CHAPTER 7

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7.1 LIMITATIONS OF THE STUDY

1. The study was performed on extracted premolar teeth that were prepared to simulate immature permanent teeth by cutting two millimeters of the apical end of the root and enlarging the canal and the apical area. This may not represent the true shape of an immature tooth.

2. The density of the calcium hydroxide was only assessed at two levels. More levels of sectioning are desirable in order to get more views of the intracanal filling of the calcium hydroxide. A cross section closer to the apical region is valuable since that is the area of interest. However, this could not be done as the thin dentinal walls at the apices were too friable to allow cross-sectioning of this area.

3. In some of the specimens, seepage of calcium hydroxide paste could be seen through the cross sections of the specimens. This can be seen either from the radiograph, the cross-sections of the specimen under the Image Analyser and also from the 3-dimensional image. The calcium hydroxide paste flowed over and thus covered the dentinal area and

presented difficulties in assessing/demarcating the area filled up with the material for the calculation of the percentage of surface area.

4. Grading/scoring of the radiographs and calculating the percentage of the surface area covered by calcium hydroxide is subjective. Only one observer was involved in analysing the data and thus only intraexaminer reliability was tested.

5. Radiodensity of Pulpdent® Tempcanal™ and colour of the material are almost similar to dentine. On the other hand, Vitapex™ is yellow in colour and the material is more radiopaque than dentine.

6. Technical artefacts such as shrinkage of the filling material and loss or fragmentation of material during separation of the cross-sections could not be ruled out in this study.

7.2 DISCUSSIONS

Endodontic management of non-vital immature permanent teeth is often complicated due to the frequently divergent root canals and open apices, thereby making debridement and obturation difficult. Induction of apical closure or apexification using calcium hydroxide has become the most widely used approach to treat these teeth. Dressing the canal with this material helps eliminate microorganisms that might otherwise prevent periapical healing (Assed *et al.*, 1996). This is most effective when there is direct contact between the material and the microorganisms (Estrela *et al.*, 2002). Calcium hydroxide should be placed as deep and compactly as possible along the entire canal (Deveaux *et al.*, 2000). Accurate placement of the material has proven to be very challenging for the clinician. Thus, this study was conducted to investigate the effectiveness of different intracanal placement methods and types of vehicles of calcium hydroxide in permanent teeth with simulated immature apices.

Earlier *in-vitro* investigations on intracanal placement of calcium hydroxide had been performed on extracted permanent teeth (Sigurdsson *et al.*, 1992, Staehle *et al.*, 1997, Deveaux *et*

al., 2000, Metzger *et al.*, 2001), simulated canals made from clear acrylic resin blocks (Rivera & Williams, 1994., Torres *et al.,* 2004, Öztan *et al.,* 2002) and also teeth from mandible of dogs which were sectioned into blocks (Estrela *et al.,* 2002). Although simulated canals made from clear acrylic resin blocks has the advantage of enabling the investigator to strictly control the factors that may influence the intracanal placement of calcium hydroxide, clear acrylic resin does not represent the exact structure of dentine, thus this may alter the passage of the material along the canal due to different structural resistance of the resin to that of dentine. Natural teeth have been used by few authors (Sigurdsson *et al.,* 1992, Staehle *et al.,* 1997, Deveaux *et al.,* 2000, Metzger *et al.,* 2001) instead of resin block in order to achieve as closely as possible the clinical situation of preparing the canal and placing the calcium hydroxide. However, none of the studies that used extracted permanent teeth had prepared the teeth in such a way to simulate immature permanent teeth with open apices. This study used extracted premolar teeth with 2mm of the apex being cut before they were mounted in clear acrylic resin using spectrophotometric curvette prior to canal preparation. Extracted premolar teeth due to orthodontic reason were used because these teeth are usually caries-free and easily available from any orthodontic clinic.

