CHAPTER 4

PRELIMINARY STUDY

CHAPTER 4

4.1 Preliminary study

A preliminary study was conducted prior to the actual study. This study was done to investigate the following:

- Methods of mounting the specimen
- Fabrication of muffle to hold the specimen
- Size of Gates-Glidden bur to prepare the apical part of the specimen in order to simulate an immature apex
- Largest file use to prepare the canal
- Methods to standardize radiograph-taking
- Exposure time for the radiograph-taking of the mounted specimen inside the muffle
- Suitable time interval after calcium hydroxide placement to separate the cross-sections of the specimen

4.1.1 Teeth selection

Teeth selected for the preliminary study were human single rooted teeth ie. premolars, which were extracted due to orthodontic purposes. Teeth were placed immediately in 10% neutral-buffered formalin. The purpose of fixation is to arrest any physical and chemical changes that could occur upon death of tissue. The teeth were then stored in 5.25% sodium hypochlorite solution for 24 hours in order to remove the organic debris and any remaining soft tissues. Teeth were cleaned under flowing water using brush. Once cleaned, teeth were kept in a fresh solution of 10% neutral-buffered formalin until use.

Pre-selection radiographs were taken to determine the anatomy of the canals. The inclusion criteria were as follows:-

- Teeth exhibit one root and one canal
- Teeth with no cracks
- Roots are free from resorption, caries and fractures

- Roots are not dilacerated

4.1.2 Preparation of tooth

Conventional endodontic coronal access cavity was prepared using transmetal bur and non-end cutting tapering bur. Pulp tissue was removed using barbed broaches, followed by copious irrigation of 2.5% sodium hypochlorite and dried with paper points. Two millimetres of the root tip was resected above the apical opening to create an open apex (Hachmeister *et al*, 2002). The actual tooth length (ATL) was determined visually after removal of 2.0mm of the apical end by using magnifying lens and a size 10 K-File was inserted into the canal until the tip of the file just emerged at the apical opening (Figure 4.1**).** The working length (WL) of the tooth was recorded at 1.0mm shorter than the ATL (Sigurdsson *et al.,* 1992**)**.

Figure 4.1: File tip just visible at the apical opening

4.1.3 Preparation of specimen

4.1.3.1 Mounting of specimen

Tooth was mounted temporarily on a block of modelling wax with approximately 2.0mm of the apical opening embedded in the wax block (Figure 4.2). A spectrophotometric clear curvette was used to mount the tooth (Figure 4.3). The base of the curvette was removed and it was cut according to the length of the tooth. The cuvette was placed directly on top of the wax block with the partially embedded tooth in the centre of the curvette (Figure 4.4). Clear acrylic (Mirapox 950-230) was carefully poured into the curvette. The clear acrylic required at least 24

hours to set. Once the acrylic has set, the wax block was removed to reveal the uncovered apical area (Figure 4.5).

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Figure 4.2: Apical part of the tooth embedded in a block of wax

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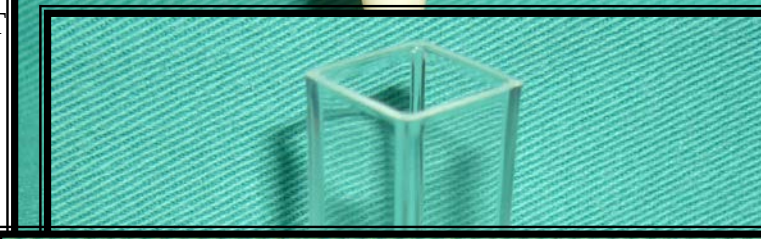

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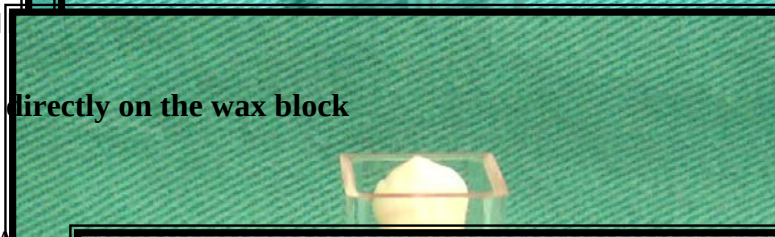

