THE CORONAL SEALING ABILITY OF AN EXPERIMENTAL NANO HYDROXYAPATITE-CONTAINING ENDODONTIC SEALER: *IN VITRO* STUDY

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by

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ABSTRAK

Keupayaan pengapan koronal bahan pengap kanal akar penting untuk kejayaan jangka panjang sesuatu rawatan kanal akar. Ini adalah kerana salah satu faktor utama yang mungkin menggagalkan rawatan endodontik klinikal adalah kerana kebocoran koronal yang menyebabkan bakteria dan bahan perangsa lain dapat masuk ke dalam sistem kanal akar.

Satu bahan nanohidrodsipatit baru yang mengandungi bahan pengap endodontik telah disediakan di Pusat Pengajian Sains Pergigian, USM, Kubang Kerian, Kelantan, Malaysia. Kajian ini bertujuan untuk membuat perbandingan keupayaan bahan pengap koronal yang baru dengan bahan pengap AH26 bebas perak sebelum dan selepas persediaan untuk tiang dan untuk mengkaji kesan persediaan tiang dan kesan prarawatan EDTA ke atas keupayaan bahan pengap koronal kedua-dua bahan pengap. Sebanyak 228 gigi manusia berakar tunggal digunakan. Pemotong tisu keras digunakan untuk memotong korona dan mendedahkan akarnya sepanjang 13mm. Setiap kanal akar dicuci dan dibentuk menggunakan ‘K-files’ dan teknik ganjak kebelakang. Kemudiannya akar tersebut dibahagi kepada 6 kumpulan eksperimen dengan 38 gigi dalam setiap kumpulan. Teknik pemadatan lateral digunakan supaya akar dalam semua kumpulan dapat diobturat. Bahan pengap nano HA digunakan dalam kumpulan pertama dan AH26 digunakan dalam kumpulan kedua. Sementara itu, bahan pengap nano HA digunakan dalam kumpulan ketiga supaya gigi dapat

diobturat dan selepas seminggu ianya akan disediakan untuk tiang. Rawatan yang sama dengan kumpulan ketiga dilakukan kepada kumpulan empat tetapi menggunakan bahan pengap AH26. Bagi kumpulan kelima akar dirawat menggunakan larutan EDTA sebelum ia diobturat menggunakan bahan pengap nano HA. Bagi kumpulan enam pula, akar dirawat dengan larutan EDTA dan diobturat menggunakan bahan pengap AH26. Kesemua kumpulan disimpan di dalam inkubator pada suhu 37°C selama 7 hari untuk pengerasan bahan pengap. Kemudiannya semua gigi akan melalui proses kitaran suhu antara 5 °C dan 55° C dengan masa rendaman selama 30 saat bagi setiap laluan dengan jumlah kitaran keseluruhan 500 kitaran. Permukaan akar ditutup sepenuhnya dengan dua lapisan varnis kuku kecuali bukaan koronal, kemudian ia ditempatkan didalam pewarna methylene biru. Selepas 24 jam, semua gigi dibasuh dengan air dan dikerat secara menegak. Kebocoran pewarna koronal dalam setiap gigi diukur dalam unit mm menggunakan mikroskop cahaya dan disokong oleh perisian pengalisis imej. Data dimasukkan kedalam perisian SPSS and dianalisis menggunakan ujian 'independent *t*'. Tiada perbezaan yang signifikan dalam kebocoran pewarna koronal antara kedua-dua bahan pengap samada sebelum atau selepas penyediaan ruang untuk tiang, dimana nilai *p* antara 0.410 dan 0.078 secara berturut telah diperolehi. Kumpulan yang disediakan untuk tiang bocor lebih signifikan berbanding kumpulan yang tidak disediakan untuk tiang di mana nilai *p* adalah 0.012 dan 0.014 untuk bahan pengap nano HA dan AH26 secara berturut. Kumpulan yang dirawat dengan EDTA mengalami kebocoran yang sedikit berbanding kumpulan yang tidak dirawat, dimana nilai *p* adalah 0.006 dan 0.031 untuk bahan pengap nano HA and AH26 secara berturut. Kesimpulannya tiada perbezaan yang signifikan antara keupayaan kedua-dua bahan pengap koronal samada sebelum dan selepas persediaan tiang. Persediaan untuk tiang mengurangkan keupayaan bahan pengap koronal kedua-dua bahan pengap; sementara itu pula prarawatan untuk kanal yang disediakan dengan EDTA meningkat keupayaan bahan pengap koronal kedua-dua bahan pengap.

THE CORONAL SEALING ABILITY OF AN EXPERIMENTAL NANO HYDROXYAPATITE-CONTAINING ENDODONTIC SEALER: *IN VITRO* STUDY

ABSTRACT

The coronal sealing of root canal filling is of crucial importance for the long-term success of root canal treatment, as the coronal leakage of bacteria and other irritants to the root canal system, is one of the main factors that may result in clinical failure of endodontic treatment.

