6-1998

An Evaluation of Root End Filling Materials Using Endotoxin

Hong-Ming Tang

LOMA LINDA UNIVERSITY

GRADUATE SCHOOL

AN EVALUATION OF ROOT END FILLING MATERIALS USING ENDOTOXIN

by

Hong-Ming Tang

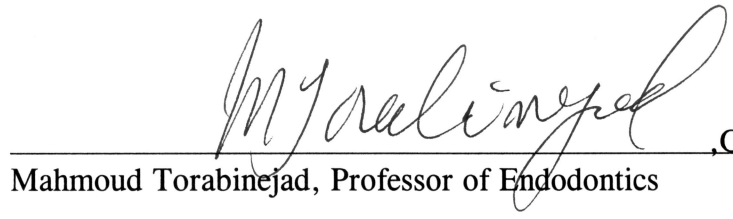
A Thesis in Partial Fulfillment

of the Requirements for the Degree Master

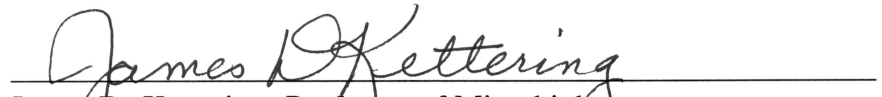
of Science in Endodontics

June 1998

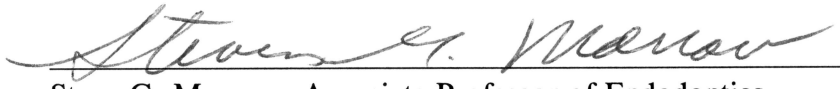
Each person whose signature appears below certifies that this thesis in their opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.



Mahmoud Torabinejad, Professor of Endodontics, Chairperson



James D. Kettering, Professor of Microbiology



Steve G. Morrow, Associate Professor of Endodontics

ACKNOWLEDGMENTS

I would like to express my appreciation to the individuals who helped me complete this study. I want to thank Dr. Torabinejad for his advice and help. I am grateful to Dr. Kettering for providing the facilities and timely advice, and I wish to thank Dr. Morrow for his advice and support. I wish to thank Mr. Aprecio for his great help and guidance in the laboratory techniques.

This work was supported in part by American Association of Endodontists Foundation.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

ABSTRACT

AN EVALUATION OF ROOT END FILLING MATERIALS USING ENDOTOXIN

by Hong-Ming Tang

Mineral Trioxide Aggregates (MTA) was recently developed at Loma Linda University. It has been shown to possess good sealing ability when tested with dye, bacteria, and a fluid filtration technique.

Endotoxin is a component of the cell wall of Gram negative bacteria. It has been proposed that it may play a role in the pathogenesis of periradicular lesions. Previous studies have demonstrated that MTA can prevent the leakage of bacteria. However, the bacterial products were not tested during these studies.

This study used endotoxin to compare the sealing ability of SuperEBA, IRM, amalgam, and MTA.

The results showed that MTA permitted less leakage than IRM and amalgam at 1, 2, 6, and 12 weeks ($p < 0.05$), and leaked less endotoxin than SuperEBA at 2 and 12 weeks ($p < 0.05$).

INTRODUCTION

The purpose of placing a root end filling during periradicular surgery is to create a hermetic seal which prevents the egress of the contents of root canals into the periradicular tissues. The sealing ability of various root end filling materials has been tested using different tracers, electrochemistry, and fluid filtration technique. The validity of these methods has been questioned by some investigators (Wu and Wesselink 1993, Torabinejad *et al.*1995a). Leakage of bacteria and their products have been proposed as an alternative for testing the sealing ability of root end filling materials (Kos *et al* 1982, Kersten and Moorer 1989, Torabinejad *et al.*1995a).

Kersten and Moorer (1989) compared the ability of four obturation methods to prevent leakage of bacteria-sized particles and endotoxin. Their findings showed that microleakage of the small dye molecules could not be prevented, whereas leakage of the bacteria-sized particles and endotoxin could be prevented with some of the obturation techniques. A recent study also suggested that endotoxin could be used as an indicator for the presence or absence of leakage (Trope *et al.* 1995).

Mineral Trioxide Aggregates (MTA) has been recently developed at Loma Linda University as a root end filling material. It has demonstrated good sealing ability when tested with dye molecules (Torabinejad *et al.* 1993; 1994). Another study (Torabinejad *et al.* 1995a) also demonstrated the superior sealing ability of MTA when tested to prevent bacterial leakage. However, their is no information on the ability of MTA to prevent leakage of bacterial byproducts.

It has been proposed that endotoxin plays a role in the pathogenesis of periradicular lesions (Dwyer and Torabinejad 1981, Pitts *et al.* 1982), has been found in root canals of necrotic teeth (Schein and Schilder 1975), and in human periapical lesions (Schonfeld *et al.* 1982). An ideal root end filling material should prevent the leakage of both bacteria and their by-products, such as endotoxin.

The purpose of this study was to investigate the ability of MTA, as a root end filling material, to prevent the leakage of endotoxin when compared to amalgam, Intermediate Restorative Material (IRM), and SuperEBA.

LITERATURE REVIEW

The sealing ability of root end filling materials has been evaluated by various techniques. These include leakage studies using different tracers, scanning electron microscopy, electrochemical method, fluid filtration technique, and *in vivo* studies.

A. Scanning Electronic Microscope Studies

Scanning electron microscopy (SEM) allows detailed study of specimens in high resolution. The rationale behind the use of SEM is the assumption that the smaller the gap or defect between the filling material and the root end cavity wall the better the seal.

The first SEM study on root end filling materials was reported by Moodnik *et al.* (1975), who examined the amalgam-dentin interface in teeth with root end fillings of amalgam. Defects ranging from 6 to 150 microns were found. It was reported that lifting of amalgam from the cut root surfaces occurred, up to 37 microns.

In another study by Tanzilli *et al.* (1980), the adaptation of retrograde amalgam, heat-sealed gutta-percha, and cold-burnished gutta-percha were compared. In this study, the cold burnish gutta-percha demonstrated the least distance, 1.8 micron, between gutta-percha and the dentinal walls. The average marginal defects of retrograde amalgam, heat-sealed gutta-percha, and the control samples were between 22 to 104 microns.

The samples are usually subjected to various procedures during the preparation stage for SEM observation. These procedures may cause cracking of the tooth

structure or the filling material. Because of the presence of artifacts in SEM samples, it has been suggested that it is best to study the samples with a replication technique. Torabinejad *et al.* (1995b) studied the marginal adaptation of MTA, EBA, IRM, and amalgam. In one group, roots were longitudinally sectioned into two halves by a slow-speed diamond saw. In the other group, resin replicas were made of the resected root surfaces. Examination of the original samples revealed numerous artifacts. In contrast, the resin replica samples did not result in any artifacts. It was reported that MTA had defects averaging 2.68 microns on the longitudinal surface and no noticeable marginal gaps on the root end resection surface prepared by the replication technique. Comparison between the size of the gaps found in this study with the data from the previous SEM studies (Moodnik *et al.* 1975; Tanzilli *et al.* 1980) underscores the importance of the replicate technique.

