MDJ

The Effect of Two Demineralizing Agents on Apical Sealing of Retrograde Filling Materials (In Vitro Study)

Dr. Bassam Karem Amin, M.Sc., B.D.S.
Dr. Raad Niama Dayem, Ph.D., M.Sc., B.D.S.
Dr. Raid Fahim Salman, Ph.D., M.Sc., B.D.S.

Abstract

The purpose of this in vitro study was to evaluate the effect of using two different demineralizing agents on the micro-leakage of different retro-grade filling, the sealing ability of the MTA, & to compare the sealing ability of the three different retro-grade fillings.

Seventy roots were selected, & the canals then instrumented utilizing crown down technique using pro-taper rotary files. All roots were obturated with non standardized gutta-percha master cones using the lateral condensation technique, apical root resections were made & root-end cavities were prepared.

The prepared roots were randomly divided into seven experimental groups with 10 specimens in each; three groups demineralized by 17% EDTA, each group of them was filled with different retrograde filling materials either MTA , Resin-modified glass ionomer cement, or amalgam. The other three groups were demineralized by 35% phosphoric acid & each group of them was filled with the same different retrograde filling materials. The seventh group which is the control was filled with MTA without demineralization agent. The specimens were then placed in 2% methylene blue solution at (37°C, & pH 7) for 72 hours. To evaluate the depth of dye penetration, each root was sectioned longitudinally in a crown-apex direction. Linear dye penetration was measured using a stereomicroscope.

Statistical analysis of the results revealed that the MTA had less leakage than the amalgam & the glass ionomer cement, & the difference was highly significant ($p>0.01$) for EDTA. In addition, the using of phosphoric acid had higher leakage than EDTA for MTA & GIC groups , however, the difference was non significant ($p>0.05$).

MTA retro-filling material had better sealing ability than other materials used and the demineralizing agents may have effect on the quality of the micro-leakage.

Introduction

Apicectomy followed by retrograde root filling is a well-established procedure to treat teeth with persistent peri-apical infections (pathology), where conventional root canal treatment has failed[1], or when unsuccessful re-treatment or if re-treatment is impossible[2]. Inefficient retrograde sealing of root canal following apicectomy is a major factor

in surgical endodontic failure and search for such a material that seals the root canal foramen is still required[1].

Throughout the dental history, a wide variety of materials have been used for retrograde fillings. Although many materials are available, no material has been found that fulfills all or most of the properties for ideal retrograde filling material, among which the sealing property. Different materials are used for retrograde filling of amputated root apex; the most commonly used are metals, posts, amalgam, cements, sealers, calcium hydroxide and MTA[3].

The use of manual, rotary and/or ultrasonic cutting instruments on root dentin surface during peri-radicular surgery produces smear layer, which is composed of organic and inorganic material and can contain microorganisms and its endo-toxins. It has been reported that the application of demineralizing agents, either acids or chelating, can improve the action of root canal dressings, adhesion and penetration of retro-filling materials, and make root surface more biocompatible, optimizing periodontal healing and not interfering with apical retro-filling seal. On the other hand, smear layer removal can increase dentin permeability, facilitating bacterial penetration and re-infection of dentinal tubules if the sealing fails.[4]

Kouvas et al at 1998, and Kennedy et al at 1995, reported that smear layer is a negative factor in root canal sealing, because this organic and inorganic material adheres easily to the sealing material and root canal wall interface reducing the adhesion of sealers.[5, 6]

There's a few research concerning the effect of using demineralizing agent for removal of smear layer on the sealing of the retro-filling materials. In this study apical seal that might be obtained from MTA is compared to that of amalgam, glass ionomer, when used as root end filling material & the effect of demineralizing agent on the sealing of these different retro-filling materials was assessed.