A new experimental nano hydroxyapatite containing endodontic sealer was developed by the School of Dental Sciences, USM, Kubang Kerian, Kelantan, Malaysia, for commercialization. The purpose of this study was to compare the coronal sealing ability of the new sealer with that of AH26 silver free sealer before and after preparation for post, and to study the effect of post space preparation, and the effect of EDTA pretreatment on the coronal sealing ability of both sealers. A total of 228 extracted single rooted human teeth were used. Crowns were amputated using a hard tissue cutter, leaving roots of 13 mm length. Each root canal was cleaned and shaped using K-files and step back technique. The prepared roots were randomly divided into 6 experimental groups, with 38 teeth in each group. Roots in all groups were obturated using the lateral condensation technique. Nano HA sealer was used in the first group, while AH26 was used in the second group. In the third group, teeth were obturated using nano HA sealer, and after one week they were prepared for post. The fourth group was treated the same as the third group, but using AH26

sealer. In group five, roots were treated with EDTA solution, before being obturated using nano HA sealer. In group six, roots were treated with EDTA solution, and then obturated using AH26 sealer. All teeth were stored in an incubator at 37°C for 7 days to allow adequate setting of sealers. After that all teeth were thermo cycled between 5°C and 55°C, with an immersion time of 30 seconds in each path, for a total of 500 cycles. Root surfaces were completely covered by two layers of nail varnish, except the coronal opening, and then they were placed in methylene blue dye. After 24 hours, all teeth were washed under running water and sectioned vertically. Coronal dye leakage in each tooth was measured in mm using light microscope supported by image analyzer software. Data was entered into SPSS software and analyzed using independent *t* test. No significant difference in the coronal leakage of dye was found between the two sealers, both before and after preparation for post, where *p* value was 0.410 and 0.078 respectively. Groups prepared for post leaked significantly more than groups not prepared for post, with *p* value 0.012 and 0.014 for nano HA and AH26 sealers respectively. Groups treated with EDTA leaked significantly less than groups with no such pretreatment, where *p* value was 0.006 and 0.031 for nano HA and AH26 sealers respectively. It was concluded that no significant difference was found in the coronal sealing ability between the two sealers, both before and after post space preparation. The preparation for post decreased the coronal sealing ability of both sealers; while pretreatment of prepared canals with EDTA improved the coronal sealing ability of both sealers.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

One of the main purposes of obturation is to provide a good coronal seal that will prevent saliva, bacteria, and bacterial byproducts in the oral cavity from invading the root canal system, and gaining access to the periradicular tissues, which may result in clinical failure of endodontic therapy (Orstavik *et al.*, 2001, Prado *et al.*, 2006). The coronal sealing ability can be influenced by many factors such as the kind of the sealer that is used (Timpawat *et al.*, 2001a), presence of smear layer in the prepared canal (Vassiliadis *et al*., 1996), and the type of coronal restoration that will be used such as post and core crown (Pappen *et al*., 2005).

Efforts to provide endodontic sealer that have the ideal physical and biological properties are on going. In the last decades a wide variety of endodontic sealers based on different formulas were commercially available and clinically used, such as zinc oxide-eugenol cements, calcium hydroxide sealers, polymers, glass ionomer cements, silicon-based sealers and others (Himel *et al*., 2006). Unfortunately considerable problems with almost all these sealers are still present, such as tissue irritation (Kim *et al*., 2004, Veloso *et al*., 2006) and being permeable to bacteria (Yucel *et al*., 2006). In the last few years, new hydroxyapatite-containing sealers were developed, such as Bioseal (Ogna, Laboratori Farmaceutici, Italy), Apatite Root Sealer Type 1 (Sankin Kogyo, Japan), and Apatite Root Sealer Type 2 (Sankin Kogyo, Japan). The fact that hydroxyapatite (HA) is a naturally occurring product, and that bone grows into and eventually replaces extruded material, makes it very acceptable biologically (Ingle *et al*., 2002).

Recently, the School of Dental Science, USM, has prepared a new experimental nano HA-filled epoxy resin based endodontic sealer. The HA nano crystals were synthesized at the School of Chemical Sciences, USM (Masudi *et al*., 2007). The composition of this experimental sealer is similar to that of various sealers of the epoxy resin based sealer type, but with different additive. This additive (nano HA) is assumed to improve the periapical healing process (Gambarini and Tagger, 1996, Masudi *et al.*, 2007) and to produce a hermetic apical seal (Alshakhshir, 2007). However, little is known regarding the coronal sealing ability of this new material, when used as a sealer in endodontic therapy.

Another factor that may have an effect on the coronal sealing ability is the presence or absence of smear layer. The need for removal of smear layer before obturation is still controversial. Several authors considered it as a problem and it is advantageous to remove it, because of the possibility that it harbors bacteria, and the colonization of bacteria can be controlled by removal of the smear layer (Yang *et al*., 2006). Secondly, the smear layer may prevent the penetration of intracanal medicaments into the dentinal tubules, and so may be detrimental to effective disinfection of the prepared canal (Torabinejad *et al*., 2002). Thirdly, the smear layer may act as a barrier between obturating materials and canal walls, and prevent the contact between them, which make it difficult to create a good seal (Clark-Holke *et al*., 2003, Farhad and Elahi, 2004).