Figure 4.4: The curvette placed directly on the wax block

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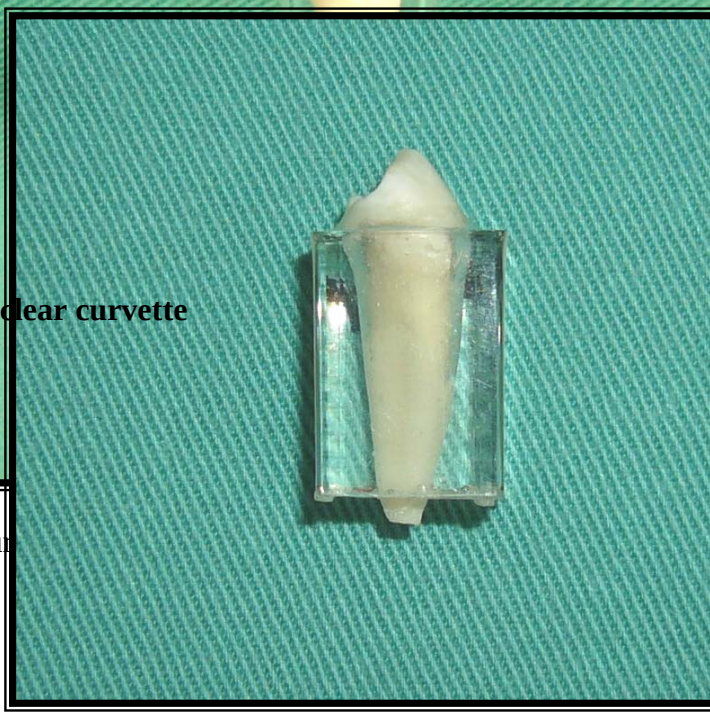

Figure 4.3: A spectrophotometric clear curvette

4.1.3.2 Preparation of the apical part of th

Gates-Glidden burs (Figure 4.6) were used t

to simulate a divergent apex of an immature perma

preparation. A periapical radiograph was taken afte

Further preparation and radiographs were repeated for bu

Figure 4.5: The specimen mounted with clear acrylic in the curvette with the apical part of the tooth uncovered

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Figure 4.6: Gates-Glidden

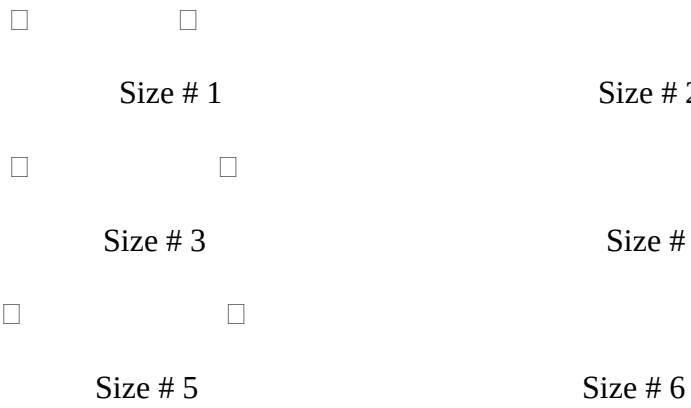

Figure 4.7: Radiographs of specimen with apical preparation using Gates-Glidden bur size 1 to 6.

4.1.3.3 Cross-sectioning of the specimens

The specimens were cross-sectioned at two levels, 10mm (level A) and 5mm (level B) from the lower end of the curvette using diamond wafering slow speed blade (ISOMET low speed saw) (Figure 4.8). In order to aid in re-assembling the cross-sections of the specimens, one of the two methods were carried out prior to the cross-sectioning procedure:-

a) a diagonal line was drawn using permanent ink marker pen on one of the outer surfaces of the specimen (Figure 4.9(a) and 4.9(b)).

b) a diagonal indentation on the same place as in Figure 4.9(a) and 4.9(b) was made using a small round bur on a high speed handpiece (Figure 4.10(a) and 4.8(b)).