Materials and Methods

Samples collection & preparation

The roots were separated from the crowns at the cemento-enamel junction with a water-cooled high speed turbine hand piece fissure bur. So the maxillary incisors, maxillary canines, palatal root of maxillary molars and the distal roots of mandibular molars were selected while the crowns were discarded.

Criteria for sample selection

The coronal orifice of the each root canal was irrigated with 1 ml. of NaOCl, then each canal was checked by size # 15 k- file to verify the canal patency, in such a way that the k- file must appear from the root apex slightly (just seen). Any root that is not fulfills this criterion (i.e. k-file not appear from the apex, were discarded and not involved in the study). Therefore, seventy roots were selected, & were stored in 50 % alcohol until the instrumentation of the specimens started. The working length was visually established by subtracting 1 mm from the length of a size #15 k-file when its tip just appeared at the apical foramen.

Instrumentation of the canals

The canals then instrumented utilizing crown down technique using pro-taper rotary files by dentsply engine at 300 rpm with torque no. 3. Before instrumentation, the canals were irrigated with 1 ml. NaOCl , then the instrumentation would be started as following; from SX up to the full working length until this file become loose , irrigating the canal with 1 ml. NaOCl, enlarging the coronal 2/3 of

the canal using S1 file (shaping file no.1) just in the coronal 2/3 in canal filled with irrigating solution which allow easy entrance of the file, using S2 file (shaping file no. 2) to just 2-3 mm. shorter than the estimated working length for each canal, irrigating the canal with the 1 ml. NaOCl, finally the apical third were prepared by using F3 file (finishing file no.3) up to the full working length by just passing the file one time to the apical area without excessive rotation of the file when it's in the full working length keeping in mind that the canal is fully filled with irrigating solution; at this stage, all the canals were prepared with a size simulating to that of k-file # 30 at apical terminal.

One ml. of NaOCl solution was irrigated after filing with every size throughout the cleaning and shaping of the canals. After completion of the instrumentation and final irrigation, canals were dried with paper point size # 35.

Root canal obturation

When the canals were dried by paper points, all roots were obturated with non standardized gutta-percha master cones using the lateral condensation technique. Finger spreader were used to adapt the gutta-percha cones laterally to the walls of the canal, & 4-6 accessory gutta-percha cones size 15, 20, & 25 were used to complete the adaptation of the cones to the walls and obtain three-dimensional obturation along the canal walls from the apical constrictor to the canal orifice coronally .

Apical resection

Apical root resections were made by removing 3 mm of the apex at a 90-degree angle to the long axis of the root with a water-cooled #57 carbide bur at high-speed turbine hand piece. The resected root-ends were smoothed with a double-sided carborundum disk. Coronally, after removing 3 mm of excess gutta-percha filling material by fissure bur, the access orifice cavities were sealed with temporary filling. The roots were then stored in a test tubes containing sterile normal saline and maintained in an incubator at 37 ± 1°C for 72 hrs.

Retrograde cavity preparation

The root-end cavities were prepared using a slow speed conventional hand piece by small carbide fissure bur with a rubber stopper placed on the shank to control the depth of the cavity which 3 mm from the tip of the bur. The cavity outline is prepared with internal round angles class I root-end cavity.

Sample grouping

The prepared roots were randomly divided into seven experimental groups with 10 specimens in each; three groups demineralized by 17% EDTA for 1 min., each group of them was filled with different retrograde filling materials as following ; MTA , Resin-modified glass ionomer cement, and amalgam. The other three groups were demineralized by 35% phosphoric acid for 15 sec. & each group of them was filled with the same different retrograde filling materials; MTA, Resin-modified glass ionomer cement, and amalgam. The seventh group which is the control was filled with MTA without demineralization agent.

Dye application

All roots′ surfaces were coated with three layers of white nail varnish. The varnish was applied onto the entire root surface, except for the area corresponding to the resected apical surface, and was left to dry, this will give complete sealing all over the root except the resected apical surface.

