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Bacterial leakage along retrofilled roots, stored in saline solution, and restored with Super-EBA or MTA: an in vitro study

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Abstract

Objectives: To compare in vitro bacterial leakage of Super-EBA and MTA as root-end filling materials.

Material and Methods: Thirty-six freshly extracted, single-rooted human teeth with mature apices were used in this study. All teeth were immersed and stored in 0.9% sterile saline solution immediately following extraction for 1 week. The teeth were randomly divided into 4 groups: group M (n=15) MTA, group E (n=15) Super- EBA, group P (n=3) positive control, group N (n=3) negative control. The prepared roots were mounted in a model system (MS) introduced by Deveaux et al and modified to be suitable for this investigation. Means and standard deviations were calculated for all groups. The Mann-Whitney test was used to evaluate the differences in the percentage of samples that demonstrated leakage. The differences related to time to bacterial leakage were evaluated using Two-Sample Kolmogorov-Smirnov test.

Results: No significant difference was observed between the types of root-end material used in the study on preventing microbial microleakage, nor was there significant difference between groups regarding time to leakage.

Conclusion: Within the limits of the present study, it can be concluded that both Super-EBA and MTA showed the same potential sealing ability of the root-end cavity against E. faecalis.

Keywords: E. faecalis, Super-EBA, MTA, sealing ability, bacterial microleakage, storage media.

Introduction

Microorganisms colonizing the root canal system play a crucial role in the pathogenesis of periradicular lesions. E.

faecalis was quantified using PCR in primary infection and in secondary infection respectively as 67.5% and 89.6% of the entire number of bacteria. [1] Since microorganisms established in the periradicular tissues are inaccessible to endodontic disinfection procedures, extraradicular infection may be a factor in the failure of endodontic therapy. [2] E. faecalis possesses the capability to form biofilms that increase its resistance to antimicrobial agents. [3-8] Intracanal biofilms might be removed to certain extent by vigorous chemomechanical preparation [9-11] or photodynamic therapy [12] while extraradicular or periradicular biofilms might necessitate surgical procedures. Surgical endodontics consists in surgically exposing the apex, resecting the apical 2-3mm, preparing a class I cavity along the long axis of the canal, and sealing the root canal system using an adequate root-end filling material. [13] The quality of the apical seal is a critical factor in evaluating potential adequacy of a root-end filling material. [14] Microleakage can be evaluated in vitro by numerous techniques. [15, 16]

A large number of restorative compounds have been proposed as potential materials for root-end fillings including amalgam, gutta-percha, zinc oxide-eugenol cements (IRM®, Super-EBA®), resin composite [17], polycarboxylate and glass-ionomer cements, mineral trioxide aggregate (MTA)[18], and bioceramics. [19] Success rates close to 95% were achieved with Super-EBA® and MTA® and were significantly higher when compared to several root-end filling materials such as amalgam and resin composite. [20]

The aim of this study was to evaluate the long-term in vitro sealing ability of Super-EBA® and MTA® retrofillings using E. faecalis .