On the other hand, some studies showed that the application of EDTA or similar material to remove smear layer will create demineralized dentin matrices (collagen matrices), that cannot be completely infiltrated by endodontic sealers, which may result in higher leakage (Tay *et al*., 2006). In addition, removal of smear layer may impair the sealer adhesion to dentin, which may reduce the sealing ability of the obturation material (Saleh *et al*., 2002). The results are controversial and again, little is known regarding the effect of the smear layer on the coronal sealing ability of hydroxyapatite containing sealers.

Another factor that may have an effect on the coronal sealing ability is the preparation for post. Preparation for post requires the removal of a considerable amount of the filling material (Wu *et al*., 1998), that will increase the chance for coronal leakage. During post space preparation the canal is exposed to the oral environment and canal contamination may result (Pappen *et al*., 2005). The different physical structure of the sealers may result in different behavior during preparation for post, for example ZOE sealer is hard and brittle while the silicon-based one is elastic. The vibration caused during the preparation for post placement can cause a disruption of the ZOE sealer, and consequent defects in the apical area (Contardo *et al*., 2005). The addition of HA to sealer may also change the physical nature of the set material that incorporates an inorganic filler (Gambarini and Tagger, 1996), and so may change its behavior during preparation for post.

1.2 Statement of the problem

Coronal leakage of endodontically treated teeth has been found to result in a high failure rate. Nano HA-containing sealer is new, and little is known about its ability to prevent coronal leakage, before and after post space preparation.

The preparation for post requires the removal of a considerable amount of the root canal filling material, and the ability of the remaining material to prevent coronal leakage is questionable.

The effect of EDTA pretreatment on the coronal sealing ability is still controversial. Little is known about the effect of smear layer on the sealing ability of the new experimental nano HA-containing sealer.

1.3 Justification of the study

This study will determine the ability of the new nano HA-containing sealer to prevent corono-apical leakage. On the other hand, comparing sealers performance after post space preparation will help in choosing the suitable sealer that performs satisfactorily.

The results of this study will help in making the decision to remove or to leave the smear layer, before obturation with nano HA-containing sealer.

Most sealers are imported, this new sealer will be locally produced, and it may become a good alternative to the imported material.

1.4 Objectives of the study

1.4.1 General Objective

The general objective is to study the ability of nano HA-containing sealer in preventing corono-apical leakage.

1.4.2 Specific Objectives

1- To compare the coronal sealing ability of nano HA-containing sealer with that of epoxy resin based sealer (AH26).

2- To compare the coronal sealing ability of nano HA-containing sealer with that of AH26 sealer, after post space preparation.

3- To determine the effect of post space preparation on the coronal sealing ability of both sealers.

4- To compare the coronal sealing ability of nano HA-containing sealer with that of AH26 sealer, after treatment of canals with EDTA.

5- To determine the effect of EDTA pretreatment on the coronal sealing ability of both sealers.

1.5 Study Hypotheses

1- Nano HA-containing sealer has a similar ability to epoxy resin based sealer (AH26) in preventing coronal leakage.

2- Nano HA-containing sealer has a similar ability to epoxy resin based sealer (AH26) in preventing coronal leakage, after post space preparation.

3- Nano HA-containing sealer has a similar ability to epoxy resin based sealer (AH26) in preventing coronal leakage, in canals treated with EDTA.

CHAPTER TWO

LITERATURE REVIEW

2.1 Endodontic Treatment

Endodontic is a specialty in dentistry which focuses on the prevention, diagnosis and treatment of diseases or injuries of the dental pulp (Glickman and Pettiette, 2006). It can be summarized as a series of procedures of cleaning, shaping, and filling of the root canal system (Karadag *et al*., 2004).

Microorgansims and their byproducts are major etiological agents of pulpal and periapical diseases (Lai *et al.*, 2001). Fabricius *et al.*, (2006) studied the influence of residual bacteria that may remain after endodontic treatment on the periapical tissue healing, and concluded that the presence of bacteria significantly correlates with non-healing apical periodontitis. Although it has been suggested that non microbial factors may be implicated in endodontic treatment failure, the literature suggests that persistent intraradicular or secondary infections, and in some cases extraradicular infections, are the major causes of failure of treated root canals (Siqueira, 2001). Therefore, it can be said that the main objective of endodontic treatment is total elimination of microorgansims from the root canal, and the prevention of subsequent reinfection (Pizzo *et al.*, 2006). This is achieved by careful cleaning and shaping followed by the complete obturation of the canal space (Lai *et al.*, 2001).

2.2 Objectives of obturation

The objective of obturation is to create a fluid-tight seal along the length of the root canal system, from the coronal opening to the apical termination (Walton and Johnson, 2002).