After storage, the specimens were placed in 2% methylene blue solution at (37°C, & pH 7) for 72 hours. During

this period the roots were shacked frequently to remove the air babbles formed on root surface and to change the direction of roots so that the apex not face the glass sides & after the specified time, the roots were removed from the dye solution, washed thoroughly, and dried with air syringe carefully.

Root sectioning

After dye application, the teeth were washed in running water for 5 min. and left drying at room temperature for 24 hrs. To evaluate the depth of dye penetration, each root was sectioned longitudinally in a crown-apex direction, getting both the buccal and lingual surfaces; the direction was approximately parallel to the long axis of the tooth and through the apex of the canal. Roots were longitudinally split by using large diamond disk and each half was fixed on a glass slide with sticky wax and each root given a special number so that data collection become easier .

Data collection

Linear dye penetration was measured using a stereomicroscope (Stemi 2000-C model, Carl Zeiss, Germany) with a 0.1-mm ocular grid at 4X magnification by using a millimeter grid digital vernier. The extension of dye penetration between the retro filling material and tooth structure along apical interfaces was assessed by two experienced specialist examiners calibrated for the technique and blinded to the groups. The measurement was made from the apex to the point where the dye no longer penetrated between the filling material and dentinal wall on both halves of each root. Accordingly, four leakage measurements were obtained for each specimen. The highest leakage values per specimen attributed by the examiners were selected and micro-leakage means recorded for the experimental groups.

Statistical analysis

The collected data were analyzed by using SPSS version 15 for windows (SPSS, Chicago, Illinois, USA), including; descriptive statistics, analysis of variance (ANOVA) test, & paired t-test.

Results

The effect of retro-filling materials

The descriptive statistic for mean values & standard deviations of the micro-leakage in mm. of the retrograde filling material at the buccal half & lingual half had been shown in the table (1).

From the table (1), the amalgam had the higher leakage average which was 1.87 mm than the other materials at lingual half, while the MTA had the lowest average value of leakage which was 1.19 mm at buccal side. By using ANOVA test, there was non-significant difference between all filling materials with using phosphoric acid at $p > 0.05$, & there was highly significant difference between all filling materials with using EDTA at $p < 0.01$, with in favor of MTA over other materials, (fig.1).

The difference between the demineralizing agents

By using t-test, there was non-significant difference between the two agents with three different retro-filling materials at $p > 0.05$, table (2), and fig. (2).

The use of demineralizing agents

The descriptive statistic for mean values & standard deviations of the micro-leakage in mm. of the MTA retrograde filling material with the using of the demineralizing agents & without using them at the buccal half

& lingual half are presented in the table (3).

From the table (3), the MTA had the higher leakage average which was 1.66 mm at buccal half, without using of the DA, while it had the lowest average value of leakage which was 1.32 mm at buccal side, with using the DA. By using t-test, there was non-significant difference between the two conditions at $p > 0.05$, at lingual side, with in favor of the without-DA condition, however, there was significant difference at $p<0.05$, at buccal side, with in favor of the without-DA condition, (fig. 3).

Discussion

Effect of different demineralizing agents

The MTA showed the least marginal dye penetration with EDTA than with phosphoric acid while GIC showed the same but, however, lesser difference while for the amalgam, the phosphoric acid gave better result with less leakage than with EDTA, although these differences are non-significant statistically. These results are somewhat similar to the findings of Yamada et al at 1983, that they reported that chelating agents are more effective than acids[7], while Takeda et al at 1999, observed no differences between 17 % EDTA and 6 % citric acid[8], while these results are disagreed with Kubo et al results because they found that using 35 % phosphoric acid for 15 sec. were leak less than 17 % and 24% of EDTA used for 3min. and 4 min. respectively.[4] This mean that there is much differences between studies because there is no a standardization of the concentration and type of demineralization solution and the time of application which were used in each study.

