Marginal adaptation of mineral trioxide aggregate (MTA) compared with amalgam as a root-end filling material: a low-vacuum (LV) versus high-vacuum (HV) SEM study

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Abstract

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Aim To compare the marginal adaptation of mineral trioxide aggregate (MTA) or amalgam root-end fillings in extracted teeth under low-vacuum (LV) versus high-vacuum (HV) scanning electron microscope (SEM) viewing conditions.

Methodology Root-end fillings were placed in 20 extracted single-rooted maxillary teeth. Ten root ends were filled with MTA and the other 10 root ends were filled with amalgam. Two 1 mm thick transverse sections of each root-end filling were cut 0.50 mm (top) and 1.50 mm (bottom) from the apex. Gap size was recorded at eight fixed points along the dentine–filling material interface on each section when uncoated wet (LV wet (LVW)) and dry under LV (0.3 Torr) in a JEOL JSM-5800 SEM and backscatter emission (LV dry uncoated (LVDU)). The sections were then air-dried, gold-coated and gap size was recorded once again at the fixed points under HV (10^{-6} Torr; HV dry coated (HVDC)). Specimen cracking, and the size and extent of the crack were noted.

Results Gap sizes at fixed points were smallest under LVW and largest under HVDC SEM conditions. Gaps were smallest in MTA root-end fillings. A General Linear Models Analysis, with gap size as the dependent variable, showed significant effects for extent of crack in dentine, material and viewing condition ($P = 0.0001$).

Conclusions This study showed that MTA produced a superior marginal adaptation to amalgam, and that LVW conditions showed the lowest gap size. Gap size was influenced by the method of SEM viewing. If only HV SEM viewing conditions are used for MTA and amalgam root-end fillings, a correction factor of 3.5 and 2.2, respectively, may be used to enable relative comparisons of gap size to LVW conditions.

Keywords: amalgam, LV SEM, MTA, root-end fillings.

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Introduction

Management of the resected root end during apical surgery, particularly the placement and sealing of the root-end filling, is critical to encourage the regeneration of the periodontium. Since its first description in the dental literature, mineral trioxide aggregate (MTA) has shown superior sealing properties to other root-end filling materials with respect to dye penetration (Torabinejad *et al.* 1994), bacterial leakage (Torabinejad *et al.* 1995a) and marginal adaptation using the scanning electron microscope (SEM; Torabinejad *et al.* 1995b). However, in this latter study, Torabinejad *et al.* (1995b) concluded that sample preparation, such as plane of section, section grinding, as well as the effects of high

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vacuum (HV) and SEM specimen preparation played significant roles in the outcome of their study.

The development and availability of low vacuum (LV), as well as environmental SEMs, have largely reduced the need for specimen pretreatment so that specimens can be viewed in near '*in vivo*' conditions. Many of the problems of measuring tooth restoration gaps are minimized using such SEMs, because the relationship between the rigid restorative materials and adjacent biological tooth structure remains stable. This has been demonstrated in qualitative LV SEM studies in dentistry of acid-etched enamel surfaces (Kodaka *et al.* 1993), and biomaterials and mammalian cells (Sammons & Marquis 1997).

In order to overcome some of the concerns raised in a similar study that compared MTA with three other conventional root-end filling materials (Torabinejad *et al.* 1995b), specimens in this study were sectioned at right angles to the long axis of the tooth and sequentially measured with standard methods using LV wet (LVW), LV dry uncoated (LVDU) and HV dry coated (HVDC) SEM viewing conditions. Sample preparation damage was minimized to simulate gap sizes at the dentine–filling interface '*in vivo*'.

Materials and methods

Selection of teeth

Twenty single-rooted, human, maxillary teeth with mature apices were selected from stocks of the School of Oral Health Sciences at the University of the Witwatersrand. Ethical clearance to use these teeth was obtained from the Committee for Research on Human Subjects, University of the Witwatersrand (Medical) – clearance number (11/5/90). Teeth with root fractures, root caries, deep-root concavities or evidence of periradicular resorptive processes were excluded. Additionally, teeth were excluded if radiographs showed multiple canals, or significant apical curvatures. The selected teeth were stored in 0.2% thymol (E. Merck, Darmstadt, Germany; Lot # – K 201 201 67) to prevent bacterial activity.

Root canal preparation

Root canal preparation was carried out on all the teeth prior to sectioning and viewing the root-end fillings in SEM. Patency of each canal was established by passing a size 15 K-file (Kerr Manufacturing Company, Romulus, MI, USA) through the apical foramen. The working length of each canal was determined where the size 15 K-file exited the apical foramen.

Root canals were instrumented with GT® rotary instruments (Tulsa Dental Products, Tulsa, OK, USA) in an Aseptico® rotary handpiece (Aseptico, Kirkland, WA, USA). The apical-third of the canals were prepared with ProFile® .04 Taper Series 29 rotary instruments (Tulsa Dental Products). To eliminate debris accumulation, approximately 2 mL of 2.5% sodium hypochlorite was used as an irrigant between file sizes. The apical foramen of each tooth was enlarged and standardized to accommodate a size 4 ProFile® Series 29 hand instrument. The instrumented canals were dried using paper points.

A master gutta-percha point (Obtura, Spartan, MI, USA) was placed in the prepared root with Roth root canal sealer (Roth Intl., Ltd., Chicago, IL, USA) and vertically condensed with Schilder pluggers (Dentsply Maillefer, Tulsa, OK, USA). The canals were then back-filled with Obtura II thermoplasticized gutta-percha (Obtura). The purpose of the gutta-percha was to provide a solid base to support condensation of the root-end filling material.

