J Mater Sci: Mater Med (2008) 19:2941–2948
DOI 10.1007/s10856-008-3429-y

Evaluation of the effect of tracer pH on the sealing ability of glass ionomer cement and mineral trioxide aggregate

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Received: 5 August 2007 / Accepted: 11 March 2008 / Published online: 25 March 2008

Abstract *Objectives* The aim of this study was to evaluate the sealing ability and physical and chemical properties of glass ionomer cement (GIC) and mineral trioxide aggregate (MTA) using Rhodamine B at different pHs as tracer. *Methods* Chemical analysis, pH and micro-hardness of GIC and MTA were performed. In addition dye leakage was assessed by tracer leakage using Tandem Scanning Confocal Microscope (TSM) after immersion of premolar teeth in a stock and a buffered fluorescent Rhodamine B for 24 h. Ultra-structural changes within the materials were evaluated by viewing under the field emission scanning electron microscope (FESEM). *Results* GIC and MTA showed elemental peaks for silicon, aluminium and calcium while MTA also had bismuth. GIC was acidic ($P = 0.001$) and caused an increase in dye pH ($P = 0$). Immersion of MTA in any of the test solutions resulted in an increase in the pH of the solution ($P < 0.05$). Use of a dye solution of lower pH than the material under test increased the cement micro-hardness. GIC demonstrated marginal leakage on TSM and both increase in marginal leakage and material porosity on FESEM. MTA was not affected by the use of acidic dye but showed a tendency to take up dye within the material shown on TSM. *Conclusions* Evaluation of marginal adaptation of dental materials was shown to be dependent on the technique used for viewing the material to tooth interface, the properties of the material under study and the pH of the dye used.

1 Introduction

Dental materials have to be impervious to microbial entry in order to be able to protect the pulp and the periapical tissues from infection. Microleakage can occur at the tooth to material interface and also within the material itself. Microleakage at the tooth to restoration interface has been extensively studied for most of the materials in use in dentistry. Glass ionomer cements (GIC) show considerable marginal leakage when assessed using dyes [1, 2] regardless the brand used [3]. Conversely other studies showed no marginal leakage during the first 6 months after placement however a small amount of leakage was observed at the incisal, occlusal and gingival margins after 18 months [4]. Shrinkage of GICs is the highest at 37°C in air [5]. Dry storage of glass ionomer leads to marginal gaps in the region of 40–80 μm [6].

Mineral trioxide aggregate (MTA) demonstrated marginal leakage which was significantly less than other root-end filling materials when tested using dyes as tracers [7–9] and also when viewing the specimens and their replicas under the scanning electron microscope (SEM) [10].

Marginal leakage can be studied by assessing leakage to India ink [11, 12], methylene blue [1, 2, 8, 9] or Rhodamine B and other fluorescent dyes [7]. Leakage measures the propagation of dye within gaps that have not been filled by the restorative material. The extent of leakage is dependant on the pH of the dye and its particle size. Methylene-blue has a low molecular weight and is more acidic than India ink, thus it tends to lead to a higher

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amount of dye leakage [13]. In contrast when used with MTA methylene blue resulted in less leakage [14]. Methylene blue has been shown to decolor when in contact with some dental restorative materials. This could lead to erroneous results in tracer leakage studies [15].

After subjecting the specimens to a dye, marginal leakage can be measured by clearing the specimens [3, 11] or sectioning them longitudinally or transversely and viewing under the SEM, light microscope or the Tandem Scanning Confocal Microscope (TSM) [7, 16, 17]. The SEM causes dehydration of the samples which often leads to formation of shrinkage gaps between the dentine and the restorative material. This can be avoided by using resin replicas. An impression of the material surface is taken with an impression rubber, which is then cast in resin. This technique avoids changes in the material caused by dehydration when using vacuum in the SEM chamber [18, 19]. Interpretation of images yielded by replicas can prove difficult due to loss of surface detail arising from a faulty impression, imperfectly cured resin, fractures of the replica and contamination by liquids like fluid exudate from cut dentine. In addition impression materials can tear and air bubbles can give rise to large voids in the replica surface [19]. The use of TSM and a fluorescent dye has several advantages namely minimal specimen preparation, no desiccation of the specimen, high resolution imaging and direct viewing and below the surface imaging when using immersion objectives. The resultant image exhibits contrast and resolution far superior to that of conventional light microscopes. Rhodamine B with a chemical formula $C_{28}H_{31}N_2O_3Cl$ is part of the family of fluorone dyes. It is frequently used as a tracer within water to determine the rate and direction of flow. Rhodamine B fluoresces when excited by red light of a particular wavelength (546 nm). The fluorescence can be easily measured with a fluorimeter. Rhodamine is toxic and is soluble in water, methanol and ethanol. Powdered Rhodamine B is green in color which turns bright pink when mixed with water. Rhodamine B has a larger molecular weight than methylene blue and decoloration will not effect its detection.

