AN *IN- VITRO* AGAR DIFFUSION STUDY COMPARING THE ANTIMICROBIAL ACTIVITY OF NANOSEAL WITH SOME OTHER ENDODONTIC SEALERS

By

ALI BURAK KAMAL

Thesis submitted in fulfilment of
the requirements of the degree of
Master of Science
(Conservative Dentistry)

School of Dental Sciences
Health Campus
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Dedication

To my beloved family, for their unconditional love, support and care…

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In the name of Allah the most gracious the most merciful

First of all, I would like to thank ALLAH for giving me the strength and courage throughout the duration of this research project. There are times when words are inadequate. I would like to express my grateful appreciation and thanks to all those who have contributed to this work.

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Dr. ALI BURAK KAMAL

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LIST OF ABBREVIATIONS

HA	= Hydroxyapatite
ADT	= agar-diffusion test
WDT	= well diffusion test
MH	= Muller-Hinton
PMN	= Polymorph nuclear lymphocytes
BPB	= Black pigmented bacteria
HIV	= Human immunodeficiency syndrome
LPSs	= Lipopolysaccharides
Ntr	= Nitrogen
arc	= Aerobic respiration regulatory
cya	= Adenylate cyclase
crp	= Catabolite repressor protein
ATCC	= American type collection culture
UV light	= Ultraviolet light
IQR	= Interquartile range
SD	= Standard deviation
USM	= Universiti Sains Malaysia
mg/ml	= milligram/milliliter
mm	= millimeter
nm	= nanometer
µl	= Microliter
ml	= Milliliter
min	= minute
ºC	= degree celcius
gm	= gram
cfu/ml	= Colony forming unit/milliliter
µg	= microgram
cm	= centimeter
hrs	= hours
M.O.	= microorganism
R.C.	= root canal

KAJIAN IN-VITRO PERBANDINGAN DIFUSI AGAR AKTIVITI ANTIMIKROBIAL BAHAN NANOSEAL DENGAN BAHAN SEALANT ENDODONTIK YANG LAIN

ABSTRAK

Penghapusan mikrob secara menyeluruh pada pulpa gigi yang dijangkiti adalah menjadi salah satu objektif utama dalam rawatan endodontik. Bahan sealant endodontik seharusnya mempunyai spektrum antimikrob yang luas dan masa tindakan yang lama disebabkan oleh ciri-ciri polimikrobial jangkitan endodontik dan ini juga penting untuk penghapusan sisa-sisa mikroorganisma yang tidak dapat dihapuskan oleh kesan penyediaan kemomekanikal dan penggunaan ubat intrakanal endodontik.

Matlamat kajian:

1) Untuk menilai aktiviti bahan antimikrobial bahan sealant endodontik -NanoSeal™ , AH26™ , Tubli-Seal™, Sealapex™ dan Roeko-Seal® keatas *Enterococcus faecalis, Pseudomonas aeruginosa, Streptococcus mutans, Streptococcus sobrinus, dan Escherichia coli* dalam tiga tempoh inkubasi iaitu 24 jam, 48 jam dan 168 jam.
2) Untuk membandingkan aktiviti antimikrobial kelima-lima bahan sealant tersebut terhadap setiap mikroorganisma itu dalam ketiga-tiga tempoh inkubasi.
3) Untuk membandingkan aktiviti antimikrobial setiap bahan sealant tersebut dengan mikroorganisma dalam ketiga-tiga tempoh inkubasi.

Bagi kajian *in-vitro* ini, ujian Difusi Agar (ADT) telah digunakan. 50 plet Agar Muller-Hinton (MH) telah ditebuk sebanyak 5 lurahan (berdiameter 4mm dan sedalam 5mm) setiap jenis satu dengan setiap jenis microorganism diberikan 10

ulangan. 200 µl kultur bakteria telah disediakan mengikut kesesuaian setiap jenis mikroorganisma. Kesemua plet kultur tersebut akan disapu dengan kapas kesat yang steril berserta dengan setiap jenis mikroorganisma yang telah disediakan. Bahan sealant endodontik yang telah disediakan kemudiannya diisi kedalam setiap satu lurahan pada plet tersebut. Plet-plet itu diperhatikan dalam tempoh inkubasi 24 jam, 48 jam dan 168 jam, bagi setiap jenis mikroorganisma.