Root-end preparation

Under continuous water spray, the apical 3 mm of each root was sectioned at 90° to the long axis of the root, with a size 701 tungsten carbide cross-cut fissure flat taper bur (Moyco Union Broach™, York, PA, USA) in a high-speed handpiece. A resection angle of 90° to the long axis of the root was used in order to minimize the number of exposed dentinal tubules.

Three-millimetre deep root-end cavities were prepared with a 170 L tungsten carbide tapered fissure bur in a high-speed handpiece with copious water spray, without ultrasonics. Five millimetres of 15% EDTA was used after preparation to remove the smear layer. A periodontal probe was used to measure preparation depth. Once cavities were completed, the root specimens were wrapped in moist gauze and stored in a closed glass bottle at room temperature (20 °C) and at 100% humidity for 12 h. Oralloy amalgam was mixed according to the manufacturers' instructions. MTA was mixed with water to a putty consistency using a powder to water ratio of 3 : 1 (Torabinejad *et al.* 1995c). All root-end preparations were sprayed with a water stream for 5 s and then dried with sterile paper points. Placement of restorative materials began immediately following mixing, and the condensation process was complete within 1 min from the start of the mix.

Each of the materials was placed and condensed into the preparations using a size P1LB endodontic perforation repair instrument and micropacker (G. Hartzell & Son, Concord, CA, USA). The materials were allowed to set for 30 s and then carefully carved to the cavosurface margin. The restored roots were wrapped in moist gauze and stored in 100% humidity in glass bottles for 48 h. This stage marked the end of the *in vitro* 'clinical' specimen preparation.

Root sectioning and section preparation

After the roots were mounted on wooden blocks with sticky wax, with their long axes at right angles, transverse sections of the roots were cut using an Isomet (Buehler Ltd., Evanston, IL, USA), low-speed, water-cooled diamond disc 0.16 mm thick.

The initial transverse section of each root, cut at 0.5 mm from the root apex of the root, was discarded. The second and third 1.0 mm thick transverse sections of each specimen, the 'top' and 'bottom' levels, respectively, were cut serially from the apex. Thus, the 'top' section surface was 0.5 mm from the apex and the 'bottom' section surface of interest was 1.5 mm from the apex. The apical surface of each section was identified through the root tapering of the transverse section. The sections were then placed for 12 h in wet gauze at 100% humidity prior to SEM viewing.

Fine abrasive paper (1200 Grit), with constant water application, was used to polish the apical surface of both the top and bottom levels. Each surface was gently polished for 1 min, using only light finger pressure. The polished surface was examined under a low-power stereolight microscope (Nikon Corporation, Tokyo, Japan) at magnification 40× to confirm a smooth, clean viewing surface.

The top and bottom levels of each specimen were mounted on aluminium SEM stubs with a thin layer of conductive tape. Once mounted, the specimens were not handled or touched again, thereby maintaining their position and orientation on the stub. To combat any dehydration, the sections were covered with wet gauze after mounting. The gauze was removed only once the specimens were placed in the SEM viewing chamber – approximately for 2 min.

Scanning electron microscopy

Specimens were examined using a JEOL JSM−5800 LV SEM (Jeol Ltd., Tokyo, Japan), which is capable of both LV and HV operations. The electron gun, column and specimen chamber were all maintained at approximately 10^{-6} Torr under HV condition, which, in LV mode, was maintained at any pressure between 0.05 and 2.0 Torr, depending on viewing conditions. Specimen imaging was by backscatter electrons (BSEs) using a BSE detector (K.E. Developments Ltd., Cambridge, UK).

A charge couple device (CCD) camera with a K.E. infrared chamberscope (K.E. Developments Ltd.) was used to visualize positioning of the specimens inside the specimen chamber. Continuous viewing of the specimen offered protection to both the specimen and the detector.

Low vacuum wet specimens

The uncoated wet specimens were placed 1 mm below the BSE detector and viewed under LV conditions of 0.3 Torr and accelerating potential of 25 kV. Tilt and focus were adjusted to ensure optimum viewing.

Assessment sites

The surface of each specimen was photographically recorded on high-density thermal paper by a Mitsubishi P90 video copy processor (Mitsubishi Electric Corporation, Tokyo, Japan) at low magnification (60–70×). The video print was processed immediately, and eight equidistant areas at the material–tooth interface were marked on the print using a custom-made stent (CorelDRAW®, Ottawa, Ont., Canada) of concentric circles with 45° divisions. The stent was placed on each section with point 1 on the mid-facial point. These eight points on the video print were used to fix the interface points examined, where the interfacial gaps were measured under the different SEM viewing conditions.

The magnification was then increased to 1000×, and the eight set points of the material–tooth interface at the top and bottom levels of each specimen were viewed and individually photographed using Ilford FP4 Plus 125 (120 mm) black and white film (Ilford Imaging UK Limited, Mobberley, UK). Thus, for each root tip, 16 sites were recorded, eight for the bottom level section and eight for the top section. The specimens were then removed from the SEM in preparation for the next viewing condition.

Low vacuum dry uncoated specimens

The specimens were allowed to desiccate in covered unsealed plastic containers for 48 h at room temperature in a dust-free environment. This time was sufficient to allow complete drying of the specimen. The dry

uncoated specimens were viewed in LV SEM at 25 kV and at 0.3 Torr. The specimens were positioned and viewed with backscatter emission in the same way as the specimens under LVW conditions. The low-power video print and the eight SEM photomicrographs taken at high power under LVW conditions were used to relocate the same points on the specimens under LVDU conditions. The interface points were rephotographed as before.