Another factor that could have allowed dye penetration in all the specimens was the structure of dentin in the apical region of human teeth as described by study of Mjo¨r et al at 2001. Their study showed that primary dentinal tubules were irregular in direction and density and some areas were devoid of tubules[9]. This explanation agreed with that of Perbhu et al at 2003 , that EDTA require more time for optimum results in the apical third in which the dentin is much more sclerotic and the number of dentinal tubules present there are less[10], so the effect of demineralizing agent were altered , and because most other studies were done in the entire root canal and not only the apical retrograde cavity so there will be some differences in the result of this study with other studies which need more in vivo studies to confirm these results.

Effect of the retrograde filling materials

The MTA give the best result by both demineralizing agent and show less marginal linear dye penetration rate followed by GIC then amalgam which give the highest leakage this results agreed with that of many studies such as, Aqrabawi at 2000, who showed that 56 % of his sample that filled with amalgam show dye leakage while MTA show none,[11] also Wu et al at 1998, found that MTA and GIC leaked less than amalgam[12], & Fogel and Marshall at 2001, found that amalgam demonstrated significantly more microleakage than MTA and intermediate restorative material[13].

GIC have been reported to have several advantageous properties for using as restorative material, such as adhesiveness to tooth structure, fluoride release, and antimicrobial activity and because of these properties GIC have also recommended for use as retrograde material[14]. There were non significant differences between the experimental groups due to the large

individual variances within each group, these individual variations occurred even though all of the root end filling were placed by the same investigator attempting to use a consistent technique and may be due to the variation in dentin permeability and the differences in the physiological old of each sample.

This good result of the MTA is due to the bond strength of the MTA to dentin which has been shown to increase significantly during the first 72 hours after placement, also the MTA lead to the formation of calcite crystals, these crystals are originating from reaction of calcium from MTA with the carbon dioxide have a role as an initiating step in the formation of a hard tissue barrier. Also MTA leak significantly less than the other materials with or without blood in the root end cavities because the humidity cause expansion of the MTA particle which assist in further sealing of the dentinal walls.[15]

The amalgam give the worst result as the amalgam is bonded mechanically and there is no chemical bonding between the amalgam and the opened dentinal tubules so in this case using of amalgam bonding agent and cavity varnish before amalgam placement may give better result , while the GIC give better result than amalgam because GIC, initially wet the tooth surface by free polyacrylic acid, followed by ionic bonding between the carboxyl group in the cement liquid and calcium ions in the tooth structure so it bond chemically to opened dentinal tubule[16].

Effect of demineralization

The results showed that there is significant differences in the using demineralizing agent which give less dye penetration, this significant result is agreed with Farhad and Elahi at 2004, found that the apical leakage was significantly increased in obturated canals with smear layer[17], in addition, Kennedy et al at 1995, also reported that removal of the smear layer reduced the apical microleakage and may improve the obturation seal[5].

References

1- **Ansari S, Qayyum Z, Khitab U.** A comparison of sealing capability of amalgam, GIC and zinc oxide eugenol cement when used as retrograde filling materials (in vitro study). *JAMC* 2003; 15(3): availableat: http://www.ayubmed.edu.pk/JAMC/PAST/15-3/shuja.htm.

2- **Rashedi A.** Treatment of peri-apical pathology with retrograde endodontic technique; 2001. www.ki.se/odont/cariologi endodonti/T10/Atousa%20Rashedi.pdf

3- **Vasudev S, Goel B, Tyagi S.** Root end filling materials- A review. *Endodontology* 2003; 15:12-18.

4- **Kubo C, Gomes A, Mancini M**. In vitro evaluation of apical sealing ability in root apex treated with demineralization agents and retro-filled with MTA through marginal dye leakage. Braz Dent J 2005; 16:187-91.