Dummer, (1997) reported the following functions for obturation:

1- To prevent percolation of periradicular exudate into the pulp space via the apical foramina and/or lateral and furcation canals.

2- To prevent percolation of gingival exudate and microorganisms into the pulp space via lateral canals opening into the gingival sulcus.

3- To prevent microorganisms left in the canal after preparation from escaping into the periradicular tissues via the apical foramina and/or lateral canals.

4- To seal the pulp chamber and canal system from leakage via the crown, in order to prevent passage of microorganisms and/or toxins along the root canal filling and into the periradicular tissues via the apical foramina and/or lateral canals.

2.3 Endodontic filling materials

After proper preparation of root canal, it should be obturated with a root canal filling material that will prevent communication between the oral cavity and the periapical tissue. The material used for this purpose should seal the root canal system both apically and coronally. Apical obturation blocks the exit to the periapical tissues for organisms that have survived in the root canal after cleaning and shaping. Coronal obturation prevents reinfection of the pulp space from the oral environment. These materials are divided generally into solid materials and sealers (Himel *et al*., 2006).

2.3.1 Core obturating materials

2.3.1.1 Function of core material

The core material occupies space and serves as a vehicle for the sealer. Sealer must be used in conjunction with the obturating material regardless of the technique or material used (Walton and Johnson, 2002). Noort, (1994) reported that the use of root canal sealers without obturating points is contraindicated. When sealer is used in bulk, it is either too soluble, or shrink extensively on setting. Additionally, it will be difficult to gauge if the canal is adequately filled or not, and there is a danger that the cement may pass beyond the root apex into the surrounding tissues.

2.3.1.2 Types of Core materials

2.3.1.2 (a) Gutta-percha

Gutta-percha is derived from a dried juice from trees of the family *Sapotaceae*. Gutta-percha points consist of about 20 percent gutta-percha and up to 80 percent zinc oxide. Metal salts and dye are usually added for color and radiographic considerations (Ingle *et al*., 2002, Walton and Johnson, 2002, Orstavik, 2005, Himel *et al*., 2006).

2.3.1.2 (b) Silver points

Insertion of small size gutta-percha points in narrow, curved canals with small taper often led to buckling and bending of the point. Silver points, flexible but quite stiff, had an advantage in that they would not buckle, and could be more easily inserted in such cases. When silver points are used, lateral condensation can be applied using gutta-percha accessory points (Ingle *et al*., 2002, Walton and Johnson, 2002, Orstavik, 2005, Himel *et al*., 2006).

2.3.1.2 (c) Resin-based core filling materials (Resilon)

Resilon is polyester core material with bioactive glass, bismuth and barium salts as fillers, with physical and handling characteristics similar to gutta-percha. The main advantage of the resin as a core material is the extent to which it will bond to the sealer used (Orstavik, 2005, Himel *et al*., 2006).

2.3.2 Endodontic sealers

Complete obturation of the root canal system is an important part of root canal treatment. This is to prevent reinfection of the cleaned pulp cavity (Georgopoulou *et al.*, 1995).

Most obturation methods are composed of solid core cemented to root canal with a sealer (Gencoglu *et al.*, 2002, Ravanshad and Khayat, 2004). *In vitro* studies have shown that the most widely used root canal filling material "gutta percha" seals significantly better when used in combination with a sealer (Ishley and ElDeeb, 1983, Skinner and Himel, 1987). Wu *et al.*, (2000) studied the sealing ability of gutta-percha alone without sealer, and the results showed that after 48 hours the root canals obturated with only gutta-percha leaked significantly more than control groups sealed with both gutta-percha and sealer.

2.3.2.1 Functions of sealer

A basic concept is that sealer is more important than the core obturating material. Sealer accomplishes the objective of providing a fluid-tight seal, which makes the physical properties and placement of the sealer important (Walton and Johnson, 2002).

The sealer fills all the space that solid core material is unable to fill. A good sealer adheres strongly to the dentin and to the core material. It should have enough strength to hold the obturation material together (Himel *et al*., 2006). Sealers act as lubricant that reduces friction with canal walls during placement of the master cone (Weine and Wenckus, 2004). In addition, it may act as a bactericidal agent, and as a marker for accessory canals, resorptive defects, root fractures and other spaces into which the main core material may not penetrate (Dummer, 1997). Sealers are responsible for the principal functions of the final root canal filling: sealing off the root canal system, entombment of remaining bacteria, and the filling of the irregularities of the prepared canal (Orstavik, 2005).

2.3.2.2 Requirements of an ideal endodontic sealer

Grossman, (1988) outlined the criteria for an ideal sealer. These criteria are as follows:

1- Exhibits tackiness when mixed, to provide good adhesion between it and the canal wall when set.
2- Establishes hermetic seal.
3- Radiopacity so that it can be seen on the radiograph.
4- Very fine powder so it can mix easily with the liquid.
5- No shrinkage on setting.
6- No staining of tooth structure.
7- Bacteriostatic, or at least does not encourage bacterial growth.
8- Exhibits a slow set.
9- Insoluble in tissue fluids.
10- Tissue tolerant; that is, nonirritating to periradicular tissue.
11- Soluble in solvents if it is necessary to remove the root canal filling.

