CHAPTER FOUR

MATERIALS AND METHOD

4.1. Collection and preparation of teeth

4.1.1. Collection of teeth

One hundred and fifty five extracted, single-rooted, mandibular premolar teeth were collected. The teeth were disinfected by using 0.5% Chloramine T trihydrate solution for one week. Soft and hard deposits were removed by using an ultrasonic scaler (Piezon® Master 400, Nyon, Switzerland). The teeth were then stored in distilled water, changed weekly, at 4ºC.

4.2. Tooth selection and preparation

4.2.1. Tooth selection

The selection procedure involved screening the teeth under a stereomicroscope (Kyowa Optical, Tokyo, Japan) at 10× magnification and discarding those teeth with gross caries and fractures. Buccolingual and mesiodistal radiographs (Eastman Kodak, Rochester, New York, USA) were taken to evaluate the integrity, number of canals and any excessively complex root canal morphology. The criteria of selection of the teeth included single, relatively straight canals, completely formed canals and patent foramina. All teeth with more than one canal, incomplete apex, obstruction within the canal system, internal or apical resorption, or pulp stone were discarded. Roots with severe curvatures were also abandoned. A total of 64 teeth were selected for the final sample.

The anatomical crowns of the selected teeth were removed with a separating disc (BEGO, Breman, Germany) under cooling water to minimize heating at the level of the cementoenamel junction (CEJ) perpendicular to the long axis of the root canal to obtain a relatively standard root canal length for all specimens. An attempt was made to select teeth with similar size and shape. Buccolingual (7.5 - 8 mm) and mesiodistal (5.0 - 5.5 mm) diameters of root canals were measured at the cementoenamel junction (CEJ) with a digital caliper (Mitutoyo/Digimatic, Tokyo, Japan). Each tooth was then mounted in impression compound (Hoffmann Harvard Dental, Berlin, Germany) to facilitate handling during root canal preparation and root canal obturation. The apical 2-3 mm of each root was left exposed to allow assessment of extrusion of root canal filling materials.

4.2.2. Preparation of root canal system

The pulp tissue was removed with a barbed broach (Dentsply Maillefer, Ballaigues, Switzerland). All the root canals were prepared with Gates-Glidden instruments (Dentsply Maillefer, Ballaigues, Switzerland) and K-files (Dentsply Maillefer, Ballaigues, Switzerland) using the crown-down pressureless technique as outlined by Morgan and Montgomery (1984).

4.2.2.1. Radicular access

For each specimen, radicular access consisted of preparing the coronal two-thirds of the root canal to remove the bulk of the canal contents and to facilitate straight-line access to the apical third of the canal.

1. The length of the coronal two-thirds of the root canal was estimated from the preoperative radiograph of the tooth.

2. The canal was sounded by placing a straight #35 file to the point of resistance without using apical force. If the file was stopped by canal narrowing, the canal was enlarged with hand instruments until the #35 file penetrated two-thirds into the canal without resistance. The radicular access length was then recorded.

3. Radicular access was completed in a wet canal with a #4 Gates-Glidden drill followed by #3 and finally with a #2 at 750-1500 rpm, each taken to the radicular access length without apical force. Irrigation with at least 3 mL of 5.25% sodium hypochlorite,

NaOCl (Clorox (M) Industries Sdn Bhd, Kuala Lumpur, Malaysia) was used to flush debris from the canal between Gates-Glidden drills and files.

4.2.2.2. Provisional working length

1. A provisional working length was established from the preoperative radiograph at a point 3 mm short of the radiographic apex.
2. A #30 file was then placed into the wet canal until resistance was first encountered. It was rotated clockwise two full revolutions without apical pressure. Next, a #25 file was placed in the canal until resistance was met and rotated twice passively.
3. This sequence was repeated with successively smaller files until the provisional working length was reached. After using each file, the root canal was irrigated with 3 mL of 5.25% sodium hypochlorite (Clorox) delivered from an endodontic luer-lok irrigation syringe with a 27-gauge needle (Monoject, Sherwood Medical, USA).