5- **Kouvas V, Liolios E, Vassiliadis L, Parissis-Messimeras S, Boutsioukis A**. Influence of smear layer depth of penetration of three endodontic sealers: An SEM study. *Endodont Dent Traumatol* 1998; 14: 191-95 cited by Živkovic S, Brkanic T, Dacic D, Opacic V, Pavlovic V, Medojevic M. Smear Layer in Endodontics. Serbian Dental J 2005; 52:7-19.

6- **Kennedy W, Walker W, Gough R**. Smear layer removal effects on apical leakage. *J Endodon* 1995; 12:21–7.

7- **Yamada R, Armas A, Goldman M, Peck S**. A SEM comparison of a high volume final flush with several irrigating solutions: Part III. *J Endodont* 1983; 9: 134-42.

8- **Takeda F, Harashima T, Kimura Y, Matsumoto K.** A comparative study of the removal of smear layer by three endodontic irrigants and two types of laser. *Int Endod. J* 1999; 32: 32-9.

9- **Mjor I, Smith M, Ferrari M, Mannocci F**. The structure of dentine in the apical region of human teeth. *Int. Endod. J* 2001; 34(5):346-53.

10- **Perabhu S, Rahim N, Mathew B**. Comparison of removal of endodontic smear layer using NaOCl, EDTA, and different concentrations of maleic acid – A SEM study. *Endodontolog* 2003; 15: 20-25.
11- **Aqrabawi j**. Sealing ability of amalgam, super EBA cement, and MTA when used as retrograde filling materials. *BDJ* 2000; 188 (5):266-68.
12- **Wu M, Kontakiotis E, Wesselink P**. Decoloration of 1 % methylene blue with dental filling materials. *Journal of dentistry* 1998; 26:585-89.
13- **Fogel H, Marshall D, Piekoff M**. Microleakage of root-end filling materials. *J Endod* 2001; 27(7):456-58.
14- **Barkhordar R, Pelzner R, Stark M**. Use of glass ionomers as retro-filling materials. *Oral surg* 1989; 67:734-39 cited by Siqueira JF Jr, Rocas IN, Abad EC,Castro AJR,Gahyva SM,Favieri A. Ability of three root –end filling materials to prevent bacterial leakage . J Endod. 2001; 2 (11):673-75.
15- **Torabinejad M, Higa R, McKendry D, Pitt Ford T**. Dye leakage of four root end filling materials: effects of blood contamination. *J Endodon* 1994; 20:159–63.
16- **Tyas M, Anusavice K, Frencken J, Mount G**. Minimal intervention dentistry – a review. *International Dental Journal* 2000; 50: 1–12.
17- **Farhad M, Elahi T**. The Effect of Smear Layer on Apical Seal of Endodontically Treated Teeth. *Journal of Research in Medical Sciences* 2004; 3: 130-33.

Table 1: The descriptive statistic of the micro-leakage of the retrograde filling materials

Materials	Mean (EDTA)	SD (EDTA)	Mean (PA)	SD (PA)
Amalgam, buccal	1.8405	±0.078	1.568	±0.058
Amalgam, lingual	1.879	±0.021	1.7155	±0.054
MTA, buccal	1.1965	±0.058	1.4525	±0.057
MTA, lingual	1.2805	±0.1	1.3965	±0.097
GIC, buccal	1.552	±0.051	1.7625	±0.085
GIC, lingual	1.523	±0.065	1.5785	±0.07

Table 2: t-test for difference between the two agents

PA-EDTA	df	T-statistic	P-value	Sign.
MTA-buccal	18	2.01	0.05	NS
MTA-lingual	18	0.73	0.23	NS
Amalgam-buccal	18	-1.77	0.09	NS
Amalgam-lingual	18	-1.38	0.09	NS
GIC-buccal	18	1.35	0.09	NS
GIC-lingual	18	0.37	0.35	NS

Table3: The descriptive statistic of the micro-leakage of the MTA with & without DA

