed residual adhesives (Figure 3.17).

For evaluation of the intra-examiner error in examination of residual adhesives on the debonded enamel surfaces, 20 randomly selected photographs were re-examined percentage of adhesive adhesives 7 days later. These measurements were compared with the values obtained for the first measurement.

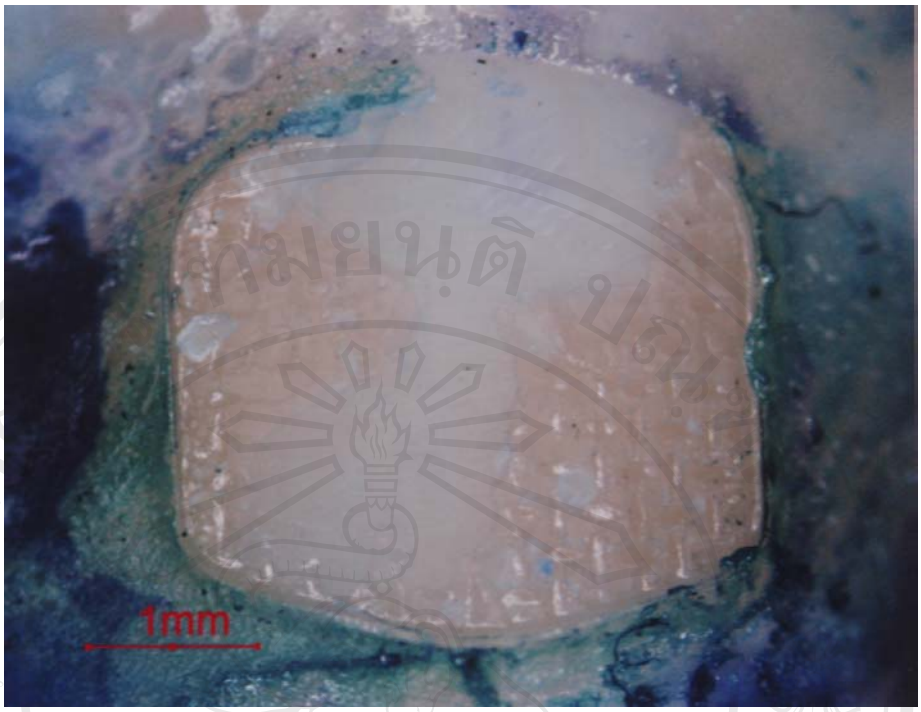

Figure 3.15 Photograph of debonded enamel surface area

Figure 3.16 The border of debonded enamel surfaces area was drawn on the transparent paper.

