CHAPTER III

MATERIALS AND METHODS

3.1 Sample collection

Twenty five lower primary incisor teeth were used for this study. The teeth were extracted from 5-9 years old healthy children due to prolonged retention (as shown in Figure 15). The teeth were stored in normal saline solution with 0.2% sodium azide and kept in refrigerator at 4 °C. All tooth specimens were experimented within 24 hours after extraction.

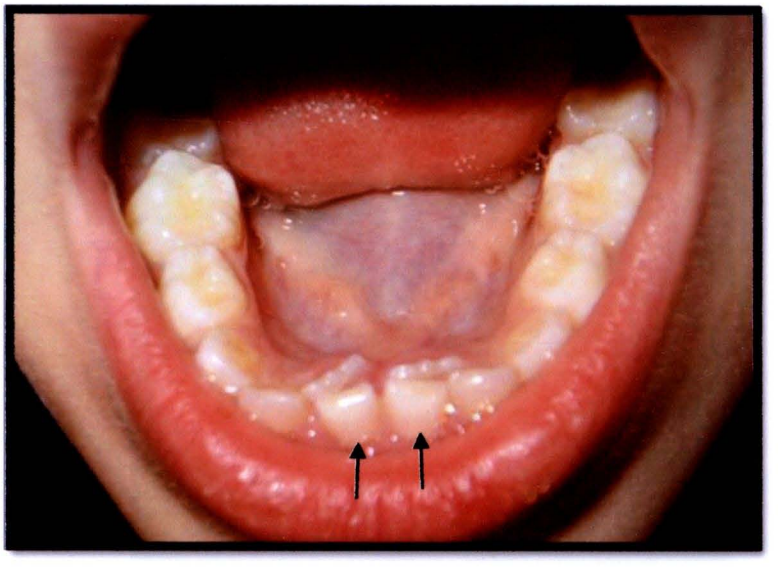

Figure 15 Prolonged retention of lower anterior primary teeth (arrow)

3.2 Tooth preparation

3.2.1 The root of the tooth was removed at the level of approximately 1mm apically to the cementoenamel junction using a high speed cylinder diamond bur (Figure 16).

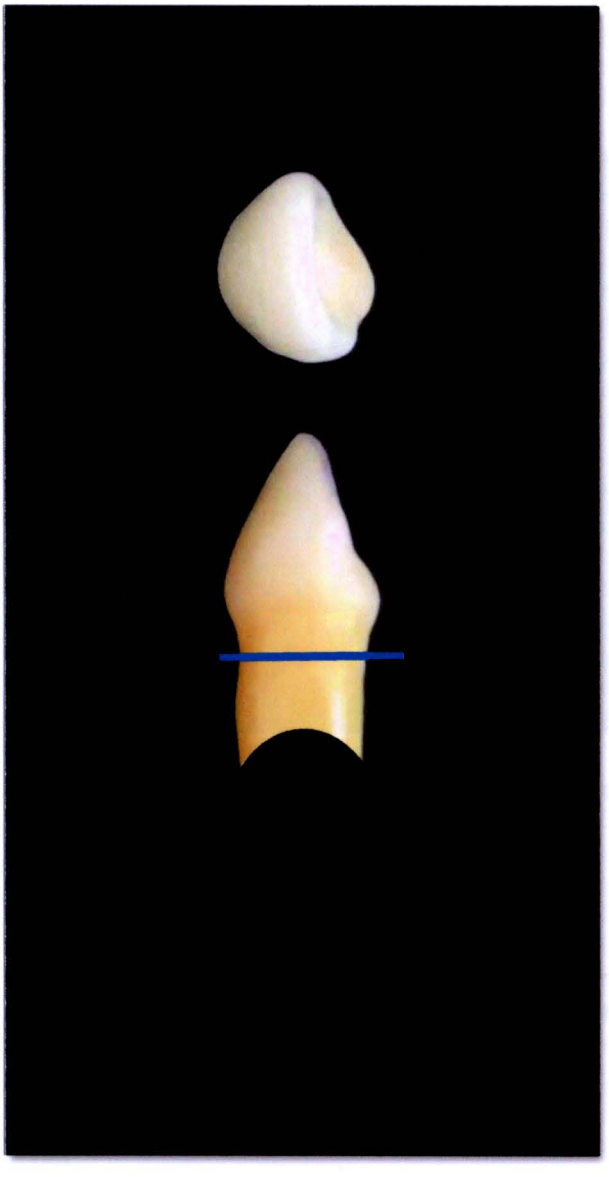

Figure 16 A root of the tooth was removed at the level of approximately 1mm apically to the cementoenamel junction.