Zon-zon antimikrobial yang terhasil telah diukur dan direkodkan. Kesemua data dianalisa dengan menggunakan ujian Kruskal-Wallis dan Mann-Whitney. Lima daripada plet MH (satu bagi setiap jenis mikroorganisma) itu telah diinokulasi sebagai sampel kumpulan kawalan positif dan diuji kerecatannya terhadap antibiotik dan lima plet lagi telah disediakan untuk kawalan negatif dan setiap jenis bakteria ini telah diinkubasi dan dibiarkan untuk berpoliferasi tanpa sebarang aditif.

Kesemua bahan yang telah diuji menunjukkan zon-zon antimikrobial aktiviti terhadap kesemua jenis mikroorganisma dalam tempoh masa kajian kecuali Roeko-Seal® yang tidak menunjukkan apa-apa zon aktiviti antimikrobial aktiviti. Terdapat perbezaan yang signifikan($P<0.001$) wujud di antara bahan –bahan sealant endodontik yang telah diuji dari segi aktiviti antimikrobial pada mikroorganisma dalam tempoh ujian ini. Namun begitu, tidak ada perbezaan yang signifikan yang diperhatikan antara NanoSeal™ dan AH26™ ($P>0.005$). Tubli-Seal™ menunjukkan kesan yang paling tinggi terhadap jenis mikroorganisma yang telah diuji diikuti dengan Sealapex™, NanoSeal™ dan AH26™. Tetapi bagi kumpulan *S. mutans* dan *S. Sobrinus,* NanoSeal™ ≈ AH26™ menunjukkan kesan yang lebih kuat berbanding Sealapex™. Bagi setiap jenis mikroorganisma yang telah diuji, *P.*

aeruginosa dan *E. faecalis* telah menunjukan kesan penentangan mikroorganisma yang paling kuat pada NanoSeal™ dan AH26™ diikuti pula oleh *E. coli, S. mutans* dan *S. sobrinus*. Namun begitu, *E. faecalis* telah menunjukan kesan lebih tinggi penentangannya terhadap Tubli-Seal™ jika dibandingkan dengan mikroorganisma yang lain. Bagi Sealapex™ pula, ia menunjukkan kesan rencatan yang paling tinggi terhadap *P.aeruginosa* diikuti *E. faecalis*, *E.coli, S.mutans,* dan *S.sobrinus.*

Kesimpulannya, aktiviti antimikrobial bahan sealant experimen NanoSeal™ adalah setanding dengan AH26™ dan bahan sealant lain kecuali Roeko-Seal®. Namun begitu, Tubli-Seal™ telah menunjukkan kesan penentangan yang paling kuat. Manakala Roeko-Seal® tidak menunjukan sebarang penentangan kepada semua strain mikroorganisma yang telah diuji. Turutan aktiviti rencatan antimikrobial bagi setiap bahan yang telah diuji adalah seperti turutan berikut: Tubli-Seal™ > Sealapex™ > NanoSeal™ ≈ AH26™ > Roeko-Seal® untuk *E.faecalis, P.aeruginosa*, dan *E.coli*, manakala Tubli-Seal™ > NanoSeal™ ≈ AH26™ > Sealapex™ > Roeko-Seal® untuk *S.mutans* and *S.sobrinus*.