SEM studies have shown direct observation of the adaptation of the root end filling materials to the root end cavity walls. The extrapolation of a smaller gap into a better seal has been questioned. Abdal and Retief (1982) evaluated the adaptation of retrograde filling materials in root-end cavities. The adaptation of the filling materials in one of each pair of teeth was evaluated directly and by resin replica of longitudinally sectioned samples. Marginal leakage at the filling-dentin interface was evaluated in the other teeth using a fluorescent dye. The results showed no correlation between maximum marginal gaps and the degree of dye penetration. The same conclusion was reported by Yoshimura *et al.* (1990), who examined the correlation between the SEM study and the pressurized fluid filtration technique in the same teeth.

However, significant correlation has been found by other investigators (Stabholz *et al.* 1985, Torabinejad *et al.* 1993, 1995b). Stabholz *et al.* compared their SEM study results with those from a previous leakage study which utilized a radionuclidic model (Shani *et al.* 1984). It was found that a good correlation between marginal adaptation and sealing ability exists. Studies by Torabinejad *et al.* (1993, 1995b) also corroborated this correlation.

B. Electrochemical Method

This technique was introduced by Jacobson and von Fraunhofer (1976) to test the leakage of root canal fillings. The mechanism of the setup works like a galvanic cell. One electrode is attached at one end to the inside of the canal with plasticine, and the other end to an ammeter. The root is then immersed in 1% potassium chloride solution. Another electrode is dipped into the electrolyte solution and connected to the same ammeter. A galvanic corrosion current in this system will flow only when there is leakage into the root canal and a continuous electrolyte path has thus been established. The current can be read from the ammeter. The time elapsed between immersion and current flow denotes the potassium chloride penetration rate, while the magnitude of the current indicates the degree of penetration.

The electrochemical method offers the advantages of obtaining quantitative measurements which can be easily compared and analyzed, the opportunity to study leakage in a continuous time period, and being able to record the time when maximum leakage occurs. The electrochemical method has been reported to be the most objective

method to measure leakage of teeth with root canal treatment (Delivanis and Chapman 1982).

As has been stated by Jacobson and von Fraunhofer (1976), this system depends on the penetration of the electrolyte solution. Total leakage of the electrolyte solution into the canal to contact the electrode is necessary to develop measurable current (Alhadainy et al 1993). This means the solution is used as the indicator of leakage, which is composed of small potassium and chloride ions and water molecules.

This method was later used by different investigators. Mattison *et al.* (1985) found that thicker (3 mm) retrofills leaked less than the thinner (1 mm) filling and varnish improved the sealing ability of amalgam. Using this method, Alhadainy et al. (1993) found that glassionomer cement had the least leakage compared to amalgam, heat-sealed gutta-perch, and zinc polycarboxylate cement.

C. Fluid Filtration Method

This technique applies positive pressure on one end of the root and determines the leakage by observing the speed of the movement of an air bubble inside a capillary tube (Derkson *et al.* 1986). Since the test is non-destructive and the measurements are quantitative, it is possible to perform the parametric analysis and repeated measurements over a period of time.

King *et al.* (1990) utilized this technique to compare the apical seal obtained by cold-burnished gutta perch, amalgam, amalgam with varnish, SuperEBA cement, and glass ionomer cement as root end filling materials over a 3-month period. Glass

ionomer allowed the most leakage, while no difference was observed between SuperEBA, amalgam, and amalgam with varnish.

Using the same technique, Gilheany *et al.* (1994) found that increasing the depth of the root end cavity significantly decreased apical leakage; there also was a significant increase in leakage as the amount of bevel increased. It was noted by the authors that both the permeability of resected apical dentin and microleakage around the retrograde filling material had a significant influence on apical leakage.

Bates *et al* (1996) used this technique to evaluate the longitudinal sealing ability of MTA, SuperEBA, and amalgam as root end filling materials. After 24, 72 hours, and 2 weeks, both MTA and SuperEBA had significantly less leakage than amalgam. At the subsequent periods of 8 and 12 weeks, no significant difference was observed among the three materials. MTA was reported to be superior to amalgam and comparable to SuperEBA in preventing leakage.

D. Radioactive Isotope Method

This method is also called the autoradiography technique. It is based on the fact that an alpha or beta particle can change the energy state of a photographic emulsion in a way that is qualitatively similar to the action of light. Thus the incident of a beta particle on the silver bromide crystals of the emulsion produces a latent image of its path, which easily may be reproduced by conventional developing procedures.

Tronstad *et al.* (1983) used ^{45} calcium to test the leakage of roots that previously had been implanted subcutaneously in rabbits. It was noted that regardless of the type

of amalgam used, the apical seal was significantly improved when a varnish was applied to the cavity prior to the placement of the retrograde amalgam filling.

Szeremeta-Browar *et al.* (1984) used the same isotope and found that lateral condensation of gutta-percha and SuperEBA had the best seal while cold-burnished gutta-percha and amalgam leaked the most. Based on their findings, the authors questioned the value of amalgam as a root end filling material.

E. Comparison of different techniques

The results from the leakage studies seem to vary and, sometimes, even contradict each other. The discrepancies found among the results stimulated some investigators to compare the different methodologies.

Matloff *et al.* (1982) compared methylene blue with ^{45}calcium, ^{14}carbon-labeled urea, and ^{125}iodine-labeled albumin. Methylene blue was found to penetrate farther up the canal than any of the isotope tracers. ^{14}Carbon-labeled urea penetrated farther than the calcium or ^{125}iodine-labeled albumin. Large variability was noticed with different molecules.

Delivanis and Chapman (1982) investigated the reliability of the electrochemical technique versus the dye leakage and autoradiographic techniques. The correlation of the electric readings with the evaluations obtained from the dye and autoradiographic techniques were $r=0.46$ and $r=0.52$ respectively. They attributed the lack of correlations to the subjective way of measuring leakage with the dye or radioisotope

techniques. Therefore, it has been suggested that the electrochemical technique is the most objective method for testing leakage.

Kersten and Moorer (1989) compared latex beads, endotoxin, butyric acid, valeric acid, with methylene blue, and found that methylene blue dye was the most penetrating material. They concluded that leakage of small molecules, such as methylene blue, through the root canal fillings cannot be stopped, whereas the leakage of bacteria-sized molecules can be prevented.

F. Bacterial Leakage Method

Most leakage studies have used either radioactive isotopes or dye molecules as indicators. The validity of these studies has been questioned in extrapolating the results to the clinical situations (Kersten & Moorer 1989).

Goldman *et al.* (1980) designed a bacterial leakage model to test the leakage of root canal fillings. The model used *Proteus mirabilis* and *Streptococcus salivarius*. An acid-responsive indicator (Phenol red) was used to demonstrate bacterial penetration. When there is bacterial leakage and growth, the pH change of the growth medium will cause a color change. The results showed that poly-HEMA root canal fillings can prevent the leakage of bacteria.

The same model was modified by Kos *et al.* (1982) and Torabinejad *et al.* (1995a), who tested for bacterial leakage in various retrofilling materials. Kos *et al.* (1982) compared poly-HEMA, amalgam, and cold-burnished gutta-percha as root end fillings. Approximately eighty to hundred percent of cold-burnished gutta-percha and

amalgam retrograde fillings had bacterial leakage in 48 hours, while poly-HEMA had almost no leakage after 38 days.