4.2.2.3. True working length and instrumentation sequence

1. A #10 K-file was introduced into the canal until it appeared at the apical foramen. The true working length was established by subtracting 0.5 mm from this measurement.
2. Afterwards, the file that reached the provisional working length was replaced by the next smaller file which was introduced into the canal and rotated twice passively in the canal. This was repeated with successively smaller files until the true working length was reached. The root canal was again irrigated with 3 mL of 5.25% sodium hypochlorite, after using each file.
3. The second instrumentation sequence began with a #35 file, which was one size larger than the file that began the previous sequence. The #35 file was then inserted, passively rotated twice, and removed. Consecutively smaller files were used similarly until a file reached the true working length. The root canal was again irrigated with 3 mL of 5.25% sodium hypochlorite, after using each file.

4. The third instrumentation sequence began with a #40 file and progressed similarly through consecutively smaller instruments down to the true working length. This was followed by a sequence beginning with a #45 file, and then one beginning with a #50 file. This sequence continued until K-file, #35, reached the working length and coronal preparation was reached to #70 file. The root canal was again irrigated with 3 mL of 5.25% sodium hypochlorite between each change of instrument.

5. After completion of the preparation, each canal was irrigated in the following sequence, initially with 3 mL 5.25% sodium hypochlorite for one minute, then with 3 mL of 17% ethylenediaminetetraacetic acid (SmearClear™, SybronEndo, Orange, USA) for one minute in order to remove the smear layer and finally, with 3 mL distilled water for one minute to ensure complete removal of the NaOCl residue. Each endodontic file and Gates-Glidden drill was discarded after preparation of five canals.

Adequate canal preparation was confirmed using the following criteria as recommended by Allison et al. (1979):

1. Recapitulation with a #10 K-file to ensure the removal of apical debris at the level of the apical foramen and to maintain patency.

2. Penetration of the apical foramen with the same file, approximately 1mm beyond the apical foramen.

3. Placement of a medium-size finger spreader (Kerr/SybronEndo, Romulus, USA) loosely to the working length.

All the specimens were then stored in distilled water to prevent dehydration of the specimens until obturation was done.

4.3. Compatibility between finger spreaders and accessory filling core materials (RealSeal™ and gutta-percha)

A medium-size finger spreader (Kerr/SybronEndo), medium-size accessory RealSeal™ core material (SybronEndo, Orange, USA) and medium-size gutta-percha core material (Kerr/SybronEndo, Romulus, USA) were selected for the cold lateral compaction technique (Figure 4.1). To assess the compatibility between the finger spreader and accessory cores, 12 specimens of medium-size finger spreaders, 24 specimens of medium-size accessory RealSeal™ cores and 24 medium-size accessory gutta-percha cores were selected and their diameters were measured at 1 mm (D1), 3 mm (D3), 6 mm (D6) from the tips using an image analysis system (Leica Qwin Colour (RGB), Cambridge, England).

4.4. Obturation of root canal system

The total sample of 64 teeth was randomly assigned into four equal groups (two experimental and two control groups). Prior to obturation, each canal was dried with paper points (Dentsply Maillefer, Ballaigues, Switzerland), and then obturated accordingly.

4.4.1. Lateral compaction of RealSeal™ (LC/R) as experimental group I

Sixteen specimens were obturated with the cold lateral compaction technique (LC) as recommended by the manufacturer (SybronEndo, Orange, USA) of the RealSeal™ obturation system (Figure 4.2):

1. A standardized RealSeal™ 0.02 taper, #35, master core that fitted to the working length with the help of an Endometer (Dentsply Maillefer, Ballaigues, Switzerland) was selected as the master core.

2. A medium-size finger spreader (Kerr) that passed down to within 1 mm of the apical terminus of the preparation was selected.

3. RealSeal™ self-etching primer was introduced into the root canal with a supersaturated paper point and excess primer was removed with a new paper point.

4. A paper point, #35, (Dentsply Maillefer) was coated with RealSeal™ sealer and then inserted immediately to the working length and the paper point was then removed.

5. The master core was coated lightly with RealSeal™ sealer and then inserted immediately to the working length.

6. The selected spreader was inserted alongside the master core with controlled apical pressure until it reached to within 1 mm from the end point of obturation. Apical pressure was applied for 10 seconds in a constant manner to achieve the appropriate compaction of RealSeal™ obturation material. Subsequently, the spreader was removed with 180º turn to prevent dislodgement of the compacted core.