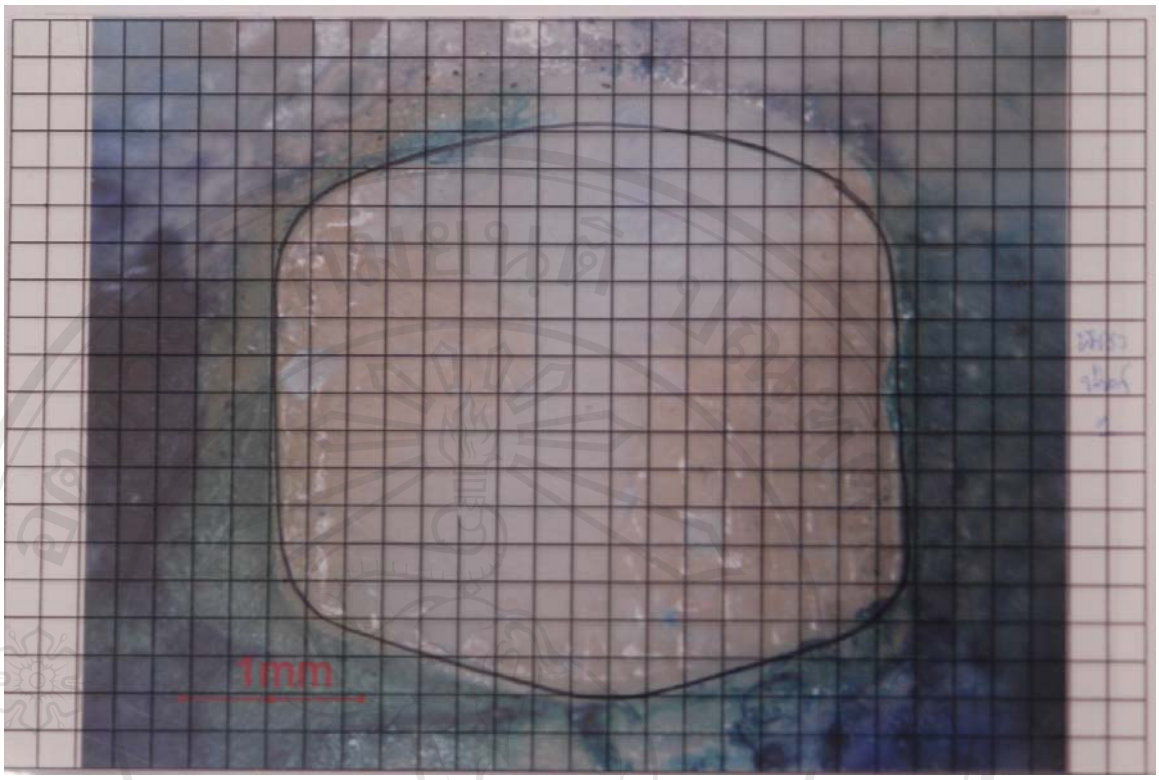

Figure 3.17 Residual adhesives on the debonded enamel surfaces were determined by using a computerized transparent grid.

Statistical Analyses

The SPSS for Windows Release 10.01 program (SPSS Inc., Chicago, USA, 1989-1999) and EpiCalc 2000 (version 1.02 written by Joe Gilman and Mark Myatt 1997, Brixton Books) were used to calculate the following analysis:

1. The shear bond strengths of three self-etching and one conventional phosphoric acid etching adhesive systems were described by means, standard deviations, and ranges.

2. One way analysis of variance (ANOVA) was used to compare the mean shear bond strengths among three self-etching and one conventional phosphoric acid etching adhesive systems.

3. Multiple comparisons test (Tukey's test) was used to identify which groups were different when there was a significant difference in the mean shear bond strengths among three self-etching and one conventional phosphoric acid etching

adhesive systems in orthodontic bracket placement as determined by the one way analysis of variance (ANOVA).

4. The Pearson Chi-square test was used to compare the percentages of failure sites among three self-etching and one conventional phosphoric acid etching adhesive systems.

5. The Z-test was used to identify which groups were different, when there was a significant difference in the percentages of failure sites among three self-etching and one conventional phosphoric acid etching adhesive systems as determined by the Pearson Chi-square test.

6. The amounts of residual adhesives on the deboned enamel surface resulting from three self-etching and one conventional phosphoric acid etching adhesive systems were described by mean rank.

7. The Kruskal-Wallis Test was used to compare the amount of residual adhesive on the deboned enamel surface among three self-etching and one conventional phosphoric acid etching adhesive systems.

8. The Mann-Whitney Test was used to identify which groups were differences, when there was a significant difference in the amount of residual adhesives on the debonded enamel surface among three self-etching and one conventional phosphoric acid etching adhesive systems as determined by the Kruskal-Wallis Test.

The 95% confidence interval was used to determine significance.

Reliability of the measurements

For evaluation of the intra-examiner error, 20 randomly selected photographs were re-examined percentage of residual adhesives on the debonded enamel surface 7 days later. The measurements were compared with the values obtained for the first measurement by Pearson's correlation test. The analysis found that correlation coefficients ($r=0.996$) at $p<0.001$ were as reported in Table 3.2. This indicated that the measurements were satisfied with a very high reproducibility.

Table 3.2 Means, standard deviations and correlation coefficients of the first and second measurements in determination the percentages of residual adhesives on the debonded enamel surface

	Number	Mean	Standard deviation	Correlation coefficients	P-value
First measurement	20	19.640	21.935	0.996	<0.001
Second measurement	20	20.055	21.812		