The results of the study by Torabinejad *et al.* (1995a) demonstrated that the median time of bacterial leakage of four different root end filling materials were: MTA - 90 days (2/10); amalgam - 28.5 days (9/10); SuperEBA - 34.5 days (8/10); and IRM - 15 days (10/10). MTA leaked significantly less than the other tested materials.

Nakata *et al* (1997) performed a bacterial leakage study with an anaerobic bacterium, *Fusobacterium nucleatum*. Perforations repaired with MTA or amalgam were evaluated for 45 days. Eight out of 18 amalgam samples leaked, while none of the MTA samples leaked. MTA was superior to amalgam in preventing leakage of *F. nucleatum* past furcal perforations repaired by these materials.

G. *In Vivo* leakage Studies

Tronstad *et al.* (1983) implanted retrofilled roots in rabbits subcutaneously. After 7, 30, and 90 days the roots were retrieved and the leakage was tested with ^{45}calcium for five minutes. It was found that varnish significantly improved the sealing ability of amalgam as a retrograde filling material. After 90 days almost no leakage could be detected.

Friedman *et al.* (1991) compared the leakage under both *in vivo* and *in vitro* conditions. In the *in vivo* part, the teeth of dogs were infected and then received apicoectomies as well as root end fillings. The healing was observed radiographically for six months. Root end fillings with amalgam were found to be more successful than

glass ionomer, and the composite resin was the least successful. In the second part of the study (*in vitro*), some of the roots were extracted for leakage study. The results showed no significant difference between the leakage of the three tested materials. It was concluded that the dye leakage did not correlate with the *in vivo* tissue response, as observed radiographically.

H. The role of endotoxin in the pathogenesis of periradicular lesions

It has been shown that the formation of endodontic lesions is due to the presence of bacteria (Kakehashi *et al.*, 1965) Improvements in anaerobic culture techniques have identified many species of anaerobic bacteria in endodontically involved teeth, most of which are Gram-negative bacteria (Sunqvist 1976, Bergenholtz 1974, Sabiston *et al.* 1976, Fabricius *et al.* 1982).

Endotoxin, or lipopolysaccharide (LPS), is the major component of the cell wall of the Gram-negative bacteria. In 1892 Richard Pfeiffer, a pupil of Robert Koch, demonstrated the existence of two toxins produced by cholera bacilli: the already known exotoxin and another toxin which he showed to be firmly bound to the cell. Endotoxin is only released into the surrounding medium if the bacteria undergo disintegration (Westphal 1993).

Endotoxin, when injected intravenously, can cause fever, granulocytosis, thrombocytopenia, disseminated intravascular coagulation (DIC), and endotoxin shock (Wolff 1973, Elin and Wolff 1976). It can activate the complement system (Morrison & Kline 1977) and increase the level of plasma cortisol and growth hormone (Wolff

1973). It may have a mitogenic effect on B lymphocytes (Moller *et al.* 1973). Also, it is the principle component of both the local and general Shwartzman Phenomenon, which may cause massive tissue damage (Elin and Wolff 1976). Endotoxin has been shown to increase the bone resorption of fetal rat bones in tissue culture (Haussman *et al.* 1970). Injection of endotoxin into the palatal gingiva of rabbits produced an acute inflammation accompanied by bone resorption (Rizza and Mergenhagen 1964). Because of the pro-inflammatory property of endotoxin, it has been suggested that it plays a role in bone resorption in periradicular tissues (Dwyer and Torabinejad 1981; Pitts *et al.* 1982).

Schein and Schilder (1975) found that teeth with radiolucent areas contained a higher level of endotoxin than teeth without radiolucent areas. Schonfeld *et al.* (1982) found endotoxin in 15 out of 20 human periradicular lesions.

In two animal studies, Dwyer and Torabinejad (1982) and Pitts *et al.* (1982) separately showed that endotoxin sealed in root canals caused more and larger lesions when examined radiographically and histologically. The results indicated that the endotoxin probably played a role in the pathogenesis of periradicular lesions. Yamasaki *et al.* (1992) found that the amount of endotoxin in the periradicular tissues of rats gradually increased with increasing time as the periradicular lesion developed.

I. Leakage study with endotoxin

Endotoxin was first utilized as a tracer in a leakage study by Kersten and Moorer (1982). In this study, 1 mg/ml latex beads, 4 microgram/ml endotoxin, 0.5% butyric

acid, 0.1% valeric acid, and 0.1% methylene blue in water were used to test the leakage of obturated root canals. The results showed that leakage of bacteria-sized particles and large-sized protein molecules could be prevented when both sealer and pressure were used in obturating root canals with gutta-percha. Microleakage of small molecules, butyric acid, and methylene blue dye could not be prevented with any of the methods of obturation.

Trope *et al.* (1995) used 100 microgram/ml endotoxin solution to evaluate the leakage of obturated root canals. Five out of 16 samples demonstrated endotoxin leakage in 21 days. They concluded that endotoxin can move through obturated root canals and can be used as an indicator for leakage studies.

J. Purpose of the study

The purpose of this study was to utilize endotoxin as a tracer to test the sealing ability of four root end filling materials: amalgam (Valiant, Vivadent USA Inc., NY, USA), IRM (L.D. Caulk, Milford, DE, USA), SuperEBA (Bosworth Company, Skokie, IL, USA), and Mineral Trioxide Aggregate (MTA).

MATERIALS AND METHODS

1. Root canal preparation

One hundred and four single-rooted extracted human teeth were used in this experiment. The soft tissues and the calculus were removed with an Amadent ultrasonic device. The apical 3 mm of each root was resected perpendicular to the long axis of the tooth with a diamond bur. The coronal portion of each tooth was resected at a point which provided a root with a total length of 15 mm.

Each root canal was instrumented with files and Gates-Glidden drills. The apical opening of each canal was enlarged to a #50 file and flared in 0.5 mm increments to a #70 file. The rest of the canal was then flared with #3 to #5 Gates-Glidden drills. The canals were irrigated with 1 ml of 5.25% sodium hypochlorite (NaOCl) solution between various sizes of files and Gates-Glidden drills and a 5 ml irrigation at the completion of instrumentation.

2. Sterilization and Detoxification

All glassware used in this experiment were detoxified with dry heat at 180°C for 2 hours.

The roots were placed in 10 ml glass test tubes filled with 5 ml of 5.25% NaOCL solution. They were placed in a Biosonics ultrasonic bath for 30 minutes. The NaOCl solution was then replaced with non-pyogenic water and changed daily for one week to wash out residual NaOCl solution.

3. Obturation

The roots were then dried with an air syringe and obturated with Obtura Gutta-percha without a sealer.

4. Mounting the tooth

A modification of the setup used by Torabinejad *et al* (1995) was utilized in this experiment. The apparatus consisted of two parts, the top and lower reservoirs. The top reservoir consisted of a capped plastic vial (Micro Cent Tube) and the lower part from a glass vial with a screw-on cap. The plastic vials were placed in the 5.25% NaOCl solution for 1 hour and then washed with non-pyogenic water.