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Figure 4.8: ISOMET low spee

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Figure 4.9(a)

Figure 4.9(a) and (b): Diagonal line drawn using a marker pen and separated cross-sections with the diagonal line

□ □

Figure 4.10(a) **Figure 4.10(b)**

Figure 4.10(a) and (b): Diagonal line made from small round bur on high speed handpiece and the separated cross-sections

4.1.3.4 Fabrication of muffle to hold the cross-sections

Two types of muffle were fabricated:-

i) Muffle fabricated from plaster of Paris and dental stone (Figure 4.11) as described by Bramante *et al.* (1987).

Figure 4.11: Muffle from dental stone and plaster of Paris at different views

ii) Muffle fabricated from clear perspex

Similar muffle was constructed using clear perspex. It comprised of two parts of perspex block which fits snugly into each other using 'key-hole' system (Figure 4.12). At the centre of the muffle, a square opening that has exactly the same size as the specimen was done in order to place and hold the specimen (Figure 4.13).

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Figure 4.13: Anterior view of clear perspex muffle

4.1.3.5 Preparation of the coronal part c

Figure 4.12: Side view of clear perspex muffle with "key-and-hole" system

Cross-sections of the specimen were reassembled and placed in the muffle (Figure 4.14(a) and (b)). With the specimen placed in the muffle, the canal of the tooth was prepared to the working length (WL) using the K-File (Dentsply, Maillefer) of different sizes (Figure 4.15) and enlarged up to size 90. Instrumentation of the canal was carried out with EDTA-containing chelating agent as lubricant (RC-Prep, Premier, PA, USA). Copious irrigation was done in between instrumentation using 2.5% sodium hypochlorite. Canal was then dried with paper points and then filled with calcium hydroxide pastes (Vitapex™ or Pulpdent® Tempcanal™).

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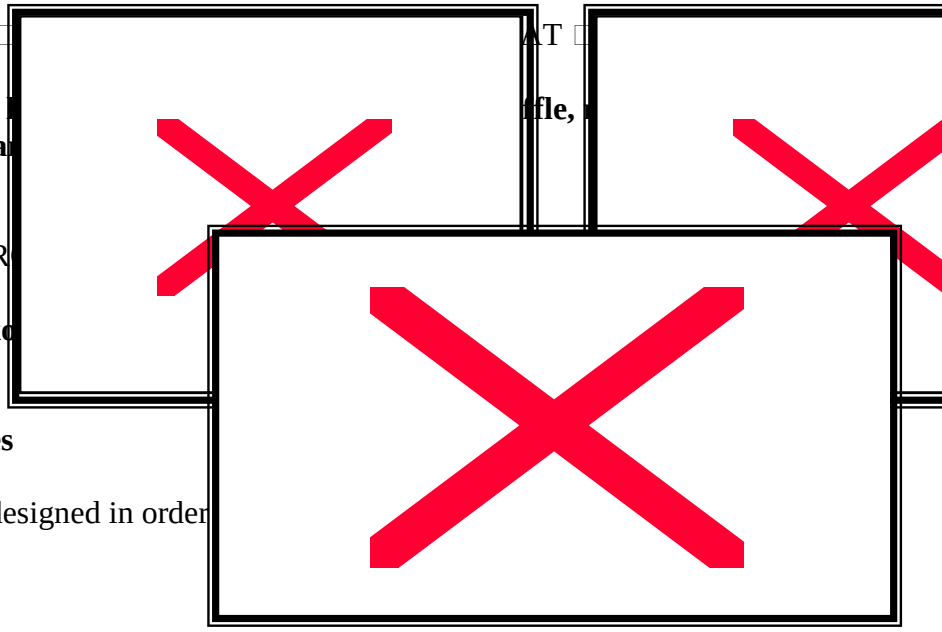

Figure 4.14(a) and (b): Anterior and ffle, for canal prepa

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Figure 4.15: K-Flex

4.1.4 Radiograph-taking techniques

Two radiographic platforms were designed in order

a) horizontal arrangement

In this technique, a conventional x-ray film holder was used and modified by placing a special attachment that can house the muffle on the bite plane of the film holder (Figure 4.16). The muffle/specimen and a periapical film were placed in the film holder using a double-sided adhesive tape (Figure 4.17).