High vacuum dry coated specimens

After LV viewing, the specimens were desiccated with silica gel for 12 h and subsequently sputter-coated with 150 Å pure fine gold (99.99%) with a Polaron E 5200 sputter coater (Polaron Equipment Limited, Watford, UK). The dry coated specimens were viewed and photographed under HVDC conditions and BSE at 1000× magnification at an accelerating voltage of 20 kV. The eight points of each specimen were located for the third and final time using the previous photomicrographs as a guide. The interface sites were rephotographed for the third time in the same way as previously mentioned.

The desiccation of the specimens from LVW to HVDC conditions caused cracking of the dentine. The number of cracks were noted at each viewing session, and were grouped into cracks which extended into the dentine–filling interface and those which did not.

Three control groups investigated the changes of marginal adaptation of root-end fillings because of different viewing conditions: the effect of atmospheric conditions on specimens in an environmental chamber (EVC group) (Fig. 1), the effect of quick drying versus slow drying of specimens (the desiccated group) and the effect of drying on the filling material without the interactive role of the dentine (epoxy group). Gaps were measured at eight equidistant points along the filling–dentine interface of the EVC and desiccated groups, and along the epoxy–filling interface of the epoxy group.

Histometric assessment

Negatives from the micrographs were printed to standard enlargement, and the gap widths at the filling–tooth interface were measured on the micrographs using a Panasonic video camera (Matsushita Communications, Yokohama, Japan), which was connected to a Kontron Image Analysing System (Kontron Bildanalyse GMBH, Eching, Munich, Germany) with appropriate software. This image analysing system was calibrated to 1 μm. The widest gap on the LVW micrograph was located and measured in micrometres. This exact point was identified and remeasured on micrographs of the same specimens taken under the different SEM conditions. The gap width measurements were recorded in micrometres and analysed with SAS (SAS for Windows Version 8.2; SAS Institute Inc., Cary, NC, USA).

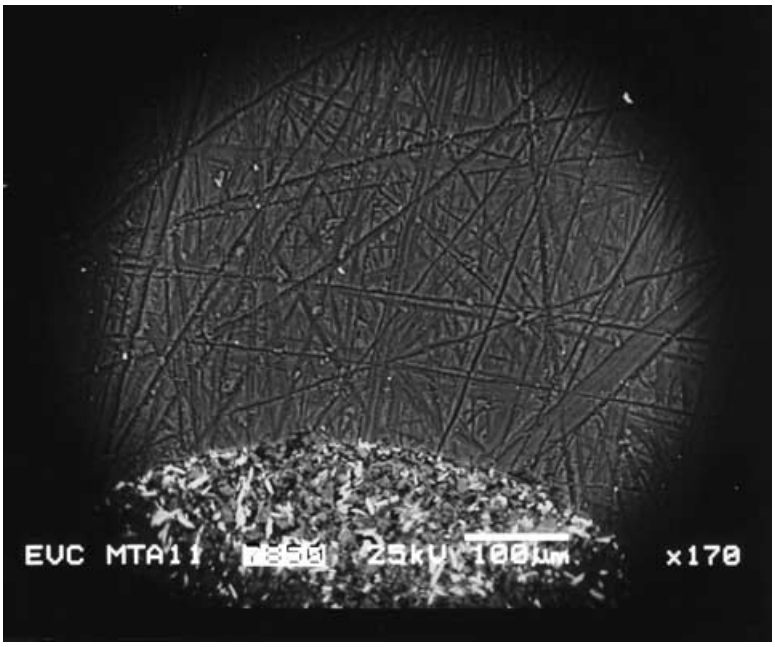

Figure 1 EVC low-power image of a MTA-filled specimen.

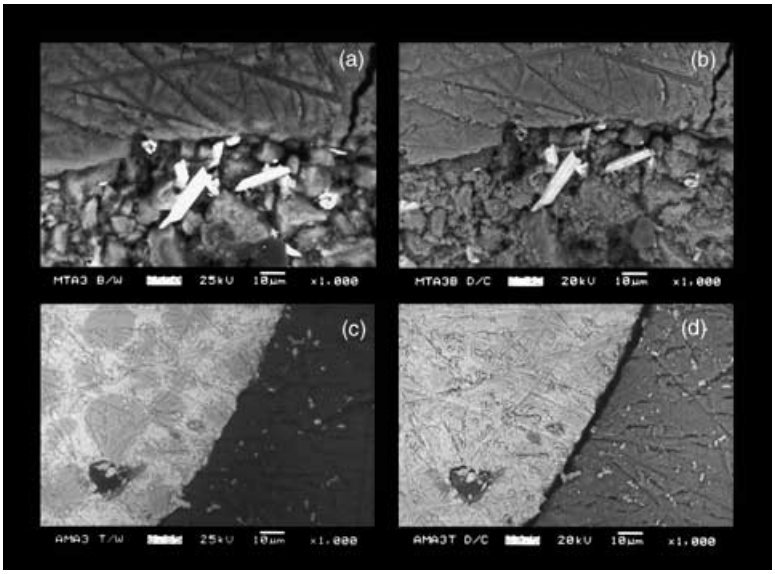

Figure 2 High-power magnification of MTA-filled specimens (LVW micrograph (a) and HVDC micrograph (b)); and amalgam-filled specimens (LVW micrograph (c) and HVDC micrograph (d)). MTA showed more resilience to these HVDC conditions (micrograph (b)) compared to amalgam (micrograph (d)).