Materials and Methods

Sample selection and preparation: Thirty-six freshly extracted, single rooted, single-canal human teeth with mature apices extracted for periodontal or orthodontic reasons were used in this study. Signs of fracture, root caries, or evidence of resorptive processes were grounds for exclusion. Root surfaces were cleaned using a piezoelectric ultrasonic unit (Suprasson® PMax, Groupe Satelec, Pierre Roland, France) with a universal scaling tip used at low frequency under a continuous flow of distilled water. All teeth were stored in 0.9% sterile saline solution immediately following extraction. Throughout the experiment, samples were kept immersed in this solution (changed weekly for infection control) and maintained at 37°C in an incubator. Clinical crowns were cut at the cementoenamel junction, using #701 fissure bur (Dentsply-Maillefer, Ballaigues, Switzerland) mounted on a high-speed handpiece under water spray. Working length was determined by placing #10 K-file (Dentsply-Maillefer) until it was visible at the apical foramen, and root canals were prepared using ProTaper® rotary files (Dentsply-Maillefer) according to the manufacturer's instructions. Irrigation was done using 2mL 5.25% sodium hypochlorite between each instrument (28G Maxi-I-Probe®, Smith & Nephew, Inc., Franklin Park, IL ,USA). All canals were dried using sterile paper points (Roeko®, Langenau, Germany) and filled with a single gutta percha cone vertically compacted with no sealer. The apical 3mm were then resected using a low-speed diamond saw (Isomet®, Buehler Ltd., Lake bluff, IL ,USA) and water coolant perpendicular to the long axis of the root. Root-end preparations were performed under 2x magnification (Orascoptic® Research Inc, Madison, WI, USA) using a piezoelectric ultrasonic unit (Suprasson®) and a zirconium nitride-coated #1 retro-tip preparation (ProUltra® , Dentsply-Tulsa Dental, Tulsa, OK, USA) was used under constant water irrigation to achieve circular 3mm-deep preparations. The teeth were then randomly divided into 4 groups :group M (n=15) filled with MTA, group E (n=15)

filled with Super EBA, group P (n=3) as positive control, and group N (n=3) serving as negative control.

Experimental groups were prepared as follows: Group M (MTA) was filled using ProRoot MTA® (Dentsply-Tulsa Dental) that was mixed according to manufacturer's instructions, and condensed into the root-end preparations with a P-1 microplugger (SybronEndo®, Orange, CA, USA), until a surplus was seen above the cavity margins. Group E (EBA) was filled with Super-EBA® (Harry J. Bosworth Co., Skokie, IL, USA). The powder/liquid ratio was set to form a thick 'dough-like' consistency paste, permitting to roll it into a thin tapered point. [21] Super-EBA® was placed into the root-end preparations using a # 7 wax spatula and condensed thoroughly with a P-1 microplugger, followed by additional material until a surplus was seen above the cavity margins.

All preparations were water sprayed for 5 seconds and then dried with sterile paper points before placement of fillings. Placement of restorative materials started immediately following mixing, and the condensation process was completed within 1min after the end of the mixing. The materials were allowed to set for 30 seconds and then excess was removed using a microcarver (MR-4 SybronEndo®) and then burnished using a ball rounded B-3 burnisher (SybronEndo®).

All specimens were replaced into vials containing the saline storage solution. The handling of MTA specimens and storage in the saline without further manipulation provided enough time for the product to set without being washed. [22] After 24 hours, all gutta-percha cones which had initially served as stopper to the root-end filling material were removed. This procedure allowed excluding any potential effect of gutta-percha on the microbial leakage through the apex.

Radiographic control

Periapical radiographs were taken to verify that the root-end filling materials did not seal more than 3mm of the root-end preparation or demonstrate radiographic voids. Any samples that were not fulfilled these criteria were discarded and replaced.

Assembly mounting

The tooth mounting system was similar to that introduced by Deveaux et al[23] but modified to assess bacterial leakage of apically-introduced microbes through root-end filling materials (Figures 1a and 1b).

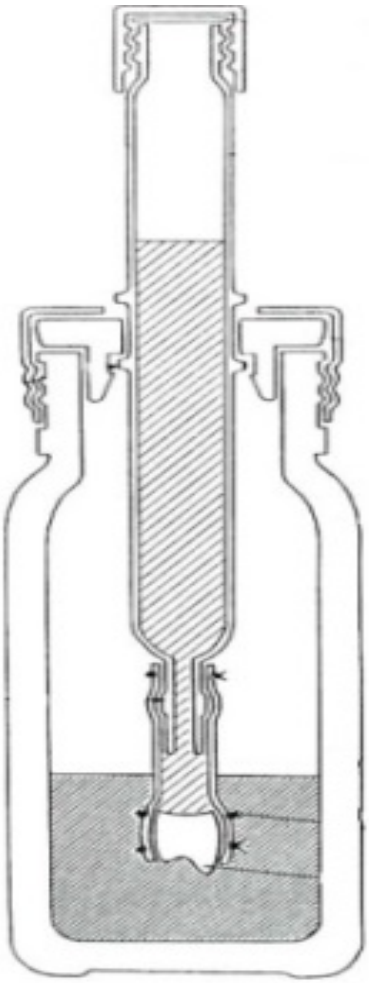

Figure 1a: Model system (MS) from Deveaux et al (1999)