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Figure 4.16: Film holder in horizontal arrangement designed from a conventional film holder and a special attachment

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Figure 4.17: Horizontal platform with specimen in the special attachment

b) vertical arrangement.

In this method, the x-ray tube is directed vertically. A book stand was then placed against the lateral sides of the tube. Two pieces of metal was then attached to the book stand to indicate the position of the x-ray tube for standardisation. The specimen secured in the muffle was then placed directly under the x-ray tube (Figure 4.18).

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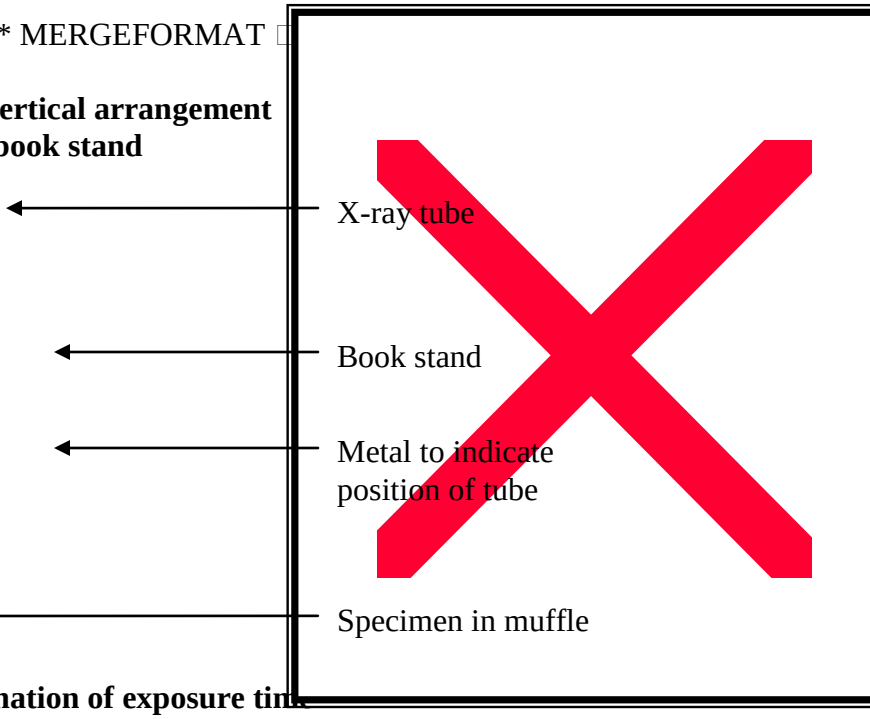

Figure 4.18: Film holder in vertical arrangement designed from a book stand

4.1.5 Image Acquisition and determination of exposure time

Radiographs of the specimen were taken using a conventional x-ray machine (Trophy Irix 70 (CCX Digital), 230 Volt, 8 mA, 70 HT kV) and a size 2 (30 mm x 40 mm) image plate known as Phosphor Storage Plate (Figure 4.19). The plate was placed in a special light protection cover

prior to exposure (Figure 4.20). Radiograph was taken after placement of Vitapex™ in the canal using 0.02s exposure time. Further radiographs were repeated for exposure time of 0.04s, 0.06s, 0.08s, 0.10s, 0.12s, 0.14a and 0.16s in order to determine the exposure time for the actual study later. Procedures were repeated for specimen with Pulpdent® Tempcanal™ in the canal.

The image plate was then removed from the light protection cover under dim light and placed into the appropriate sized foil cassette (Figure 4.21) and scanned immediately after exposure using the image plate scanner Dürr VistaScan Intra 2130-60 (Dürr Dental Gmblt & Co. KG) (Figure 4.22) at a resolution of over 15 line pairs per mm (LP/mm) according to the manufacturer's recommendations. The image is processed with DBSWIN version 3.2 software and saved as JPEG images.