The aim of this study was to evaluate the sealing ability and physical and chemical properties of GIC and MTA using Rhodamine B at different pHs as tracer.

2 Materials and methods

Two cement types were used in this study. GIC (Ketac Molar, 3M ESPE, Seefeld, Germany) and white mineral trioxide aggregate (White MTA, Tulsa Dental Products, Tulsa, OK, USA). Both materials were mixed according to manufacturer's instructions. The glass ionomer (Batch Number: 756464/02) was mixed in a powder to liquid ratio of 3.0:1 proportion by weight. The MTA (Batch number: 02093081) was mixed with 35% water provided by the manufacturer. Rhodamine B (Sigma Aldrich Dorset, UK) 0.25% concentration was prepared (Rh) by mixing 0.25 g of Rhodamine B powder to distilled water. Two other solutions Rhodamine 7 (Rh 7) and Rhodamine 10 (Rh 10) were prepared by mixing the Rhodamine B powder to buffers pH 7 and pH 10 (Hanna Instruments, Padova, Italy). The buffers were made up of a solution based on sodium bicarbonate. The buffers were used to maintain the pH of the dye solution stable regardless additions of acidic or alkaline substances. The pH of the solutions was measured with a pH meter (Jenway Scientific, Felsted, UK).

2.1 Chemical constitution of cement powders

Energy Dispersive Analysis by X-ray (EDAX) was performed under the scanning electron microscope (SEM; S3500, Hitachi, Wokingham, UK). A thin layer of powder was dispersed over carbon double-sided tape attached to an aluminium stub (Agar Scientific, Stansted, UK). The stubs were carbon coated (Emitech K250, Emitech, Ashford, UK) for electrical conductivity. The specimens were then viewed under the SEM and X-ray analysis was carried out to determine the constituent elements of the powders. Two stubs were made for each material and the analysis was performed twice for each sample.

2.2 pH

One gram of cement was mixed on a glass slab according to manufacturer's instructions. After mixing the materials were compacted into metal moulds producing discs 10 mm in diameter and 2 mm thick. Six discs were made for each material. The materials were allowed to cure in the moulds for 24 h at 37°C and 100% humidity. After removal from the moulds, the discs were placed in sealed containers filled with 5 ml of either distilled water or stock or buffered fluorescent dye for 24 h. The discs were then removed from the solutions, washed under running water and were then allowed to acclimatize at 100% humidity and 37°C for 14 days. The pH of the storage solution was measured using a pH meter (Jenway Scientific, Felsted, UK). In order to measure the pH of the cements the surface was first rubbed with a fine grit abrasive paper using water slurry. The surface pH of the cement could thus be measured.

2.3 Micro-hardness testing

Discs made of, GIC and MTA 10 mm in diameter and 2 mm thick, were cast, cured for 24 h at 37°C and 100% humidity, were then removed from the moulds and stored in water at 37°C for 28 days. The discs were then immersed in either water, or stock or buffered Rhodamine B solution for 24 h after which micro-hardness testing was performed using a diamond shaped indenter. Vickers Hardness number (VHN) was recorded. Six teeth for each cement type were prepared for each immersion solution.

2.4 Tracer leakage experiment

Intact premolar teeth extracted for reasons other than caries and pulpal involvement were used in this study. After extraction the teeth were stored in water. Small occlusal cavities 3–4 mm wide and 2–3 mm deep were prepared using a high speed hand-piece and a small fissure bur. The cavity walls were finished using a low speed fissure bur. No calcium hydroxide lining or dentine conditioner was used under the GIC restorations. The materials were then mixed according to manufacturer's instructions on a glass slab and delivered using a flat plastic instrument. The material was plugged in place with small and medium sized pluggers. The restorations were carved with a Wards 2 carver. The materials were allowed to set for 6 h at 37°C to allow the MTA to hydrate fully after which the teeth were immersed in water and were cured at 37°C in water for 28 days. Six teeth for each cement type were prepared for each immersion solution.