Statistical analysis

A General Linear Models Analysis of the data from the experimental group was carried out with gap width as the dependent variable, and material, SEM viewing condition, section level, measurement point and extent of crack as independent variables. A Tukey's Studentized Range Test on gap size followed this. The level of statistical significance was set at $P < 0.05$.

Reproducibility of measurement was determined by making four repeated gap width measurements at the eight measuring points on both top and bottom levels of four specimens, followed by calculation of the 95% confidence intervals.

Results

Morphological appearance of specimens under the three SEM conditions

When the top and bottom levels of each specimen were viewed at low power (60–65 ×), the filling–dentine interface was clearly visible. Root-end cavities showed undamaged dentine with cracking (a separation within the dentine itself) and well-adapted fillings under LVW conditions. The filling material remained intact in all specimens under all three SEM conditions.

The unique morphological characteristics of each assessment site enabled the exact relocation of points viewed under all three SEM conditions at 1000 × magnification. Gaps at the MTA–dentine interface were smaller than those at the amalgam–dentine interface under all three conditions (Fig. 2).

Gaps at the filling–tooth interface in both MTA and amalgam groups increased from LVW to HVDC conditions (Table 1). Cracks in dentine extended to the filling–dentine interface as dehydration of the specimen increased, i.e. from LVDU to HVDC conditions (Table 2).

Histometric data

Gap width measurements in the experimental group (in micrometres)

Gap sizes were measured at 953 of the 960 points; seven points were omitted for technical reasons. The results are listed in Table 1. Gaps were narrower alongside MTA compared with amalgam, and were increased in both groups with dehydration and increased vacuum (Table 1).

Table 2 shows the gap with results subdivided according to the presence of cracks reaching to the filling–tooth interface. Few cracks to the interface were found under LVW conditions. However, the number of cracks in the dentine to the interface increased from LVW to HVDC conditions for both MTA and amalgam-filled specimens. More cracks to the interface were found in the LVW condition of the MTA specimens compared to that of amalgam (Table 2).

Mean and maximum gap width sizes were greater in the specimens with cracks to the interface under all three conditions compared to the specimens which

Table 1 Mean, SDs, minimum and maximum gap sizes of specimens in the experimental group, including specimens with or without cracks to the filling–tooth interface (in micrometres)

Material	Condition	*N*	Mean	SD	Minimum	Maximum
Amalgam	LVW	159	1.064	1.520	0	9.032
	LVDU	157	2.025	2.704	0	14.617
	HVDC	159	3.825	4.272	0	23.247
MTA	LVW	159	0.523	0.781	0	4.344
	LVDU	159	0.750	1.013	0	5.829
	HVDC	160	1.190	1.421	0	9.070

had no crack to the interface. The minimum gap width measurement of the MTA-filled specimens with cracks progressing to the interface under all three conditions was 0.00 μm. However, in the amalgam-filled specimens, a minimum gap width of 4 μm was found where cracks reached the filling–dentine interface. There was a difference in numbers between cracked and noncracked specimens between amalgam and MTA. Forty-eight specimens cracked to the interface for amalgam and 61 for MTA.

Gap width measurements in the control groups (in micrometres)

EVC group

The EVC control group showed lower mean gap sizes than the experimental group (Fig. 3; Table 3). The amalgam-filled specimen showed a decrease in mean gap size under all three conditions of the EVC control group (EVC = 0.67 ± 0.68 μm; LVDU = 1.45 ± 1.25 μm; HVDC = 2.18 ± 1.78 μm) compared to the specimens in the experimental group (LVW =1.06 ± 1.52 μm; LVDU = 2.03 ± 2.7 μm; HVDC = 3.83 ± 4.27 μm). The MTA-filled specimen of the EVC control group (EVC = 0.41 ± 0.51 μm; LVDU = 0.70 ± 0.87 μm; HVDC = 1.13 ± 1.06 μm) showed slightly smaller mean gap sizes under all three conditions compared to the experimental group (LVW = 0.52 ± 0.78 μm; LVDU = 0.75 ± 1.01 μm; HVDC = 1.19 ± 1.42 μm).

Desiccated group

Amalgam specimens were more affected by HV SEM preparation procedures than MTA specimens. A maximum gap size of more than 6 μm in amalgam, and a higher mean gap size compared to MTA, indicated the detrimental effect of HVDC conditions (Fig. 4; Table 3).

The mean gap sizes were significantly smaller in the desiccated group (amalgam: LVW = 0.62 ± 0.45 μm, HVDC = 1.62 ± 1.94 μm; MTA: LVW = 0.26 ± 0.39 μm, HVDC = 0.54 ± 0.73 μm) compared to the mean gap sizes recorded in the experimental group (amalgam:

Table 2 Mean, SDs, minimum and maximum gap sizes of specimens in the experimental group subdivided by cracks into the interface or not reaching the interface (in micrometres)

Material	Condition	Crack	*N*	Mean	SD	Minimum	Maximum
Amalgam	LVW	i	3	6.486	2.510	4.013	9.032
		n	156	0.960	1.301	0.000	8.635
	LVDU	i	11	9.280	3.134	4.590	14.617
		n	146	1.478	1.702	0.000	9.024
	HVDC	i	34	10.191	5.036	4.218	23.247
		n	125	2.094	1.540	0.000	7.679
MTA	LVW	i	13	1.364	0.775	0.000	2.961
		n	146	0.448	0.739	0.000	4.344
	LVDU	i	22	1.610	1.570	0.000	5.829
		n	137	0.612	0.821	0.000	3.966
	HVDC	i	26	2.465	2.043	0.000	9.070
		n	134	0.943	1.118	0.000	4.640

i, to interface; n, no crack to interface.