The images were displayed on a Dell E153FP 17-inch digital flat panel LCD monitor set at a resolution of 1024x768 pixels and true colour (32-bit).

(a) **(b)**

Figure 4.19: Intraoral Phosphor Storage Plate (a) and special container (b) that

can held up to 4 image plates

Figure 4.20: Phosphor Storage Plate placed in light protection cover

Figure 4.21: Foil cassette to hold the exposed image plate

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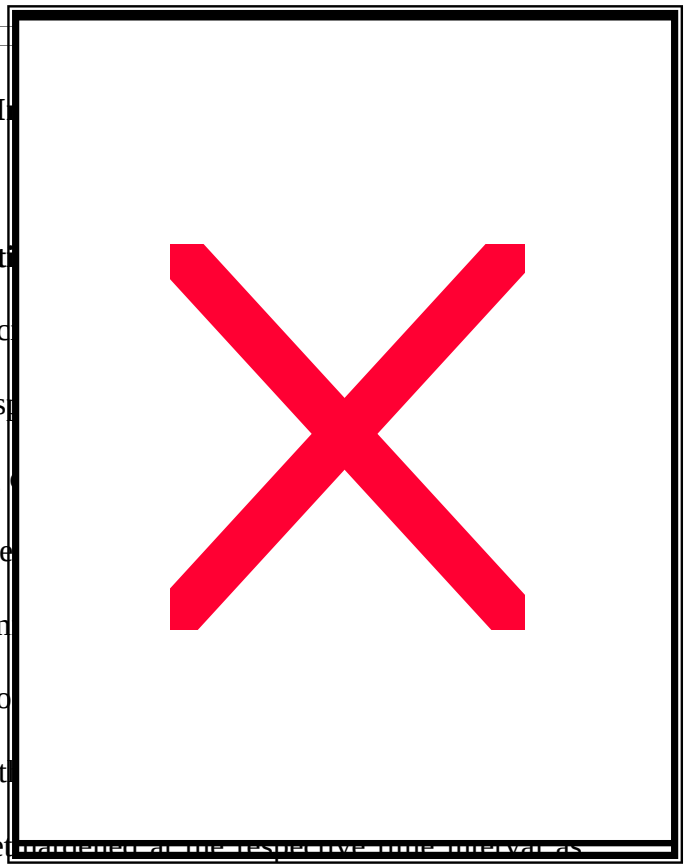

Figure 4.22: Dürr VistaScan I

4.1.6 Time interval to separate the cross-secti

Separation of the cross-sections of the spec
specimen were taken. The cross-sections of the s
microscope and Image Analyser. Since non-setting
interval after calcium hydroxide placement was nee
the muffle. Thus, achieving an appropriate harden
desirable. Small blobs of pastes with diameters o
smooth surface and kept at room temperature and t
period of times to determine whether they have set
shown in Table 4.1.

Table 4.1: Model of visual inspection of calcium hydroxide at different time interval

Time interval for visualization of hardening of materials□□1

□1

1

hr□2

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hrs□4

4

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6

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4.1.7 Removal of the uncovered apical part

After calcium hydroxide placement and radiographic taking were done, the specimen was removed from the muffle and the uncovered apical part was removed using a conventional slow speed handpiece and diamond disc.

4.2 Findings of preliminary study

4.2.1 Preparation of specimen

4.2.1.1 Preparation of the apical part of the canal

In order to simulate a usual divergent shape of the apical part of an immature permanent tooth, Gates-Glidden bur was use in retrograde approach. From the series of radiographs shown in Figure (4.7), it was noted that a divergent apical part of the canal that best simulates an open apex was obtained when size 5 Gates-Glidden bur was used. Fragmentation of the apical part occurred when size 6 Gates-Glidden bur was used. Thus, it was decided that apical preparation in the actual study would be done using Gates-Glidden burs up to size 5.