According to Weine and Wenckus, (2004), the two most important properties among these properties, are the ability to seal, and the minimal toxicity, and it is obvious that unless the sealer has the ability to seal, it is useless.

2.3.2.3 Types of sealer

Today, numerous root canal sealers are available, based on various formulas (Lai *et al.*, 2001). Table 2.1 provides some examples of the sealers with their composition.

Table 2.1 Overview of sealers: chemical types and examples (Orstavik, 2005)

Type	Brand	Principle components	Manufacturer
ZnO-eugenol	Roth	ZnO-eugenol, colophony, Bi- and Ba salts	Roth Inc., Chicago, IL, USA
	Kerr PCS	ZnO-eugenol, thymol, silver	Kerr, Romulus, MI, USA
	ProcoSol	ZnO-eugenol, colophony, Bi- and Ba salts	Den-tal-ez, Lancaster, PA, USA
	Bioseal	Hydroxyapatite, barium sulfate, di-iodothymol, natural resin, zinc oxide, calcium hydroxide	Ogna, Laboratori Farmaceutici, Milan, Italy
Resin	AH Plus	Epoxy-bis-phenol resin, adamantine	Dentsply Maillefer, Ballaigues, Switzerland
	AH26	Bismuth (III) oxide , Hexamethylenetetramine, epoxy resin	Dentsply De Trey GmbH, Germany
	Epiphany	BisGMA, UDMA and hydrophilic methacrylates	Pentron, Wallingford, CT, USA
	EndoRez	UDMA	Ultradent, South Jordan, UT, USA
	Acroseal	Epoxy-bis-phenol resin, metheneamine, enoxolone, calcium hydroxide	Septodont, Saint-Maur des Fosse´s, France

Table 2.1 Continued

Type	Brand	Principle components	Manufacturer
Glass ionomer	Ketac-Endo	Polyalkenoate cement	3M ESPE, St. Paul, MN, USA
Silicone	RoekoSeal	Polydimethylsiloxane, silicone oil, zirconium oxide	Roeko/Colte`ne/Whaledent, Langenau, Germany
	GuttaFlow	Polydimethylsiloxane, silicone oil, zirconium oxide, gutta-percha	Roeko/Colte`ne/Whaledent, Langenau, Germany
Calcium hydroxide	Sealapex	Toluene salicylate, calcium oxide	Kerr, Romulus, MI, USA
	Apexit	Salicylates, calcium hydroxide	Ivoclar Vivadent, Schaan, Liechtenstein

Although in the last few decades the root canal filling material was considered mainly as an inert filler of the canal space, it is slowly emerging as an active element of the endodontic therapy. The expanding use of calcium hydroxide has prompted investigators to test its application for enhancing periapical healing. Although the exact mechanism whereby a calcium hydroxide containing sealer may act is not clear, this compound was added empirically (Gambarini and Tagger, 1996).

Recently, several calcium hydroxide-based sealers have become commercially available, such as Sealapex and Apexit. These sealers are promoted as having therapeutic effects because of their calcium hydroxide content (Himel *et al.*, 2006). Figueiredo *et al.*, (2001) studied the tissue response to four endodontic sealers that were implanted in the oral mucosa of white New Zealand rabbits, and concluded that the calcium hydroxide-containing sealers had enhanced healing when compared to other sealers. Bernath and Szabo, (2003) conducted a similar study in the root canals of monkeys and reported that sealers with different chemical compositions, initiated different histological reactions. Sonat *et al.*, (1990) found that hard tissue formation was more pronounced after root filling with Sealapex sealer than with pure calcium hydroxide.

However, Tagger *et al.*, (1988) reported that the “disintegration of Sealapex indicated that solubility may be the price for increased activity”. In other words, these sealers must dissolve in order to release their calcium hydroxide (Ingle *et al*., 2002, Himel *et al*., 2006). This will ruin the function of the sealer, because it will disintegrate in the tissue (Himel *et al*., 2006). It then becomes a question of which will happen first, cementification across the apex or leakage into the exposed canal? Is there a substitute for calcium hydroxide that may have its stimulating effects, but not its drawbacks? The answer may be hydroxyapatite (Ingle *et al*., 2002).

2.3.2.3 (a) Hydroxyapatite containing sealers

Natural bone is composed of organic compounds (mainly collagen) and inorganic compound (mainly partially carbonated HA on the nanometer scale) (Wei and Ma, 2004). Because the main periapical healing reaction is calcific one (formation of bone and cementum), other substances that may enhance bone apposition have been tried too, such as collagen gel, calcium phosphate, hydroxyapatite (HA) and others (Gambarini and Tagger, 1996).