Groups	No. of samples	Mean	SD
With DA-buccal	20	1.3245	±0.07
Without DA-buccal	10	1.6615	±0.13
With DA-lingual	20	1.3385	±0.09
Without DA-lingual	10	1.332	±0.16

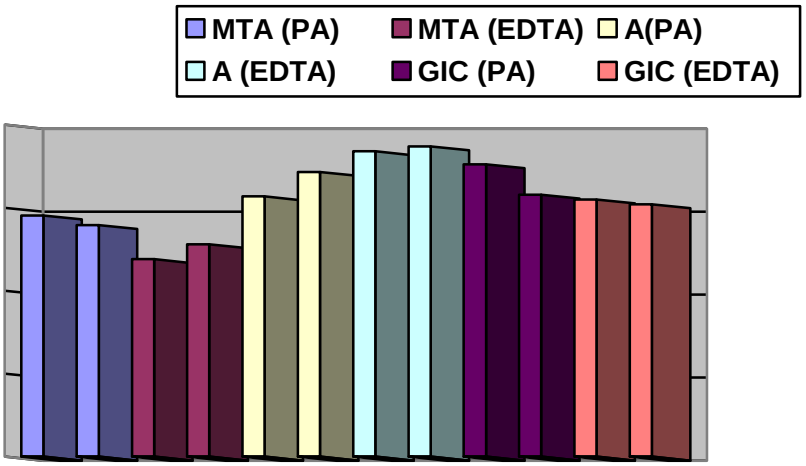

Fig.1: Bar chart for difference between all filling materials

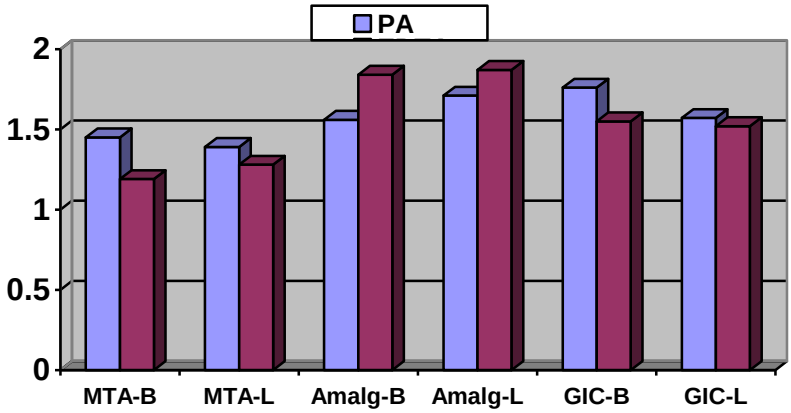

Fig.2: Bar chart for the difference between the two agents

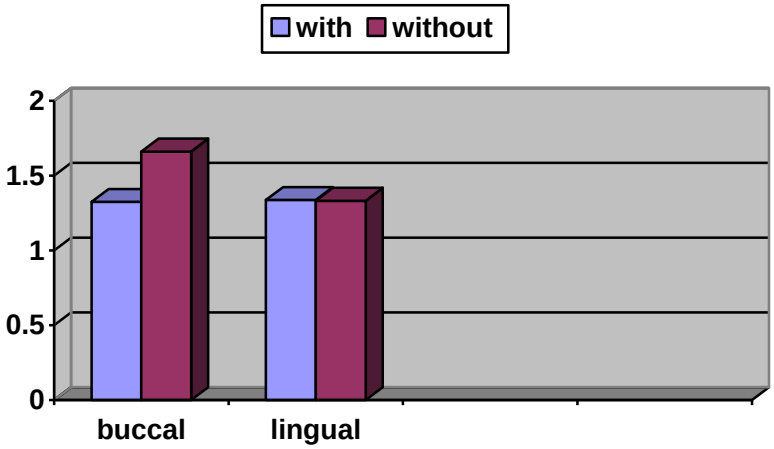

Fig. 3: Bar chart for the difference between the two conditions