4.2.1.2 Reassembling the cross-sections of the specimen

Although a permanent ink marker pen was used, problems were still encountered in reassembling the cross-sectional of the specimens as the line tended to fade away and this made the rearrangement of the cross-sections of the specimen difficult. On the other hand, when the diagonal line was made using a small round bur on a high speed handpiece, the mark was left permanently. Therefore, for the actual study, diagonal line would be made using a bur.

4.2.1.3 Muffle to hold the specimen

The quality of the radiograph seen was poor when the radiographs were taken with the specimen placed in a muffle fabricated from dental stone and plaster of Paris (Figure 4.23). A better and superior image was obtained when the Perspex muffle was used (Figure 4.24).

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Figure 4.23: Radiograph taken using dental stone/plaste

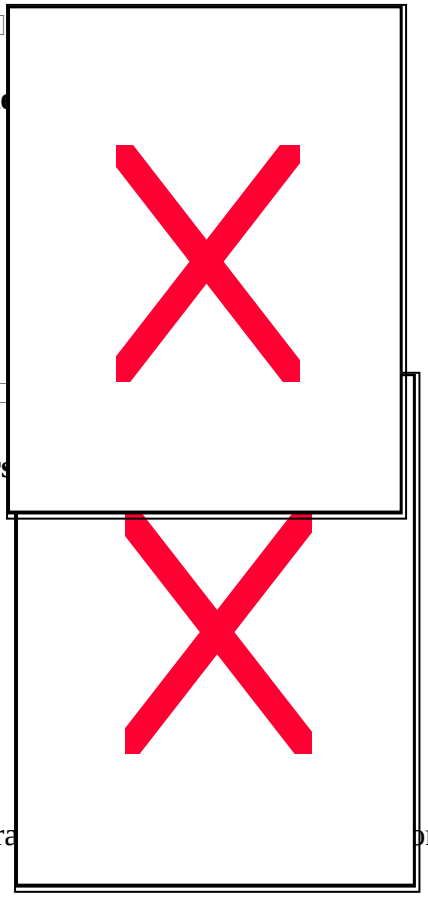

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Figure 4.24: Radiograph taken using Pers

4.2.2 Radiographic taking techniques

A few problems were encountered when horizontal arra orm as shown in Figure (4.17) was used:-

- holder was not stable on the x-ray cone since the muffle was quite heavy.
- holder with the muffle and specimen dropped while radiograph was taken due to its weight.

These problems were not encountered with the vertical arrangement (Figure 4.18). In addition, the following advantages were noted with the latter:-

- The x-ray film, muffle and the "holder" could be placed on any flat surface. Therefore, there will be no risk of it to drop as what happened in the first method described before.
- Distance from the x-ray cone to the x-ray film is standardized by placing the x-ray cone just until the metals placed in the midline of the book stand.
- The empty space on the middle of the book stand can also act as the guideline as where to place the x-ray cone.
- This method is cheap, easy and easily reproducible.

Thus, the vertical arrangement platform was chosen as the radiographic platform in the actual study.

4.2.3 Exposure time

The image of the specimens taken at different exposure times after placement of Vitapex™ and Pulpdent® Tempcanal™ in the canal are as shown in Figures 4.25 and 4.26 respectively.

Generally the radiographic contrast obtained was better with Vitapex™ than Pulpdent® Tempcanal™. For Vitapex™, it was observed that excellent radiographic contrast was obtained with 0.12s.

For Pulpdent® Tempcanal™, the contrast between dentine and material could not be easily visualized and it was judged that exposure time of 0.14s gave the most acceptable image.