AN *IN- VITRO* AGAR DIFFUSION STUDY COMPARING THE ANTIMICROBIAL ACTIVITY OF NANOSEAL WITH SOME OTHER ENDODONTIC SEALERS

ABSTRACT

Complete elimination of microbes in teeth with an infected dental pulp is one of the main objectives of endodontic treatment. Endodontic sealers should have a wide antimicrobial spectrum and action time because of the polymicrobial characteristics of endodontic infections and to eliminate residual microorganisms unaffected by the effects of chemomechanical preparation and intracanal medication of endodontic treatment.

The objectives of this study were

1) To evaluate the antimicrobial activity of NanoSeal ™, AH26 ™, Tubli-Seal ™, Sealapex ™ and Roeko-Seal ® against *Enterococcus faecalis, Pseudomonas aeruginosa, Streptococcus mutans, Streptococcus sobrinus, and Escherichia coli* in three incubation periods of 24 hours, 48 hours and 168 hours .

2) To compare the antimicrobial activity of the five sealers towards each type of microorganism in the three periods.

3) To compare the antimicrobial activity between the tested sealers towards each type of microorganism in the three periods.

Agar diffusion test (ADT) was used. In this study 50 Muller- Hinton agar plates were punched with 5 standard wells (4 mm in diameter and 5 mm in depth) on each a plate. Ten replicates were made for each type of microorganism. Two hundred microliters of bacterial suspension was made of appropriate turbidity for each type of

microorganism and all plates were streaked with sterile cotton swab. Freshly mixed endodontic sealers were dispensed in the wells of each plate. The plates were observed after 24 hours, 48 hours and 168 hours incubation for each type of microorganism.

Zones of inhibition produced were measured and recorded. Data were analysed by using Kruskal-Wallis test. *P* value <0.05 was considered statistically significant of difference. Mann-Whitney test (*Post hoc* test for Kruskal-Wallis test) was used to compare the zones of inhibition between each two sealers towards each type of microorganism. *P* value < 0.005, based on *Bonferroni correction* to avoid type 1 error rate, was considered statistically significant of difference.

Five MH agar plates were prepared and inoculated (one plate for each type of microorganism) to serve as a positive control group and tested for their susceptibility to antibiotics and another five plates were prepared to serve as negative control as the bacteria were incubated and allowed to grow alone without any additives.

All tested materials exhibited inhibition zones towards the tested microorganisms for all the tested periods except for Roeko-Seal® as it showed no inhibition zones. Significant difference was found between the tested sealers (*P*<0.001) towards each microorganism at all times with regard to their antimicrobial activities. There was no significant difference (based on *Bonferroni correction* to avoid type 1 error rate) observed between NanoSeal™ and AH26™ (P>0.005). Tubli-Seal™ showed the greatest inhibitory effect towards the tested microorganisms followed by Sealapex™, NanoSeal™ ≈ AH26™, and Roeko-Seal®. For *S.mutans* and *S.sobrinus,*

NanoSeal™ ≈ AH26™ showed greater effect than Sealapex™. With regard to the tested microorganisms, *P.aeruginosa* and *E. faecalis* were the most resistant strains to NanoSeal™ and AH26™ then followed by *E.coli, S.mutans,* and *S.sobrinus*. On the other hand, *E. faecalis* was more resistant to Tubli-Seal™ than the other microorganisms. While for Sealapex™, it showed the greatest inhibitory effect towards *P.aeruginosa* then *E. faecalis*, *E.coli, S.mutans,* and *S.sobrinus* respectively.

In conclusion, the antimicrobial inhibitory activity of the new experimental sealer NanoSeal™ was comparable to AH26™ and as good as other sealers, except for Roeko-Seal®. However, Tubli-Seal™ exhibited the greatest inhibitory effect and Roeko-Seal® exhibited no inhibitory effect against all test strains. The order of antimicrobial inhibitory activity of the tested materials could be expressed in the following sequence: Tubli-Seal™ > Sealapex™ > NanoSeal™ ≈ AH26™ > Roeko-Seal® for *E.faecalis, P.aeruginosa*, and *E.coli.* On the other hand it was Tubli-Seal™ > NanoSeal™ ≈ AH 26™ > Sealapex™ > Roeko-Seal® for *S.mutans* and *S.sobrinus*.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

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

Therefore, complete elimination of the microbes in teeth with an infected dental pulp is one of the main objectives of root canal treatment (Molander *et al.*, 1998). In this respect, endodontic sealers with antimicrobial properties can be advantageous as they would cope better with persisting residual infection and the microorganisms re-entering from the oral cavity (Eldeniz *et al.*, 2006).

