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Interaction of resin-modified glass-ionomer cements with moist dentine

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KEYWORDS

Resin-modified glass-ionomer cement;
Dentine; Interface;
Water movement;
Permeability

Summary Objectives. The objective of this study was to report on a novel phenomenon that occurs when resin-modified glass-ionomer cements (RMGICs) are bonded to moist human dentine.

Methods. Dentine surfaces from extracted third molars were abraded with 180-grit SiC paper. Ten teeth were prepared for each of the two RMGICs tested (Fuji II LC, GC Corp. and Photac-Fil Quick, 3M ESPE). RMGIC buildups were made according to the manufacturers' instructions. After storage at 37 °C, 100% humidity for 24 h, the bonded specimens were cut occlusogingivally into 0.9 × 0.9 mm beams. Dentine surfaces bonded with the two RMGICs were examined along the fractured RMGIC/dentine interfaces. Additional beams fractured within the RMGICs and at 3 mm away from the interfaces were used as controls. The fractured beams were examined using scanning electron microscopy (SEM), field emission-environmental SEM (FE-ESEM) and transmission electron microscopy (TEM).

Results. SEM and FE-ESEM revealed numerous solid spherical bodies along the RMGIC/dentine interfaces. By contrast, no spherical bodies could be identified within the RMGIC fractured 3 mm distant from the bonded interface. TEM and energy dispersive X-ray analyses performed on carbon-coated ultrathin sections showed that these solid spherical bodies consisted of a thin aluminum and silicon-rich periphery and an amorphous hydrocarbon core within the air voids of the original resin matrix.

Conclusion. The spherical bodies probably represent a continuation of GI reaction and poly(HEMA) hydrogel formation that results from water diffusion from the underlying moist dentine. Their existence provides evidence for the permeation of water through RMGIC/dentine interfaces.

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Introduction

Resin-modified glass-ionomer cements (RMGICs) were developed in 1988 by adding polymerisable hydrophilic resin to the conventional glass-ionomer

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formulation.^{1,2} It was first available commercially as a liner material, and later as a direct restorative material. These materials were created to overcome some of the problems of moisture sensitivity and low early mechanical strengths associated with conventional GICs, while maintaining their clinical advantages.³ The basic RMGIC consists of ion-leachable glass, polyalkenoic acid or a modified polyalkenoic acid with a photocurable side chain grafted onto the polymer backbone, a photocurable monomer such as 2-hydroxyethyl methacrylate (HEMA) and water.⁴ Unlike conventional GIC, RMGIC has a dual setting reaction. The primary setting reaction consists of free-radical polymerisation of the monomeric components, which is activated by exposure to visible light in the region of 470 nm. The second setting reaction is the classic acid-base reaction that commences upon mixing of the cement and continues after light-activation. The final set cement contains an interpenetrating network of polyalkenoate salts and a poly(HEMA) matrix.⁵

Little information is available on the exact mechanism of bonding of RMGICs to dentine. The adhesion of RMGIC has been reported to be both an ionic interaction between the cement and the dentine surface,^{3,6} and a micromechanical interlocking of the polymer with the polyacrylic acid-conditioned tooth substrates.⁶ Hybrid-like layer formation has been reported at the RMGIC/dentine interfaces when conditioning was carried out prior to application of the cement.^{7,8-10} Tandem scanning confocal microscopy has revealed the existence of an 'absorption layer' between RMGICs and dentine.¹¹⁻¹³ This absorption layer develops over time after setting of RMGIC and is thought to be due to water sorption from dentine to the maturing cement. The absorption layer was only observed when RMGICs were applied to deep dentine, and was absent from either superficial dentine or enamel.¹¹ The consequence of such water movement on the ultrastructure of the RMGIC/dentine interface is unknown.