The root apices were blocked with light cured composite resin (Star Flow Microhybrid, Danville Materials Inc. USA). The teeth were then coated completely with varnish except for the restoration, restoration margins and 1 mm tooth circumference around the restoration. They were then immersed in either Rhodamine B 0.25% concentration (Sigma Aldrich Dorset, UK), buffered Rhodamine (Rh 7, Rh 10) or in water for 24 h after which they were washed with water in an ultrasonic bath (Biosonic, Coltene/Whaledent Inc. Cuyahoga Falls, Ohio, USA) for 5 min. The teeth were then sectioned longitudinally from mesial to distal using a hard tissue microtome (Labcut 1010, Agar scientific, Stansted, UK) using a diamond wafering blade (Buehler, Wisconsin, USA). The sectioned teeth were ultrasonically washed again for 5 min.

The specimens were mounted on a glass cover-slip with plasticine and pressed in place to achieve planarity. The samples were viewed under the Tandem Scanning Confocal Microscope (TSM Noran Instruments, Middleton, WI, USA) using oil immersion objectives in conjunction with a green filter (wave length 546 nm). Rhodamine B gave red/orange fluorescence when excited with green light of 546 nm wavelength. The presence of dye within the material was confirmed by blocking the reflected light from within the sample with a red filter with a wavelength of 610 nm. Magnification was at 20× with a 0.8 numerical aperture (NA). The extent of dye uptake within the cements was evaluated by 2 operators independently. A scoring method was used to evaluate both the marginal adaptation of the materials and assess leakage within the material if any. Divergence of opinion was resolved by viewing the specimen by both operators jointly and resolving the difference in score. Images were recorded with a Nikon 5000 digital camera.

2.5 Marginal adaptation

0: No marginal leakage
1: Marginal leakage with dye penetration up to 1–2 mm
2: Marginal leakage with dye penetration up to 2–3 mm
3: Complete marginal leakage with penetration to the cavity floor.

2.6 Material porosity

0: No material porosity
1: Dye penetration within the material up to 1–2 mm
2: Dye penetration within the material up to 2–3 mm
3: Complete dye penetration within the material to the cavity floor

2.7 Field emission scanning electron microscopy

After subjecting the cements to normal and buffered dye, and water for 24 h, the other half of the sectioned specimens were first polished using progressively finer grit sizes of abrasives using water slurry. After polishing the specimens were washed under water to remove all the fine particles stuck to the surface. The sections were then stored at 6°C fully hydrated. Ultra-structural changes in the cements were evaluated using a field emission scanning electron microscope (FESEM: FEI Quanta 200F, Eidenhoven, The Netherlands). The specimens were then viewed fully hydrated at an accelerating voltage of 10 kV under 7.0 Torr pressure using a gaseous secondary electron detector. The low temperature and full hydration ensured 100% relative humidity in the chamber thus avoiding specimen dehydration. Magnification was set at 500×. The tooth to restoration interface was evaluated together with changes in material porosity. Both fields were evaluated and any changes noted qualitatively. The specimens were evaluated by 2 operators independently. Divergence of opinion was resolved by viewing the specimen by both operators together and resolving the difference in jointly.

2.8 Statistical analysis

The data was evaluated using Statistical Package for the Social Sciences (SPSS) software. The distribution was first evaluated to determine what kind of statistical test would be performed. The data was plotted and the distribution curve was analyzed together with the Kolmogorov–Zmirnov test with $P = 0.05$. $P > 0.05$ signified a normal distribution thus parametric tests could be performed accordingly. With normally distributed data Analysis of Variance (ANOVA) with $P = 0.05$ was first performed to evaluate any variation between the means. Then two-tailed independent sample *t*-test at 95% confidence level with $P = 0.05$ was used to analyze the data.

3 Results

3.1 Chemical constitution

The GIC had larger peaks for calcium, silicon and aluminium with smaller peaks for phosphorus, fluorine, sodium and lanthanum (Fig. 1a). MTA was composed of calcium, silicon, aluminium, iron and bismuth (Fig. 1b).