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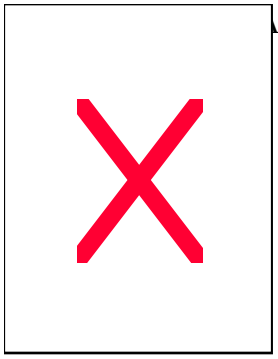

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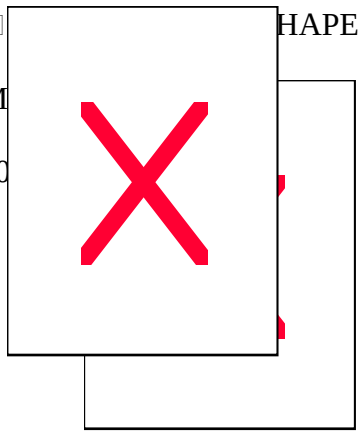

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0.1

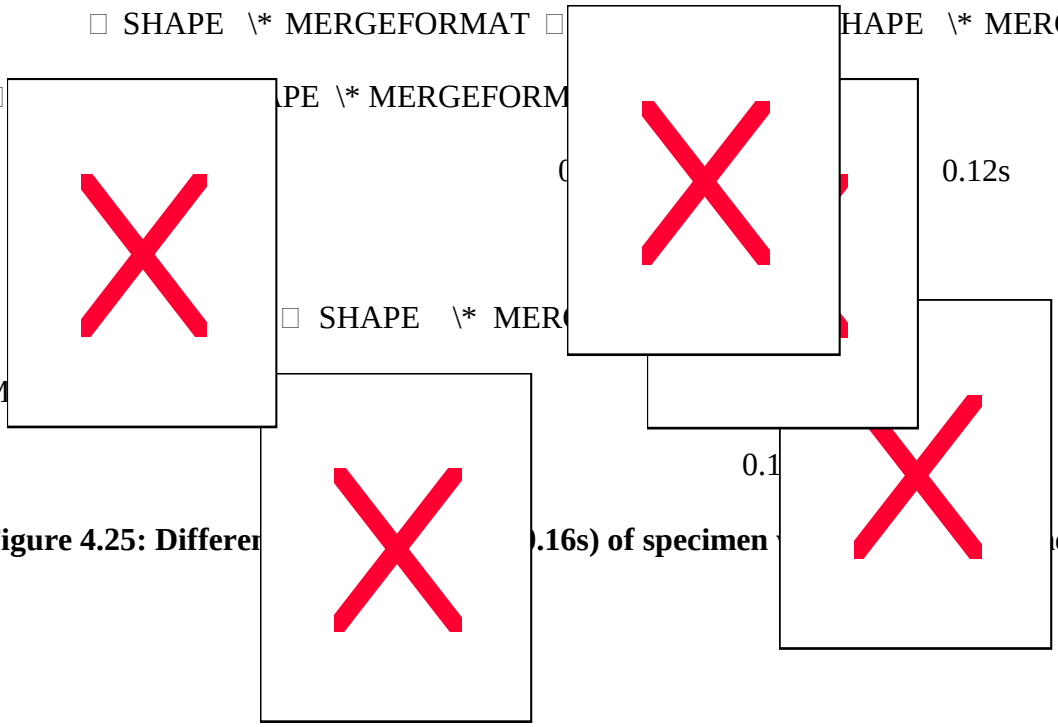

Figure 4.25: Differen .16s) of specimen e canal

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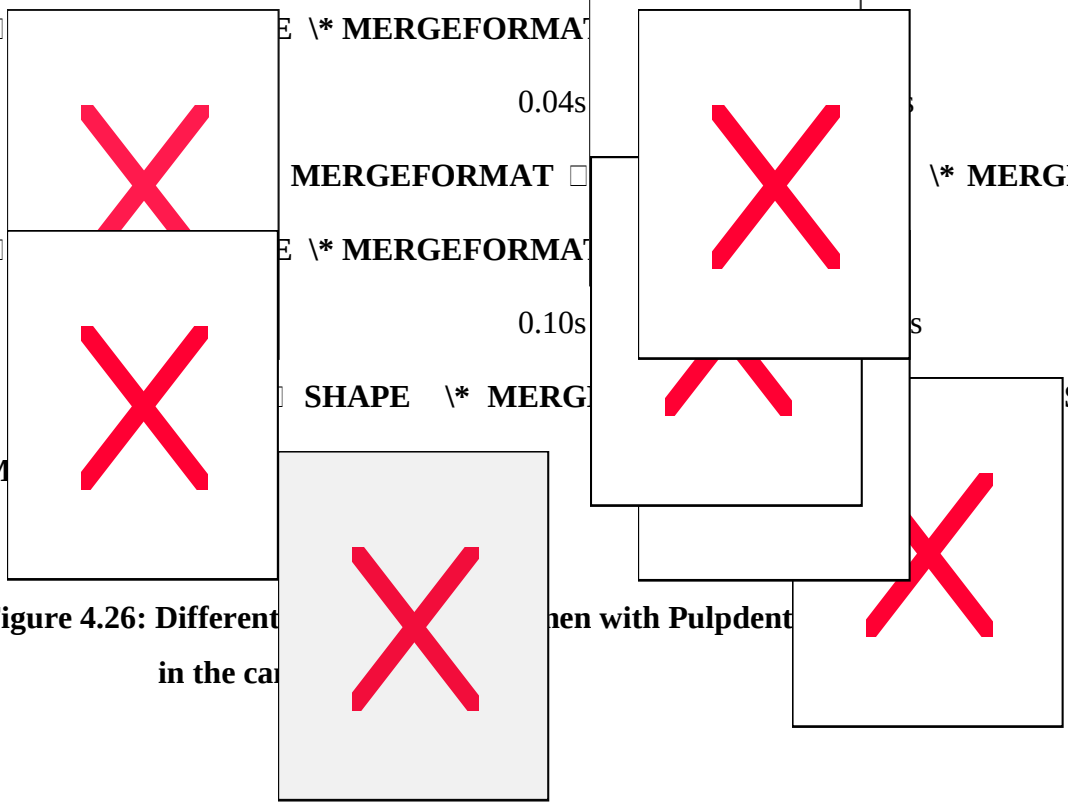

Figure 4.26: Different nen with Pulpdent

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4.2.4 Removal of the uncovered apical part

It was difficult to remove the 2mm uncovered apical “whole” due to small size and thin dentine left. Most of the time, the uncovered apical part was fractured, resulting in fragmentation of the tooth and the calcium hydroxide.

4.2.5 Time interval to separate the cross-sections of the specimen after calcium hydroxide placement

The results of the hardening process of the two materials are as shown in Table (4.2).

Table 4.2: Time lapse to achieve hardening of materials

Type of Ca(OH)$_2$□Time interval □□□1
Time interval □□□1
□□1
□1

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2
hrs□4
4
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6
hrs□8
8
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24

hrs□□Vitapex™□_□_□_□_□_□_□+□□Pulpdent® Tempcanal™□_□_□_□_□+□+□+□□
□Vitapex™□_□_□_□_□_□_□+□□Pulpdent® Tempcanal™□_□_□_□_□+□+□+□□
Vitapex™□_□_□_□_□_□_□+□□Pulpdent® Tempcanal™□_□_□_□_□+□+□+□□
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The (-) signs indicated that the material has not hardened/set from visual and tactile examination. The (+) signs showed that the material has hardened/set after the time interval indicated. For Vitapex™, the material showed good visual and tactile hardening/set 24 hours after placement, whereas for Pulpdent® Tempcanal™, a shorter time ie. 8 hours after placement was required. Thus, for the convenience of the researcher, it was decided to separate the cross-sections of the specimen from the muffle for the purpose of examining it under microscope and Image Analyser at least 24 hours after calcium hydroxide placement.

4.3 Summary and conclusions from the preliminary study

From the preliminary study, it was decided that:-

- Specimens would be mounted in clear acrylic and spectrophometric cuvette
- Diagonal line on the specimen would be made using round bur
- Apical preparation of canal would be prepared with Gates Glidden bur up to size 5
- Perspex muffle would be used to hold the specimen
- Radiographic platform in vertical arrangement would be used

- Exposure time to be used for Vitapex is 0.12s, and 0.14s for Puldent® Tempcanal™
- Cross sections of the specimen would be separated after 24 hours
- Removal of uncovered apical part would not be carried out