Furthermore, microbial persistence and growth in dentineal tubules, lateral canals and apical ramifications have also been proven and therefore the residual microorganisms, together with those reentering from the oral cavity, if the access cavity is not sealed adequately will rapidly repopulate the empty canals between appointments and can induce or sustain apical periodontitis (Pizzo *et al.*, 2006).

In addition to that, Leonardo *et al.*, (2000) stated that equally important is the antimicrobial activity of endodontic sealers that can prevent recurrent root canal infection and aid the repair process of apical and periapical tissues. Besides that, they should have a wide antimicrobial spectrum and action time because of the polymicrobial characteristics of endodontic infections.

Moreover, when root canal treatment failed, the cause was generally believed to be intracanal infection resisting treatment or microorganisms invading the canal via coronal leakage of the root filling (Molander *et al.*, 1998).

Thus, there are three primary functions for the endodontic sealer:

1. Sealing against ingrowth of microorganisms from the oral cavity.
2. Entombment of remaining microorganisms.
3. Complete obturation at a microscopic level to prevent stagnant fluid from accumulating and serving as nutrients for microorganisms from any other source (Orstavik *et al.,* 2005).

Several properties are required for an ideal endodontic sealer and among them antimicrobial activity which plays an important role in the success rate of root canal therapy, besides the biocompatibility and sealing ability (Siqueira *et al.*, 2000).

In this respect, endodontic sealers should have antimicrobial activity or at least they should not encourage microbial growth. Therefore, sealers with antimicrobial effects have significant and beneficial importance, particularly when pulpal or periapical infections are present as they may help to eliminate residual microorganisms unaffected by the effects of chemomechanical preparation and intracanal medication (Siqueira *et al.*, 2000; Miyagak *et al.*, 2006; and Pizzo *et al.*, 2006).

Moreover, it should be considered that when selecting a material for root canal therapy, biocompatibility is as important as the antimicrobial properties. As the endodontic sealers may come in direct contact with periapical tissues or may leach

through dentine, only those should be used which have been proved to possess an at least acceptable biocompatibility (Pizzo *et al.*, 2006).

Therefore, and because the antimicrobial agents of endodontic sealers do not have selective antimicrobial activity against microorganisms, they may exert toxic effects on the host tissues as a non-selective antimicrobial action which may result in tissue toxicity. Loss of antimicrobial activity during setting will result in reducing the toxic effect of endodontic sealers (Eldeniz *et al.*, 2006).

Moreover, freshly mixed sealers have different and usually more potent antimicrobial activity than 24 hours or one week-old materials. Therefore, Fuss *et al.*, (2000) suggested that the antimicrobial activity of endodontic sealers depends on the time interval between the mixing and the test.

For creating and maintaining a three- dimensional seal of the entire root canal system (i.e. apically, laterally and coronally), sealers should have adhesiveness, be dimensionally stable, be insoluble to oral and tissue fluids and have an adequate flow rate. Sealers that possess both optimum antimicrobial and flow ability properties might theoretically eliminate microorganisms located in the confined areas of the root canal system such as irregularities, isthmus, fins and ramifications (Siqueira *et al.*, 2000).

As microbes are considered to be the primary etiologic agents in the endodontic diseases (pulpal and periapical diseases), there have been an increasing number of

reports of anaerobes infecting root canals, especially in which the infection is long standing (Menezes *et al*., 2004).