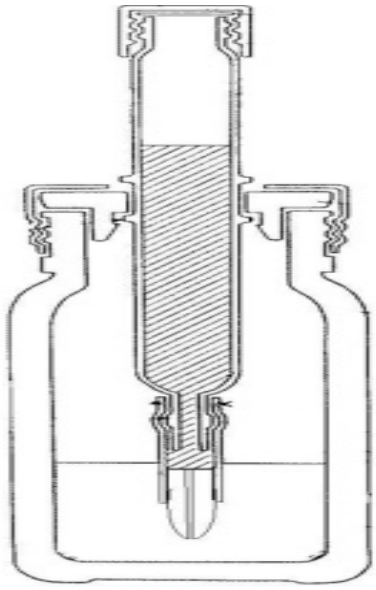

Figure 1b: Model system (MS) from Deveaux et al modified

Two layers of nail polish were applied to the external surface of all root sections and allowed to dry. Samples were numbered M1 to M15, for group M, E1 to E15 for group E, P1 to P3 for group P, and N1 to N3 for group N.

All samples were then double-packed and sterilized with ethylene oxide gas. Within 12 hours, all samples were opened aseptically under a laminar flow hood for testing. The tooth-tubing-tube assemblies were filled with 5 ml of freshly prepared brain-heart infusion medium (BHI) and tetracycline (0.2µg/mL), lightly tapped to remove trapped air bubbles. The flasks were then filled with 40ml of the same medium, inoculated with 5mL of a 48h culture of single colonies of tetracycline-resistant E. faecalis (10^7~bacteria/mL) (ATCC 29212, American Culture Collection, Rockville, MD), and incubated at 37°C.

Two days later, samples were removed from each flask and tube and 0.5 mL of theses samples were spread on freshly prepared BHI agar plates (12g BHI agar broth and 250mL distilled water). The inoculated plates were then incubated at 37°C for 48 hours. Throughout the study, 1/3rd of the BHI broth were removed from the flasks and the tubes and replaced every 4 days. Also every 2 days samples from each flask and tube were spread on BHI agar plates to detect bacterial leakage and contamination if it was present. Flasks were inoculated with 5 ml of a 48 hours culture of E. faecalis every 2 days all over the study to sustain colony viability.

All laboratory procedures were done by a specialized team from the Health Faculty of the Lebanese University that was blinded to the filling material.

Parameters evaluated

After 48 hours, the purity of the plate cultures removed from the flasks were verified by visual examination and light microscopy at15 x after Gram stain catalase reaction combined to specific markers. Samples were removed from the study if bacterial presence other than E. faecalis was detected. The presence of E. faecalis in any culture plate from tubes indicated that the root-end filling material in this sample was leaking. The time to leakage was noted for the corresponding sample. In the absence of bacterial leakage, bacterial sampling was continued for 6 months. (Figure 2)