3.2 pH

The pH of the water and the normal and buffered dye solution prior to immersion of the cements is shown in Fig. 2. The water was neutral. The Rhodamine B was very acidic with a pH of 1.3. The use of buffers allowed a rise in pH of the Rhodamine B. The results for the pH readings of both cements and soaking solutions after a 24 h contact are shown in Fig. 2. There was no statistically significant change in the pH of the water, and buffered Rhodamine solutions after immersion in GIC ($P > 0.05$), but a rise in pH of un-buffered Rhodamine B was noted ($P = 0.001$). Immersion of MTA in water, and buffered and un-buffered Rhodamine resulted in a large increase in pH ($P < 0.05$). In the case of the un-buffered Rhodamine B a 24 h immersion of MTA caused the pH to rise from 1.3 to 10.2 thus from very acidic to extremely alkaline. After immersion in the dye GIC showed a reduction in pH when immersed in un-buffered Rhodamine B ($P = 0.001$) and in Rhodamine B buffered to pH 7 ($P = 0.002$). Immersion of MTA in Rhodamine B did not cause a change in pH of the cement ($P = 0.482$), however immersion in buffered Rhodamine caused a reduction in cement pH ($P = 0.001$).

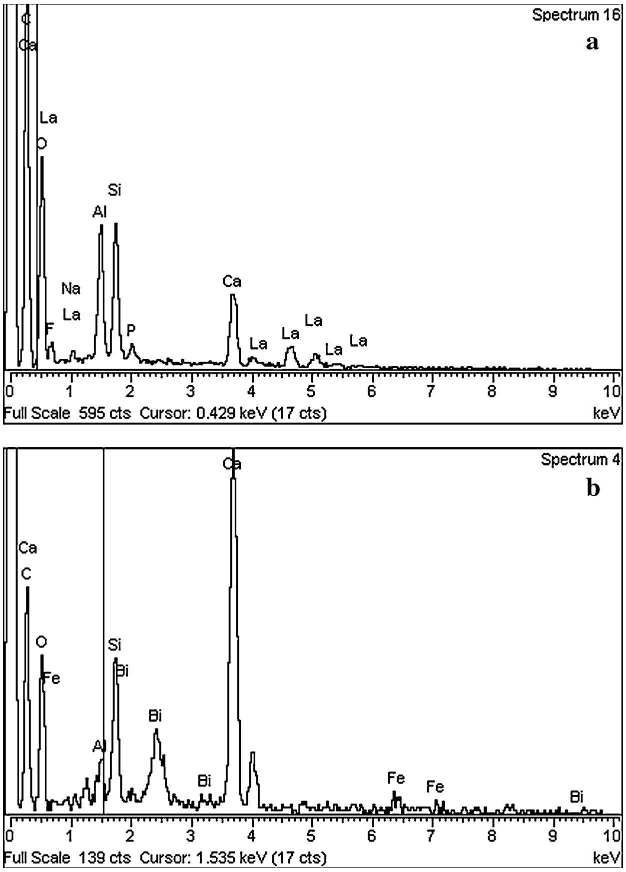

Fig. 1 EDAX of (**a**) GIC and (**b**) MTA showing the elemental constitution

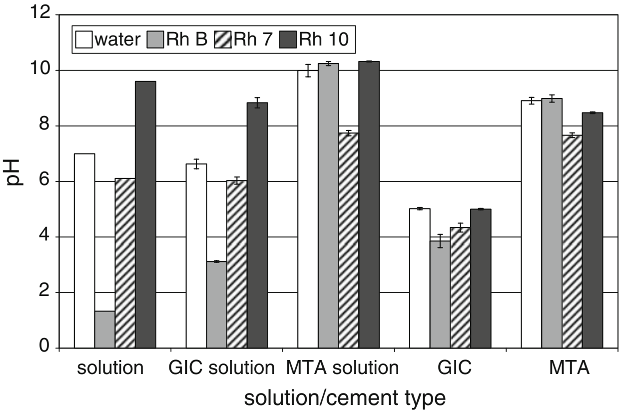

Fig. 2 pH of various solutions before and after immersion of glass ionomer cement and MTA for 24 h and pH of cements after immersion in solution ±SD ($n = 6$)

3.3 Micro-hardness testing

The results for micro-hardness testing of GIC and MTA are shown in Fig. 3. The results obtained (VHN) for each material were compared. GIC and MTA had a similar surface hardness when immersed in water for 24 h. Immersion in un-buffered Rhodamine B increased the surface hardness of the GIC ($P = 0.015$) compared to the result of the surface hardness when the material was immersed in water. Immersion of MTA in Rhodamine B buffered to pH 7 resulted in an increase in micro-hardness of MTA ($P = 0.001$) compared to the surface hardness of the material immersed in water.