Anaerobic microorganisms may be especially well adapted to survive in the environment of necrotic pulp and in dentineal tubules in which blood supply and oxygen is limited or even nonexistent. Facultative anaerobic microorganisms may interact with strict anaerobic microorganisms, causing changes in the nutritional relationships and shifts in the redox potential and oxygen tension, which determine microbial-survival relationship (Lai *et al*., 2001).

In view of the great prevalence of facultative anaerobes and strict anaerobes in unsuccessful endodontic therapy, antimicrobial activity of endodontic sealers on these microorganisms may help to eliminate the residual microorganisms unaffected by the effects of chemomechanical preparation, irrigants, intracanal medications and help in controlling the infection (Leonardo *et al.*, 2000; and Cobankara *et al*., 2004).

In general, in the early stages of pulpal infection, facultative anaerobes like streptococci as well as staphylococci may be found. With time, pulpal necrosis and periapical lesion may be developed and many obligate anaerobes may be found like *Porhyromonas* and *Prevotella spp*. (Lai *et al.*, 2001).

Furthermore, other microorganisms like *Enterococcus feacalis*, *Pseudomonas aeruginosa*, *Lactobacillus casei* and *Escherchia coli* may be found and considered to be resistant to several antimicrobial agents and have also been involved in some persistent and therapy resistant endodontic infections (Eldeniz *et al.*, 2006).

Therefore, the antimicrobial activity of endodontic sealers and the effectiveness of their antimicrobial agents or components play an important role in the activity of endodontic sealers to prevent re-colonization by microorganisms, recontamination of the canal system to prevent the growth of residual microorganisms in the dentineal tubules, lateral and accessory canals and to eliminate gaps between the core of the filling materials and the canal walls (Gomes *et al.*, 2004).

Efforts to provide endodontic sealers that have the ideal physical and biological properties are in progress. 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; and Veloso *et al*., 2006) and being permeable to microorganisms (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 (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 filler. The nano HA filler is assumed to improve the periapical healing process (Gambarini and Tagger, 1996; and Masudi *et al.*, 2007) and to produce a hermetic apical seal. However, little is known regarding the antimicrobial activity of this new material, when used as a sealer in endodontic therapy.

1.2 Statement of the problem

1. Residual microorganisms are unaffected by the effects of chemo-mechanical preparation and intracanal medication (Siqueira *et al.*, 2000; Miyagak *et al.*, 2006; and Pizzo *et al.*, 2006).

2. Re-colonization and re-contamination of the root canal system by microorganisms, growth of residual microorganisms in the dentineal tubules, lateral and accessory canals and the gaps between the core of the filling materials and the canal walls (Gomes *et al.*, 2004).

3. Coronal recontamination by saliva and seeping of periradicular tissue fluids into the root canal system provide the nutrient supply to the remaining microorganisms. Therefore, root canal obturation plays an important role in both prevention and control of endodontic infection (Siqueira *et al.*, 2000).

4. None of the current available sealers possesses ideal properties but some do have better clinical properties than others (Walton and Johnson, 2002; Johnson and Gutmann, 2006). Therefore, there will be no gold standard sealer to compare the new product with.

1.3 Justification of the study

1. This research will demonstrate the antimicrobial activity of five endodontic sealers based on different basic chemical formulations served as bases for endodontic sealers and the result of the test will help to determine the most favourable to be used clinically.

2. To evaluate the antimicrobial activity of this new experimental nano HA containing epoxy resin-based sealer named NanoSeal and to be used as an alternative to other currently used brands of resin based sealers such as AH26.

3. Most of the currently used brands of endodontic sealers are international brands. Therefore, if the antimicrobial activity of this new experimental sealer can be proven then it can be locally manufactured, and it may become a good replacement to those brands.

1.4 Objectives of the study

1.4.1 General Objective

The general objective of this study is to evaluate the antimicrobial activity of endodontic sealers towards aerobic and facultative anaerobic microorganisms.