3.2.2 The remaining pulpal tissue in the coronal portion was removed with barbed broach under water for preventing air bubble.

3.2.3 The tooth was attached to Perspex collar (i.d. 4 mm, o.d. 8 mm, height 6 mm) using self cure acrylic resin (Figure 17).

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

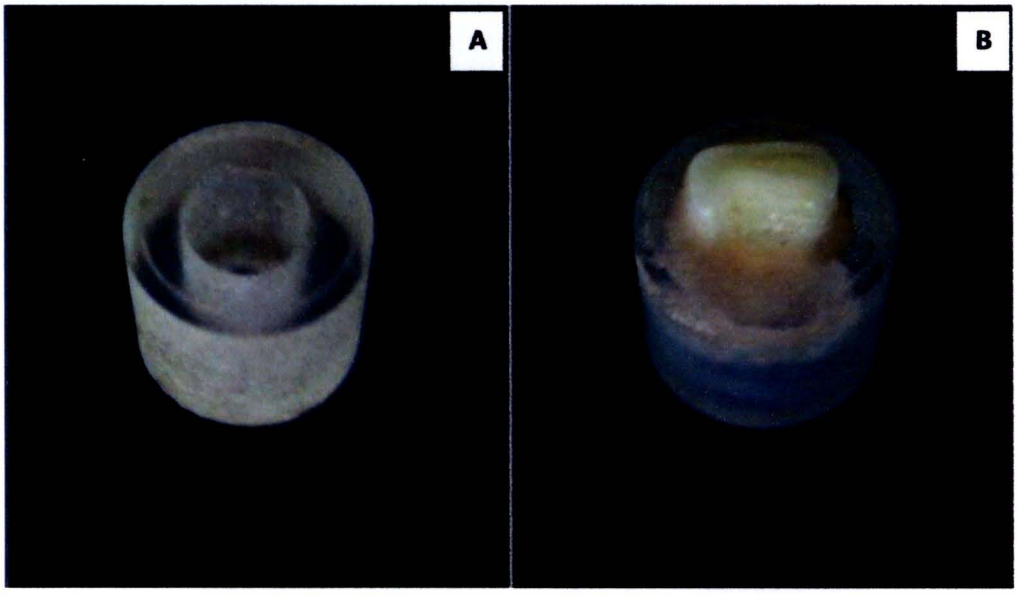

Figure 17 The tooth was attached to Perspex collar (A) using self cure acrylic resin and cut the incisal edge until exposed dentin (B).

3.2.4 The high speed cylinder diamond bur was used for cutting the incisal edge until exposed dentin under water coolant. Then, further 1 mm dentin was removed using the same bur.

3.2.5 Intrapulpal pressure was set to 0, 15, 30 and 45 cmH_20 consecutively. Care was taken to avoid tapping air bubble in the system (Figure 18).

