CHAPTER III

MATERIALS AND METHODS

3.1 Population and Sample

3.1.1 Target population: Radicular dentin surface

3.1.2 Study population: Radicular dentin surface of straight rooted teeth

3.1.3 Sample: Radicular dentin surface of straight rooted teeth, single rooted teeth of anterior or premolar teeth and complete root formation, no crack or fracture, no caries and root resorption.

3.1.4 Variables

3.1.4.1 Independent variables

Type of irrigants: 2% chlorhexidine, 2.5% sodium hypochlorite, 17% EDTA and sterile water

Root canal filling material: Epiphany® and Resilon®

3.1.4.2 Dependent variables: Leakage

3.1.4.3 Confounding factors:

Volume of irrigants, Contact time of irrigants, Rate of irrigation, Size of needle, Depth of needle, Freqeuncy of irrigation, Technique of irrigation, Concentration of irrigants, Technique of preparation, Technique of application primer/sealer, Type of primer/sealer, Size of root canal, Apical size, Obturation technique

3.2 Materials

3.2.1 Extracted human single-rooted teeth

3.2.2 Resilon® master cone (Pentron Clinical Technologies)

3.2.3 Resilon® accessory cones (Pentron Clinical Technologies)

3.2.4 Epiphany® sealer and primer (Pentron Clinical Technologies)

3.2.5 0.2% thymol solution

3.2.6 5.25% sodium hypochlorite solution

3.2.7 17% ethylene-diaminetetraacetic acid solution

3.2.8 2% chlorhexidine solution

3.2.9 0.1% benzoic acid solution in 50 mM phosphate buffer

3.2.10 1 mol L^{-1} glucose solution in 0.1% benzoic acid solution

3.2.11 D-(+)-Glucose anhydrous sigmaultra (S.M. Chemical Supplies CO., LTD.)

3.2.12 Glucose liquicolor (Human, S.E. SUPPLY LTD., PART.)

3.2.13 Low speed saw (IsoMet®, Buehler)

3.2.14 An operating microscope (Carl Zeiss Surgical, Inc., Thornwood, NY)

3.2.15 K-file (Dentsply Maillefer, Ballaigues, Switzerland)

3.2.16 K-Flex-o-files (Dentsply Maillefer, Ballaigues, Switzerland)

3.2.17 Gate Glidden drills (Dentsply Maillefer, Ballaigues, Switzerland)

3.2.18 ProTaper rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland)

3.2.19 Needle irrigate (gauge 27)

3.2.20 Syringe irrigate (size 5 ml, 10 ml)

3.2.21 Paper points (Dentsply Maillefer, Ballaigues, Switzerland)

3.2.22 Glass slab metal spatula

3.2.23 Metal spatula

3.2.24 Blade No.15

3.2.25 Spreader size D11Ts (Dentsply Maillefer)

3.2.26 Endodontic plugger

3.2.27 Long glass tube (17-cm)

3.2.28 Sterile centrifuge tube

3.2.29 Rubber cap

3.2.30 Silicone tube

3.2.31 Vacuum pump (Bio-Rad Laboratories, 3300 Regatta Boulevard, Richmond, CA 94804)

3.2.32 Three-way, Double oblique, Pressure, Solid glass plug, Stopcock size 2 mm (Fortune Scientific Co., LTD).

3.2.33 Incubator (set 37°C)

3.2.34 Curing light plus Optilux radiometer model

3.2.35 Pipette tip (size 100 µl, 1 ml)

3.2.36 Cuvette

3.2.37 Stop watch

3.2.38 Silicon sealant L6301, clear color (Sony Chemical & Information Device Corporation)

3.2.39 Curing light machine (ESPE Elipar® TriLight)

3.2.40 Spectrophotometer (Biomat, Becthai Bangkok Equipment & Chemical CO., Ltd.)

3.3 Methods

3.3.1 Selection and preparation of teeth

A total of 70 extracted human single-rooted teeth and straight root were used. The teeth were stored in 0.2% thymol solution until use. The teeth were immersed in 5.25% sodium hypochlorite for approximately 15 min to remove organic material from the root surfaces. Any remaining tissue was mechanically removed using a curette with intention not to damage the root surface. The teeth were stored in distilly water for 24 hours before use to eliminate traces of thymol. Each tooth was decoronated to give approximately 15 mm of root length from the coronal surface to the apex of the root with low speed saw (IsoMet®, Buehler). An operating microscope (Carl Zeiss Surgical, Inc.,Thornwood, NY) was used to inspect the roots for cracks under 25× magnification.