After removing the apical 10 mm of the plastic vial, the root was inserted into the vial with the apex of the root sticking out the end of the vial. The space between the vial and the root was sealed with sticky wax. Except the resected surface of the root, the rest of each root was coated with two layers of sticky wax (Fig. 1).

A hole was drilled through the cap of the glass vial to accommodate the root and the plastic vial setup. The root and plastic vial setup was attached to the cap of the glass vial through the hole with sticky wax (Fig. 2).

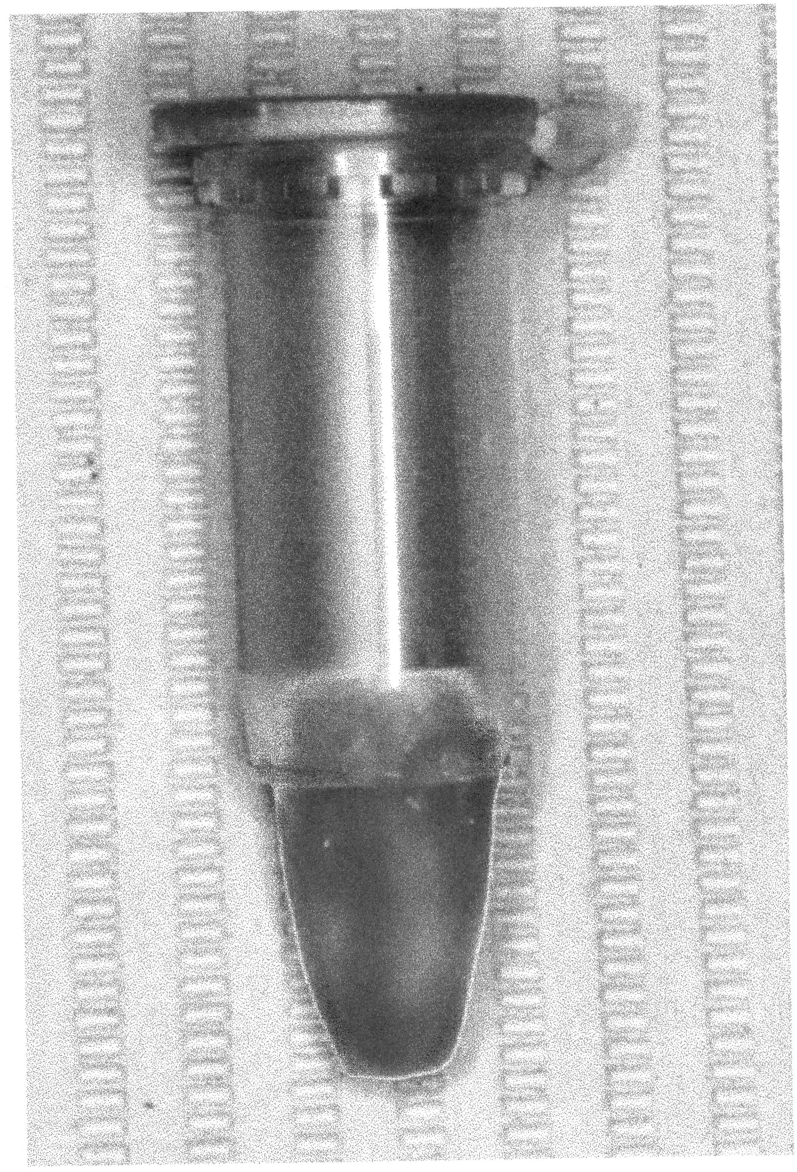


Fig 1. Assembly of the root and the Micro Cent Tube.

Fig 2. Assembly of the root-vial and the cap.

5. Root end preparation

The root end preparation was performed with a #557 carbide bur providing a preparation of 3 mm in depth and 1 mm in diameter.

6. Root end filling

Four root end cavity preparations received Obtura gutta-percha without a root canal sealer and served as positive controls. Four roots were entirely filled with sticky wax and served as negative controls. Another four roots were prepared as positive controls and served as blank controls. The blank controls were used as indicators for the presence of endotoxin prior to the beginning of the experiment or endotoxin contamination during the experiment.

The remaining 92 roots were randomly divided into four separate experimental groups of 23 samples each. The root end cavities were filled with either SuperEBA, IRM, amalgam, or MTA according to the instructions provided by their manufacturers. Each setup, consisting of the root, the plastic vial, and the cap, was screwed onto the glass vial, which served as the lower reservoir (Fig. 3). The top and the lower reservoirs were filled with non-pyogenic water.

7. Measurement of endotoxin leakage

After one week, a 0.5 ml of sample from the lower reservoir was examined for the presence of endotoxin with the QCL-1000 endotoxin testing kit

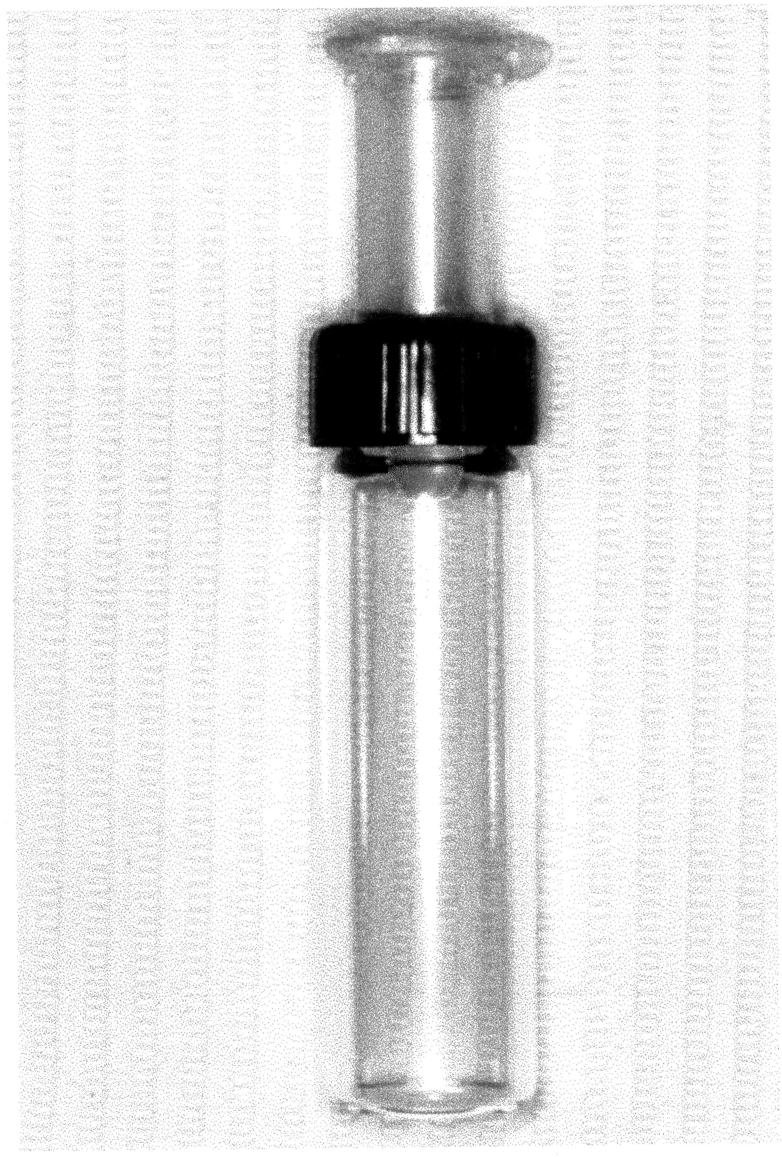


Fig 3. The final assembly of the model used in present study.