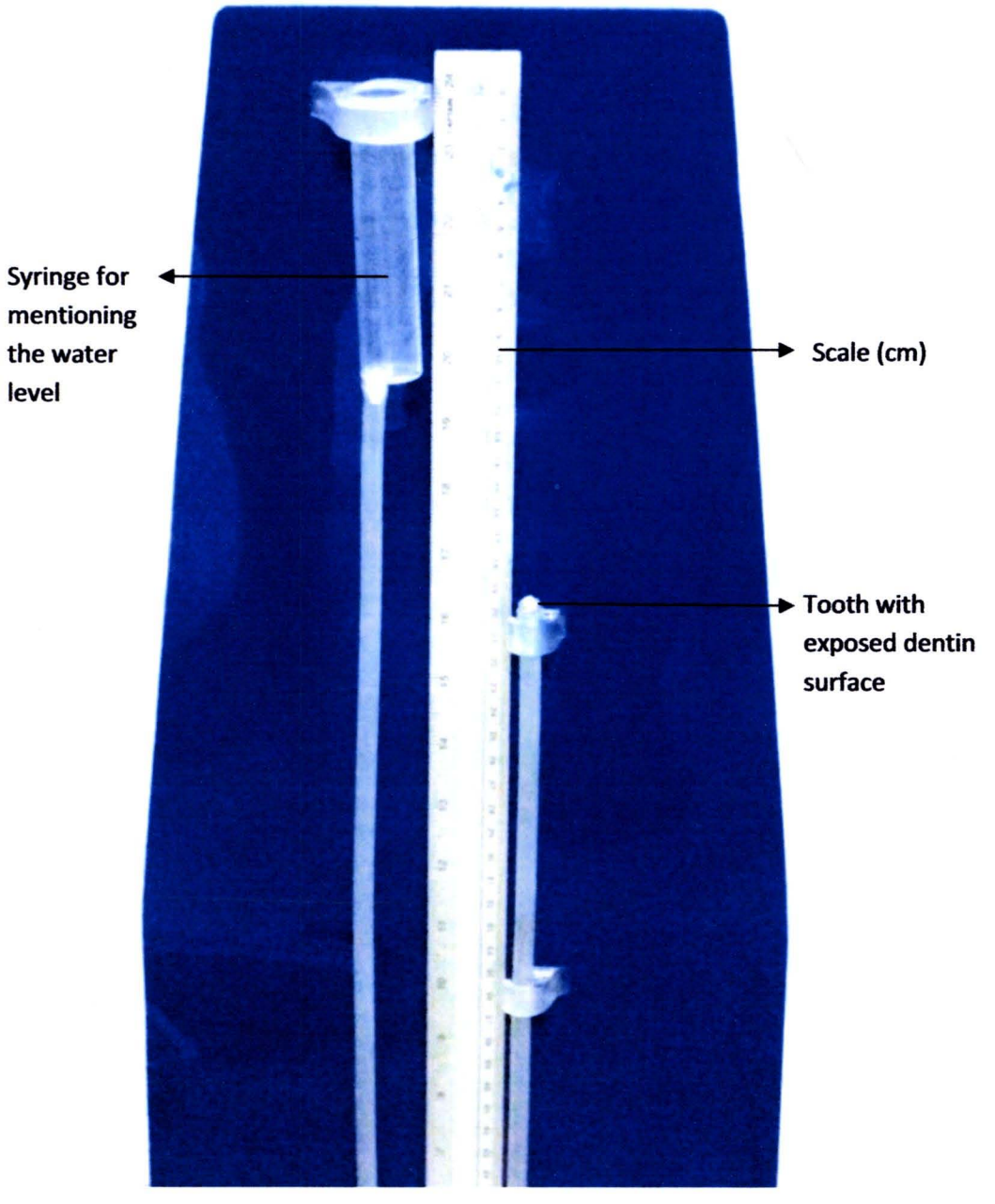

Figure 18 Connecting the collar to water manometer

3.3 Preparation of dentin surface

3.3.1 The exposed dentin surface was equally divided into 2 parts. One half was apply with 35% phosphoric acid gel (3M ESPE Scotchbond™, 3M ESPE,

U.S.A.) for 15 seconds, then rinse with distilled water and mop dry with cotton pallet, while the other half is left unetched.

3.3.2 The hydrostatic pressure was applied to the pulp cavity by setting manometer at 0, 15, 30 and 45 cmH_2O in turn. For example, for setting hydrostatic pressure at +45 cmH_2O, the water level at the open end was set to be higher than the tooth surface for 45 cm.

3.3.3 A hydrophobic silicone impression material (Xantopren® VL plus: Heraeus, Kulzer, Germany) was used for taken the impression of dentin surface after mop dry and left for 30 seconds after setting the pressure (Figure 19, 20).

Figure 19 Perspex cap for loading a hydrophobic, silicone rubber material in recording the surface of cut dentin.

