

**CHAPTER 5**  
**MATERIALS AND METHOD**

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**5.1 Collection and storage of specimen**

A total of 100 extracted sound human premolar teeth were collected. Storage after collection, cleaning of the teeth and pre-selection radiographs were done as described previously in section 4.1.1. A total of sixty-six teeth were selected according to the inclusion criteria for use in the study. These teeth were then stored in 10% neutral-buffered formalin until use.

## 5.2 Preparation of specimens

Two millimetres of the root tip was removed above the apical opening. This was followed by apical preparation using Gates-Glidden bur up to size 5. The tooth was then mounted, cross-sectioned and instrumented using K-File up to size 90 as described in the preliminary study. The clear muffle was used to hold the specimen while canal preparation was done.

The specimens were then randomly divided into three groups according to the methods of placement of calcium hydroxide. Each group (22 specimens) was further subdivided into 2 sub-groups (11 specimens each) according to the type of calcium hydroxide used. One specimen from each sub-group (total of 6 specimens) would be randomly selected later for 3-dimensional imaging using microcomputed tomography (SkyScan 1172), leaving 60 specimens for radiographic and cross-sectional investigation and analysis (Table 5.1).

**Table 5.1: Sample according to method of placement and material**

Group	Method of placement	Sub-group	Material

□

Group A  
(n=22) □

Syringe

□ Group A1

Group A1

(n=11) □ Pulpdent® Tempcanal™ □ □ □ □ Group A2  
Pulpdent® Tempcanal™ □ □ □ □ Group A2

□ □ □ Group A2

□ □ Group A2

□ Group A2

Group A2

(n=11) □ Vitapex™ □ □

Vitapex™ □ □

□

Group B  
(n=22) □

Syringe +

Lentulo spiral □ Group B1

Group B1

(n=11) □ Pulpdent® Tempcanal™ □ □ □ □ Group B2  
Pulpdent® Tempcanal™ □ □ □ □ Group B2

□ □ □ Group B2

□ □ Group B2

□ Group B2

Group B2

(n=11) □ Vitapex™ □ □

Vitapex™ □ □

□

Group C  
(n=22) □

Lentulo spiral □ Group C1

Group C1

(n=11) □ Pulpdent® Tempcanal™ □ □ □ □ Group C2  
Pulpdent® Tempcanal™ □ □ □ □ Group C2

□ □ □ Group C2

□ □ Group C2

□ Group C2

Group C2

(n=11) □ Vitapex™ □ □

□

### 5.3 Method of calcium hydroxide placement

Calcium hydroxide paste (Vitapex™ or Pulpdent® Tempcanal™) was placed into the canal while the specimen was secured within the muffle. Both types of calcium hydroxide were placed according to the assigned method as described below:

#### a) Syringe method

For this method a commercially prepared calcium hydroxide paste preloaded in a syringe was injected into the prepared canal. Two types of calcium hydroxide paste were used in this study ie. Vitapex™ (oil suspension calcium hydroxide) and Pulpdent® Tempcanal™ (aqueous suspension calcium hydroxide). Vitapex™ (Figure 5.1) is supplied with a special disposable plastic syringe tip which can be directly connected onto the tube. On the other hand, Pulpdent® Tempcanal™ (Figure 5.2) is packed in a 3 ml syringe with an 18, 22 and 25 gauge, blunt tip, metal hub needle.

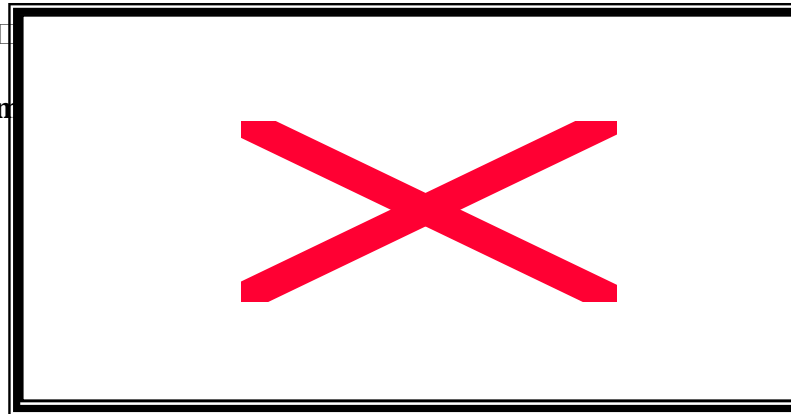
Similar method was used for both materials. For Vitapex™ placement, a rubber stopper was placed on the syringe needle at a level of one fifth of the working length (WL) from the apical part (as in the manufacturer's instruction). The needle was then inserted into the canal to the length as determined by the rubber stopper. The piston of the syringe was then slowly pressed out. The filling amount could be noticed from the graduation gauge. The tip should be slowly pulled out as the paste reversed back into the pulp chamber.