(BioWhittaker, Walkersville, MD, USA). Then 0.5 ml non-pyogenic water was resupplemented into the lower reservoir.

QCL-1000 utilizes a modified Linulus Amebocyte Lysate (LAL) and a synthetic color producing substrate to detect endotoxin chromatogenically. Immediately before the test, standard solutions containing 1, 0.75, 0.50, and 0.25 EU/ml were prepared from the endotoxin sample supplied according to the manufacturer's manual. Substrate solution and LAL solution were reconstituted with non-pyogenic water. Twenty five percent acetic acid solution was prepared by diluting 100% acetic acid with non-pyogenic water. It was used as a stop agent to halt the reaction. The 96-well microplate method was used.

Two persons worked together to perform the assay. One person added the solutions to the wells of the microplate and the other used the stop watch to make sure the timing and rate of adding solution was accurate. The tests were performed in a walk-in incubator with temperature set at 37^0C. A 50 μl of sample was dispensed into the appropriate microplate well. Each series of determinations included a blank (non-pyogenic water) plus the four endotoxin standard solutions run in duplicate. All reagent additions and incubation times were identical.

At 0 minute, 50 μl of LAL was added to the column of the microplate serially using a 8-channel pipettor. The rate of adding solution was maintained consistent. After the LAL had been dispensed into all microplate wells, the plate was tapped on the side repeatedly to facilitate the mixing.

At 10 minutes, 100 μl of substrate solution was added. The substrate solution was pipetted in the same manner as adding LAL and consistent rate was maintained. The plate was tapped repeatedly after adding the solution.

At 16 minutes, 100 μl of stop agent was added. The agent was pipetted in the same manner as before. The plate was tapped repeatedly on the side after adding the stop agent. The absorbance was read at 405 nm (EIA 400, BioWhittaker, Walkersville, MD, USA). The concentration of the samples were calculated using linear regression (WinSTAR release 1.72, Anderson-Bell Corp, USA).

Except in the blank control group, the non-pyogenic water in the top reservoirs were replaced with 1 ml of endotoxin solution (*Escherichia coli* 055:B5, 10 μg/ml).

The leakage of endotoxin into the lower reservoir was examined at 1, 2, 6, and 12 weeks. Random samples from the top reservoirs were also tested at the end of the experiment to determine the endotoxin activity.

The number of samples with leakage was recorded at each time interval and the statistical difference was analyzed using chi-square analysis.

RESULTS

Examination of the baseline endotoxin levels in the lower reservoir before the addition of endotoxin to the upper reservoirs (0 week) showed presence of endotoxin below 0.1 EU/ml.

All positive controls showed leakage greater than 1 EU/ml at one week. All negative and blank controls showed endotoxin leakage below 0.2 EU/ml at the end of the study. Random samples from the top reservoir tested at the end of the study still showed endotoxin activity greater than 1 EU/ml (Table 1).

The number of samples that didn't have any leakage and the percentage are shown in Table 2. Chi-square analysis for different time periods are shown in the Appendix.

In one week, MTA was significantly better than amalgam and IRM ($p=0.01$, $p=0.04$). No significant difference was observed between MTA and SuperEBA ($p=0.35$). Also, no significant difference was observed among SuperEBA, IRM, and amalgam.

In two weeks, MTA leaked significantly less than amalgam ($p=0.00$), IRM ($p=0.00$), and SuperEBA ($p=0.02$). No significant difference was observed among SuperEBA, IRM, and amalgam.

In six weeks, MTA leaked significantly less than amalgam ($p=0.00$) and IRM ($p=0.01$), but no significant difference was observed between MTA and SuperEBA ($P=0.06$). Also, no significant differences were observed among SuperEBA, IRM, and amalgam.

In twelve weeks, MTA leaked significantly less than amalgam ($p=0.00$), IRM ($p=0.00$), and SuperEBA ($p=0.01$). No significant differences were observed among SuperEBA, IRM, and amalgam.

Table 1. Concentration of endotoxin in lower reservoir (Endotoxin Unit per milliliter). AMAL = amalgam, MTA = mineral trioxide aggregate, IRM = intermediate restorative material, EBA = SuperEBA, POS = positive control, NEG = negative control, BLK = blank control, '-' = no test was performed.

Number	Sample	0 week	1 week	2 weeks	6 weeks	12 weeks
1	MTA	0.08	3.78	-	-	-
2	MTA	0.10	3.53	-	-	-
3	MTA	0.07	0.00	0.08	0.06	0.06
4	MTA	0.07	0.11	0.08	0.13	0.13
5	MTA	0.08	2.95	-	-	-
6	MTA	0.09	0.00	0.12	0.00	0.00
7	MTA	0.10	0.11	0.09	0.04	0.13
8	MTA	0.07	0.11	0.11	0.05	0.06
9	MTA	0.09	3.05	-	-	-
10	MTA	0.08	0.12	0.14	0.07	0.05
11	MTA	0.07	1.78	-	-	-
12	MTA	0.09	0.11	0.11	0.02	0.02
13	MTA	0.07	0.11	0.11	0.02	0.02
14	MTA	0.08	0.06	0.05	0.10	0.00
15	MTA	0.08	0.07	0.05	0.09	0.06
16	MTA	0.08	0.06	0.06	1.18	-
17	MTA	0.08	2.30	-	-	-
18	MTA	0.08	0.07	0.05	0.09	0.05
19	MTA	0.08	0.06	0.05	0.09	0.70
20	MTA	0.08	0.06	0.06	0.09	0.00
21	MTA	0.08	0.08	0.06	-	-

Table 1 (continued)

Number	Sample	0 week	1 week	2 weeks	6 weeks	12 weeks
22	MTA	0.09	0.14	0.07	-	-
23	MTA	0.08	0.08	0.06	-	-
24	EBA	0.09	0.13	0.81	-	-
25	EBA	0.08	1.59	-	-	-
26	EBA	0.08	0.11	0.10	0.00	0.05
27	EBA	0.07	2.59	-	-	-
28	EBA	0.08	2.65	-	-	-
29	EBA	0.09	0.10	0.24	-	-
30	EBA	0.08	0.37	1.18	-	-
31	EBA	0.08	0.10	0.16	0.00	0.04
32	EBA	0.07	1.47	-	-	-
33	EBA	0.08	0.36	0.42	-	-
34	EBA	0.08	0.15	0.12	0.02	0.06
35	EBA	0.09	0.09	0.10	0.57	1.99
36	EBA	0.07	0.09	0.14	0.00	0.04
37	EBA	0.08	0.30	0.66	2.17	-
38	EBA	0.08	0.07	0.05	0.1	0.00
39	EBA	0.08	0.06	0.07	1.93	-
40	EBA	0.08	2.17	-	-	-
41	EBA	0.13	1.71	-	-	-
42	EBA	0.09	0.07	0.05	0.38	-
43	EBA	0.08	2.28	-	-	-
44	EBA	0.08	0.08	0.28	-	-
45	EBA	0.10	0.11	0.23	-	-
46	EBA	0.09	0.12	0.89	-	-