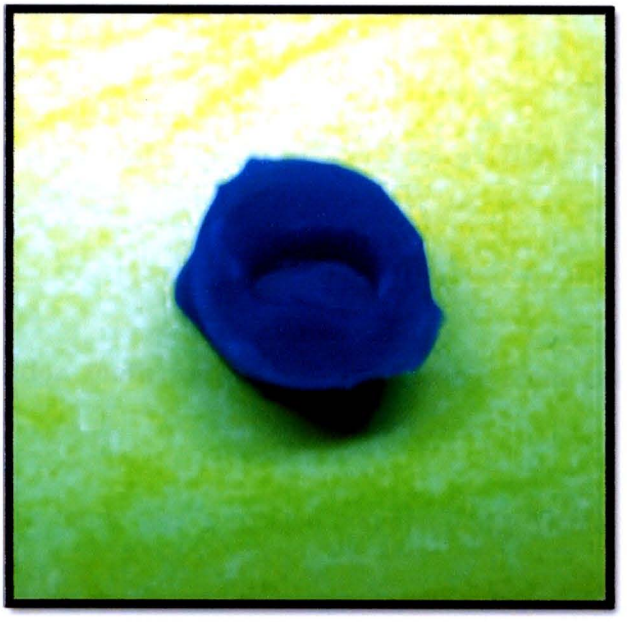

Figure 20 Impression from recording cut dentin surface.

3.3.4 The impression material was loaded into the Perspex cap just enough to fill up the cap to prevent the excess material which may compress the droplet on dentin surface.

3.3.5 Gently press the cap with impression onto the collar for recorded surface of the dentin and left in place until the impression material was fully set.

3.3.6 The replica was made by casting the impression with low viscosity epoxy resin within an hour and left overnight.

3.3.7 The replica was examined in a Scanning Electron Microscope (JEOL® JSM-5410LV; JEOL, Tokyo, Japan).

3.4 Preparation of dry dentin surface

3.4.1 At the end of experiment, the dentin surface of each tooth specimen was examined in a Scanning Electron Microscope (JEOL® JSM-5410LV; JEOL, Tokyo, Japan).

3.5 Preparation tooth for measuring distance from cut dentin surface to dental pulp

3.5.1 The tooth was separated longitudinally (labial to lingual) into 2 sections using dental chisel and hummer.

3.5.2 The dentin surface of the separated tooth was examined in a Scanning Electron Microscope (JEOL® JSM-5410LV; JEOL, Tokyo, Japan).

3.6 Processing for Scanning Electron Microscope

3.6.1 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 21, 22).

3.6.2 All specimens were examined in a Scanning Electron Microscope (JEOL® JSM-5410LV; JEOL, Tokyo, Japan) (Figure 23) at the Institute of product quality and standardization Maejo University.

3.6.3 Digital photomicrographs were taken at a magnification of x 50, 3500 for later analysis.

3.6.4 The size of the droplets and dentinal tubules and the distance from cut dentin surface to dental pulp were measured by Scion Image Program (Release-Alpha 4.0.3.2).