For Pulpdent® Tempcanal™, intracanal placement was also done according to the manufacturer's instruction. Size 25 gauge needle was selected. A rubber stopper was placed on

the needle up to the WL. The needle was inserted into the canal and the screw plunger was turned slowly to allow the material to flow through the needle. The canal was filled completely by turning the plunger while slowly withdrawing the needle. To stop the flow, the plunger was turned counter-clock-wise.

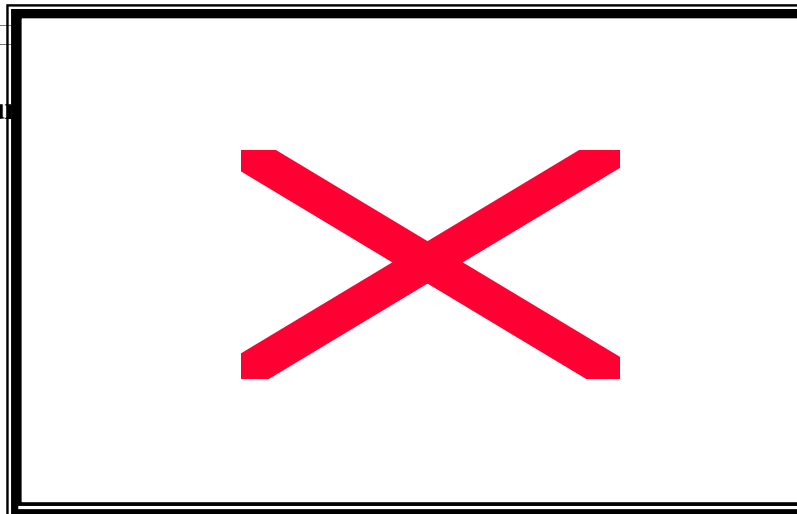
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**Figure 5.1: Oil suspension calcium**



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**Figure 5.2: Aqueous suspension calcium**



**b) Syringe and followed by lentulo spiral**

The syringe method was done as described above, but after the paste was injected, a size 35 lentulo spiral was inserted up to working length in a clockwise direction with a slow-speed handpiece, rotated at 500 rpm. The lentulo spiral was removed and reinserted to the working length three times while turning the instrument (Sigurdsson *et al.*, 1992).

**c) Lentulo spiral**

Small amounts of calcium hydroxide paste were placed on the whorls of the Lentulo spiral of ISO size 35. The paste was introduced into the root canal using a slow-speed handpiece, rotated at 500 rpm up to the working length. Once the Lentulo spiral was placed into the root canal, it was slowly rotated out of the canal, under light pressure against the root canal walls. This was repeated until the paste could be seen at the entrance of the root canal system. No additional packing was done.