1.4.2 Specific Objectives

1. To evaluate the antimicrobial activity of a new experimental epoxy resin-based endodontic sealer called NanoSeal and four conventional endodontic sealers AH 26, Tubli-Seal, Sealapex and Roeko-Seal against five aerobic and facultative anaerobic microorganisms (*Enterococcus faecalis*, *Pseudomonas aeruginosa, Streptococcus mutans*, *Streptococcus sobrinus*, and *Escherichia coli*) within the time interval factor involved (24 hours, 48 hours and 168 hours).

2. To compare the antimicrobial activity (zone of inhibition) of the five tested sealers towards each type of microorganism within the time interval factor involved (24 hours, 48 hours and 168 hours).

3. To compare the antimicrobial activity (zone of inhibition) between the tested sealers towards each type of microorganism within the time interval factor involved (24 hours, 48 hours and 168 hours).

1.5 Study Hypotheses

1. All endodontic sealers tested have antimicrobial activities towards the tested microorganisms.

2. The new experimental NanoSeal is an effective antimicrobial sealer towards the tested microorganisms.
3. Comparatively, all endodontic sealers tested do not necessarily have the same antimicrobial activity against the tested microorganisms.

CHAPTER TWO

LITERATURE REVIEW

2.1 Endodontic Treatment

Endodontics is essentially a clinical discipline concerned with the prevention and control of root canal infection by eliminating all bacteria, bacterial by-products and complete tissue debridement from the root canal system by means of chemo-mechanical cleaning and shaping, irrigation and as well as the use of intracanal medications to disinfect the root canal system which has been advocated to enhance the success rate of root canal treatment (Ercan *et al.*, 2006).

Cleaned and shaped root canal must be three dimensionally filled, eliminating any empty space, which has the potential to be infected or reinfected, by creating a fluid-tight seal canal apically, laterally and coronally. Therefore, root canal obturation plays an important role in both prevention and control of endodontic infection (Siqueira *et al.*, 2000).

Microorganisms and their by products 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 *et al.*, 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, irrigation and shaping followed by the complete obturation of the canal space (Lai *et al.*, 2001).

The importance of using an antimicrobial irrigant is to reduce the bacterial load during root canal treatment. The bacteria located in areas such as isthmuses, ramifications, deltas, irrigularities and dentineal tubules will not be eliminated by mechanical means alone. Therefore, the use of chemical disinfectants such as root canal irrigants and antimicrobial medications between appointments are significantly effective for elimination of these bacteria and for disinfection of the root canal system (Mohammadi and Abbott, 2009).

This antimicrobial effect can be a direct chemical effect or indirectly by facilitating the mechanical disinfection through lubrication, tissue dissolving and flushing of contaminated debris accumulated during root canal preparation. In addition, endodontic irrigant should be biocompatible with oral tissues (Van der Sluis *et al.*, 2006).

2.2 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).

2.2.1 Functions of obturation

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.

2.2.2 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 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.

2.3 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. 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).

Most root canal filling techniques use core materials associated with endodontic sealers. Core obturating material such as, gutta percha; usually occupy space, whereas endodontic sealers enhance the possible attainment of an impervious seal by serving as filler for root canal irregularities and minor discrepancies between the root canal wall and the core material (Siqueira *et al.*, 2000).

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

2.3.1.2.1 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 and Bakland, 2002; Walton and Johnson, 2002; Orstavik *et al.,* 2005; and Himel *et al*., 2006).

2.3.1.2.2 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, have 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 and Bakland, 2002; Walton and Johnson, 2002; Orstavik *et al.,* 2005; and Himel *et al*., 2006).

2.3.1.2.3 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 *et al.,* 2005; and 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 into root canal with a sealer. Therefore, 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 (Gencoglu *et al.*, 2002).