3.4 Tracer leakage experiment

The results for the marginal adaptation of the cements under study are shown in Table 1 and the results for the

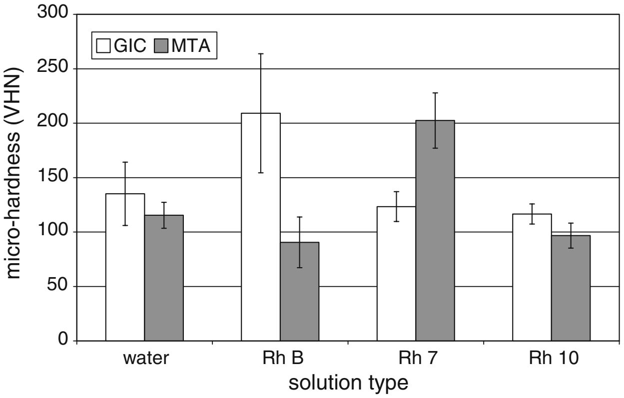

Fig. 3 Micro-hardness testing of glass ionomer cement and MTA after immersion in different solutions for 24 h ±SD ($n = 6$)

Table 1 Mean ± SD of marginal adaptation of different materials

Material	Rhodamine B	Rhodamine 7	Rhodamine 10
GIC	2.5 ± 0.5	2.67 ± 0.5	2.67 ± 0.5
MTA	0	0	0

Table 2 Mean ± SD of material uptake of dye of different cements

Material	Rhodamine B	Rhodamine 7	Rhodamine 10
GIC	0	0	0
MTA	3 ± 0	2.83 ± 0.4	2.67 ± 0.5

material uptake of dye are shown in Table 2. Glass ionomer showed sizeable gaps at the material to tooth interface (Score 2.5–2.67). No dye was taken up by the material regardless of the pH of the solution (Score 0; Fig. 4). MTA showed a high susceptibility to penetration into the material (Score 2.67–3) rather than to the tooth-material interface (Score 0; Fig. 5). There was a significant difference between the marginal leakage ($P = 0.001$) and also dye penetration within the material ($P = 0.001$) for GIC and MTA.

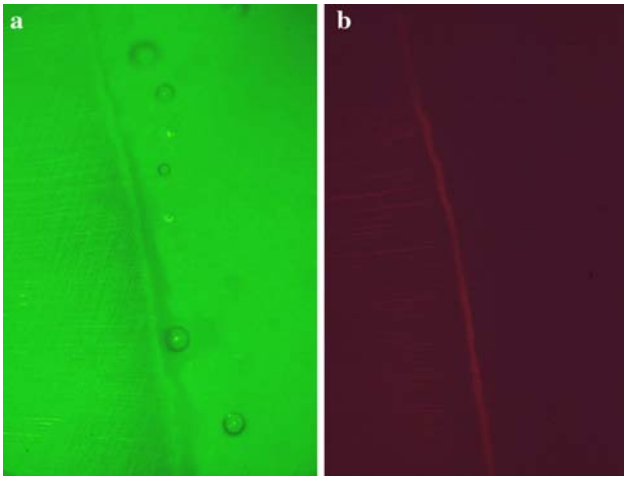

Fig. 4 Material to tooth interface leakage of glass ionomer cement viewed under the TSM using oil immersion objectives (**a**) using a 610 nm filter and (**b**) 610 nm emission filter excited with a 546 nm red filter showing dye uptake at the tooth to material interface (20× magnification)

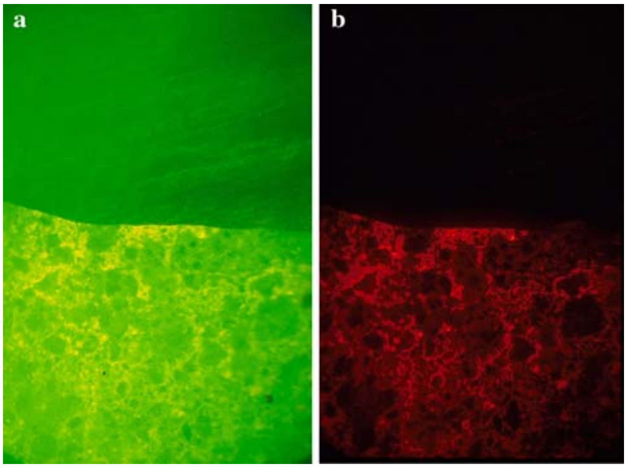

Fig. 5 Material leakage of mineral trioxide aggregate viewed under the TSM using oil immersion objectives (**a**) using a 610 nm filter and (**b**) 610 nm emission filter excited with a 546 nm red filter showing dye uptake within the material (20× magnification)