3.3.2 Instrumentation and obturation of root canals

The working length was determined visually by subtracting 1 mm from the length of a size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) at the apical foramen. The middle and coronal thirds were prepared using ISO size 1, 2, 3, and 4 Gate Glidden drills (Dentsply Maillefer, Ballaigues, Switzerland). All teeth were instrumented with a crown-down technique, using a set of ProTaper rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) as follows: S_1 (tip diameter 0.17 mm; variable taper) was taken into the canal just short of depth at which hand file was taken previously. The other files were used to working length in the following sequence, S_2 (tip diameter 0.20 mm;

variable taper), F_1 (tip diameter 0.02 mm; 7% taper), F_2 (tip diameter 0.25 mm; 8% taper), and F_3 (tip diameter; 0.30 mm; 9%). The apical portion of the canal was instrumented to size 40 master file with K-Flex-o-files (Dentsply Maillefer). The purpose of this preparation regimen was to create a uniform size of canal and to overcome the variations in natural morphology. Each canal was irrigated with 3 ml 2.5% sodium hypochlorite solution with a 27-gauge needle after each instrument and ensured patency by extrusion of the file beyond the apical foramen. The needle was inserted as deep as possible into the canal without binding. All canals were irrigated with 5 ml 17% EDTA solution for 1 minute to remove the smear layer

All root canals were enlarged by only one operator to minimize operator variation.

Twenty teeth were assigned to three experimental groups. Five teeth were assigned to the control groups.

Groups	Irrigant	Number of teeth
Group 1	Sterile water	20
Group 2	2% chlorhexidine	20
Group 3	2.5% sodium hypochlorite + 2% chlorhexidine	20

After preparation was complete

Group 1 was irrigated with 5 ml sterile water.

Group 2 was irrigated with 5 ml 2% chlorhexidine.

Group 3 was irrigated with 5 ml 2.5% sodium hypochlorite followed by 5 ml 2% chlorhexidine.

Each canal was dried with paper points.

Root canal obturation

Group 1, 2, 3: root canals were filled with Epiphany® and Resilon® (Pentron Clinical Technologies)

A self–etching primer (Epiphany® primer; Pentron Clinical Technologies) was placed into the canal with paper point. One drop of the primer was used for each root. Excess primer was removed using paper point, leaving the internal surfaces moist with primer. The remaining solvent evaporated with a gentle air spray for 5 seconds. Epiphany® root canal sealer was dispensed onto a mixing pad and placed with a master cone. The canal was then filled with Resilon® cone using the lateral condensation technique, which using a spreader size D11Ts (Dentsply Maillefer) and accessory resilon® cones (size fine). The tip of each accessory cone was lightly coated with sealer. When the Resilon® filling was completed, endodontic plugger was used for vertical compaction and the coronal surface was light cured for 40 seconds to polymerize. The deeper resin sealer then polymerizes by chemical curing during the following 30 to 60 minutes.

Positive control group

Root canals were filled using laterally compacted Resilon® cones without any sealer.

Negative control group

Root canals were sealed using laterally compacted Resilon® and Epiphany® sealer and completely covered with silicone.

After the obturation, all root canal specimens were examined with a microscope at 25x magnification to ensure that there were no cracks or craze lines in the roots. Moreover, in all groups postoperative radiographs were taken to ensure that all root canal specimens were properly obturated without voids. Root canal specimens were not adequately filled or with cracks were excluded and replaced by a new specimen.

After obturation, the all root canal specimens were stored at 37°C and 95% humidity for 1 week to allow the materials to set completely.

3.3.3 Glucose penetration model – preparation and measurement (Fig.16)

Each root was connected to a 17-cm-long glass tube and sealed with silicone sealant (clear color, L6301, Sony Chemical & Information Device Corporation). The all root canal specimens were coated with silicone, except for the 4 mm of apical part in

order to allow glucose penetration via apical region. The assembly was then placed in a sterile centrifuge tube with a rubber cap. 2 ml of 0.1 % benzoic acid solution in 50 mM sodium phosphate buffer solution (pH 7.0) was dispensed into the sterile centrifuge tube. The 4 mm apical portion of root canal specimens was immersed in the solution. Benzoic acid was used to inhibit the growth of microorganisms that might influence the glucose readings. The tracer used in the present study was 1 mol L^{-1} glucose containing 0.1% benzoic acid solution in 50 mM sodium phosphate buffer solution (pH 7.0).