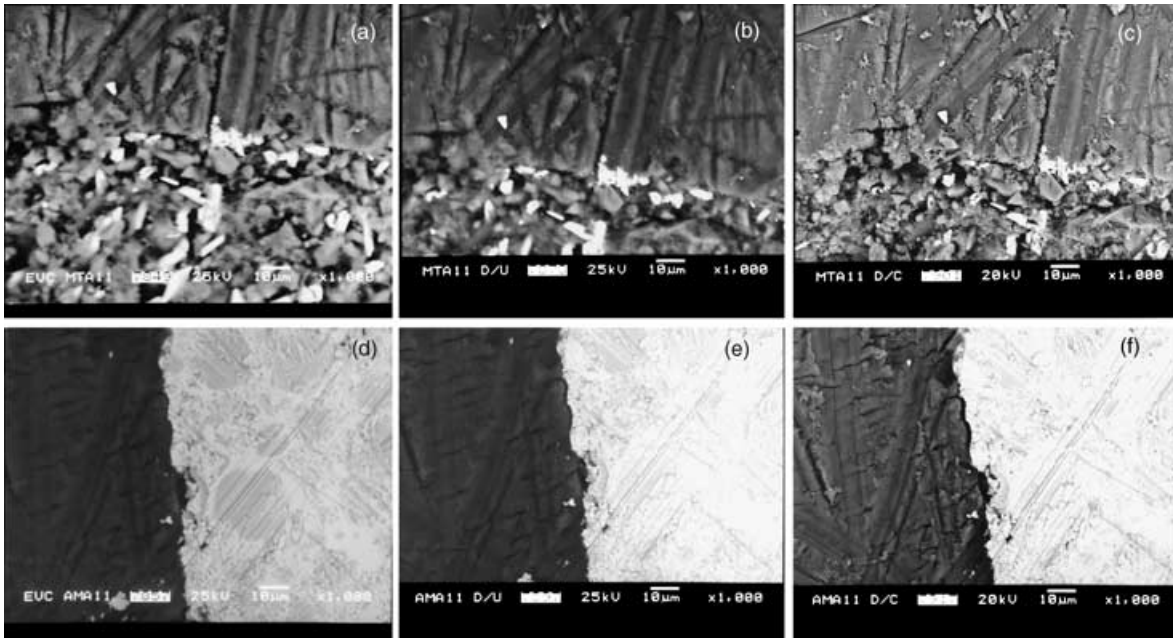

Figure 3 High-power magnification of MTA-filled (micrographs (a–c)) and amalgam-filled (micrographs (d–f)) specimens in the EVC control group.

LVW = 1.06 ± 1.52 μm, HVDC = 3.83 ± 4.27 μm; MTA: LVW = 0.52 ± 0.78 μm, HVDC = 1.19 ± 1.42 μm).

However, the ratio of increase of mean gap sizes between LVW and HVDC was similar in both groups, namely, a 300 and 200% increase in mean gap sizes from LVW to HVDC conditions of the amalgam and MTA-filled specimens, respectively, in both the experimental and the desiccated control groups. These results indicated the detrimental effect on the specimen from drying over a short period of time and the gold coating of the specimen.

Epoxy resin group

Table 3 showed the effect of dentine deformation and/or cracking because of SEM preparation on the marginal adaptation of root-end fillings. The amalgam-filled

Table 3 Mean, SDs, minimum and maximum gap sizes of specimens in the control groups (in micrometres)

Groups	Material	Condition	*N*	Mean	SD	Minimum	Maximum
EVC	Amalgam	EVC	8	0.666	0.677	0	1.905
		LVDU	8	1.448	1.253	0	4.073
		HVDC	8	2.183	1.784	0.897	6.392
	MTA	EVC	8	0.408	0.513	0	1.241
		LVDU	8	0.696	0.866	0	2.310
		HVDC	8	1.125	1.059	0	2.471
Desic	Amalgam	LVW	16	0.618	0.448	0	1.239
		HVDC	16	1.624	1.938	0	6.084
	MTA	LVW	16	0.256	0.387	0	0.987
		HVDC	16	0.540	0.728	0	1.918
Epoxy	Amalgam	LVW	8	1.992	1.695	0	5.270
		LVDU	8	1.994	1.696	0	5.276
		HVDC	8	2.070	1.660	0.352	5.380
	MTA	LVW	8	0.544	0.187	0.222	0.868
		LVDU	8	0.859	0.116	0.717	1.081
		HVDC	8	0.879	0.115	0.732	1.093