The objective of this study was to examine, with scanning electron microscopy (SEM), field emission-environmental SEM (FE-ESEM), and transmission electron microscopy (TEM), the ultrastructure of RMGIC/bonded, moist human dentine. Dentine surfaces bonded with two RMGICs and sealed immediately to prevent environmental water gain or loss were examined along the fractured RMGIC/dentine interfaces. As water movement can only occur from the dentine side of the bonded specimens, additional specimens fractured within the RMGIC at 3 mm away from the interfaces were used as controls. The null hypothesis tested was that

there is no difference in the ultrastructure of RMGICs fractured at different locations away from the bonded dentine.

Materials and methods

Tooth preparation

Twenty extracted human third molars were collected after the patients' informed consent had been obtained under a protocol reviewed and approved by the institutional review board from the University of Hong Kong. The teeth were stored in a 1% chloramine T solution at 4 °C and used within 1 month after extraction. Prior to the bonding experiments, the teeth were retrieved from the disinfectant solution and stored in distilled water, with four changes of the latter within 48 h to remove the disinfectant. The occlusal enamel and superficial dentine were removed using a slow-speed saw with a diamond-impregnated disk (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under water cooling. The roots and the dental pulp of each tooth were not removed, in order to preserve the hydrated status of the exposed dentine. Dentine surfaces were abraded with 180-grit silicon carbide paper to create a clinically relevant smear layer¹⁴ on the surface of the dentine. Bonding was performed on the occlusal surface of deep coronal dentine.

Experiment design

Ten teeth were prepared for each of the two RMGICs tested: Fuji II LC Capsule (GC Corp., Tokyo, Japan) and Photac-Fil Quick Aplicap (3M ESPE, St Paul, MN, USA). Fluoroaluminosilicate glass (FASG) filler particles are present in the powder component of both RMGICs. The liquid component in Fuji II LC contains 2-hydroxyethyl methacrylate (HEMA), polyacrylic acid, 2,2,4-trimethyl hexamethylene dicarbonate, triethylene glycol dimethacrylate and water, while that of Photac-Fil Quick contains HEMA, polyethylene polycarbonic acid, urethane dimethacrylate, magnesium-HEMA ester and water.

The dentine surfaces were treated with 10% polyacrylic acid (Dentine Conditioner; GC Corp.) for 20 s immediately before bonding with the RMGICs. After rinsing off the conditioner with distilled water for 10 s, each tooth was blot-dried and left inverted on a piece of wet, lint-free tissue to prevent the loss of moisture from the dentine surface during the mixing and dispensing of the RMGICs. The teeth

were bonded with the RMGICs according to the manufacturers' instructions, to form 6-mm high cores over the surfaces of the moist dentine. After initial setting and/or light-activation, the exposed RM-GICs were coated with a thin layer of light-cured, oxygen inhibition layer-free, glaze resin (BisCover, Bisco Inc., Schaumburg, IL, USA), to prevent them from desiccation, or water-sorption via external sources. After storage at 37 °C and 100% relative humidity for 48 h, the bonded specimens were cut occlusogingivally into 0.9×0.9 mm beams, and further protected by the BisCover resin. The beams were sealed in bottles at 100% humidity and examined within 48 h after sectioning.

SEM and FE-ESEM preparation

For SEM examination, 10 beams were randomly selected from the teeth bonded with each RMGIC, and fractured along the RMGIC/dentine interface by three-point bending. A custom-made aluminum device was used with a 5-mm wide loading span between the two fixed end-supporting beams. A load was applied via the blunt end of a razor blade along the RMGIC/dentine interface until the beam broke. Ten additional bonded beams were intentionally fractured, by first creating a nick with a sharp scapel at a location within the RMGIC that was approximately 3 mm distant from the RMGIC/dentine interface. Each beam was similarly placed on top of the end-supporting beams of the three-point bending device. Intentional fracture was produced within the RMGIC beneath the nick by applying the blunt end of the razor blade along the nick. Both the dentine and RMGIC sides of the fractured beams were dehydrated by leaving the fractured beams to air-dry in a desiccator, sputter-coated with gold/palladium, and examined using a scanning electron microscope (Cambridge Stereoscan 360, Cambridge, UK) operating at 10–20 kV.