Among the available HA containing sealers are Sankin apatite type I, Sankin apatite type II, and Sankin apatite type III (Sankin Trading Co., Tokyo, Japan) (Telli *et al.*, 1999). Bioseal (Ogna, Laboratori Farmaceutici, Milan, Italy) is another example.

Gambarini and Tagger, (1996) compared the sealing ability of two sealers, Bioseal and Pulp Canal Sealer, using dye penetration method. Both of these sealers were zinc oxide eugenol based, but the Bioseal contained hydroxyapatite powder additive. Results showed that there was no significant difference between the sealing ability of the two sealers. The author concluded that the addition of HA had no adverse effect on the seal.

Sugawara *et al*., (1990) studied the sealing ability of a calcium phosphate cement (CPC), and reported that the CPC formed hydroxyapatite as the final product. The authors also concluded that the CPC resulted in less dye leakage as compared to Grossman's root canal sealer (a zinc oxide-eugenol-based sealer).

Kim *et al.*, (2004) studied the biocompatibility of two calcium phosphate-based root canal sealers (Capseal I and II), with that of a zinc oxide-eugenol-based sealer (Pulp Canal Sealer), and reported that these calcium phosphate-based sealers showed a lower tissue response in all the experimental periods.

2.3.2.3 (b) Experimental nano HA-filled epoxy resin based endodontic sealer

The nanometer size of the inorganic component in natural bone is considered to be important for the mechanical properties of the bone. Recent research in this field also suggested that better osteoconductivity would be achieved if synthetic HA could resemble bone material more in composition, size and morphology. In addition, nano sized HA may have other special properties due to its small size, and huge specific surface area (Wei and Ma, 2004).

Moreover, the small size of sealer particles may result in a deeper penetration in the dentinal tubules. Karadag *et al.*, (2004) concluded that the resin based sealer AH26 penetrated into the dentinal tubules better than the glass ionomer sealer Endion, which may be attributed to the smaller size of its particle and its viscosity. They reported that the microstructure of the sealer in the dentinal tubules and the degree of the tubules closure may be the most important factor for a tight obturation.

Dolci *et al.*, (2001) studied the efficacy of different gels, suspensions, solutions, and toothpastes containing nano HA on dentine permeability, and reported that all the formulations containing nano HA used in this study led to a dramatic fall in the dentinal permeability. The author reported that nano HA small particle size, and the reduced agglomerate diameter, resulted in higher penetration and reaction efficiency, leading to lower dentine permeability.

Only few sealers in the market are based on the nano HA, therefore the School of Chemical Science together with School of Dental Sciences, USM, decided to prepare a new experimental nano HA-filled epoxy resin based endodontic sealer using a simple and inexpensive technique. The composition of experimental sealer is similar to that of various sealers of the epoxy resin based sealer type but with different additive. This additive (HA) is assumed to improve the periapical healing process and to produce a hermetic seal (Alshakhshir, 2007, Masudi *et al.*, 2007).

2.3.2.4 Problems with sealers

Unfortunately, none of the currently available sealers possesses all the ideal properties, but some have more than others (Walton and Johnson, 2002, Johnson and

Gutmann, 2006). It is now well accepted that the current generation of root canal sealers are unable to provide a hermetic seal (Noort, 1994).

Problems with sealers are still present, for example, dimensional changes of root canal sealers over time may introduce gaps and channels along the sealer-dentin or sealer-gutta percha interface, these channels may be large enough to permit micro-organsims to pass along the spaces (Orstavik *et al.,* 2001).

Some sealers may cause discoloration of the tooth structure. Partovi *et al.*, (2006) studied the crown discoloration from commonly used endodontic sealers, and reported that all sealers caused a degree of tooth discoloration.

Some studies reported that sealers may dissolve over time, leaving unacceptable voids, so it is suggested that the amount of sealer should be minimized as much as possible and it is assumed that a thinner sealer layer results in a better seal (Georgopoulou *et al.*, 1995).

Veloso *et al.*, (2006) studied in rats the biocompatibility of three commonly used sealers, Sealapex, Apexit and Sealer26, and concluded that all these sealers were irritant although the intensity varied between them.

2.4 Methods of obturation

2.4.1 Single gutta-percha cone

Standardised files are used for instrumentation, and a single standard gutta-percha cone corresponding to the file size is cemented in the canal with sealer (Grossman, 1988, Chestnutt and Gibson, 2002). Although the technique is simple, it has several disadvantages and cannot be considered as one that seals canals completely. After preparation, root canals are seldom round throughout their length, except possibly for the apical 2 or 3 mm. Therefore, the single-cone technique, at best, only seals this portion (Wesselink, 2003). Another disadvantage is that in complex root canal anatomy, it frequently results in voids (Chestnutt and Gibson, 2002).

2.4.2 Lateral condensation

Lateral condensation is a common method for obturation that can be used in most clinical situations, and it provides a good control of obturation length (Johnson and Gutmann, 2006). It is relatively uncomplicated, requires a simple armamentarium (Walton and Johnson, 2002).