3.5 Field emission scanning electron microscopy

Glass ionomer specimens cured in water showed no material porosity with little marginal leakage (Fig. 6). The

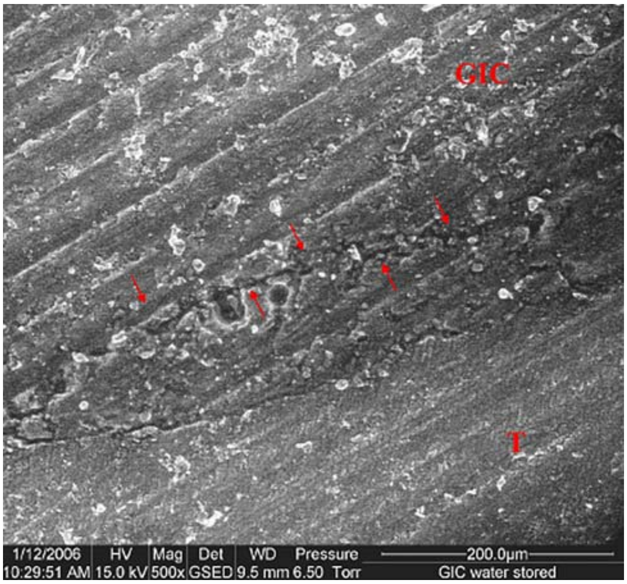

Fig. 6 Electron micrograph of glass ionomer cement (GIC) restoration in extracted tooth (T) stored in water viewed under FESEM (magnification 500×) showing minimal marginal leakage (red arrows) and no material porosity

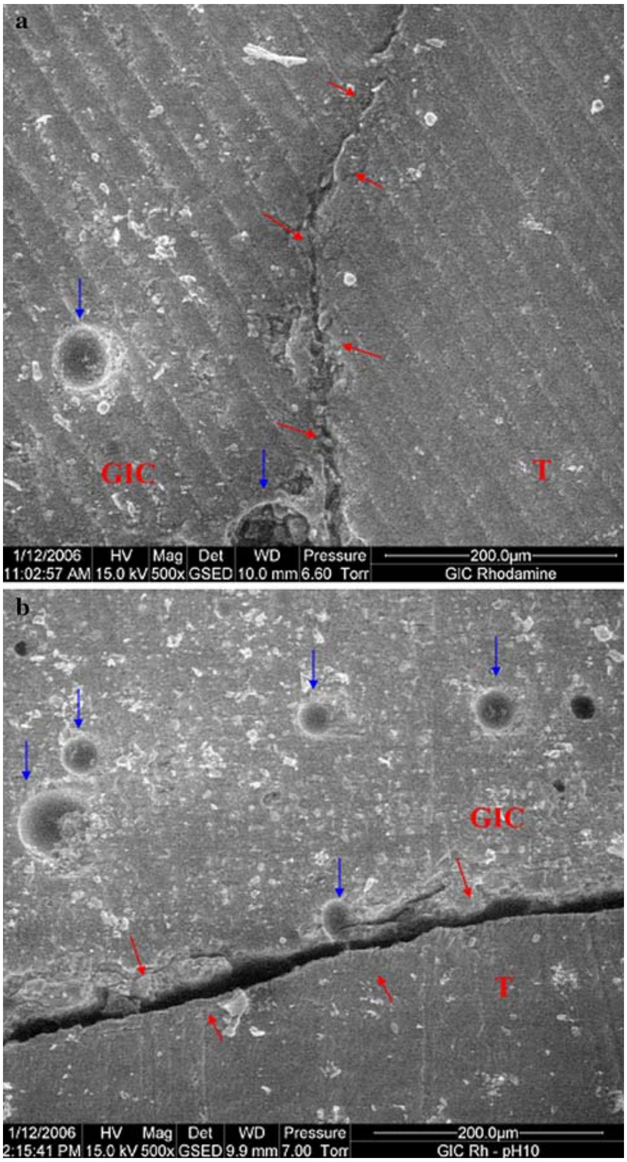

Fig. 7 Image of glass ionomer cement (GIC) restoration in an extracted tooth (T) immersed in Rhodamine viewed under FESEM (magnification 500×) showing (**a**) minimal marginal leakage (red arrows) and some material porosity (blue arrows) when immersed in un-buffered Rhodamine and (**b**) more material porosity (blue arrows) and increased marginal leakage (red arrows) when immersed in buffered Rhodamine pH 10

material was well apposed to the tooth. Immersion of glass ionomer in Rhodamine B resulted in an increase in both material porosity and marginal leakage (Fig. 7a). Both porosity and marginal leakage increased further with a higher dye pH (Fig. 7b). Immersion of MTA in Rhodamine B did not show any changes in the material ultra-structure (Fig. 8a) when compared to the material immersed in water. Immersion in buffered Rhodamine B at pH 10 resulted in the deposition of crystals over the cement surface (Fig. 8b).