7. The selected spreader was then cleaned with a piece of gauze and reinserted into the canal as described above.

8. The first medium-size accessory core was held with locking tweezers (NORDENT 2 Stainless, Germany) at the point equivalent to the length of the spreader that was inserted into the canal. After coating the core with RealSeal™ sealer, it was inserted without delay into the space created by the spreader.

9. The sequence of spreader application and RealSeal™ insertion was continued until the spreader could only penetrate 2-3 mm below the cementoenamel junction (CEJ). The System B side of the Elements™ Obturation Unit (SybronEndo, Orange, USA) was used to sear off the cores at the level of cementoenamel junction (CEJ). After that, the RealSeal™ in the coronal portion was vertically compacted using a root canal plugger, #11 (Hu-Friedy, Chicago, USA). When the RealSeal™ filling was completed, the coronal surface was light cured with a light curing unit (Spectrum™ 800, Dentsply Caulk, Milford, USA) for 40 seconds to create an immediate coronal seal.

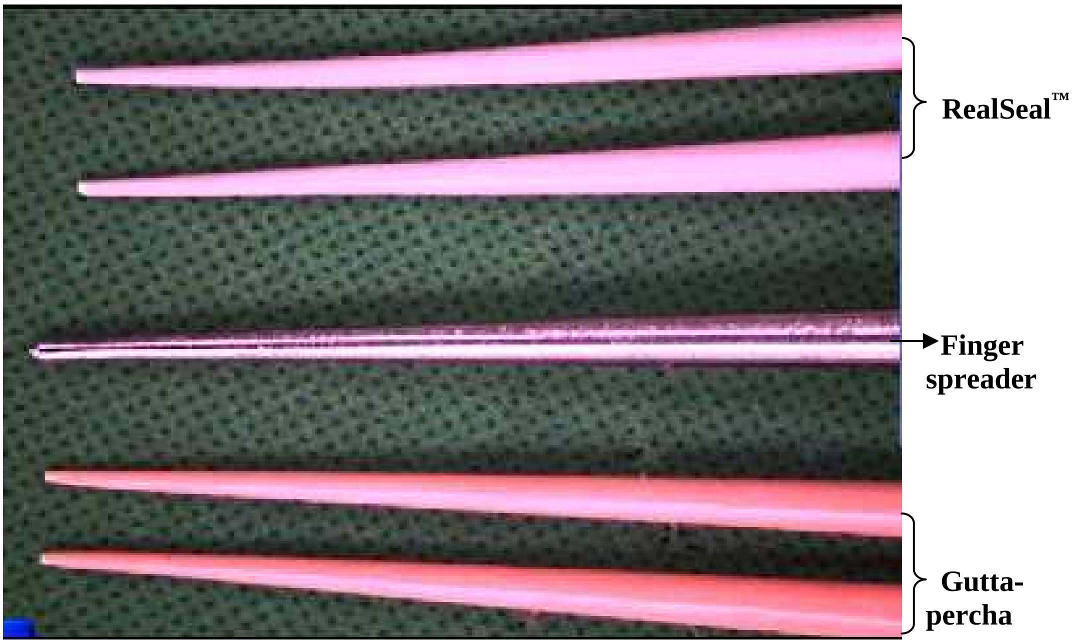

Figure 4.1 Medium-size finger spreader and accessory cores (RealSeal ™ and gutta-percha).

Figure 4.2 RealSeal™ obturation system.

4.4.2. Lateral compaction of gutta-percha (LC/GP) as control group I

The cold lateral compaction technique, as described by Gutmann and Witherspoon (2002), was used to obturate another 16 specimens.

Briefly, a standardized gutta-percha 0.02 taper, #35, master core (Kerr/SybronEndo) that fitted to the working length with the help of an Endometer was chosen as the master core. AH-Plus™ sealer (Dentsply De Trey, Konstanz, Germany) was then placed into the root canal with a paper point. The core was then coated lightly with AH-Plus™ and seated to place. Lateral compaction was accomplished, followed by insertion of medium-size gutta-percha accessory core using the instruments and technique as described for experimental group I. The System B side of the Elements™ Obturation Unit was used to sear off the cores at the level of cementoenamel junction (CEJ). After that, the gutta-percha in the coronal portion was vertically compacted using a root canal plugger, #11.