Following canal preparation, sealer is applied to canal walls, and the master cone is fitted in place. A space is created by using a spreader, and unfilled spaces are obturated around the master gutta-percha cone with accessory cones (Walton and Johnson, 2002, Johnson and Gutmann, 2006).

Peng *et al.*, (2007) compared the outcome of root canal obturation by warm gutta-percha versus cold lateral condensation, and reported that a greater incidence of overextension was seen in the warm obturation group. The obturation quality and long-term outcome were similar between the two techniques.

Aqrabawi, (2006) conducted a similar study *in vivo*, and concluded that no significant difference was found between both techniques, except for the cases presented with periapical lesions, where vertical compaction showed better results.

Gilbert *et al.*, (2001) compared the coronal sealing ability between 3 obturation techniques; Thermafil, lateral compaction and vertical compaction. The dye penetration study showed no significant difference among these techniques.

As compared to other obturation techniques, a satisfactory treatment outcome can be obtained using the lateral condensation technique.

2.4.3 Warm vertical condensation

The technique involves fitting a master cone short of the working length (0.5 to 2 mm). A root canal spreader is heated and then plunged into the gutta-percha. The softened material is condensed apically with a series of pluggers. The coronal part is back-filled using small pieces of gutta-percha 3 to 4 mm in length (Johnson and Gutmann, 2006). This technique includes difficulty of length control, and it is a more complicated procedure. In addition, a somewhat larger canal preparation is needed to allow manipulation of instruments (Walton and Johnson, 2002).

2.4.4 Hybrid technique

It is a combination of lateral and vertical condensation (Chestnutt and Gibson, 2002). The apical and middle thirds of the canal are obturated using warm lateral condensation technique, while in the coronal third, warm and plasticized gutta-percha is vertically compacted with root canal pluggers (Mittal *et al.,* 2002).

2.4.5 Continuous wave technique

It is a variation of vertical compaction. This technique employs an electric heat carrier, like System B unit, with pluggers of different tapers. The tip of a cold plugger is applied against the gutta-percha. Heat is started and a firm pressure is applied. The heat is inactivated while pressure is maintained for 5 to 10 seconds. After gutta-percha has cooled, the plugger is removed (Johnson and Gutmann, 2006).

2.4.6 Warm lateral condensation

Similar to cold lateral condensation, except that a warmed spreader is used (Chestnutt and Gibson, 2002). Endotec II device with its tips are usually used for obturation with this technique (Johnson and Gutmann, 2006).

2.4.7 Inverted cone method

When the apical foramen is extremely wide, as in the upper anterior teeth of young people, a gutta-percha cone is inserted butt end first, and additional cones are then inserted around the inverted cone as in lateral condensation method (Grossman, 1988).

2.4.8 Rolled cone method

When the root canal is wide and the walls are parallel, the tapered shape of gutta-percha cones will not fit adequately in the root canal. In such case 3 or more gutta-percha cones are rolled together on a warm glass slab in order to make a thick cone of even diameter (Grossman, 1988).

2.4.9 Thermoplastic injection technique

The gutta-percha is heated outside the tooth and then injected into the canal with a device that works as a caulking gun. The Obtura II and Ultrafiol 3D systems are examples (Walton and Johnson, 2002, Johnson and Gutmann, 2006).

2.4.10 Carrier based gutta-percha

These systems usually use a central carrier (stainless steel, titanium, or plastic) coated with gutta-percha. The canal is lightly coated with sealer. The obturator is heated in a special oven and firmly placed to working length. The carrier is sectioned above the orifice (Walton and Johnson, 2002). Thermafil (Dentsply-Tulsa Dental, Tulsa, OK), Successfil (Coltene/Whaledent, Inc) and Simplifill (Lightspeed Technology Inc., San Antonio, TX) are examples (Johnson and Gutmann, 2006).

2.4.11 Thermomechanical condensation

This technique employs an instrument with flutes similar to Headstrome file but in reverse. When activated in a low speed hand piece, the instrument will create friction, soften gutta-percha, and move it apically (Johnson and Gutmann, 2006).

2.4.12 Solvent techniques

Gutta-percha can be plasticized by solvents such as chloroform, eucalyptol, and xylol (Johnson and Gutmann, 2006) and halothane (Walton and Johnson, 2002). Disadvantages include shrinkage, voids, inability to control the material, and irritation to the periapical tissue (Johnson and Gutmann, 2006).

2.5 Obturation related failure

Walton and Johnson, (2002) reported the following ways for obturation related failures:

1- Apical seal: Bacteria and other irritants are usually not totally removed from the canal during cleaning and shaping, which may result in inflammation. Sealing these irritants during obturation may prevent their escape into the surrounding tissues. On the other hand, tissue fluids may seep into the unfilled root canal space and degrade to irritating chemicals; these irritants may then diffuse back into the periapical tissue and cause inflammation.