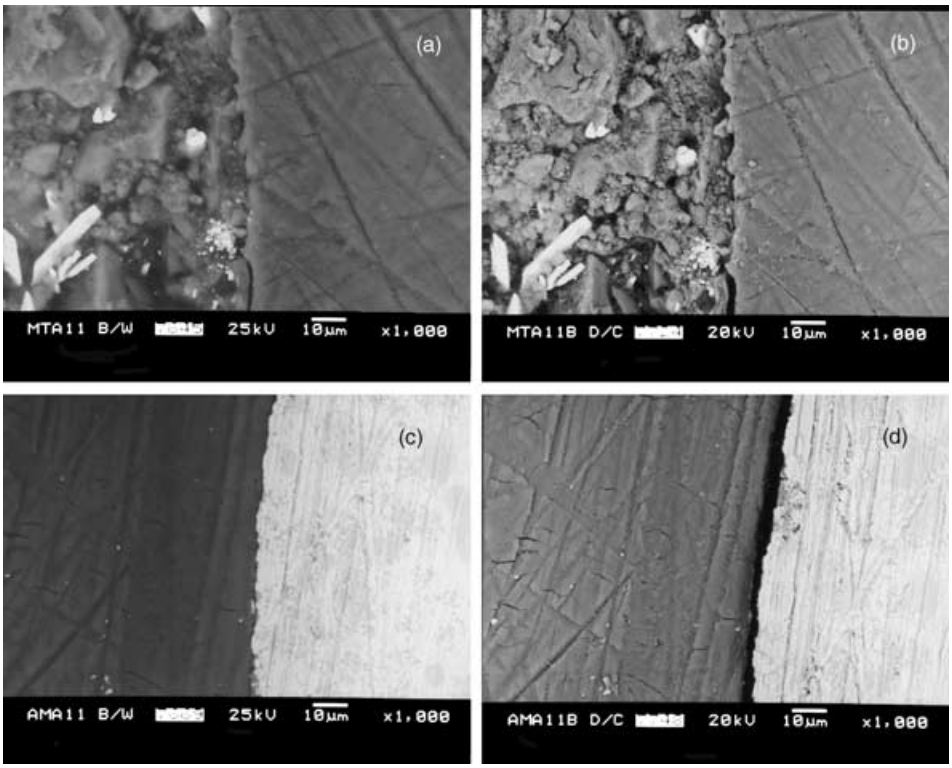

Figure 4 High-power magnification of MTA-filled (a,b) and amalgam-filled (c,d) specimens in the desiccated control group.

specimens showed no significant difference in the mean gaps of the amalgam–epoxy interface under all three SEM conditions (LVW = 1.99 ± 1.70 μm; LVDU = 1.99 ± 1.70 μm; HVDC = 2.07 ± 1.66 μm). These results showed the stability of the amalgam under all three SEM conditions (Fig. 5; Table 3).

The MTA-filled specimens in this control group showed an increase in mean gap sizes from LVW to HVDC SEM conditions (MTA: LVW = 0.54 ± 0.19 μm; LVDU = 0.86 ± 0.12 μm; HVDC = 0.88 ± 0.12 μm). The mean gap sizes in this control group were smaller than the mean gap sizes of the experimental group because of the noninteractive role of the dentine. The experimental group had a 200% increase in gap size between LVW and HVDC SEM conditions, while the epoxy group showed a 60% increase in gap sizes between the LVW and HVDC conditions.

Statistical results

Reproducibility of the measurement technique was shown by a mean gap width of 1.749 μm with a 95% confidence limit of 0.305 μm on either side of the mean.

General Linear Models Analysis of the experimental group

The General Linear Models Analysis showed that four of the five independent variables, namely, extent of crack, material, SEM condition and measurement point had statistically significant effects on gap width (Table 4). A crack that extended to the material–dentine interface ($F = 420.73$) had the greatest affect on gap size, followed by material ($F = 163.62$) and SEM condition ($F = 27.73$), while the point of measurement had a weak, although significant effect. The Tukey's Studentized Range Test showed that for gap size, all three SEM conditions differed significantly from each other; measurements at points 1, 4 and 6 also differed significantly from each other.

Discussion

Under restricted access conditions *in vivo*, bur-prepared cavities may be misaligned relative to the long axis of the root. For these cases, ultrasonic instruments have been used to improve the access, alignment, depth and overall quality of the root-end cavity. A favourable outcome of apical surgery performed with use of ultrasonics