There are many ways in which different preparation techniques and filing systems may be used to achieve specific preparation aims. For this study, a standardized preparation of apical-coronal technique was used in order to prepare the root canal by enlarging it sequentially to a selected size up to the working length (Walton & Rivera, 1996). The canal was prepared till size 90 up to the working length. For this procedure, K-files were used, which are twisted instruments made from square or triangular blanks, depending on their ISO size and manufacturer (Schäfer, 1997).

Quality assessment of calcium hydroxide filling in simulated immature permanent teeth was expected to be rather subjective. In this study, it has been shown that a completely filled canal is not always achieved. Clinically, it is advocated that the material should be placed at the apex or about 1-2mm from the apex (Mackie, 1998). This would maximise the effect of the calcium hydroxide and at the same time would not affect the surrounding viable tissues at the apical region. If the material is placed short of the apex, the maximum effect of the material at the apical region may not be achieved and hence proper periapical healing may be impaired. Evaluation of the quality of filling using different methods of placement from the radiographs demonstrated that when only syringe method was used, the material was either at the apex (≤2mm from the apex - apex) or short of the apex (> 2mm from the apex). In contrast, extrusion of the material was seen in most of the specimens when lentulo spiral±syringe method of placement was carried out. Webber (1984) felt that extrusion of the calcium hydroxide paste is of no concern because the paste is reabsorbed and may even stimulate the deposition of hard tissue. Nevertheless, few studies advocated that extrusion of calcium hydroxide beyond the apex may be a cause for the lack of healing of periapical lesions (Verneiks & Messer, 1978, De Moor & De Witte, 2002 and Felippe *et al.*, 2005). Carrotte (2004) also reported that in clinical situation, care should be taken to prevent the extrusion of material into the periapical tissues in order not to cause further damage to the surrounding viable tissues.

The quality of calcium hydroxide filling was assessed using a scoring system that was modified from Rivera and Williams (1994). In their study, they evaluated the distance of calcium hydroxide from the working length as; (a) short, (b) at, or (c) long beyond the apex.

However, based on the recommendations of the National Clinical Guidelines in Paediatric Dentistry (United Kingdom) in 1998 on management and root canal treatment of non-vital immature permanent incisor, the filling should be placed in the canal completely up to the working length ie. 1-2mm short of the apex (Mackie, 1998). Thus a filling is considered short when it was found to be more than 2mm from the apex, and a filling that was 2mm or less to the apex are assessed as "at the apex".

In the present study, it has been shown radiographically that the syringe technique is a satisfactory method of placement of calcium hydroxide. In contrast, it was found that extrusion of the material beyond the apex occurred in the majority of the specimens using lentulo spiral alone or in conjunction with syringe. This could be due to the ease of flow of the material in anterior teeth which have large and straight canals and wide and flaring apices. The lentulo spiral may however, be very useful in a smaller or curved canal and this is supported by Sigurdsson *et al.* (1992) in their study on molar teeth with minimal curved canal who found that lentulo spiral is an adequate instrument to place calcium hydroxide paste in minimally instrumented canals to full working length.

Few authors reported that visual observation of radiographs revealed the presence of some empty spaces when lentulo spiral was used (Staehle *et al.* 1997, Deveaux *et al.* 2000). Results from this study showed that there was no significant difference in quality of calcium hydroxide filling assessed radiographically using different methods of placement and different types of vehicles of calcium hydroxide ($p>0.05$). However, images from 3-dimensional views suggested that better quality of filling was noted when lentulo spiral ±syringe method was used.

It was shown from the 3-dimensional views that when the syringe method of placement was carried out, voids were noted along the long axis of the tooth, which may represent the shape of the syringe used for both types of calcium hydroxide.