2- Coronal seal: Coronal seal is probably as important as or more important than the apical seal in long-term success. If bacteria and irritants from oral cavity can gain access to periradicular tissues, they may cause inflammation and failure of endodontic treatment.

3- Lateral seal: Making a good seal in the middle part of the canal is important, to prevent passage of irritants through lateral canals.

4- Overfill: Overfill may result in inflammation in the periapical tissue that may lead to failure in the treatment.

5- Underfill: The filling should be 1 to 2 mm short of the apex. Obturation shorter of this length leaves irritants in the apical canal that may result in inflammation.

6- Lateral canals: Irritants in the root canal may leak to the periodontium through these canals and cause inflammation.

7- Vertical root fracture: Lateral forces created during obturation may cause vertical root fracture due to the wedging action.

2.6 Leakage

"Leakage" is defined as "the action of leaking, admission or escape of water or other fluid through a hole in a vessel, or loss of fluid by this means". The addition of the prefix "micro" suggests that such leakage is microscopic in amount or is measured in units one millionth (i.e., x 10^{-6}) that is used for "leakage" (Jensen *et al.*, 2007).

In dentistry, it has been defined as the passage of ions, molecules, fluids or bacteria between a prepared tooth surface and the restorative material applied (Kidd, 1976, Seppa and Forss, 1991, Srinivasan *et al.*, 2005).

2.6.1 Methods for assessment of leakage

Many methods have been used to assess the coronal and apical sealing abilities of different obturating materials and techniques. Shahravan *et al.*, (2007) in a Meta analysis study, have systematically reviewed the literature to determine the effect of smear layer on leakage. They found that among the 65 published leakage studies, 44 used dye leakage test, 7 used the fluid filtration test, 7 used the electrochemical test, and 6 used the bacterial leakage test. Only one study used volumetric dye leakage test to assess the effects of smear layer removal. The author concluded that the dye leakage test was the favorite method in leakage studies.

2.6.1.1 Methods that use dye

2.6.1.1 (a) Passive dye penetration

The methodology using tooth immersion in various types of dyes (eosin, methylene blue, black India ink, Procion brilliant blue, and others) is perhaps the most widely used method (Verissimo and do Vale, 2006). After obturation, the external root surfaces are covered by 2 or 3 coats of nail polish or another isolating material, and then submerged in dye solution. The teeth are then rinsed under water, and sectioned longitudinally, transversely, or cleared (Figure 2.1). The linear penetration of the dye is then recorded (Pommel *et al.*, 2001, Camps and Pashley, 2003, Verissimo and do Vale, 2006). One of the disadvantages of the clearing method is that the particles of methylene blue may dissolve during decalcification and clearing process (Ravanshad and Torabinejad, 1992). Ahlberg *et al*., (1995) pointed out that immersion in acids such as nitric acid and alcohol (during clearing process) for a long period of time may cause dye dissolution in this technique.

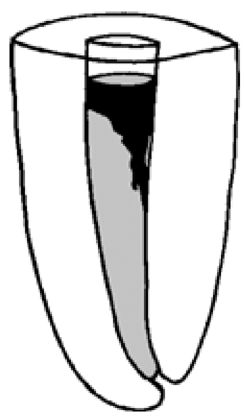

Figure 2.1 The coronal leakage measurement is taken from the top of the gutta-percha to the deepest extent of dye penetration (Vivacqua-Gomes *et al.*, 2002)

2.6.1.1 (b) Dye penetration with vacuum application

This method is the same as in passive dye penetration, but a vacuum pump is used to decrease pressure in the dye container. The reduced pressure may result in enhancement in the penetration ability of the dye through voids (Wimonchit *et al.*, 2002). However, it has been reported that the use of such reduced pressure is not clinically relevant and is a severe test of leakage (Masters *et al.*, 1995, Roda and Gutmann, 1995). This method remains too distant from the actual clinical practice, where no vacuum method is used (Vassiliadis *et al.*, 1996). Antonopoulos *et al.*, (1998) concluded that there is no need to use negative pressure for evaluating the sealing ability of root canal fillings *in vitro*.

2.6.1.1 (c) Dye extraction method

In the dye extraction or dissolution method, teeth are dissolved in acids that release all the dye from the tooth, and the optical density of the solution is measured by a spectrophotometer (Camps and Pashley, 2003, Verissimo and do Vale, 2006).

2.6.1.2 Bacteria and toxin infiltration method

The filled root is mounted between an upper chamber containing a test bacterium, and a lower chamber that contains sterile medium (Figure 2.2). With time bacterial growth may occur in the lower chamber, indicating that the test organism has passed along the root canal filling. These bacterial studies are qualitative rather than quantitative. If only one bacterium passes through the obturated canal, it may multiply in the lower chamber resulting in turbidity (Orstavik, 2005, Verissimo and do Vale, 2006). This method is complex, and if the sealer has antibacterial action, it is unfeasible to use the bacteria method (Verissimo and do Vale, 2006).