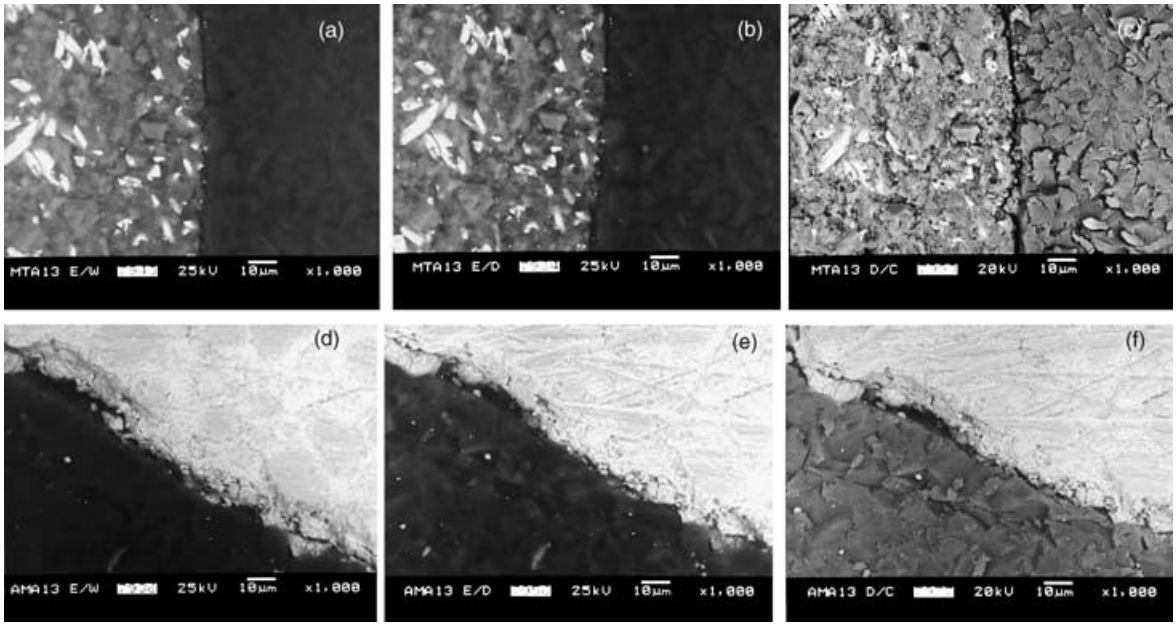

Figure 5 High-power magnification of MTA-filled (a–c) and amalgam-filled (d–f) specimens under all three SEM conditions in the epoxy resin control group.

Table 4 General Linear Models Analysis for gap width

Independent variable	*F*	*P*
Extent of crack	420.73	0.0001
Material	163.62	0.0001
Condition	27.73	0.0001
Point	2.11	0.0398
Level	0.63	0.4265

has been reported in several studies (Zuolo *et al.* 2000, Rubinstein & Kim 2002, Chong *et al.* 2003). However, Saunders *et al.* (1994) and Layton *et al.* (1996) observed cracks extending down the canal after ultrasonic root-end preparations. Lloyd *et al.* (1997) also associated the use of sonic tips with chipping of the root-end cavity margins. Burs were used in this study as crack formation, especially in the thinnest walls surrounding the root-end preparation, may be more frequent with ultrasonic tips than with burs (Abedi *et al.* 1995). A resection angle of 90° to the long axis of the root was used to minimize the number of cut dentinal tubules (Gagliani *et al.* 1998).

While some *in vitro* studies have shown good sealing of the root-end with amalgam (Tronstad *et al.* 1983, Inoue *et al.* 1991), others have indicated that amalgam's sealing qualities are poor (Kaplan *et al.* 1982, Szeremeta-Browar *et al.* 1985). In these studies, a variety of materials, procedures and microleakage assessment techniques were used, which may account for the diversity of results and conflicting conclusions.

MTA showed better marginal adaptation to the root-end cavity wall than Oralloy amalgam. This may be intrinsically linked to the nature of the material. MTA powder consists of fine hydrophillic particles, the principal compounds of which are tricalcium silicate, dicalcium silicate, tetracalcium aluminoferrite, calcium sulfate dihydrate and tricalcium aluminate. Bismuth oxide is present to make the aggregate radiopaque. The MTA absorbs water during hydration of the powder. Therefore, the material expands during solidifying, which must have played a role in its superior adaptation to dentine. This expansion of MTA may play a role in the increased incidence of cracks to the interface compared to the amalgam specimens (Table 2).

The chosen amalgam, Coltene® Oralloy, is a ternary nongamma-2 amalgam with irregular-shaped spheroidal and spherical particles. It was used in this study as it has favourable physical properties that include a high copper and low zinc content, namely, 59% silver, 28% tin and 13% copper. The particles are flake-like or spheroidal, which are formed by atomizing or spraying the alloy liquefied by heat into a cold environment and allowing the particles to freeze against a cold surface. The morphology of the particles so treated permits

closer packing and causes the preset mix to condense in a manner similar to that of a fine-cut alloy (Leinfelder 1981).

Amalgam has been used for root-end fillings and is therefore commonly used as the control when testing new or potential root-end materials. The biocompatibility of amalgam has been shown to be acceptable (Maher *et al.* 1992). However, several authors have questioned the use of amalgam as a root-end filling material, particularly in comparison with other available materials (Dorn & Gartner 1990, Frank *et al.* 1992, Chong *et al.* 1994, Pitt Ford *et al.* 1994).

If amalgam was to be introduced as a new root-end filling material in the year 2004, its relative toxicity, delayed expansion and corrosion, potential for tissue staining and marginal leakage would probably argue against its clinical testing and use. Nelson & Mahler (1990) investigated delayed expansion and concluded that low-copper zinc-containing amalgams should not be used as a root-end filling. A high-copper amalgam (12% copper or more) does not contain the corrosion-prone γ_2-phase. Zinc-containing amalgams have been shown to exhibit setting expansion and corrosion (Phillips 1982), and should probably not be used for retrograde root fillings. These facts were the basis for the selection of the amalgam used in the current study.

In this study, three SEM methods enabled in-depth evaluation of gap size. In particular, a LV SEM allowed the examination of moist specimens without preparation or coating. This method virtually eliminates surface charging problems experienced in HV SEM, extending imaging to samples difficult to examine or incompatible with conventional SEM methods (Wight & Zeissler 1993). Backscatter electron imaging was used in LV SEM to improve contrast, thereby facilitating measurement.

An environmental SEM could permit observation of specimens in a more hydrated state, but observation of specimens is extremely time-consuming and more costly than LV SEM. It was thus impractical to use in a quantitative study of this nature. The LV SEM is a relatively inexpensive alternative, which has a number of potentially useful applications in the biomaterials research field.

Procedural controls were undertaken to evaluate the effect of specimen preparative techniques and viewing conditions on specimen morphology and histometry. Even though the EVC control group (Table 3) showed less mean gaps than the LVW condition of the experimental group (Table 1), it could be said that viewing wet specimens under LVW conditions may result in similar gap width measurements in comparison to specimens under EVC conditions.