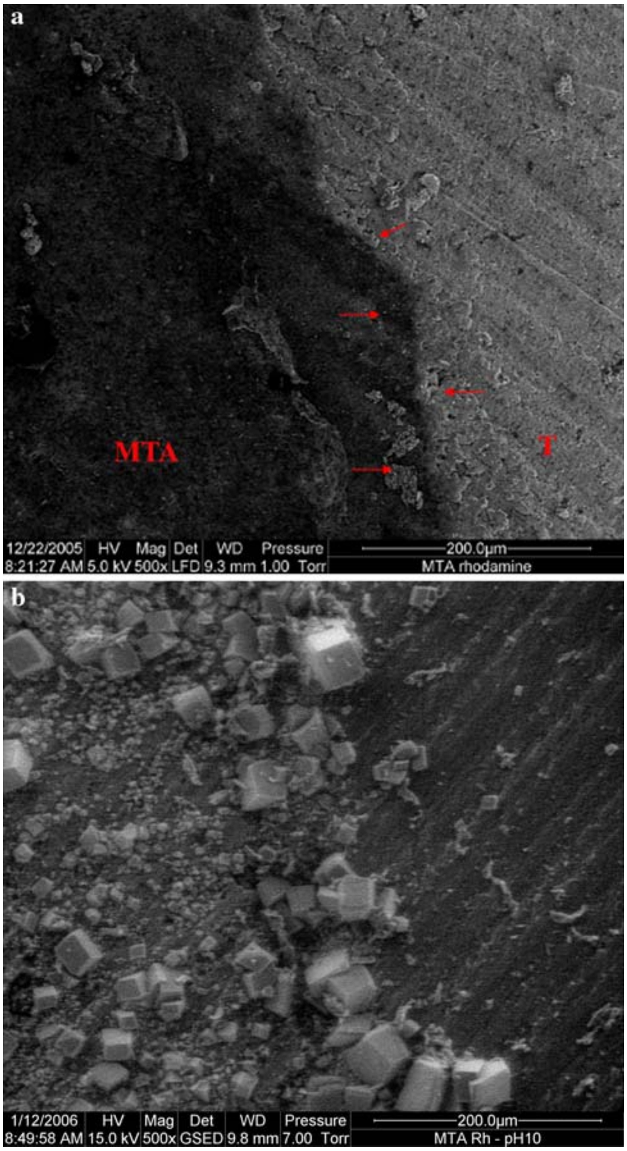

Fig. 8 Electron micrographs of MTA immersed for 24 h in (**a**) Rhodamine B pH 1.3 showing good marginal adaptation (red arrows) and no material porosity and (**b**) buffered Rhodamine B pH 10 showing crystal deposits over the material surface (magnification 500×)

4 Discussion

Several studies have used fluorescent dye as a tracer in studies evaluating the marginal adaptation of dental materials. Fluorescent dyes are used in conjunction with the TSM as Rhodamine B gives red/orange fluorescence when excited with green light of 610 nm wavelength. The presence of dye leakage within the material is confirmed by blocking the reflected light from within the sample with a red filter with a wavelength of 546 nm. The use of TSM and a fluorescent dye has several advantages namely minimal specimen preparation, no desiccation of the specimen, high resolution imaging and direct viewing and below the surface imaging when using immersion objectives. None of the studies report the pH of the dye used. Ahlberg et al. [13] have shown that methylene blue gave a higher amount of leakage when compared to India ink due to its acidity and low molecular weight. In addition the dye tends to be decolored in contact with some restorative material which also tends to give erroneous results [15].

Marginal adaptation of MTA has been evaluated by the use of SEM [20]. SEM desiccates samples thus evaluation of marginal adaptation of materials can be erroneous. Low vacuum SEM results in smaller gap sizes than high vacuum SEM. Gap sizes at fixed points were smallest under low vacuum and largest under high vacuum SEM conditions [21]. The use of resin replicas in conjunction with the SEM overcomes the problem of specimen dehydration however the method is very technique sensitive and also interpretation of the surface structures on resin replicas can be erroneous due to faults in impression taking and casting [19]. The field emission electron microscope (FESEM) does not desiccate the specimens as it uses water as a medium for transmission of electrons in contrast to SEM. In addition interpretation of the images yielded by FESEM is easier and the technique dos not involve the use of other materials and additional specimen preparation.