Clinically, the quality of root canal fillings is usually assessed by radiographs. In addition to radiographic analysis discussed early, the present study also allows direct analysis of fillings at cross sectional levels. This was achieved by cross-sectioning the specimen before preparation of the canal and placement of calcium hydroxide in order to avoid damaging the material in the canal. The cross-sections were then reassembled and secured in a muffle. The muffle was first introduced by Bramante *et al.* (1987). It was made of plaster stone and was designed to aid in studying the anatomical morphology of root canals, before and after instrumentation. Modifications of Bramante's muffle had been subsequently presented by few authors, enabling a three-dimensional view of the canal pre- and post-instrumentation (McCann *et al.,* 1990, Tamse & Pilo, 1998 and Hülsmann *et al*., 1999). McCann *et al.* (1990) fabricated a muffle for the same purpose using acrylic resin first, than used it as patterns for flasking, burn-out and casting it in ticonium metal. In 1998, Tamse & Pilo fabricated a new muffle model that was composed of a metal stand, four pins and a single Teflon mold that was used to invest all the teeth. Another muffle system was designed by Hülsmann *et al.* in 1999 which consisted of three separate parts made from plastic that can be mounted together and fixed with three metal screws. The common features to all of these techniques are an external two/three-piece holding mold and external indexing of the tooth block (either made from stone or acrylic resin). In this study, a muffle was fabricated from clear Perspex. Clear Perspex was used to make the muffle since radiograph would be taken while the specimen was in the muffle. This would cause minimal

alteration to the image of the specimen in comparison if the muffle is fabricated from stone or metal.

In an immature tooth, the most crucial area of placement of material is at the apical area. However, during the preliminary study, it was found that removing the 2mm apical part of the specimen after canal preparation and filling was not easy. Due to the thin dentine, the apical part was easily fractured during cross-sectioning. In addition, fragmentation of the material during the removal of the 2mm apical end also occurred. ISOMET low speed saw could not be used to cut the 2mm apical end during the preliminary study since this machine needs a special lubricant for cutting which may affect the texture of the calcium hydroxide and may cause dissolution of the material. Thus, it was decided that the quality of filling could only be assessed at only two levels ie. level A (10mm from the base of the curvette) and level B (5mm from the base of the curvette). The same problem was also reported by Staehle *et al.* (1997) who assessed the quality of calcium hydroxide filling using different methods of placement from radiographs as well as from the cross-sections. In their study, the specimens were cross-sectioned at more levels than the present study ie. 12, 8, 4 and 1 mm from the apex. Although they had attached the cross-sectioned of the specimen to an acrylic resin at the respective cutting plane in order to avoid loss of filling material, the authors reported that the outcomes were still doubtful as fragmentation of the material during the cross-sectioning procedure was unavoidable. Thus, the present study only cross-sectioned the specimen at fewer levels and the cross-sectioning was done before preparing and filling the canal with calcium hydroxide.

The quality of filling from the cross-sectional views was examined at two levels, 10mm and 5mm from the base of the spectrophotometric curvette. This study showed that there was no significance difference in the quality of filling at the two horizontal levels irrespective of types of vehicle of calcium hydroxide ($p>0.05$) ie. either aqueous suspension (Pulpdent® Tempcanal™) or oil suspension (Vitapex™). However, significant difference was noted between different methods of calcium hydroxide placement at level B, which is the level closer to the apex (5mm from the base of the curvette) ($p<0.05$). At this level, it was observed that when syringe technique was used in combination with lentulo spiral, the quality of filling was significantly better than using syringe technique ($p<0.05$). Smaller diameter of the canal closer to the apex may cause the ease of filling of the material laterally in comparison to the area towards the coronal area (level A, 10mm from the base of the curvette) which has bigger diameter. The utilisation of lentulo spiral after filling up the canal with calcium hydroxide using syringe would aid in spreading the material throughout the canal. Thus, combination of syringe and lentulo spiral would give better quality of filling at the level closer to the apical area, irrespective of types of vehicle of the calcium hydroxide paste. This was supported by the percentage of surface area obtained from the 3-dimensional cross-sectional views where lentulo spiral ±syringe method showed better quality of filling irrespective of types of vehicles of calcium hydroxide used. Similar result was also reported by Torres *et al.* (2004) in their study where they compared three methods of placement on curved canal instrumented up to size #40, where they found that calcium hydroxide filling in the curved canal was significantly better using lentulo spiral only, especially at 1 mm from the canal terminus. Better results with lentulo spiral could be due to the structure of lentulo spiral itself, which is more flexible than syringe, thus allowing a free movement of the instrument up to 1mm from the apex.