Specimens in the EVC control group showed increased gap widths at the filling–dentine interface under LVDU and HVDC conditions compared to specimens under EVC conditions (Fig. 3; Table 3). Similarly, the specimens in the desiccated control group showed increased gap widths at the filling–dentine interface under HVDC conditions compared to specimens under LVW conditions (Fig. 4; Table 3). These results confirmed the effect of dentine dehydration on the marginal adaptation of root-end filling materials because of dry SEM conditions. The ratio of increase in gap width sizes at the filling–dentine interface was similar in the desiccated and EVC control groups to that of the experimental group.

However, the root-end filling material may also be affected by the desiccating and coating process used for HV SEM viewing. This was observed in the epoxy resin control group, whereby MTA–resin interface increased from LVW to HVDC conditions (Fig. 5; Table 3). This was because of the evaporation of water out of MTA from LVW through to HVDC conditions. The amalgam showed a constant gap size under all conditions in this control group, which indicated the resilience and stability of this material from drying and HV conditions under LVDU and HVDC conditions, respectively.

Studies that investigate the sealing ability and marginal adaptation of root-end filling materials have so far not produced clear results. The true importance of leakage is unknown, and, until it is known, as well as how much leakage is important, it is prudent to use materials and techniques that provide the most effective seal. Therefore, although *in vitro* tests cannot completely simulate *in vivo* conditions, a material that has excellent *in vitro* adaptation to dentine with zero gap width may achieve the best sealability.

Gap width measurements of the specimens under HVDC conditions in this study were comparable with those of Torabinejad *et al.* (1995b), who reported MTA with a mean gap of 2.68 ± 1.35 μm. This study under HVDC conditions showed MTA to have a mean gap of 1.19 ± 1.42 μm (Table 1).

MTA showed superior adaptation compared to amalgam under all three conditions. However, it is interesting to note that amalgam had a mean gap width of 1.06 ± 1.52 μm and a minimum gap of 0.00 μm under LVW SEM conditions. This could explain the possible clinical success of cases treated with amalgam as a root-end filling *in vivo*.

Gap size increased for both MTA and amalgam-filled specimens from LVW to HVDC SEM conditions. The gap size in the MTA-filled specimens increased because of the water loss and shrinkage of both the MTA and the dentine in the dry SEM conditions. Amalgam did not change because of dehydration and therefore did not deform. These properties created tensions within the dentine, which resulted in larger gaps found in the amalgam-filled specimens compared to the MTA-filled specimens under LVDU and HVDC SEM conditions (Table 1).

Perhaps, the slower rate of drying the specimens for viewing under LVDU conditions in this study (the wet specimens were allowed to dry at room temperature over 48 h before desiccating with silica gel and coating with gold) reduced the potential damage of the dentine by allowing stresses to be dissipated without cracking of dentine.

A General Linear Models Analysis of the experimental group for gap width showed four of the five variables, namely, cracks to the filling–dentine interface, material, condition and point had significant effects on gap size (Table 4). The Tukey's Studentized Range Test showed that for gap size, all three conditions differed from each other. It also showed that points 1, 4 and 6 differed from each other. Cracks at these points were located adjacent to thicker dentine compared to other points of the same specimen. In addition, more cracks reaching the interface were evident at the bottom levels under HVDC conditions where there was more tooth structure because of the tapering morphology of the root end. However, the level did not have a significant effect on gap size under LVW SEM conditions, confirming the meticulous placement of these root-end filling materials.

The preponderance of cracks in the wider areas of dentine suggest that cross-section studies of root ends should be preferred above a single longitudinal section where cracking could be hidden below the visual plane. This could lure the investigator to wrong conclusions with artifacts influencing measurements rather than the experimental procedure.

Table 2 illustrated the prevalence of cracks to the interface in both MTA and amalgam-filled specimens under HVDC conditions. However, only a few cracks to the interface were evident under LVW conditions (3 specimens in amalgam and 13 specimens in MTA). However, the cracks to the interface from LVW to HVDC conditions in amalgam-filled root ends increased proportionately compared to MTA-filled root ends. Importantly, in MTA-filled root ends with cracks to the interface, mean gap widths remained low from LVW to HVDC SEM conditions.

Mean gap sizes were smaller in the specimens where the crack did not reach the interface under all three SEM conditions. The maximum gaps at the interface were always found at the location of a crack that reached the filling–tooth interface. These results clearly showed that cracks to the interface, which resulted from stresses in the dentine from the dehydration procedures under LVDU and HVDC conditions, caused an increase in gap sizes at the filling–tooth interface.

The difference in gap widths between the three different SEM conditions indicated that a correction factor may be used when investigating the marginal adaptation of root-end fillings in similar SEM studies. If only HV SEM is used, a correction factor of 3.5 and 2.2 may be used for amalgam and MTA root-end fillings, respectively, to enable relative comparisons to LVW conditions. Similarly, if specimens are not maintained wet under LV conditions (LVDU conditions), a correction factor of 1.8 and 1.5 may be used for amalgam and MTA root-end fillings, respectively. Therefore, in order to obtain a better indication of the marginal adaptation of amalgam and MTA root-end fillings in previous and future SEM studies, the correction factors suggested above may be used.

Conclusions

Mineral trioxide aggregate showed better marginal adaptation than Oralloy amalgam to the root-end cavity wall under all three SEM conditions. HV SEM conditions had a detrimental affect on the specimen and did not give an accurate recording of the 'true' marginal adaptation of the root-end filling. LV SEM was successfully used to evaluate marginal adaptation, and offers promise in future endodontic studies.

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