# CHAPTER 3

MATERIALS AND METHODS

3.1 Sample collection

Forty lower primary incisor teeth were used for this study. The teeth were extracted from 5-8 years old healthy children due to prolonged retention as shown in Figure 2. The teeth were stored in normal saline solution with 0.1% sodium azide and kept in refrigerator at 4 °C. Saline was used for storage of the freshly extracted teeth. This is a well-known procedure, as it does not influence the chemical and physical properties of human dentin (Ciucchi *et al.*, 1995; Lee *et al.*, 2007). All tooth specimens were experimented within 24 hours after extraction.
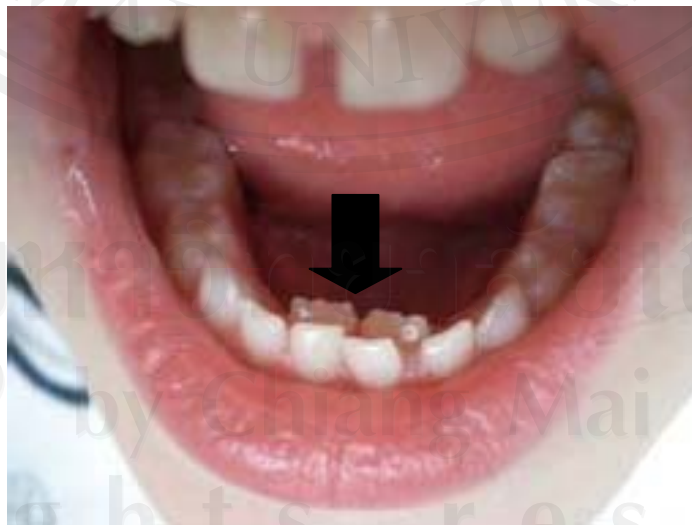
Figure 2 Prolonged retention of lower anterior primary teeth (arrow).

3.2 Tooth preparation

3.2.1 The root of the teeth is removed at the level of approximately 1 mm apically to the cementoenamel junction using a high speed cylinder diamond bur (Intensiv®, Swiss Dental Products, Switzerland) with water spray (Figure 3).

Figure 3 A root of the tooth was removed at the level of approximately 1 mm apically to the cementoenamel junction.

3.2.2 The tooth was stuck to Perspex collar (i.d. 8 mm, o.d. 10 mm, height 10 mm) using self cured acrylic resin (Figure 4).

3.2.3 The remaining pulpal tissue in the coronal portion is removed with barbed broach under water to prevent air bubble formation.
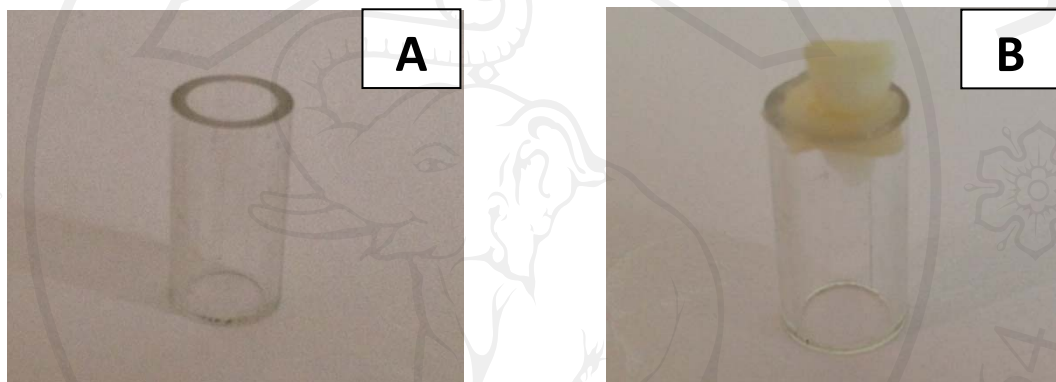

Figure 4 The tooth was stuck to Perspex collar (A) using self cure acrylic resin and cut the incisal edge until exposed dentin (B).

3.2.4 The pulp cavity was then filled with distilled water and the collar was connected to manometer.

3.2.5 The high speed cylinder diamond bur was used for cutting the incisal edge until exposed dentin under water coolant. Then, 1 mm of exposed dentin (check by calipers) was further removed using the same bur.