4.4.3. Warm vertical compaction of RealSeal™ as experimental group II

Sixteen specimens were obturated using a warm vertical "continuous wave" technique (WVCW) and warm vertical compaction of injectable RealSeal™ (WVCI) as recommended by the manufacturer:

1. Root canal pluggers, #7, #9 and #11 (Hu-Friedy) (Figure 4.3) were pre-fitted in the prepared root canal space. The rubber stop was then adjusted 2-3 mm short of the binding point in preparation for the back fill sequence. These pluggers were then placed aside to be used later in the back fill phase of the root canal system.

2. A medium-size Buchanan plugger (SybronEndo, Orange, USA) was fitted into the root canal (usually 3-4 mm from working length). The rubber stop was then adjusted 2-

3 mm shy of the binding point to minimize direct contact on dentine. The selected Buchanan plugger was then placed aside until use.

3. A medium-size non-standardized RealSeal™ core was fitted into the root canal until tug-back at the working length was achieved. The core tip was trimmed with a scalpel blade (No. 15) (Miltex, York PA, USA) until tug-back was achieved 0.5 mm short of the apical foramen.

4. RealSeal™ self-etching primer was introduced into the root canal with a supersaturated paper point and excess primer was removed with a paper point.

5. RealSeal™ sealer was dispensed onto a mixing pad. The sealer's viscosity was adjusted by using RealSeal™ thinning resin.

6. RealSeal™ sealer was then placed into the root canal with a paper point and the paper point was then removed.

7. The selected RealSeal™ core was then coated lightly with sealer and then inserted immediately to the working length. The inserted core was then downpacked with the selected Buchanan plugger using the System B side of the Elements™ Obturation Unit (Figure 4.4) at a temperature of 150ºC and a power setting of (10) as recommended by the manufacturer.

8. The switch was placed on touch mode.

9. The System B plugger was activated and driven through the centre of the RealSeal™ core material in a single motion into the canal to the level determined by the rubber stop.

10. The power was then deactivated and the plugger was pushed to the binding point and was held there for 10 seconds.

11. One second touch of heat was applied and after waiting a further one second, the plugger was removed.

12. When the heat carrier was removed, the surplus RealSeal™ material came out with it leaving a clean canal space. A hand plugger, #5, (Figure 4.3) was then used to compact the softened RealSeal™ and to confirm that it had not been dislodged.

13. RealSeal™ sealer was first applied to the walls of the canal before the backfilling.

14. Backfill of the canal was accomplished by an injectable RealSeal™ (Elements RealSeal™ obturation cartridge, SybronEndo, Orange, USA) (Figure 4.5) from the Extruder side of the Elements™ Obturation Unit (Figure 4.4) which was set at 150ºC.

15. The Extruder applicator tip was placed into the root canal against the apical RealSeal™ for 5 seconds.

16. It was allowed to warm the RealSeal™ and then a 4-6 mm increment was delivered. Whilst still soft, the RealSeal™ material was compacted using a selected plugger; the compacting cycle began with a small plugger followed by bigger pluggers with the application of each increment.

17. The mass of RealSeal™ was allowed to "push" the needle coronally to the cementoenamel junction (CEJ) and the needle was removed after a pause of one second. The selected root canal plugger, #11, was then inserted into the canal to firmly compact the mass of RealSeal™. When the RealSeal™ filling was completed, the coronal surface was light cured for 40 seconds to create an immediate coronal seal.

4.4.4. Warm vertical compaction of gutta-percha as control group II

A medium-size non-standardized gutta-percha core was fitted into the root canal until tug-back at the working length was achieved. The core tip was trimmed with a scalpel blade (No. 15) until tug-back was achieved 0.5 mm short of the apical foramen. AH-Plus™ sealer was then placed into the root canal with a paper point. The selected gutta-percha core was then coated lightly with sealer and then inserted immediately to the working length. The inserted core was then downpacked with the selected Buchanan plugger using the System B side of the Elements™ Obturation Unit at a temperature of

200ºC and then backfilled with the Extruder side of the same unit using gutta-percha (Elements gutta-percha obturation cartridge, SybronEndo, Orange, USA) (Figure 4.6) which was set at 200ºC as described for experimental group II. The mass of gutta-percha was allowed to force the needle coronally to the CEJ and the needle was removed after a pause of one second. The selected root canal plugger, #11, was then inserted into the canal to firmly compact the mass of gutta-percha.