All specimens were subjected to reduce pressure before the glucose solution was injected into the glass tube. The glass tube was connected to vacuum pump (Bio-Rad Laboratories, 3300 Regatta Boulevard, Richmond, CA 94804) by using Three-way, Double oblique, Pressure, Solid glass plug, Stopcock size 2 mm (Fortune Scientific Co., LTD). The air was removed from the root canal specimen and glass tube until the pressure in the system was stable at 20 inch Hg measured by pressure gauge that connected to the vacuum pump. After maintaining the pressure of 20 inch Hg for 5 minutes, the glucose solution was released into the glass tube by opening the three-way stop cock, without allowing air to enter the glass tube.

About 5 ml of the glucose solution, containing 0.1% benzoic acid in 50 mM sodium phosphate buffer solution was released into the glass tube until the solution was 14 cm above the root canal specimens. This level of glucose solution created a hydrostatic pressure of 1.5 KPa or 15 cm H_2O (Xu *et al.* 2005).

All specimens were stored in the incubator at 37°C and 95% humidity through observation period. A solution of 50 µl was drawn from the centrifuge tube using a micropipette at 1, 7, 14, 21 and 28 days. The same amount of 0.1% benzoic acid was added to the centrifuge tube reservoir to maintain a constant volume of 2 ml. The sample was then analyzed with a glucose liquicolor (Human, S.E. SUPPLY LTD., PART.) in a spectrophotometer at a wavelength of 500 nm. Concentration of glucose in the centrifuge tube was presented in mmol L^{-1}.

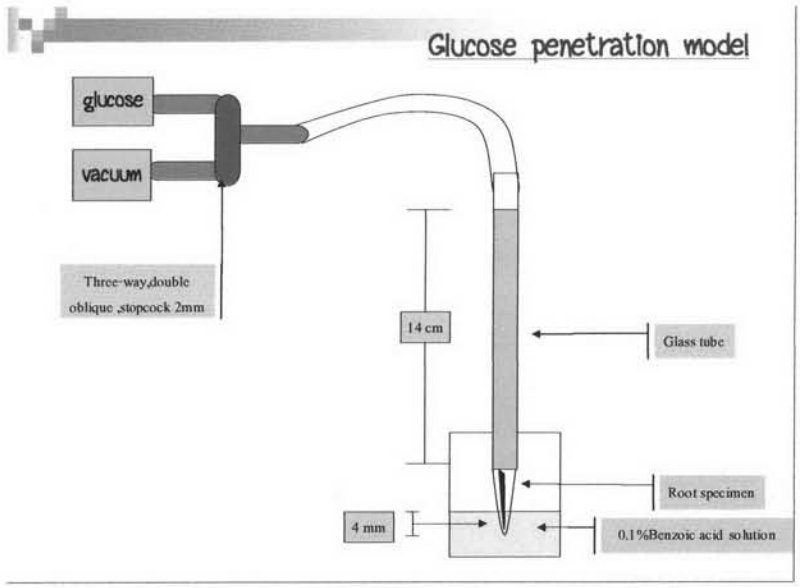

Fig. 16 Glucose penetration model (Modified from Xu *et al.*, 2005)

3.4 Outcome Measurement

Repeated measurement: blinded technique

1. Glucose penetration model
2. Spectrophotometer (wavelength of 500 nm)

The samples were analyzed with glucose liquicolor (Human, S.E. SUPPLY LTD., PART.) in spectrophotometer Spectrophotometer (Biomat, Becthai Bangkok Equipment & Chemical Co., Ltd.) at a wavelength of 500 nm. Concentrations of glucose in centrifuge tube were calculated by using standard curve between absorbance value and glucose concentration and presented in mmol L^{-1} at each time interval following root canal obturation.

3.5 Data analysis

Data concerning leakage of glucose concentration were checked for normality by using the Kolmogorov-Smirnov test. Data of glucose concentration (mM) that leakaged through the filled root canals were not normally distributed. Therefore, they were statistically analyzed by the nonparametric test (Friedmann test and Kruskal-Wallis test). The level of significance was set at $\alpha = 0.05$ (SPSS, version 12.0.1).