Table 1. (continued)

Number	Sample	0 week	1 week	2 weeks	6 weeks	12 weeks
47	IRM	0.08	0.11	0.08	0.06	0.17
48	IRM	0.09	3.60	-	-	-
49	IRM	0.08	3.35	-	-	-
50	IRM	0.08	0.19	0.96	-	-
51	IRM	0.09	3.37	-	-	-
52	IRM	0.08	3.27	-	-	-
53	IRM	0.08	0.12	0.11	0.15	1.43
54	IRM	0.08	3.48	-	-	-
55	IRM	0.07	3.29	-	-	-
56	IRM	0.09	3.29	-	-	-
57	IRM	0.10	3.63	-	-	-
58	IRM	0.08	0.05	0.09	0.04	0.05
59	IRM	0.08	0.11	1.97	-	-
60	IRM	0.08	2.37	-	-	-
61	IRM	0.08	0.07	0.05	2.13	-
62	IRM	0.08	2.21	-	-	-
63	IRM	0.07	2.29	-	-	-
64	IRM	0.08	0.93	-	-	-
65	IRM	0.08	0.93	-	-	-
66	IRM	0.08	0.07	0.06	0.10	2.02
67	IRM	0.08	0.08	0.28	-	-
68	IRM	0.08	2.52	-	-	-
69	IRM	0.08	0.07	0.06	-	-
70	AMAL	0.07	1.74	-	-	-
71	AMAL	0.08	3.06	-	-	-

Table 1. (continued)

Number	Sample	0 week	1 week	2 weeks	6 weeks	12 weeks
72	AMAL	0.08	3.48	-	-	-
73	AMAL	0.08	4.13	-	-	-
74	AMAL	0.09	3.90	-	-	-
75	AMAL	0.08	0.11	0.18	0.08	2.01
76	AMAL	0.07	3.85	-	-	-
77	AMAL	0.07	3.50	-	-	-
78	AMAL	0.08	3.31	-	-	-
79	AMAL	0.08	3.49	-	-	-
80	AMAL	0.09	3.04	-	-	-
81	AMAL	0.09	0.08	1.43	-	-
82	AMAL	0.08	0.06	0.05	1.15	-
83	AMAL	0.07	2.34	-	-	-
84	AMAL	0.08	2.32	-	-	-
85	AMAL	0.07	2.30	-	-	-
86	AMAL	0.08	0.06	0.06	2.36	-
87	AMAL	0.08	0.06	1.58	-	-
88	AMAL	0.08	0.11	0.18	0.086	2.01
89	AMAL	0.09	3.09	-	-	-
90	AMAL	0.09	0.08	0.08	-	-
91	AMAL	0.09	2.59	-	-	-
92	AMAL	0.08	2.71	-	-	-
93	POS	0.08	2.38	-	-	-
94	POS	0.09	2.50	-	-	-
95	POS	0.09	2.27	-	-	-
96	POS	0.08	2.31	-	-	-

Table 1. (continued)

Number	Sample	0 week	1 week	2 weeks	6 weeks	12 weeks
97	NEG	0.08	0.16	0.19	0.15	0.18
98	NEG	0.08	0.07	0.07	0.10	0.09
99	NEG	0.07	0.08	0.08	0.08	0.00
100	NEG	0.08	0.08	0.08	0.07	0.08
101	BLK	0.08	0.07	0.08	0.06	0.08
102	BLK	0.08	0.08	0.09	0.10	0.08
103	BLK	0.07	0.08	0.09	0.08	0.08
104	BLK	0.08	0.08	0.08	0.07	0.07

Table 2. Number of samples without any leakage in each time interval.

	1 week	2 weeks	6 weeks	12 weeks
EBA	13/23 (56.5%)	8/23 (34.8%)	6/20 (30%)	4/20 (20%)
IRM	9/23 (39.1%)	6/23 (26.1%)	4/20 (20%)	2/20 (10%)
AMALGAM	7/23 (26.9%)	4/23 (17.4%)	1/20 (5%)	0/20 (0%)
MTA	17/23 (73.9%)	17/23 (73.9%)	13/20 (65%)	13/20 (65%)

DISCUSSION

Studies have shown that the presence of bacteria are necessary for the development of pulpal disease and periradicular lesions. Several studies have shown that the presence of bacteria in the root canal results in the formation of periapical lesions (Moller 1981; Korzen *et al.* 1974; Kakehashi *et al.* 1965). In addition, other studies have shown placement of bacterial byproducts, such as endotoxin, results in the formation of periradicular lesions (Dwyer & Torabinejad 1982; Pitts *et al* 1982;Mattison *et al.* 1992).

This study used endotoxin as a tracer to evaluate the leakage of three commonly used root end filling materials and a new material, MTA. Absence of endotoxin leakage in negative controls and its presence in the positive controls showed that the test design was reliable. In this study, penetration of endotoxin through the Obtura Gutta-perch root canal filling without a sealer corroborates the findings of Kersten and Moore (1989) as well as Trope *et al.* (1995). Trope *et al.* (1995) found that endotoxin can leak through the root canal fillings and can be used as an indicator of the sealing ability of various root canal filling materials.

Based on the results of this study, it appears that MTA provides the best sealing ability, followed by SuperEBA, IRM, and amalgam. Statistically, MTA provides the best seal in almost all the time intervals, except in the first and six weeks periods, in which no difference was observed between MTA and SuperEBA. Bates *et al* (1996), using the fluid filtration technique, reported that MTA was comparable to SuperEBA as a root end filling material in preventing leakage. The differences between the two

studies may be due to the use of different tracers or differences in tooth preparations in these investigators.

Previous leakage studies using dye or bacteria showed that MTA provided the best seal as a root end filling material when compared to amalgam, SuperEBA, or IRM (Torabinejad *et al* 1993, 1994, 1995a). In an *in vivo* study in dogs, when amalgam and MTA root end fillings were exposed to the oral flora, MTA samples showed less inflammation and more cementum formation compared to the amalgam samples (Torabinejad *et al.* 1995e). This study confirms the results found in the in vivo and in vitro studies by showing that MTA seals better than commonly used materials as root end filling materials.

Amalgam has long been used as the standard of the root end filling material. However, its complete sealing ability has been questioned. Most studies have shown that amalgam cannot prevent leakage. Szeremeta-Browar *et al.* (1984) as well as Bramwell and Hicks (1986) suggested that the amalgam did not seal the root end cavity preparation efficiently. Frank *et al.* (1993) also has shown cases where amalgam as root end filling may have short-term success results. However, half of the cases failed when they were followed for more than ten years. Therefore, they suggested that amalgam may not have long-term sealing ability. As shown in this study, amalgam allowed leakage most often in every time period, and almost all the samples allowed leakage before six weeks.

Because of the proposed shortcomings observed in amalgam, IRM and SuperEBA have been recommended as the alternatives to this material as a root end filling material.