As it is impossible to prevent artefactual crack formation within the RMGIC matrices when desiccated specimens are examined under the high vacuum conditions of a conventional SEM, FE-ESEM was further employed for examining wet, non-dehydrated specimens in order to confirm the SEM results. We retained the use of a conventional SEM because of the limitation in taking low magnification micrographs of the entire fractured beam surface once the FE-SEM was fitted with a gaseous secondary electron (GSE) detector. Five additional beams from each group were used for FE-ESEM examination. The beams were fractured immediately before placement into the microscope chamber to prevent the loss of moisture and the creation of artefacts that could be caused by

breaking the seal of the resin-coated beams. After on-site fracture, each beam was mounted on carbon tape, inserted immediately into the Peltier (cooling) stage of a field emission-ESEM (Philips XL-30 ESEM-FEG, Eindhoven, The Netherlands), and examined wet and without coating at 20 kV using the GSE detector. The temperature of the specimen chamber was fixed at 5 °C and the vapour pressure was maintained at 6.6 Torr to achieve a 100% relative humidity.¹⁵

Although the use of tandem scanning confocal microscopy with fluorescent markers has been effective in capturing the dynamic fluid interactions between materials and tooth substrates with minimal sample preparation,^{16–18} its limited resolution does not allow submicron characterisation of the restoration-tooth interfaces.^{19,20} FE-ESEM examines the surfaces of moist, unfixed specimens with depth, resolution and magnification equivalent to that acquired by SEM.²⁰ The crucial difference between SEM and FE-ESEM is the differential pumping system of FE-ESEM, that can maintain different water pressure regimes inside the microscope to achieve the desired relative humidity.^{21,22} The presence of moisture in the specimen chamber helps to reproduce the actual clinical condition more accurately and eliminates the problems associated with dehydration in conventional high vacuum SEM. It is particularly suitable for the study of water-based RMGIC and its interaction with moist dentine.²³

TEM preparation

Five additional beams were randomly selected for each RMGIC. Intact beams (i.e. without fracturing) were supported with epoxy resin, but without infiltration, to facilitate specimen handling during ultramicrotomy, according to the TEM protocol for GIC examination described by Tay et al.²⁴ Undemineralised sections of 90–120 nm thickness were collected with single-slot, carbon- and formvar-coated copper grids (Electron Microscopy Sciences, Fort Washington, PA, USA), and examined without staining, using a transmission electron microscope (Philips EM208S, Eindhoven, The Netherlands) operating at 80 kV.

After TEM examination, energy dispersive X-ray (EDX) analysis was used to determine the elemental composition of various structural phases identified by TEM along the fractured RMGIC/dentine interfaces, similar to the protocol described by Hatton and Brook for EDX analysis of GICs.²⁵ No freeze substitution was employed, as the restorative material examined was already resin-based. Previously examined TEM grids containing the features

of interest were further coated with carbon and examined with another TEM (Philips Technai 12) equipped with an EDX analyser (EDAX Inc., Mahwah, NJ, USA) at 80 kV. Spot analyses were performed using the EDX software TEM Quant Biological (EDAX Inc.). As required by the software, the elemental compositions of the grids and the formvar films were first analysed in sequence, prior to spot analyses of the tooth-material sections.

Results

Representative SEM micrographs taken from dentine bonded with Fuji II LC are shown in Fig. 1. When fractures occurred adjacent to the cement/dentine interface, distinct spherical bodies could be observed in almost every air void that was trapped within the resin matrix (Fig. 1a). At a higher magnification, partially fractured spherical bodies revealed their solid internal structure (Fig. 1b). The average diameter of these spherical bodies was about 5–10 μm . Artefactual dehydration cracks that were formed as a result of desiccation and examination under high vacuum conditions could also be seen within the resin matrices (Fig. 1b). No spherical bodies were observed in specimen beams restored with the same RMGIC that were deliberately fractured within the RMGIC at 3 mm away from the bonded dentine surface. Only empty air voids were present within the resin matrices (Fig. 1c).