The quality of fillings reported in this study was determined by the presence of calcium hydroxide at the two cross-sectional levels. Thus, the presence of void would affect the percentage of surface area of calcium hydroxide. The presence/absence of voids was recorded from the two cross-sections using the image analyser. If voids were present, the site of voids, whether within the material or at the canal wall, was also recorded. Clinically, it is more important to have material that is well adapted to the wall. This would ensure the effectiveness of the antibacterial effects of the calcium hydroxide at the dentinal tubules and also to the lateral canals if present. This study showed that when syringe method was used to place the calcium hydroxide, voids were mostly present at the wall of the canal for both levels A and B (10mm and 5mm from the base of the curvette) irrespective of the types of vehicle of calcium hydroxide (aqueous suspension (Pulpdent® Tempcanal™) or oil suspension (Vitapex™)). Clinically, insufficient material along the canal wall may prevent proper healing as the calcium hydroxide may not act efficiently on the lateral canals, if present. Theoretically, lateral canal may form a communication between the pulp and the periodontal ligament, permitting either irritant from the necrotic pulp to irritate and inflame the lateral periodontal ligament or in the case of a periodontal lesion, toxic substances to enter the pulp to induce pathological changes (Scarfe *et al.*, 1995). Furthermore, lateral canals harbouring inflamed and/or infected material may cause pain during the treatment (Weine, 1984). Presence of voids within the material was noted in only a small number of specimens, irrespective of the methods of placement, types of vehicle and levels of cross-sectioning. However, this is not significant clinically as compared to presence of voids at the wall of the canal. This study showed that when lentulo spiral±syringe method was used for placing the material, voids were not seen in the majority of the specimens at

both levels, irrespective of types of calcium hydroxide vehicles. This observation was also supported by cross-sectional views from the 3-dimensional images. In contrast, Staehle *et al.* (1997) reported that fewer air inclusions or porosities (voids) were seen with syringe system compared to the lentulo spiral when aqueous suspension of calcium hydroxide was used.

The quality of radiographic image is dependent on many factors and efforts were taken throughout the study in order to standardise the conditions during acquisition of the image including the use of a radiographic platform. Several radiographic platforms had been designed and used by various researchers in endodontic studies (Southard *et al.*, 1987, Sydney *et al.*, 1991, Hülsmann *et al.*, 1999, Jardine & Gulabivala, 2000). These radiographic platforms used an apparatus that enable one to standardize the radiographic taking. In this study, a simple radiographic platform in vertical arrangement was fabricated from a bookstand which enabled an easy and standardized method of radiographs taking.

Problems were encountered when conventional developer was used to develop the radiographs. It was difficult to standardize the fixer and developer solutions as the solutions were prepared by different clinical staff once in two weeks. Furthermore, the radiographs had to be properly dried before it could be scanned. The process of developing the radiograph, drying and scanning was too time-consuming. Thus, a digital radiograph scanning machine was used (Dürr VistaScan Intra 2130-60 (Dürr Dental Gmblt & Co. KG)), which is easier to handle and very simple.

There are few formats for saving images from the computer such as Windows Bitmap (BMP), Tagged Image File Format (TIFF) and Joint Photographic Experts Group (JPEG). BMP is compatible with other Microsoft Windows program. However, there is no compression of the images which results in a very large file. Thus, saving the images would require large file and consume longer time. TIFF is not supported by web browsers and not all TIFF files are compatible with all programs that support the baseline TIFF standard (Tran *et al.,* 2000). Thus, for this study, JPEG format was used to save the radiographic images from the computer to pendrive for analysis purposes. JPEG is designed for compressing either full-colour or gray-scale images. The main reason of using JPEG is photo compression which means that JPEG can make the image files smaller (Tran *et al.,* 2000). Images are saved by grouping similar pixels together rather than treating each one individually. This capability makes a faster transmission of files cross networks and for storing images. Another advantage of JPEG is its ability to retain all the information and what appears on the screen is the real picture (Tran *et al.,* 2000). The decision to use JPEG images, despite their glossy format was purely due to practical reason. TIFF and Bitmap files are larger and use more memory.