3.2.6 Intrapulpal pressure was set to -30, 0 and 30 cmH$_2$0 consecutively. Care was taken to avoid tapping air bubble in the system (Figure 5).
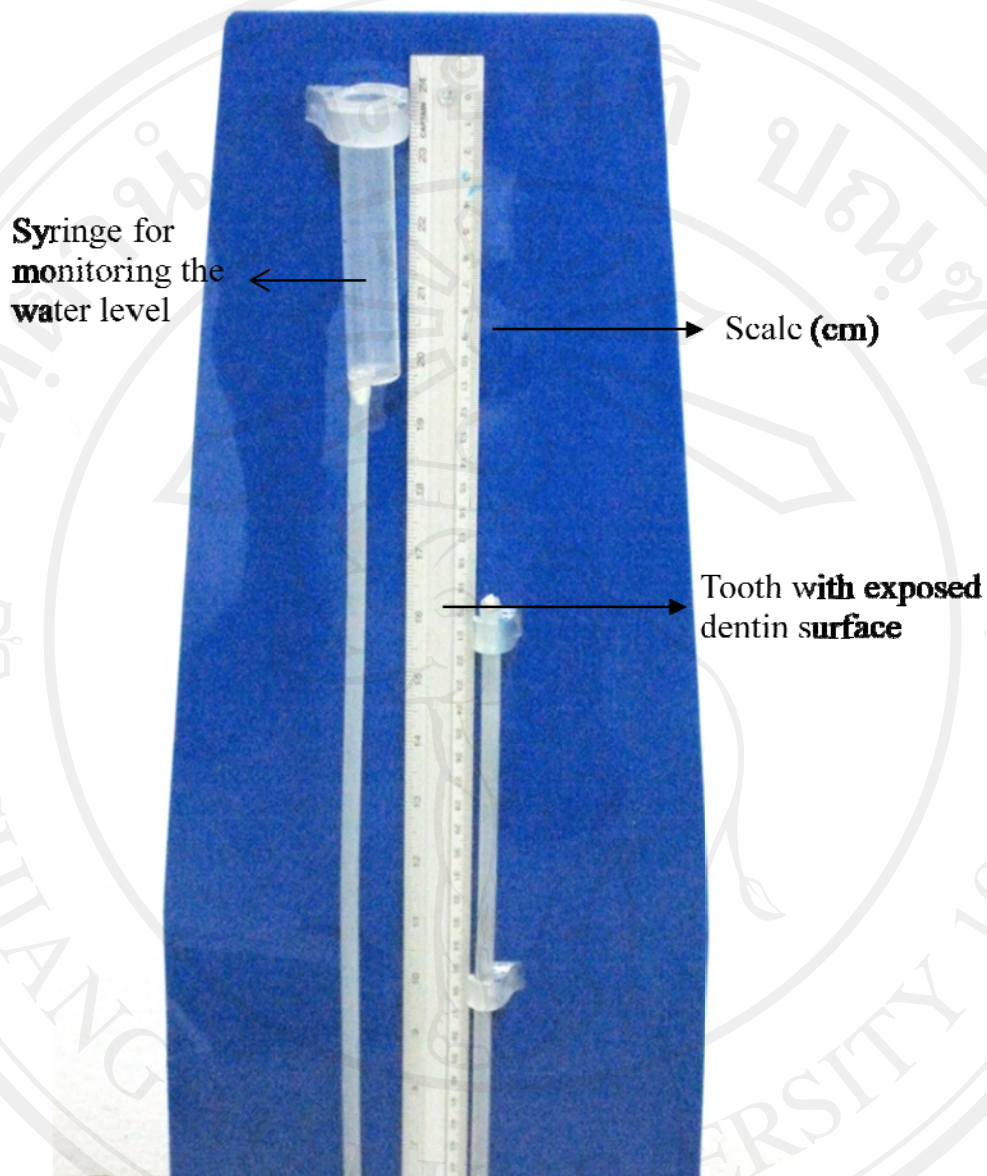
Figure 5 Connecting the collar with experimented tooth to water manometer.

3.3 Preparation of dentin surface

3.3.1　　While the hydrostatic pressure was applied to the pulp cavity by setting

manometer at -30, 0 and 30 cmH$_2$O, the exposed dentin surface was applied with

35%　phosphoric acid gel (Scotchbond™ Etchant #7423, 3M ESPE, U.S.A) for

15 seconds, then rinsed with distilled water and blotted the excess water with a cotton pellet.