4.5. Radiographic evaluation

For all four groups, after canal obturation, each tooth was removed from its impression compound mounting. Radiographs were then taken from the buccal and mesial aspect of each tooth to assess the quality of the root canal filling. If the root canal filling was considered unsatisfactory, a new specimen was prepared using the same materials and method. The canal coronal to the root filling was restored with IRM (Dentsply Caulk, Milford, USA).

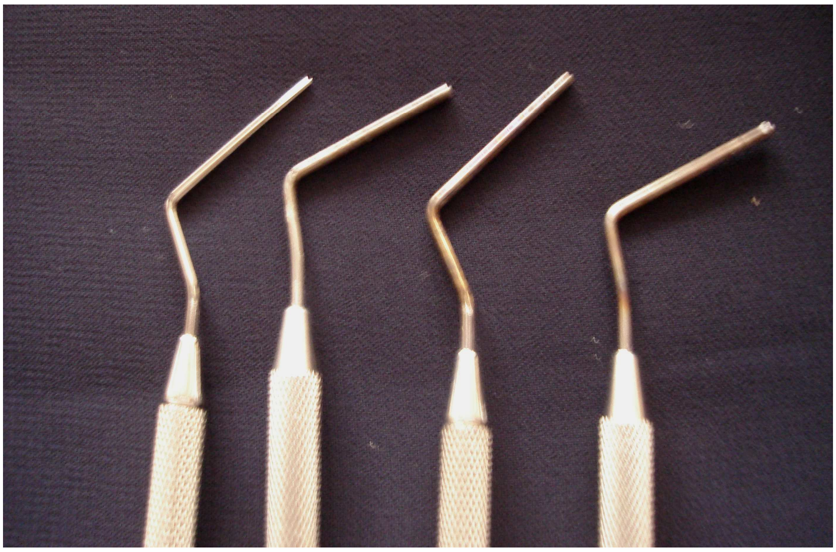

Figure 4.3 Root canal pluggers #5, #7, #9 and #11 from left to right.

Figure 4.4 Elements™ obturation unit.

Figure 4.5 Elements RealSeal™ obturation cartridge.

Figure 4.6 Elements gutta-percha obturation cartridge.

4.6. Evaluation of root canal obturation

4.6.1. Time taken during obturation

Time was measured from the beginning until the end of the whole obturation procedure. Time was recorded with a stopwatch (Citizen, Tokyo, Japan).

4.6.2. Assessment of extrusion of root filling materials

The presence or absence of extruded root filling materials through the apical foramen was assessed through a stereomicroscope (Kyowa Optical) at 10× magnification. It was recorded on a dichotomous scale: “Yes” or “No”.

4.6.3. Assessment of obturation quality

4.6.3.1. Assessment of cross-sections

4.6.3.1(a). Sectioning of specimens

All specimens were then kept in an incubator (Memmert, Schwabach, Germany) at 37ºC for one week in 100% humidity to simulate oral environment. Each specimen was then fixed by using baseplate wax in a plastic cuvette (Dispolab-Kartell II, Milano, Italy) and embedded in epoxy resin (Mirapox A and B, Miracon, Kuala Lumpur, Malaysia). The epoxy resin was allowed to set for 24 h. Each root was then ground from the tip in a grinding machine (Grinder/Polisher, Metaserv®, Buehler Ltd, Illinois, USA) until the obturation material could be seen. Each specimen was then sectioned horizontally in a low-speed sectioning machine (ISOMET™, Buehler® Ltd, Evanston, USA) (Figure 4.7).

Cutting of the first section was done with a 0.3 mm thick diamond rotary blade (ISOMET™) with copious coolant lubricant. Since the thickness of the blade was 0.3 mm, the first cut was made 1.3 mm from the obturated canal terminus. The second one was made at 3.3 mm and the third one was made at 6.3 mm from the obturated canal terminus as illustrated in Figure 4.8.

Figure 4.7 ISOMET™ Low speed saw.