Oynick and Oynick (1978) have reported the successful use of EBA in two hundred cases for 14 years, even though they could only recall 60 patients. Two periapical tissue samples from the cases were examined histologically and by scanning electronic microscope. Histologically, the periapical tissues healed with mild inflammation. Collagen fibers seemed to grow into the crevice in the material when examined under scanning electronic microscope. The clinical experiences were corroborated by recent animal usage studies. Trope *et al.* (1996) found that SuperEBA, though not significantly different from IRM, was consistently the best material tested when compared with the other root end filling materials, including two formulations of glassionomer cement, amalgam with varnish, and a light-cured composite resin. Pitt Ford *et al.* (1995) also reported that SuperEBA as a root end filling material is acceptable and considerably more favorable than amalgam. In both studies, root canals were infected and no disinfection procedures were performed. A retrospective study by Dorn and Gartner (1992) showed that teeth which had SuperEBA and IRM as root end filling materials had better prognosis (95% and 91% respectively) than those with amalgam (75%) as root end filling material. No significant difference in sealing ability was observed between IRM and SuperEBA. Results from these clinical reports confirmed those from in vitro leakage studies by other investigators (Szeremeta-Browar *et al.*, 1985; Bondra *et al.*, 1989).

Based on the results of this study and previous investigations which have shown good sealing ability and biocompatibility for MTA (Torabinejad *et al.* 1993,1994,1995a), it appears MTA is an acceptable alternative to the commonly used root end filling materials such as amalgam, SuperEBA, and IRM.

Because the root end filling materials come in contact with the periradicular tissues, the biocompatibility of these materials is as important as their sealing ability. As discussed previously, amalgam is not considered a good choice for root end filling due to its inferior sealing ability, despite its good biocompatibility (Marccote *et al.* 1975; Olsen *et al.* 1994; Pitt Ford *et al.* 1994; Torabinejad *et al.* 1995c). SuperEBA and IRM have been demonstrated to have good biocompatibility and favorable sealing ability (Szeremeta-Browar *et al.* 1985; Bondra *et al.* 1989; Olsen *et al.* 1994; Pitt Ford *et al.* 1994; Pitt Ford *et al.* 1995a; Trope *et al.* 1995). Therefore, the use of SuperEBA and IRM as root end filling materials may still be justified. Our results showed, though not statistically significant, that SuperEBA is superior to IRM, which makes the SuperEBA the preferred choice as a root end filling material over IRM. In a cytotoxicity study using the agar overlay technique and radiochromium method, it was also shown that amalgam was the least toxic, followed by MTA, SuperEBA, and IRM (Torabinejad *et al.* 1995c).

Because MTA has both good sealing ability and biocompatibility (Torabinejad *et al.* 1993; Torabinejad *et al.* 1994; Torabinejad *et al.* 1995a; Torabinejad *et al.* 1995b; Torabinejad *et al.* 1995c; Torabinejad *et al.* 1995d; Torabinejad *et al.* 1995e), and it also promotes the regeneration of periradicular tissues (Pitt Ford *et al.* 1995b;

Torabinejad *et al* 1995e, Torabinejad *et al*. 1997), it appears to be more suitable as a root end filling material compared to the presently available materials.

APPENDIX

Table 3. One week chi-square analysis ('*' = $p < 0.05$)

	AMALGAM	IRM	EBA
MTA	0.01*	0.04*	0.35
AMALGAM		0.76	0.14
IRM			0.38

Table 4. Two weeks chi-square analysis ('*' = $p < 0.05$)

	AMALGAM	IRM	EBA
MTA	0.00*	0.00*	0.02*
AMALGAM		0.46	0.17
IRM			0.75

Table 5. Six weeks chi-square analysis ('*' = $p < 0.05$)

	AMALGAM	IRM	EBA
MTA	0.00*	0.01*	0.06
AMALGAM		0.34	0.10
IRM			0.72

Table 6. Twelve weeks chi-square analysis ('*' = $p < 0.05$)

	AMALGAM	IRM	EBA
MTA	0.00*	0.00*	0.01*
AMALGAM		0.47	0.11
IRM			0.66

BIBLIOGRAPHY

Abdel AK, Retief DH. The apical seal via the retrosurgical approach. Oral Surg Oral Med Oral Pathol 1982;53:614-21.

Alhadainy HA, Elsaed HY, Elbaghdady YM. An electrochemical study of the sealing ability of different retrofilling materials. J Endodon 1993;19(10):508-511.

Bates CF, Carnes DL, Del Rio CE. Longitudinal sealing ability of MTA as a root end filling material. J Endodon 1996;22(4):205 Abs OR67.

Bergenholtz G. Microorganisms from necrotic pulp of traumatized teeth. Odont Revy 1974;25:347-358.

Bondra D, Hartwell G, MacPherson M, Portwell F. Leakage in vitro with IRM, high copper amalgam, and EBA cement as retrofilling materials. J Endodon 1989;15:157-60.

Bramwell JD, Hicks ML. Sealing ability of four retrofilling techniques. J Endodon 1986;12(3):95-100.

Delivanis PD, Chapman KA. Comparison and reliability of techniques for measuring leakage and marginal penetration. Oral Surg Oral Med Oral Pathol 1982;53:410-6.

Derkson GD, Pashley DH, Derkson ME. Microleakage measurement of selected restorative materials: a new in vitro method. J Prosth Dent 1986;56:435-40.

Dorn SO, Gartner AH. Retrogade filling materials: A retrospective success-failure study of amalgam, EBA, and IRM. J Endodon 1990;16(8):391-393.

Dwyer TG, Torabinejad M. Radiographic and histologic evaluation of the effect of endotoxin on the periapical tissues of the cat. J Endodon 1981;7:31-5.

Elin RJ, Wolff SM. Biology of endotoxin. Ann Rev Med 1976;27:127-141.

Fabricius L. *et al.* Predominant indigenous oral bacteria isolated from infected root canals after varied time of closure. Scand J Dent Res 1982;90:134.

Frank AL, Glick DH, Patterson SS, Weine FS. Long-term evaluation of surgically placed amalgam fillings. J Endodon 1992;18:391-8.

Friedman S, Rotstein I, Koren L, Trope M. Dye leakage in retrofilled dog teeth and its correlation with radiographic healing. J Endodon 1991;17(8):392-395.

Gilheany PA, Figdor D, Tyas M. Apical dentin permeability and microleakage associated with root-end resection and retrograde filling. J Endodon 1994;20:22-6.

Goldman LB, goldman M, Kronman JH, Letourneau JM. Adaptation and porosity of poly-HEMA in a model system using two microorganisms. J Endodon 1980;6:683-6.

Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures on dental pulps in germ free and conventional laboratory rats. Oral Surg 1965;20:340.

Kerstein HW, Moorer WR. Particles and molecules in endodontic leakage. Int Endod J 1989;22:118-24

King KT, Anderson RW, Pashley DH, Pantera EA. Longitudinal evaluation of the seal of endodontic retrofilling. J Endodon 1990;16:307-10.

Korzen B, Krakow A, Green D. Pulpal and periapical tissue response in conventional and monoinfected gnotobiotic rats.