In the present study marginal adaptation of both GIC and MTA are evaluated using two different microscopy techniques. The TSM in conjunction with the use of a fluorescent dye showed that GIC had sizable gaps at the tooth to material interface. This is in accordance with previous studies on the material [22–24] using the TSM and fluorescent dyes. When the specimens were viewed under the FESEM the gaps at the material to tooth interface were again in evidence in conjunction with an increase in material porosity which was not demonstrated on TSM. When GIC was immersed in water and viewed under the FESEM there was no evidence of either material porosity or marginal leakage thus the changes in the material must have been caused by the use of an acidic dye. The use of buffered dyes seemed to increase the gap at the tooth to material interface and increase the cement porosity as demonstrated on FESEM. Ultra-structural changes were observed within GIC due to inability to maintain solution pH. GIC reaction with water produced acidic cement (pH 5) in accordance with a previous study [25]. Immersion of glass ionomer in water produced an acidic elution (pH 6.6) which was however more basic than the cement itself. The use of Rhodamine B with a pH of 1.3 caused a reduction in the cement pH which could affect the hydration mechanism and also adaptation of the GIC to the tooth.

Marginal adaptation of MTA was shown to be adequate regardless the technique used and the pH of the dye. Evaluation of the tooth to material interface using the TSM and Rhodamine B as dye showed good marginal adaptation of MTA in accordance with previous experiments [7]. Similar findings were observed under the FESEM again in accordance with previous experiments using the SEM [21]. Thus both techniques seem to be suitable for evaluating marginal adaptation of MTA. Marginal leakage of MTA was shown to be dependent on the dye used. Restorations made of MTA leaked more when exposed to Rhodamine B than when methylene blue was used as a tracer [14]. It has been reported that an acid environment does not hinder the sealing ability MTA but rather enhances it [26]. This is in accordance to what was shown in this study where the acidic dye did not seem to affect the marginal adaptation of MTA. MTA showed a susceptibility to take up dye within the material. In a previous experiment where Rhodamine B was used to measure marginal adaptation of MTA using the TSM and Rhodamine as tracer, no changes in the material were reported [7]. This is in contrast to the present study where a large quantity of dye was taken up by the cement. Reaction of MTA with water produced alkaline cement (pH 8.9) and an even more alkaline elution on immersion in water for 24 h (pH 10). This is in accordance to a previous study reporting MTA alkalinity [27]. MTA was not affected by the dye but it made the dye alkaline. This is caused by the liberation of calcium hydroxide during the hydration reaction [28]. With the use of a buffered dye at pH 10 there was crystal deposition on the MTA surface. No crystals were observed when the material was immersed in the un-buffered Rhodamine B and in the Rhodamine B buffered to pH 7. Similar crystal deposition has already been reported in other studies where MTA surfaces were observed under the SEM [29]. These crystals were believed to be calcium carbonate crystals in calcite form which were deposited over the material surface by carbonation of the calcium hydroxide produced as a by-product of the hydration reaction of MTA. The carbonate ion in this study was derived from the buffer solution.

When the cements were subjected to a higher pH than that normally found in material elutions, an increase in cement micro-hardness was registered. Thus a change in medium pH affects the micro-hardness of the cements.

5 Conclusions

Evaluation of marginal adaptation of dental materials was shown to be dependent on the technique used for viewing the material to tooth interface, the properties of the material under study and the pH of the dye used. GIC was more susceptible to changes in the pH and the technique used to evaluate the tooth to material interface. Changes at the tooth to material interface seemed to be related more to the use of dye The dye in contact with GIC resulted in increased material porosity and a wider restoration to tooth interface. MTA was less affected by changes in dye pH, but there was an increased susceptibility of leakage within the material rather than at the tooth to material interface.

Acknowledgments The following should be thanked: Mr. Peter Pilecki at Microscopy and Imaging, Department of Biomaterials Research of King's College London Dental Institute at Guy's, King's and St Thomas' Hospitals', London for his assistance with the confocal microscopy and Dr. Tony Brain and Mr. Ken Brady of the Centre for Ultra-structural Imaging, King's College London for the field emission scanning electron microscopy. The Department of Metallurgy, Faculty of Engineering, University of Malta, for access to equipment.

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