

Bacterial leakage of mineral trioxide aggregates and Portland cement

Mohammad Asnaashari^{1*} DDS, MS, Saeed Asgary² DDS, MS, Asef Khatami³ DDS

1. Associate Professor of Endodontics, Dental School, Shaheed Beheshti Medical University, Tehran, Iran.

2. Professor of Endodontics, Iranian Center for Endodontic Research, Dental School, Shaheed Beheshti Medical University, Tehran, Iran.

3. General practitioner, Tehran, Iran.

Abstract

Introduction: The purpose of this study was to compare the ability of ProRoot mineral trioxide aggregate (PMTA) and Root mineral trioxide aggregate (RMTA) as root-end filling materials, and Portland cement (PC) to prevent bacterial leakage through filled root end cavities.

Materials and Methods: Fifty-one extracted human single rooted teeth were cleaned and shaped using a step back technique. The root-ends were resected and a 4 mm deep root-end preparation was made with fissure bur. The teeth were randomly divided into three experimental groups (n=15) and a further six teeth served as controls. Three root-end cavities were filled with gutta-percha without a root canal sealer (positive control) and three remaining were filled with sticky wax, covered with two layers of nail polish (negative control). Root-end cavities in each experimental group were filled with PMTA, RMTA or PC. After attaching the teeth to plastic caps of 9 ml vials, the teeth and the caps were sterilized using Gamma ray. Then the caps with teeth were placed on the vials containing Phenol Red Lactose broth. A tenth of microliter of Trypticase Soy broth containing *Staphylococcus Epidermidis* (SE) was placed into the root canals of the teeth. Every 48 hours inoculation of 0.1 ml of the SE broth culture into each root canal was performed.

Results: All positive controls leaked within 3 days, none of the negative controls leaked. Bacterial leakage occurred in 33% of samples in the PMTA group and in 40% of samples in RMTA and PC groups. The results indicated no statistical difference between three test materials after 35 days.

Conclusion: It was concluded that PMTA, RMTA and PC demonstrated a similar ability to seal root end cavities.

Keywords: Bacterial leakage, MTA, Portland cement, Root end filling

Received February 2006; accepted July 2006

*Correspondence: Dr Mohammad Asnaashary, Associate Professor of Endodontics, Dental School, Shaheed Beheshti Medical University, Evin, Tehran, Iran. E-mail; mo_asna12@yahoo.com

Introduction

When a non-surgical root canal therapy fails or is contraindicated, periradicular surgery with a root-end filling material is necessary. The most important factor in determining the success of a periradicular surgery is the efficiency of the apical seal. The apical seal inhibits leakage of residual irritants from the root canal into the periradicular tissues. Periradicular lesions of endodontic origin have been associated with the presence of bacteria and their by-products

in an infected root canal system (1). Root-end filling materials seal the content of the canal preventing egress of bacteria or toxic materials into the periradicular tissues.

The sealing ability of root-end filling materials has been assessed by different methods such as dye or bacterial penetration, electrical methods, fluid filtration technique, radioisotope tracing, and marginal adaptation by SEM. Dye penetration and Radioisotope techniques have been the most frequently used methods to

evaluate the sealing ability of various root-end filling materials (2).

Numerous materials have been recommended to be used as root-end filling materials such as amalgam, gutta-percha, composite resins, glass ionomers, mineral trioxide aggregate (MTA), and some other restorative materials.

Sealing ability of these materials have been compared in many studies, and most of them have shown that ProRoot MTA (PMTA) have significantly less leakage than other root-end filling materials, and is considered as a high quality root-end filling material (3-4).

Recently a new similar material, Root MTA (RMTA), has been developed in Iran, which is made of the same components of PMTA (5). In various *in vivo* and *in vitro* studies, it has been proved that RMTA has so many similarities with PMTA (6-8).

Newly the chemical, physical and biological properties of Portland cement (PC) have been analysed. It was shown that PC contained the same principal chemical elements as MTA, except for bismuth oxide (9-10). Wucherpfenning and Green reported that MTA and PC were almost macroscopically and microscopically identical, when evaluated by X-ray diffraction analysis (11). Other researchers have also reported that MTA and PC had similar properties (12-13).

The purpose of the present *in vitro* study was to compare the sealing ability of PMTA, RMTA and PC in extracted human single rooted teeth, using a bacterial leakage model.

Materials and Methods

Fifty-one extracted human single rooted teeth were selected. After initial radiographs, standard access cavities were prepared. Using the step back technique, all teeth were instrumented to a #70 k-files (Mani, Japan). During cleaning and shaping, copious irrigation was performed with sodium hypochlorite solution (2.5%). At 3 mm from the apical end of the root, an apical resection at 45 degrees to the long axis of the tooth was made under water spray with a fissure bur (D&Z, Germany). Standardized class I root-end preparation were made to a depth of 4 mm using 008 fissure burs (D&Z, Germany) under spray water. The prepared teeth were randomly divided into

three experimental groups of 15 teeth each, and two control groups of 3 teeth each. Before placing the root end fillings, the cavities were dried using paper points (Aryadent, Iran).