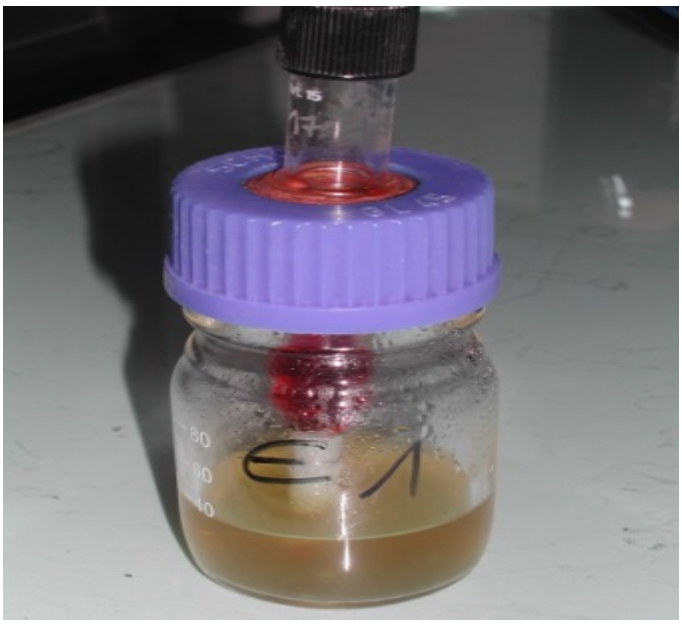

Figure 2. Model System E1 showing two compartments: tube with only vial and flask containing vial inoculated with E. faecalis

Data collected

Mean day to bacterial leakage was recorded for the 2 groups. The Mann-Whitney test (non parametric mean of ranks) at $p<0.05$ was used to evaluate the differences between material groups. The length of time until bacterial leakage (BL) had occurred in the tube was recorded for each sample in the 2 material groups. The following 7 time intervals was selected: 0-15 days 16-30 ,days, 31-45 days, 46-60 days, 61-90 days, 91-120 days, 121-150 days, and 151-180 days. The percentage of samples with TL in each time interval was calculated. The differences between groups related to time were evaluated using Two-Sample Kolmogorov-Smirnov Test.

Results

Bacterial leakage occurred in group M in 2 samples on day 34 and the last sample exhibited microleakage on day 64. The mean day to leakage was 44.13 with a standard deviation of 9.27 days. In group E, the first samples leaked on day 36 and the last on day 60. The mean day to leakage was 46.93 with a standard deviation of 8.07 days. In group P, bacterial penetration occurred on day 1 in all teeth, whereas group N showed no leakage throughout the experimental period (Table 1, 2, 3).

MS	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15
BDL	42	36	60	64	36	48	44	42	34	54	34	44	40	42	36
MD	44.1							SD	9.3						

Table 1: Day to Bacterial Leakage (DBL), Mean Day (MD) and Standard Deviation (SD) for Model System (MS) with MTA

MS	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15
DBL	60	36	42	52	42	48	42	52	56	36	48	48	60	36	46
MD	46.9							SD	8.1						

Table 2: Day to Bacterial Leakage (DBL), Mean Day (MD) and Stadard Deviation (SD) for Model System (MS) with EBA

MS	P1	P2	P3	N1	N2	N3
DBL	1	1	1	0	0	0

Table 3: Day to Bacterial Leakage (DBL) for the Positive (P) and Negative (N) control Model System (MS)

Results of the statistical analysis using the Mann-Whitney test indicated that there was no significant differences between Super EBA® group and MTA® group ($p>0.05$) (Table 4).

Statistical test	Number of days
Mann-Whitney U	86.5
Wilcoxin	206.5
Z	-1,088

Asymp.Sig. (2-tailed)	0.276
Exact. Sig. [2*(1-tailed sig.)]	0.285*

Table 4:Mann-Whitney Test to evaluate the difference between the material groups

The frequencies of leakage in different time interval for group MTA showed similar distribution (Table 5).

	Groups		Rank	Ranka
Number of Days	1	15	17.23	285.5
	2	15	13.77	206.5
	Total	30		

Table 5: Two-sample Kolmogorov-Smoirnov Test

Discussion

The bacterial model was used previously by Deveaux et al [24] for in vitro coronal leakage. In the present investigation, the same mounting device was modified and used to assess bacterial leakage of apically-introduced microbes through root-end filling materials. [23] We did not need to discard any of the 36 samples, confirming the validity of our model system and each assembly served as its own control. The quick augmentation in the three positive controls in liquid volume in the tube against gravity gradient while fixing the device to the flask also validated the experimental model system. Although the use of an in vitro bacterial leakage model is more relevant than dyes or isotopes models, it has some inherent drawbacks. The inherent antibacterial activity of the material itself may affect the outcome.