3.3.2   The dentin adhesive (Adper™ Single Bond 2-step total-etch adhesive 3M ESPE, U.S.A) was applied 2-3 times by gently agitation of the enamel and exposed dentin for 15 seconds using a fully saturated applicator, then gently air blow for 5 seconds to evaporate the solvents and light cured for 10 seconds.

Figure 6 shows Scotchbond™ Etchant #7423 (3M , ESPE) and Adper™ Single Bond 2-step total-etch adhesive (3M, ESPE).

Table 2 Mode of application, compositions, and manufacturer of tested adhesive.

Material	components	Mode/steps of application	Manufacturer
Single Bond Total-etch Self-priming	1. 35% phosphoric acid 2. Adhesive: 2-hydroxyethylmethacrylate; water; ethanol; Bis-GMA; dimethacrylates; amines; methacrylate-functional; copolymer of polyacrylic and polyitaconic acids.	Etch for 15 seconds. Rinse with water spray for 10 seconds, leaving tooth moist. Apply two consecutive coats of the adhesive with a fully saturated brush tip. Dry gently for 2-5 seconds. Light cure for 10 seconds.	3M ESPE, St.Paul, MN, USA.

3.3.3 The prepared dentin surfaces were attached to composite rods 1 mm in diameter. Each rod was prepared from cured-Z-350 resin-based composite (Filtek™ Z350, 3M ESPE, U.S.A) which was molded from the 1 mm in diameter and 1 cm in height of perforated plastic rod.

Z-350 resin-based composite

Figure 7 Samples were prepared for microtensile bond strength test. (A) Composite (Filtek™ Z350, 3M ESPE, U.S.A) was used to prepared composite rods. (B) The prepared dentin surface was attached to composite rod.

3.3.4 Specimens were stored in distilled water at 37°C for 24 hours.

3.3.5 The specimens were fixed to the tensile bond testing apparatus and a test machine (Universal Testing Machine, Instron®, Instron (Thailand) Limited, U.S.A) which stressed in tension at a cross-head speed of 1 mm/min.

Figure 8 shows Universal Testing Machine (UTM) using for microtensile bond strength test.

Figure 9 shows Universal Testing Machine (UTM) with the tensile bond testing apparatus.

Figure 10 shows a specimen was fixed to the tensile bond testing apparatus.

3.3.6   The tensile force was calculated at failure and converted to a tensile stress (MPa).

3.3.7   All fractured specimens were determined the modes of failure by using a Scanning Electron Microscope (JEOL® JSM-5410LV; JEOL, Tokyo, Japan) and were classified according to one of three types (Gupta and Tewari, 2006).

  a. Type 1: adhesive mode of failure was recorded if the restorative material was completely detached from the tooth surface.

  b. Type 2: cohesive mode of failure was recorded if the bond failure occurred entirely within dentin or the restorative material.

c. Type 3: mixed mode of failure was recorded if the bond failure was

a combination of the adhesive and cohesive modes of failure.

3.4 Preparation tooth for determining the modes of failure

3.4.1 The tooth was separated longitudinally (labial to lingual) into 2

sections using diamond disc in bonding area.

Figure 11 shows separated teeth which prepared for examining in a Scaning

Electron Microscope (SEM).

3.4.2 The dentin surface of the separated tooth was examined in a Scanning

Electron Microscope (JEOL® JSM-5410LV; JEOL, Tokyo, Japan).

Figure 12 shows a Scanning Electron Microscope

(JOEL® JSM-5410LV; JEOL, Tokyo, Japan).

3.5 Processing for Scanning Electron Microscope

3.5.1   The samples were fixed to stubs with the conductive adhesive tape and

coated with gold-palladium under vacuum (JEOL® JFC1200 Fine Coater; JEOL,

Tokyo, Japan) (Figure 14).

Figure 13 shows a gold palladium coater machine.

Figure 14 Samples were coated with gold palladium prior SEM examination.

3.5.2   All specimens were examined in a Scanning Electron Microscope (JEOL® JSM-5410LV; JEOL, Tokyo, Japan) (Figure 12) at the Institute of product quality and standardization, Maejo University, Chiang Mai, Thailand.

3.5.3   Digital photomicrographs were taken at a magnification of ×1,000 for later analysis.

3.6 Statistical Analysis

Data are statistically analyzed using ANOVA with the statistical software SPSS version 16.0 (SPSS; Chicago, IL, USA). One-way ANOVA is performed to compare bond strength of adhesive at different intrapulpal pressures (-30, 0, 30 cmH$_2$O and dry teeth) *P<0.05* is considered as significant difference.