Determination of radiographic exposure time for both materials was investigated in the preliminary study. This was needed in order to get the best image of the radiograph. Exposure time was investigated starting from the lowest exposure (0.02s) to 0.16s for both materials. For Vitapex™, it was quite easy to get the best image since Vitapex™ has iodoform as one of its contents, which is a radiopaque material. Although Pulpdent® Tempcanal™ contains barium sulphate, it is still not as radiopaque as Vitapex™. Thus for Pulpdent® Tempcanal™, it was decided that the radiographic image taken using the best exposure time had to be embossed first

in order to get a better outline for analysis purposes. This was done using Adobe® Photoshop® Version 6.0 software. This technique would give a better outline of the image and aids in analysing the radiograph.

More recently, three-dimensional images using microcomputed tomography system has gained popularity. Microcomputed tomography system is a new way for quantitative evaluation of various interest such as root canal instrumentation (Bergmans *et al.,* 2001, Bergmans *et al.,* 2002a, Bergmans *et al.,* 2003), cervical external root resorption (Bergmans *et al.,* 2002b), osseointegration of dental implants (Jung *et al.,* 2003, Park *et al.,* 2005), bone loss (Bodic *et al.,* 2004) and dentine-adhesive-composite interfaces (Santis *et al.,* 2005). However, the cost of acquiring the system is rather prohibitive. Currently, the system is unavailable at the Dental Faculty, University of Malaya and the specimens had to be sent overseas. Due to the high cost, three-dimensional images for only one sample from each group were obtained using the microcomputed tomography system. The present study used microcomputed tomography system called SkyScan 1172. SkyScan 1172 represents a new generation in x-ray microcomputed tomography systems in which the sample stage and the x-ray camera can both move during magnification adjustments, allowing a combination of spatial resolution, image quality, sample size accommodation and scanning speed. It was noted that this mode of assessment provides a more reliable and accurate views than the conventional radiographic images. Microcomputed tomography system visualizes the morphological characteristics of teeth in a detailed and accurate manner. A large amount of information could be obtained from such system where the slices of images can be recreated in any plane and the data can be represented as either two-dimensional or three-dimensional images (Kim *et al.,* 2007). In addition, the internal and

external anatomy could be demonstrated simultaneously/separately and the images could be assessed both qualitatively and quantitatively. Conventional method only allows a two-dimensional views and it is obtained by overlapping of the structures of the specimen. Hence, it does not represent the true internal and external images of the specimen. Radiographic views taken from conventional method also do not allow observation of the specimen at any horizontal or sagittal planes as microcomputed tomography system do. Thus, microcomputed tomography is a highly accurate and non-destructive method for evaluation of endodontic studies (Jung *et al.*, 2005).

In the present study, it was noted that for the specimens that had been filled with Vitapex™, the percentage of surface area calculated from the image analyser were almost similar to the percentage of surface area obtained from the microcomputed tomography. However, the percentage of surface area calculated from the image analyser was much higher in the specimens that were filled with Pulpdent® Tempcanal™. This difference could be attributed to the colour of the material; Pulpdent® Tempcanal™ is quite similar to that of dentine, thus it presented difficulty in distinguishing the exact demarcation between the dentine and the filling material. These could directly affect the calculation of percentage of surface area of the cross-sectional views, which explains why the results from the image analyser differ from microcomputed tomography. Similar problem was also encountered for Pulpdent® Tempcanal™ group when radiographic analysis was carried out (see page 120) where the radiographic image had to be embossed to facilitate the analysis. This problem was not encountered in Vitapex™ group as the material is yellow in colour, thus it is readily distinguishable from dentine, hence more accurate demarcation of the material could be carried out.