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 a sealer

A basic concept is that a sealer is more important than the core obturating material. A sealer accomplishes the objective of providing a fluid-tight seal, which makes the 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 dentine 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 *et al.,* 2005).

2.3.2.2 Requirements of an ideal endodontic sealer

Grossman (1988) outlined the criteria for an ideal sealer. These criteria are as following (Orstavik *et al.,* 2005):

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.

2.3.2.3 Types of sealers

Numerous root canal sealers are available today, 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 *et al.*, 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
	Endomethasone	ZnO-eugenol, paraformaldehyde	Septodont, Saint-Maur des Fosse´s, France
	Tubli-Seal	Base: ZnO, Ba sulfate, modificator and others. Catalyst: Canada balsam, others.	SybronEndo,CA,USA
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
Glass ionomer	Ketac-Endo	Polyalkenoate cement	3M ESPE, St. Paul, MN, USA
Silicone	Roeko-Seal	Polydimethylsiloxane, silicone oil, zirconium oxide	Roeko/Colte`ne/Whale dent, Langenau, Germany
	GuttaFlow	Polydimethylsiloxane, silicone oil, zirconium oxide, gutta-percha	Roeko/Colte`ne/Whale dent, Langenau, Germany
Calcium hydroxide	Sealapex	Toluene salicylate, calcium oxide	Kerr, Romulus, MI, USA
	Apexit	Salicylates, calcium hydroxide	Ivoclar Vivadent, Schaan, Liechtenstein

2.3.2.3.1 Zinc oxide and Eugenol based sealers (ZnOE)

Most of the ZnOE sealers are based on Grossman's formula which is a modification of the original Rickert sealer. Its commercial products include Tubli-Seal (SybronEndo, CA, USA), Pulp Canal Sealer (Kerr, Romulus, MI, USA) and the less readily available one is Roth sealer (Roth Inc., Chicago, IL, USA). All ZnOE sealers are cytotoxic and their toxicity may last longer than those produced by other materials. Moreover, they have shown to be mutagenic in extremely high doses (Dummer, 1997).

However, these problems are not apparent when the material is used clinically. They are probably used more than the other sealers combined and give satisfactory results. The various products have different ranges of setting times and flow rate characteristics. Therefore, each case should be given some thought before a sealer to be chosen and used. For example, difficult canals which require more time to fill require a sealer with an extended working time (Dummer, 1997).

With respect to the antimicrobial activity of ZnOE sealers, they show antibacterial activity against all microorganisms due to the fact that ZnOE sealer contains eugenol, zinc oxide and rosin which are potent antimicrobial agents and eugenol contributes a major part of the antimicrobial activity of ZOE-based sealers (Eldeniz *et al.*, 2006; and Bodrumlu and Semiz, 2006).

2.3.2.3.2 Epoxy resin based sealers

By far the most successful of resin-based sealers has been AH series. AH26 is an epoxy amine resin based sealer. It was recommended by Schroeder in 1995 as root canal cement (Whitters *et al.,* 1999).

Epoxy resin based sealers have comparatively good sealing properties, good mechanical properties and as well as excellent adhesion and adaptation to dentine. No adverse effects are expected with using AH26, allergic reactions are apparently rare, cytotoxicity is moderate to low and mutagenicity is mainly observed shortly after mixing and no risk is expected for the patient (Bergenholtz, 2003).

The antimicrobial activity of epoxy resin-based sealer is related to its hexamethylenetetramine content which in an acidic environment decomposes to ammonia and formaldehyde. The release of formaldehyde upon polymerization process is beneficial because there has been an evidence to show that bacteria were killed when in contact with formaldehyde (Eldeniz *et al.,* 2006).

The presence of silver in AH26 may lead to tooth discoloration due to formation of black silver sulfide and other preparations are available without silver while bismuth oxide is added for radioopacity. The setting reaction of AH26 lasts about two days at the body temperature and during the polymerization process formaldehyde is released but its concentration is more than 300-fold less than that of formaldehyde-releasing ZnOE formulation (Bergenholtz, 2003).