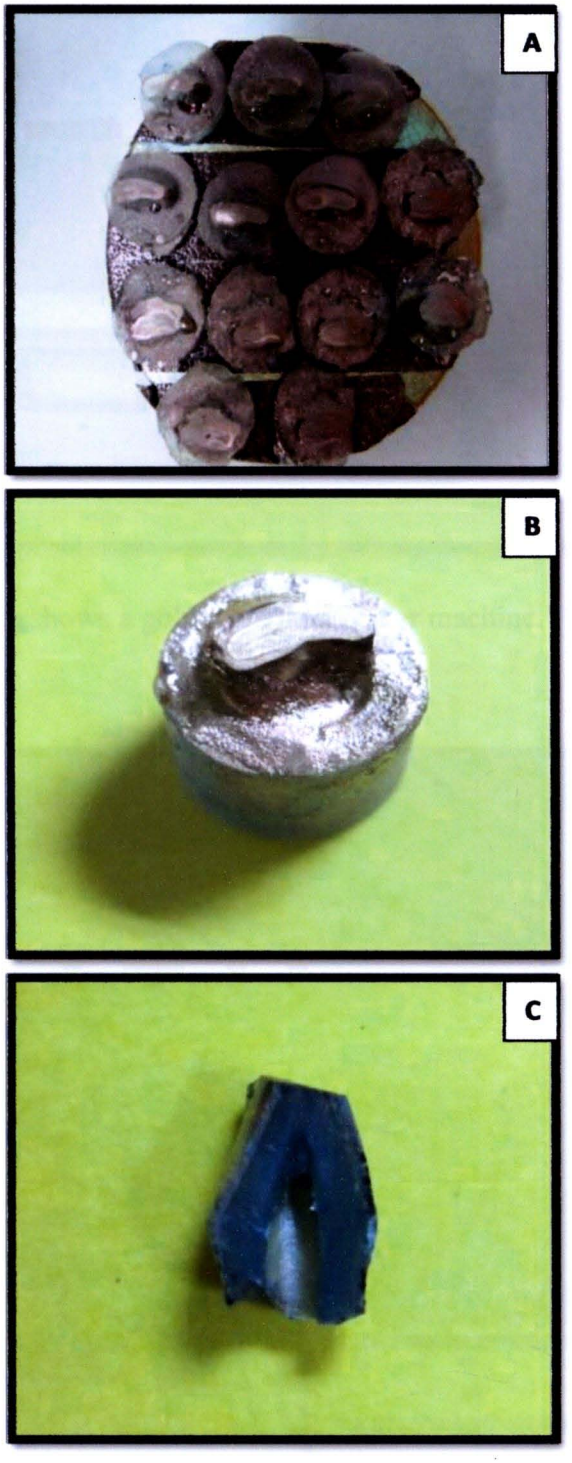

Figure 21 Samples were coated with gold palladium prior SEM examination.

(A) a replica of the tooth in epoxy resin

(B) a dry tooth

(C) a separated tooth

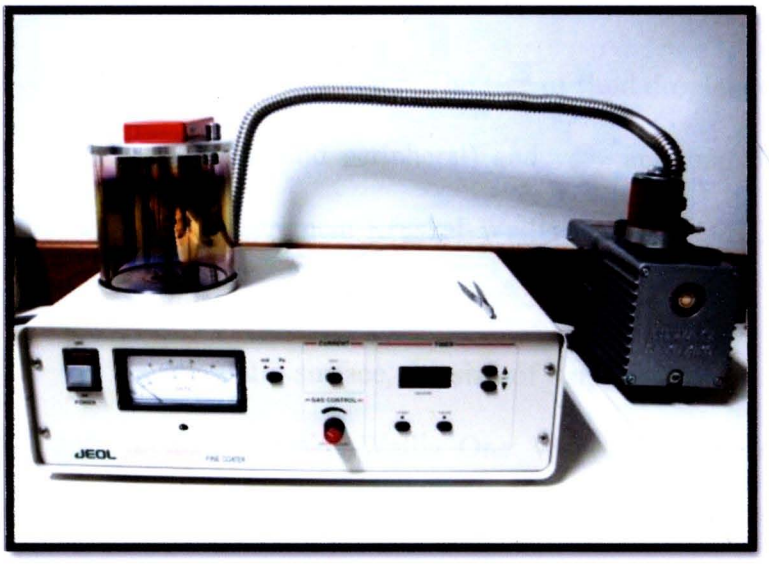

Figure 22 The picture shows a gold palladium coater machine.

Figure 23 The picture shows a Scanning Electron Microscope (JEOL® JSM-5410LV; JEOL, Tokyo, Japan)

3.7 Statistical Analysis

3.7.1 On unetched dentin surface, The size of fluid droplets was comparing both areas of sampling (central and peripheral) and vary applying pressures using paired t-test and repeated measurement Kruskal-Wallis One Way ANOVA on Ranks with Dunn's multiple comparison consecutively.

3.7.2 On etched dentin surface, the size of dentinal tubules was compared using repeated measurement Kruskal-Wallis One Way ANOVA on Ranks with Dunn's multiple comparison. No comparison between central and peripheral was tested.

3.7.3 The size of dentinal tubules at central and peripheral of etched dentin surface of the dry teeth was compared using paired t-test.