## **5.4 Post insertion radiograph**

### **5.4.1 Three-dimensional view**

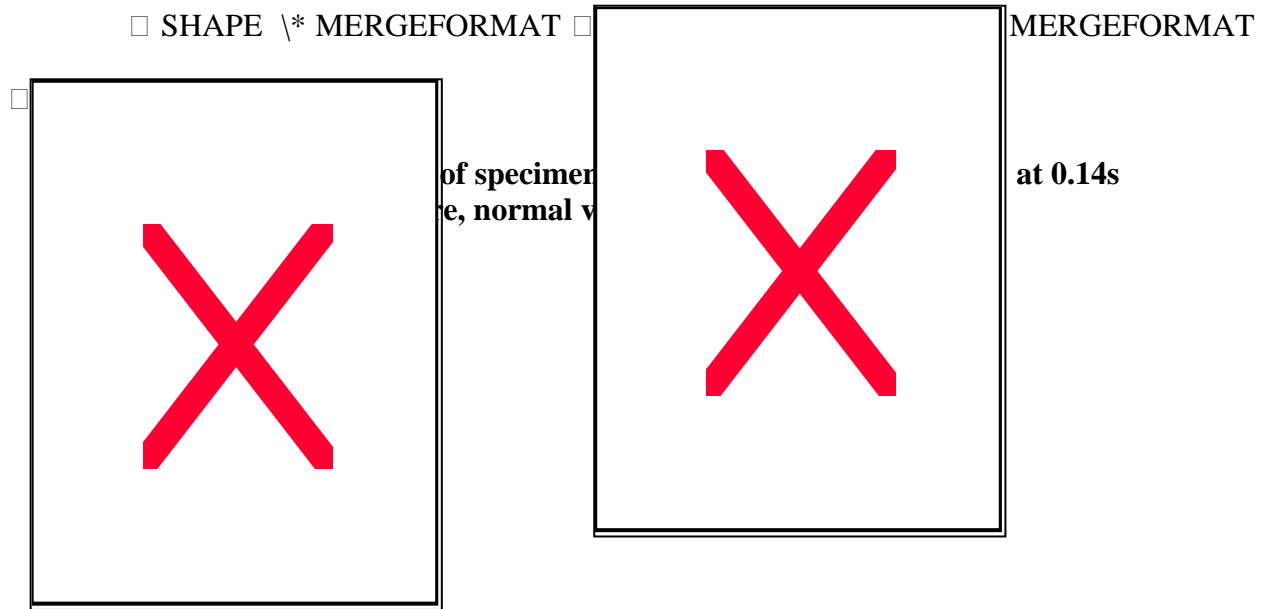
Following the insertion of the calcium hydroxide pastes, one specimen from each subgroup was randomly selected and these 6 specimens were sent for 3-dimensional imaging using microcomputed tomography (SkyScan 1172).

### **5.4.2 Two-dimensional view**

Radiographs of the specimens were taken at anterior view using the standardized vertical radiographic platform as described and shown in section 4.1.4 and Figure 4.18. Radiographs were taken at 0.12s exposures for specimen filled with Vitapex™ and 0.14s exposures for specimens with Pulpdent® Tempcanal™ using conventional x-ray machine (Trophy Irix 70 (CCX Digital), 230 Volt, 8 mA, 70 HT kV) and special image plate (VistaScan, Phosphor Storage Plates). Radiographs were taken and scanned as described previously in section 4.1.5. The images of the radiographs, which were scanned using the Dürr VistaScan Intra 2130-60 into the DBSWIN software, were imported in a JPEG command into a pendrive. The images were then exported into Leica QWin software, for which the analysis of the images was carried out.

Due to the poor radiographic contrast between the dentine and Pulpdent® Tempcanal™, the radiograph had to be embossed prior to analysis (Figure 5.3). This was carried out by using Adobe® Photoshop® Version 6.0 software. The images were embossed under those commands:

Filter-> Stylize-> Emboss and embossment was standardized with an angle of 125°, a height of 30 pixels and amount of 200%. This technique would give a better outline of the image and aids radiographic analysis of the images.





### 5.5 Investigation of the cross-sections using Image Analyser

After the radiographs were taken, the specimens were kept inside the muffle at room temperature for 24 hours. After 24 hours, the muffle was unlocked and the cross-sections of the specimens were carefully separated (Figure 5.4(a)).

□ □ □

□

**Figure 5.4 (a)**

□ □

□

**Figure 5.4 (b)**

**Figure 5.4(a) and (b): Cross-sections of the specimen showing surfaces A and B**

Surfaces A and B of the cross-sections of each specimen (Figure 5.4 (b)) were examined using Image Analyser. The Image Analyser consists of a stand with measurement in centimetres on it, a macrolens and a flat platform to place the specimen. It is connected to the computer and the images were captured and stored in Leica Qwin software (Leica Imaging Systems Ltd., Cambridge, England) (Figure 5.5).

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**Figure 5.5: Image Analyser (Leica Imaging Systems Ltd., Cambridge, England)**



Prior to examination, the cross-section of the specimens was placed on a jig or special holder. It was made from clear acrylic with a central hollow column and a moveable platform (Figure 5.6 and 5.7). Each cross section was placed centrally on the column and the screw was turned clockwise until the surface to be examined was at the same level as the top part of the jig (Figure 5.8). This would ensure that the distance between the macrolens to the surface of the section to be examined is kept constant for all the specimens.