2.3.2.3.3 Glass Ionomer cement sealers (GIC)

They consist fluoroalumino-silicate glasses which react with polycarboxylic acid. They show low shrinkage on setting and possess the virtually unique ability to bond directly to dentine and enamel. These properties should make GIC a good root canal sealer. However, it was not until early 1990 when glass ionomer cement was developed specifically as a root canal sealer (Noort, 1994).

Its antibacterial activity is based on fluoride-ion release and can be regarded as a disinfectant material with a potential and strong but short antibacterial effect on the affected dentine because of its excellent flow rate and the diffusion of its antibacterial components. It is also possible that the antibacterial components of Ketac-endo are less soluble in aqueous milieu relative to the other sealers' components (Cobankara *et al.*, 2004).

GIC has some drawbacks including short working time, difficulty in transport to root canal, difficulty in adaptation to root canal wall, lack of radiopacity and problem regarding its biocompatibility status when it comes in contact with the apical tissues. However, these problems have now been largely overcomed by incorporating an x-ray contrast agent and reducing the glass particle size to less than 25µm (Noort, 1994).

2.3.2.3.4 Silicone based sealers

Silicone as a root canals sealer was introduced in 1984. The first product was based on a condensely polymerized silicone (C–silicone). After subcutaneous

implantation in rats, this material was initially a mild irritating material and virtually non- toxic on the long term (Whitters *et al.*,1999).

In comparison with calcium hydroxide and ZnOE sealers it was proven to be the material with the least tissue irritation. Recently, a root canal sealer with an additional polymerized silicone (A–silicone) became available. A–Silicones used as impression material are known to be more dimensionally stable than C–silicones which release ethanol during polymerization. (Noort, 1994).

Roeko-Seal is a polydimethylsiloxane based sealer which has been recently introduced into the dental community and according to a study by Cobankara *et al.,* (2004), it was stated that Roeko-Seal contained antibacterial activity only in the freshly mixed samples, but this result might be from insolubility and no diffusion of material in the agar medium.

More recent formulations of Roeko-Seal would polymerize without shrinkage, with platinum as a catalyzing agent as well as they show impressive biological performance (Orstavik *et al.,* 2005).

2.3.2.3.5 Calcium hydroxide based sealers $Ca(OH)_2$

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 and found that hard tissue formation was more pronounced after root filling with Sealapex sealer than with pure calcium hydroxide.

Calcium hydroxide based sealers depend for their antimicrobial activity on the concept of ionization that releases hydroxyl ions $(OH)^-$ and causing an increase in pH. A pH >9 may reversibly or irreversibly inactivate cellular membrane enzymes of the microorganism, resulting in a loss of biological activity of the cytoplasmic membrane or leading to the destruction of phospholipids or unsaturated fatty acids that results in a loss of cytoplasmic integrity (Eldeniz *et al.*, 2006).

Sealapex sealer shows only slight toxicity in the fresh state; however it exhibits increasing cytotoxicity in the set state. This might be the reason why Sealapex could exhibit the antimicrobial activity (Lai *et al.*, 2001).

2.3.2.4 New types of sealers

2.3.2.4.1 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 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, and Milan, Italy) is another example of HA containing sealer. 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.

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.

2.3.2.4.2 Experimental nano HA-filled epoxy resin based endodontic sealer (NanoSeal)

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 dentineal tubules. Karadag *et al.*, (2004) concluded that the resin based sealer AH26 penetrated into the dentineal 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 dentineal 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 have led to a dramatic fall in the dentineal permeability. The author reported that nano HA small particle size, and the reduced agglomerate diameter, have resulted in higher penetration and reaction efficiency, leading to lower dentine permeability.

This new experimental nano HA-filled epoxy resin based endodontic sealer (NanoSeal) has similar composition to that of other epoxy resin based sealers but