Kos WL, Aulozzi DP, Gerstein H. A comparative bacterial microleakage study of retrofilling materials. J Endodon 1982;8:355-8.

Marccotte LR, Dowson J, Rowe NH. Apical healing with retrofilling materials amalgam and gutta-percha. J Endodon 1975;1:63-5.

Matloff IR, Jensen J, Singer L, Tabibi A. A comparison of methods used in root canal sealability studies. Oral Surg 1982;53(2):203-208.

Mattison GD, von Fraunhofer JA, Delivanis PD, Ander AN. Microleakage of retrograde amalgams. J Endodon 1985;11(8):340-345.

Mattison GD, Haddix JE, Kehoe JC, Progulske-Fox A. The effect of Eikenella corrodens endotoxin on periapical bone

Moller AJR, Fabricius L, Dahlen G, Ohman A, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scan J Dent Res 1981;89:475.

Moller G, Sjoberg O, Anderson J. Immunogencity, tolerogenicity, and mitogenicity of lipopolysaccharides. The Journal of Infectious Diseases 1973;128 supp:S52-S56.

Moodnik R, Levey M, Bensen M, Borden B. Retrograde amalgam filling: A scanning electron microscopic study. J Endodon 1980;1(1):28-31.

Nakata TT, Bae KS, Baumgartner JC. Perforation repair comparing mineral trioxide aggregate and amalgam. J Endodon 1997;25(4):259 Abs OR40.

Olsen FK, Austin BP, Walia H. Osseous reaction to implanted ZOE retrograde filling materials in the tibia of rats. J Endodon 1994;20:389-394.

Oynick J, Oynick T. A study of a new material for retrograde fillings. J Endodon 1978;4:203-6.

Pitt Ford TR, Andreasen JO, Dorn SO, Kariyamwasam SP. Effect of IRM root end filling after replantation. J Endodon 1994;20:381-5.

Pitt Ford TR, Andreasen JO, Dorn SO, Kariyamwasam SP. Effect of Super-EBA as a root end filling after replantation. J Endodon 1995a;21(1):13-15.

Pitt Ford TR, Torabinejad M, McKendry DJ, Hong CU, Kariyawasam SP. Use of mineral trioxide aggregate for repair of furcal perforations. Oral Surg, Oral Med, Oral Path, Oral Radio Endod 1995b;79:756-762.

Pitts DL, Williams BL, Morton TH. Investigation of the role of endotoxin in periapical inflammation. J Endodon 1982;8:10-18

Rizzo A, Mergenhagen S. Histopathologic effects of endotoxin injected into rabbit oral mucosa. Arch Oral Biol 1964;9:659-70.

Rud J, Munksgaard EC, Andreasen JO, Rud V, Asmussen E. Retrograde root filling with composite and a dentin bonding agent. 1. Endod Dent Traumatol 1991;7:118-25.

Sabiston CB Jr, Gribsby WR, Segerstrom N. Bacterial study of pyrogenic infections of dental origin. Oral Surg 1976;41:430-435.

Schein B and Schilder H. Endotoxin content in endodontically involved teeth. J Endodon 1975; 1:19-21

Schonfeld SE, Greening AB, Glick DH, Frank AL, Simon JH, Herles SM. Endotoxic activity in periapical lesions. Oral Surg 1982;53:82-7.

Shani J, Friedman S, Stabholz A, Abed J. A radionuclidic model for evaluating sealability of retrograde filling materials. Int J Nucl Med Biol 1984;11:46-52.

Stabholz A, Shani J, Friedman S, Abed J. Marginal adaptation of retrograde fillings and its correlation with sealability. J Endodon 1985;11:218-223.

Sunqivst G. Bacteriological studies of necrotic dental pulps. PhD thesis. Umea University Odontol Dissertation. No. 7. 1976.

Szeremeta-Brower TL, Van Cura JE, Zaki AE. A comparison of the sealing properties of different retrograde techniques: an autoradiographic study. Oral Surg Oral Med Oral Pathol 1985;59:82-7.

Tanzilli JP, Raphael D, Moodnik RM. A comparison of the marginal adaptation of retrograde techniques: a scanning electron microscopic study. Oral Surg Oral Med Oral Pathol 1980;50:74-80.

Torabinejad M, Waston TF, Pitt Ford TR. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. J Endodon 1993;19:591-5

Torabinejad M, Higa RK, McKendry DJ, Pitt Ford TR. Dye leakage of four root end filling materials: effects of blood contamination. J Endodon 1994;20:159-63.

Torabinejad M, Rastegar AF, Kettering JD, Pitt Ford TR. Bacterial leakage of mineral trioxide aggregate as a root-end filling material. J Endodon 1995a;21:109-112.

Torabinejad M, Petra WS, Kettering JD, Pitt Ford TR. Comparative investigation of marginal adaptation of mineral trioxide aggregate and other commonly used root-end filling materials. J Endodon 1995b;21(6):295-299.

Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD. Cytotoxicity of four root end filling materials. J Endodon 1995c;21(10):489-492.

Torabinejad M, Hong CU, Pitt Ford TR, Kariyawasam SP. Tissue reaction to implanted Super-EBA and mineral trioxide aggregate in the mandible of Guinea pigs: A preliminary report. J Endodon 1995d;21:569-571.

Torabinejad M, Hong CU, Lee SJ, Monsef M, Pitt Ford TR. Investigation of mineral trioxide aggregate for root-end filling in dogs. J Endodon 1995e;21:603-608.

Torabinejad M, Pitt Ford TR, McKendry DJ, Abedi HR, Miller DA, Kariyawasam SP. Histologic assessment of mineral trioxide aggregate as a root end filling material in monkeys. J Endodon 1997;23(4):225-228.

Tronstad L, Trope M, Doering A, Hasselgren G. Sealing ability of dental amalgam as retrograde fillings in endodontic therapy. J Endodon 1983;9:551-3.

Trope M, Chow E, Nissan R. In vitro endotoxin penetration of coronally unsealed endodontically treated teeth. Endod Dent Traumatol 1995;11:90-94.

Trope M, Lost C, Schmitz HJ, Friedman S. Healing of periapical periodontitis in dogs after apicoectomy and retrofilling with various filling materials. Oral Surg, Oral Med, Oral Path, Oral Radiol Endod 1996;81(2):221-8.

Westphal Otto. An old timer's reflections on endotoxin. In Bacterial endotoxin: Recognition and effector mechanisms. J.Levin, C.R. Alving. R.S. Munford, P.L. Stutz, eds. PP 17-36.

Wolff SM. Biological effects of bacterial endotoxins in man. The journal of infectious diseases 1973;128(supp.):s259-264.

Wu MK, Wesselink PR. Endodontic leakage studies reconsidered. Part I. Methodology, application and relevance. Int Endod J 1993;26:37-43.

Yamasaki M, Nakane A, Kumazawa M, Hashioka K, Horiba N, Nakamura H. Endotoxin and gram-negative bacteria in the rat periapical lesions. J Endodon 1992;18(10):501-504.

Yoshimura M, Marshall FJ, Tinkle JS. In vitro quantification of the apical sealing ability of retrograde amalgam fillings. J Endodon 1990;16:9-12.