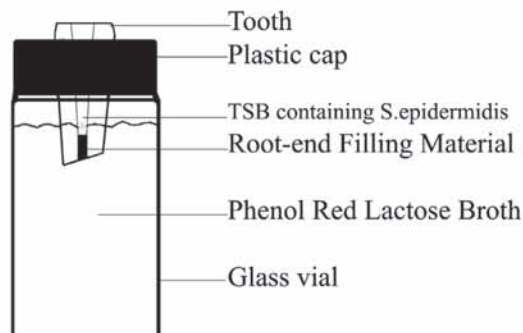


Fig 1. Diagram representing the microbial test system used to determine bacterial leakage

The root end filling materials were inserted as the following manner: PMTA in group 1 (ProRoot MTA, Dentsply, Tulsa, OK, USA), RMTA in group 2 (Root MTA, Salamifar Co, Tehran, Iran), and PC in group 3 (Simane Abyek, Qazviv, Iran) were prepared according to the manufacture's directions and placed into the root end cavities. Three root-end cavities filled with sticky wax and covered with two layers of nail polish, served as negative controls. Positive controls were three root-end cavities filled with gutta-percha without sealer. Two layers of nail polish were applied to the external surfaces of all roots to prevent bacterial leakage through the lateral and or accessory canals.

Nine milliliter vials (Hepatitis vaccine vials) were used to suspend the prepared teeth. The snap on caps were punctured. The teeth were placed into holes of the caps and sticky wax was used to seal the gaps between teeth and caps. This complex was sterilized using Gamma ray. Phenol Red Lactose (PRL) broth was placed in each vial to a level of 2 to 3 mm above the resected root-ends (Fig 1). A sterile Insulin syringe was used to inoculate 0.1 ml of an over night broth culture of SE into the root canal of each tooth via the coronal access cavity preparation. Any leakage of the SE from the root canals into the PRL broth would become evident when the color of the broth changes from red to yellow as a result of acid production from bacteria growth. The entire

apparatus was then placed into an incubator, maintained at a constant 37°C. After sterilizing the teeth with Gamma ray, all of the works and procedures were performed under a biological safety hood with strict adherence to aseptic technique. Inoculation of SE was performed every 48 hours. The PRL broth vials were monitored every day for color changes. If a color change occurred, a sample of yellow medium was plated on TSB and then on blood agar with presence of Novobiocine antibiogram disks, and incubated at 37°C to confirm the bacterial presence as SE. The experiment was run for 35 days.

A Kruskal Wallis and Chi-Square tests were used to determine the statistical differences between various groups.

Results

All specimens of the positive control group showed color change in the PRL broth within 3 days of incubation. No evidence of color change in the PRL broth occurred in the negative control group during the experimental period.

Five of the 15 samples of the group 1 (PMTA) were fully contaminated within 5 to 25 days. Six of the 15 samples of the group 2 (RMTA) were fully contaminated within 5 to 27 days. Six of the 15 samples of the group 3 (PC) were fully contaminated within 5 to 30 days. In all cases when color changes occurred, the bacteriological tests showed that the bacteria present in the growth medium was SE.

The data obtained were statistically analyzed with the Kruskal Wallis and Chi-Square tests which showed no statistically significant difference between the three experimental materials ($P > 0.05$).

Discussion

Dye leakage studies have been used for many years to evaluate the sealing ability of endodontic materials. Dye penetration studies tend to overestimate leakage as the size of dye molecule is smaller than bacteria (2). Despite the ease of use, dye leakage studies have several disadvantages: a) like radioisotope traces, the molecular size of most dye particles is smaller than bacteria, so it causes dissimilar leakage patterns. b) Most dye leakage studies

have measured the degree of leakage in one plane, making it impossible to evaluate the total leakage, and c) compared with clinical conditions, in vitro dye studies are static and do not reflect the dynamic interactions between the root canals and periradicular tissues (15).

Studies using bacterial cultures have been used widely to test the sealing ability of endodontic materials and may be considered to have more biological relevance than dye leakage tests. Obviously, bacterial leakage studies have much resemblance with oral environment and condition, bacterial flora of the mouth, and also leakage pattern rather than other techniques (16).

In this study we evaluated the sealing ability of a 4 mm thickness of PMTA, RMTA (as root-end filling materials), and PC (as an alternative material) using a bacterial leakage model. Positive and negative controls responded as expected. The entire positive control group exhibited a color change which indicates that a root canal sealer is needed to improve gutta-percha as a root end filling material.

Incubation of bacteria started 48 hours after placing the plastic caps and teeth, on the PRL broth vials. The absence of color changes in this period of time indicates that we could provide a contamination free environment, for the test of root end filled samples.

Considering that PMTA contains the same principal chemical elements as PC (9-13), as well as RMTA (5), they probably have a similar mechanism of action and thus the results of biological investigation are similar.