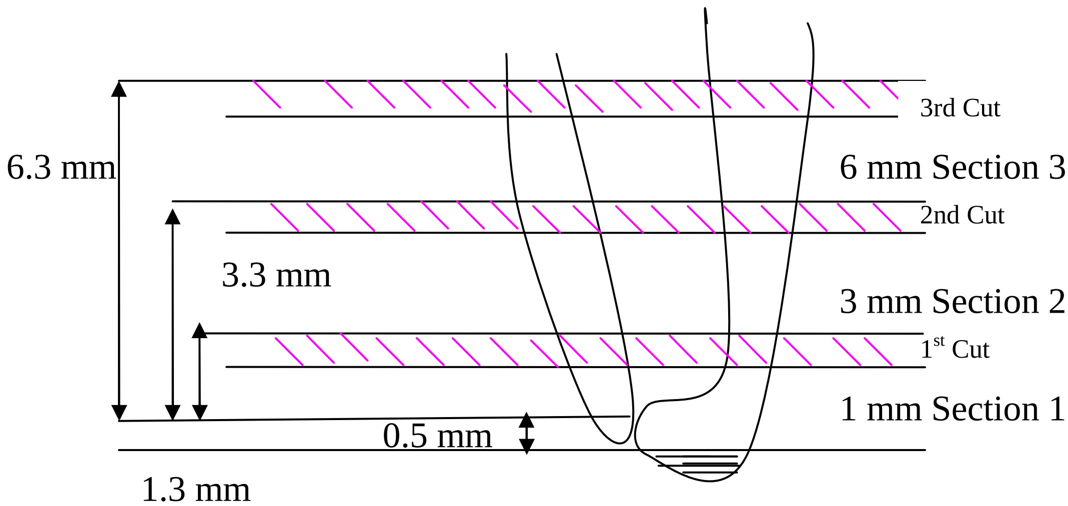

Figure 4.8 Schematic diagram of root canal sections.

4.6.3.1(b). Measurements of cross-sections

Fifteen teeth from each group were used to investigate the percentages of canal area occupied by RealSeal™ core material, sealer and voids, and the area occupied by gutta-percha, sealer and voids at the three cross-sections. Images of each section were acquired with a video camera (Digital ½ inch CCD, JVC, Yokohama, Japan) and zoom microscope (Edmund Industrial Optics, Tokyo, Japan) (Figure 4.9).

Shapes of root canals in all cross-sections were examined using an image analysis system (Leica Qwin Colour). To determine whether the shape of each canal is round or irregular, the buccolingual and mesiodistal canal diameters were measured.

Percentages of area occupied by RealSeal™ filling core material, sealer and voids were measured and compared with the areas of gutta-percha, sealer and voids by using an image analysis system (Leica Qwin Colour). To measure percentages of area occupied by filling core materials for each image, the operator drew around the region of interest by clicking the left mouse button and dragging the mouse. At the release of the mouse button, the region representative of the area was highlighted. Subsequently, areas occupied by sealers and voids were traced in the same manner as for the areas of RealSeal™ core material (Figure 4.10) and gutta-percha core material (Figure 4.11). Once all areas were mapped out, the image analysis system would compute itself by counting the number of pixels of the representative area. Areas of RealSeal™ core, sealer and voids or gutta-percha, sealer and voids were expressed as percentages of the total area.

4.6.3.1(c). Reliability measurement

All the cross-sections were then stored in distilled water until reproducibility of measurements and accuracy of the method was done. Intra-examiner reliability was assessed by re-evaluating randomly 10% of all cross-sections on two occasions at one-week intervals and without the knowledge of the previous readings. The areas occupied by RealSeal™ core material, sealer, and voids of nine randomly selected cross-sections and areas occupied by gutta-percha, sealer, and voids of nine randomly selected cross-sections were measured. Data collected was statistically analyzed. Based on the variance of each mean, the variation for each repeated measurement was calculated and then the total variation was obtained.

4.6.3.2. Scanning electron microscopic observation

The last specimen from each group (randomly chosen) was examined in a field-emission gun scanning electron microscope (FESEM) (Low Vacuum Operating Mode, Quanta 200 F, FEI, Hillsboro, USA) (Figure 4.12). A central line was drawn along the longitudinal axis of the specimen embedded in the epoxy resin. Each specimen was then longitudinally sectioned so that the dentine-filling interface could be evaluated. It was mounted on the aluminum stub using carbon tape as adhesive and was subsequently mounted on the 7-holder specimen stage inside the chamber. The specimens were not coated prior to analysis. Imaging was done under 10 and 15 kV accelerating voltage at magnification between 200× to 4000× using both the secondary electron and back-scattered electron signals.

Figure 4.9 Video camera and zoom microscope.