□

**Figure 5.6: Anterior view of the jig with central hollow column and a screw to move the platform**

□

**Figure 5.7: Lateral view of the jig**

□

**Figure 5.8: Top view of the specimen placed inside the jig, ready to be examined under Image Analyser**

## **5.6 Determination of the accuracy of length from radiographs (quality of filling)**

The radiographs were scanned using VistaScan into a computer using DBSWin software. The quality of filling was evaluated from the images by giving a score modified from Rivera & Williams (1994) as bellow:-

- Score 1 –  $\leq 2$ mm from the apex – apex (hereafter referred as at the apex)
- Score 2 –  $>2$ mm from the apex (hereafter referred as short of the apex)
- Score 3 – extrusion / long beyond the apex

## **5.7 Determination of the quality of filling/surface area from the cross-sections of the specimens**

Using the Image Analyser, the shape demarcating the boundaries of the inner wall of the canal was drawn to obtain the surface area of the surface A or B (a). This was followed by demarcating the shape of the material present to obtain the surface area (b). This was a straight forward procedure if there was no voids within the material or if presence, the voids were along the inner wall of the canal. Any voids present within the material were demarcated as well to get the surface area (c), and these values would be subtracted from values (b) to get the exact surface area occupied by the material. The percentages of the surface area occupied by the material were obtained by dividing (b) or ((b)-(c)) by (a) and multiply it with 100. This was done for each of the surfaces of the cross-section of the specimens.

Example:-

i) Surface area of canal (a) = 55mm<sup>3</sup>

Surface area of material (b) = 45mm<sup>3</sup>

Percentage of filling =  $45\text{mm}^3/55\text{mm}^3 \times 100 = 81.82\%$

ii) Surface area of canal (a) = 60mm<sup>3</sup>

Surface area of material (b) = 55mm<sup>3</sup>

Surface area of voids within the material (c) = 15mm<sup>3</sup>

Percentage of filling =  $(55\text{mm}^3 - 15\text{mm}^3)/60\text{mm}^3 \times 100 = 66.67\%$

The absence/presence of voids and the location of voids from the cross sections was recorded as well, whether it was within the material or at the canal wall. Scoring system was used whereby Score A – no voids, Score B – presence of voids within the material and Score C – presence of voids at the wall of the canal.

## 5.8 Data Analysis

The Statistical Package for Social Science (SPSS) Version 11.5 was used for data analysis as it provides multivariate tests of the main effects and interactions. Summary of statistics (mean, standard deviation, median and percentage) were obtained for the data set. The data were also subjected to SPSS DESCRIPTIVES in the evaluation of assumption.

The intra-examiner consistency was calculated for the measurements of 10% of the specimens at two weeks interval. The intra-examiner consistency was assessed using a weighted

kappa statistic and it was noted that the percent agreement was 90%, giving a favourable indication of the consistency of the evaluation.

### **5.8.1 Analysis of quality of filling from the radiographs**

Categorical data is typically analyzed by calculating chi-square values testing the hypothesis of independence. When tables consist of more than two variables, the best test is a log-linear model. This model which is an extension of two-way contingency table was first introduced by Goodman in 1970's. For this part of the study, there is one dependent variable (DV), that is the quality of filling (score) and two independent variables (IVs) that are the types of calcium hydroxide and the methods of placement. Thus, the table would consist of more than two variables. The data was initially analysed by using the two-by-two contingency using chi-square test, to test the interaction for types of calcium hydroxide and methods of placement.

### **5.8.2 Analysis of quality of filling from the cross sections**

For this part of the study, there are two IVs and two DVs.

The two independent variables (IVs) are :-

- a) intracanal placement methods
  - i) syringe
  - ii) syringe, followed by lentulo spiral
  - iii) lentulo spiral
- b) types of calcium hydroxide according to vehicles
  - i) Pulpdent® Tempcanal™ (aqueous vehicle)

- ii) Vitapex™ (oil vehicle)

The two dependent variables (DVs) are:-

- a) percentage of surface area of calcium hydroxide at level A (10mm from the base of the curvette)
- b) percentage of surface area of calcium hydroxide at level B (5mm from the base of the curvette)

Thus, a 2x2x3 factorial multivariate analysis of variance (MANOVA) is necessary for data analysis. MANOVA is used to ascertain whether:-

- a) the quality of filling at the two levels of cross-sectioning varies as a function of differences in methods of placement.
- b) the quality of filling at the two levels of cross-sectioning varies as a function of differences in types of vehicles of calcium hydroxide.
- c) the quality of filling has interaction with both methods of placement and types of vehicle of calcium hydroxide.

### **5.8.3 Analysis of presence/location of voids**

This part of the study involves categorical data of more than two variables. Thus, similar analysis as for the analysis of quality of filling from the radiographs (section 5.8.1) was used (chi-square and extension of chi-square by means of log-linear model).