It was shown that the mechanism of action of PMTA and PC were similar. Both materials contain calcium oxide that forms calcium hydroxide when mixed with water. The reaction of the calcium hydroxide and the carbon dioxide from the pulp tissue produces calcite crystals (12,17). These findings strongly support the role of calcite crystals and fibronectin as an initiating step in the formation of a hard tissue barrier.

Shokouhinejad et al. compared the sealing ability of White and Gray PMTA, RMTA, and PC as root-end fillings using bacterial leakage method and reported comparable results of the test materials (8). Similar finding was also shown by other researchers (7, 18).

The results of the present study support those described previously (7-13, 17-18) suggesting that PMTA, RMTA and Portland cement are almost similar.

Based on these results, it seems that there is no significant difference between PMTA, RMTA and PC and considering all other characteristics of these three materials, it is possible to replace and substitute PMTA, with RMTA and even Portland cement. But just because the Portland cement is a non medical product, the use of this industrial material is not recommended.

Conclusion

Under the conditions of this *in vitro* study, the bacterial leakage patterns of ProRoot mineral trioxide aggregate, Root mineral trioxide aggregate, and Portland cement in root-end fillings were similar over a period of 35 days.

Acknowledgement

This study was supported by Iranian Center for Dental Research, Shaheed Beheshti University of Medical Sciences.

References

- 1- Gartner AH, Dorn SO. Advances in endodontic surgery. Dent Clin North Am. 1992; 36: 357-78.
- 2- Wu MK, Wesselink PR. Endodontic leakage studies reconsidered. Part I. Methodology, application and relevance. Int Endod J. 1993; 26: 37-43.
- 3- Scheerer SQ, Robert SH, Cohen J. A comparative evaluation of three root end filling materials: An *in vitro* leakage study, using *Prevotella nigrascens*. J Endod. 2001; 27: 40-2.
- 4- Fischer EJ, Arens DE, Miller CH. Bacterial leakage of Mineral Trioxide Aggregate as compared with zinc-free Amalgam, Intermediate Restorative Material and super-EBA as a root end filling material. J Endod. 1998; 24: 176-9.
- 5- Eghbal MJ, Asgary S, Parirokh M. An SEM study and comparative microanalysis of two root end filling material. Beheshti Univ Dent J. 2005; 23: 206-13. (In Persian)
- 6- Asnaashari M, Blourchi E, Eslami B, Sattari M. Tissue Reaction to MTA and Root MTA to rat's connective tissue. Beheshti Univ Dent J. 2004; special issue. 601-13. (In Persian)
- 7- Nourian M, Mortazavi YJ, Bolhari B. Bacterial leakage of ProRoot MTA and Root MTA as root end filling materials. Rafsanjan Dental School 2001; Thesis No: 82.
- 8- Shokouhinejad N, Zarrabian M, Aligholi M. Bacterial leakage of four root end filling materials; White ProRoot MTA, Root MTA, Gray ProRoot MTA and Portland cement. Tehran Dental School 2004; Thesis No: 488.
- 9- Estrela C, Bammann LL, Estrela CR, Silva RS, Pe'cora JD. Antimicrobial and chemical study of MTA, Portland cement, calcium hydroxide paste, Sealapex and Dycal. Braz Dent J. 2000; 11: 3-9.
- 10- Asgary S, Parirokh M, Eghbal MJ, Brink F. A comparative study of white mineral trioxide aggregate and white Portland cements using X-ray microanalysis. Aust Endod J. 2004; 30: 89-92.
- 11- Wucherpfening AL, Green DB. MTA vs Portland cement (Two biocompatible filling materials). J Endod. 1999; 4: 304-11.
- 12- Holland R, de Souza V, Nery MJ, Faraco JR, Bernabe PFE, Otoboni, Filko JA, Dezan E. Reaction of Rat connective tissue to implanted dentin tube filled with MTA, Portland cement or Calcium Hydroxide. Braz Dent J. 2001; 12: 3-8.
- 13- Safavi k, Nichols FC. Secretion of PGE2 from monocytes exposed to MTA or Portland cement. J Endod. 2000; 9: 540-5.
- 14- Walton RE, Torabinejad M. Principles and Practice of endodontics, second edition 1996; chap 19: pp 398-401.
- 15- Torabinejad M, Fallah Rastgar A, Kettering JD, Pittford TR. Bacterial leakage of Mineral Trioxide Aggregate as root end filling material. J Endod. 1995; 21: 109-12.
- 16- Mattison GD, Von fraunhofer A, Deliranis PD, Anderson AN. Microleakage of retrograde Amalgam. J Endod. 1985; 11: 340-5.
- 17- Holland R, de Souza V, Murata SS et al. Healing process of dog dental pulp after pulpotomy and pulp covering with mineral trioxide aggregate or Portland cement. Braz Dent J. 2001; 12: 109-13.
- 18- Mozayeni MA, Asnaashari M, Farzim MR. Comparison of the bacterial leakage of three root end filling materials. Beheshti Univ Dent J. 2003; 20: Special Issue. 658-65. (In Persian)