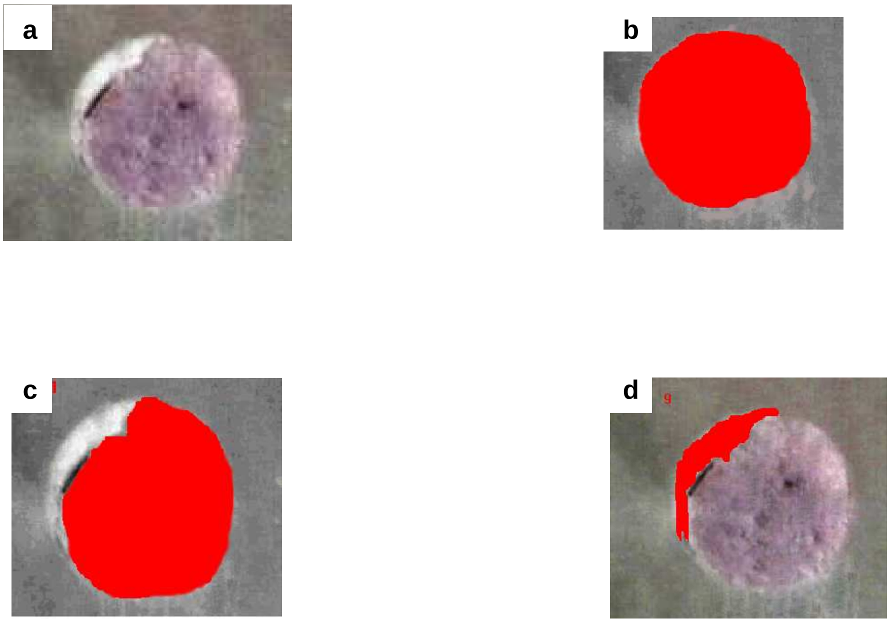

Figure 4.10 (a, b, c, d) Measurement of percentages of canal area occupied by RealSeal™ core material and sealer.

a. A cross-section at L1.

b. Highlight of the entire canal area at L1.

c. Highlight of the region representative of the area of RealSeal™ core.

d. Highlight of the region representative of the area of sealer.

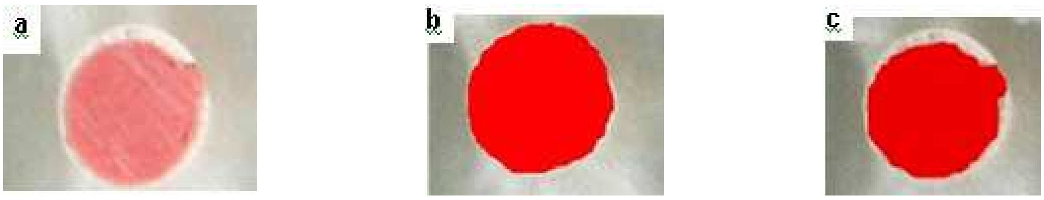

Figure 4.11 (a, b, c) Measurement of percentage of canal area occupied by gutta-percha.

a. A cross-section at L1.

b. Highlight of the entire canal area at L1.

c. Highlight of the region representative of the area of gutta-percha core.

Figure 4.12 Field-emission gun scanning electron microscope (FESEM).

4.7. Data analysis

The data were subjected to statistical analysis in SPSS, version 12 (SPSS Inc., Chicago, USA) as follows:

4.7.1. Time taken for obturation

The mean time taken to obturate the canals was analyzed using the one-way analysis of variance (ANOVA). If there was a significant difference among the groups, the multiple comparisons (Dunnett T3) was carried out. If the p-value was <0.05, it meant that the difference was statistically significant and vice versa.

4.7.2. Extrusion of filling materials through apical foramen

A chi-square test was done to analyze association of extrusion with types of filling materials and techniques used.

4.7.3. Percentages of filling core materials, sealers and voids

The quality of filling materials at each level using the same technique was subjected to statistical analysis using the independent sample t-test. However, the homogeneity of variance assumption was not similar. Thus the non parametric analysis test (Mann-Whitney U test) was applied. To compare the effect of both materials and both techniques, the two-way analysis of variances (ANOVA) was used. To show the distribution of the percentages of filling core materials (RealSeal™ and gutta-percha), sealers and voids at all levels, the Kolmogorov-Smirnov (α) test was applied.