The qualitative evaluation method in our study gave a dichotomous response to whether bacteria were leaking from the lower to the upper compartment. Bacterial models used are generally chosen to satisfy several demands as availability, easy handling, and affordability. [25] The use of species not normally founded in the apical part of the teeth led to many discrepancies among the results of several previous studies. Furthermore, the bacterial size, the motility, the reduction-oxidation potential, the pathogenicity and other characteristics of the species could play a fundamental role in the behavior of root-end filling materials including the sealing ability against the bacterium chosen.[22] In our study, we used E. faecalis, a gram-positive facultative anaerobe, as the most prevalent species found in primary infection and in canals of root-filled teeth associated with periapical lesions.[1, 26, 27] E. faecalis possesses several virulence factors.[28, 29] Its ability to cause periradicular disease stems from its aptitude to survive the effects of root canal treatment and persist as a pathogen in the root canals and dentinal tubules of teeth, by forming biofilms and survive starvation. [3, 6, 7, 11]

Based on the study of Portenier et al [30], the growth curve of E .faecalis showed 3 phases :growing cells (6 hours), stationary phase (6 to 24 hours) and starvation phase (24 to 96 hours). The starvation phase is the phase where the bacterium is the most virulent and resistant. In clinical situation, the starvation phase is the most dominant phase where eradication of E. faecalis by physical and chemical techniques is often inefficient. [39]

In this study we wanted to challenge the 2 materials while the bacteria were in starvation phase. For that purpose, we changed the medium broth every 4 days and inoculated new bacteria every 2 days to sustain colony viability while E. faecalis was in starvation phase.

Present methods of sterilizing and storing extracted teeth include steam autoclave, freezing, gamma radiation, liquid chemicals and gaseous chemical. [31, 32] The 2003 Center

for Disease Control (CDC) recommendations advocate autoclave sterilization but acknowledge that its effects on microchemical relationships between dentin and biomaterials are unknown. Alternatively, a 2-week immersion of extracted teeth in 10% formalin was also recommended as an effective method of internal and external high-level disinfection of extracted teeth. However, formalin a commonly used tissue fixative has also been shown to cause histologic structural changes in dentin. As the teeth need to be sterilized or disinfected and stored until testing, they may undergo changes in structure and composition during the treatments, which can interfere with results. [33] The potential effect of storage media that may have on extracted teeth used in endodontic research has received little attention .

In this study, the use of MTA® or Super-EBA® as root-end filling materials combined with a careful technique led to a same result concerning bacterial leakage. Our results are in contrast to those of previous bacterial leakage studies. Fischer et al [34] found that MTA® withstands bacterial penetration better than Super-EBA®. Another study found that MTA® showed better adaptation than Super-EBA®. [13] They used S. salivarius as marker, but formalin was used as a storage media.

On the other hands our results agree with Scheerer et al [35] who showed that MTA® and Super-EBA® exhibited the same sealing ability against bacterial leakage. Also our results agree with Amezcua et al [36] using turbidity of the medium to compare bacterial leakage of MTA® and Super-EBA®.

In vivo studies showed a high success rate healing when using MTA® or reinforced zinc oxide eugenol cements as root-end filling materials. [37] Also, these in vivo studies showed no statistical difference in histologic and radiographic results between MTA® and reinforced zinc oxide eugenol. The good results obtained with MTA® and Super-EBA® would suggest that failures were not related to the choice of material but were largely because of other factors: cracks, reservoir of infection not eradicated by conventional therapy prior to endodontic surgery. [13-15, 17, 18, 20, 21]

In conclusion, and within the limitations of this study, both Super-EBA® and MTA® showed the same sealing ability of the root-end cavity against E. faecalis .

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