Representative FE-ESEM micrographs taken from dentine bonded with Photac-Fil Quick are shown in Fig. 2. Similar spherical bodies were observed along the fractured RMGIC/dentine interfaces. An intact, perfectly spherical body that was closely adapted to the surrounding resin matrix is illustrated in Fig. 2a. It could easily be differentiated from the adjacent angular FASG fillers. Under FE-ESEM examination, partially fractured spherical bodies were also found to be solid in appearance (Fig. 2b). Cracks were also observed within the resin matrices (not shown), even when the specimens were fractured on site and examined at 100% relative humidity. Spherical bodies were completely absent, and only empty air voids could be found in bonded RMGICs that were fractured within the RMGIC at a distance of 3 mm away from the bonded interfaces (Fig. 2c).

TEM micrographs taken from intact beams of RMGIC bonded dentine are shown in Fig. 3. Along the cement/dentine interface, an 8–10 μm thick, resin-rich absorption layer that was devoid of FASG fillers could be seen between the partially

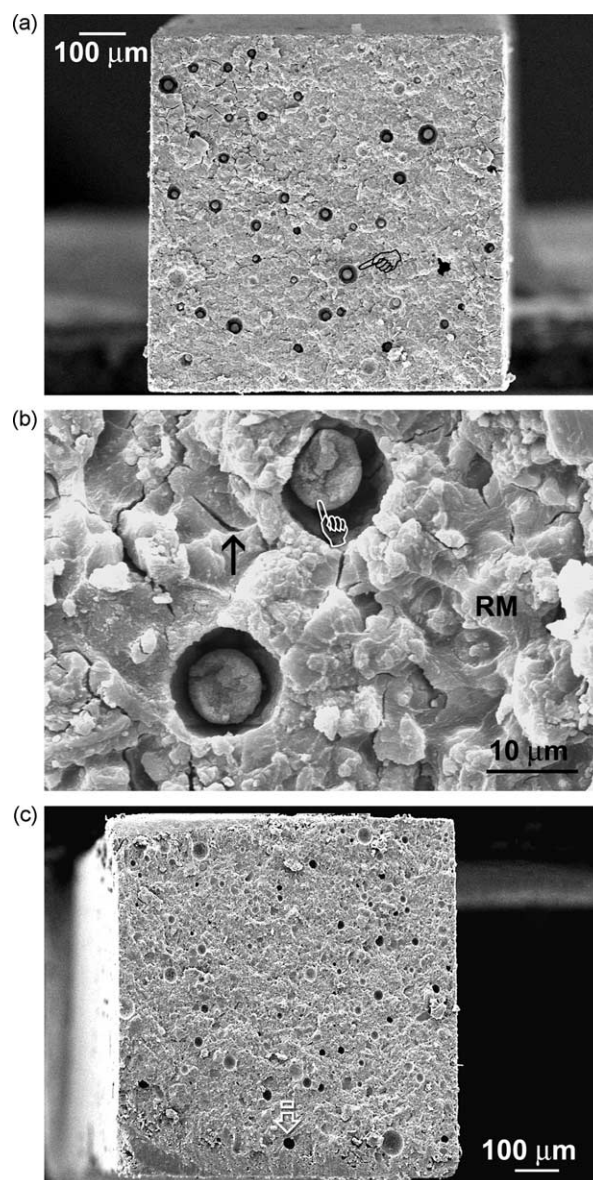


Figure 1 SEM micrographs of Fuji II LC bonded to moist dentine. The RMGIC side of fracture beams was shown in these micrographs. (a) When fracture occurred naturally adjacent to the RMGIC/dentine interface, spherical bodies (pointer) could be seen within almost every air void of the fractured RMGIC. (b) A high magnification view of a specimen that was fractured adjacent to the RMGIC/dentine interface, showing partially fractured, solid spherical bodies (pointer) within the air voids. Artefactual dehydration cracks (arrow) could be seen within the resin matrix (RM). (c) RMGIC side of dentine bonded with Fuji II LC that was deliberately fractured 3 mm distant from the bonded interface. No spherical body could be identified within the air voids (open arrow) were exclusively observed.

demineralised hybrid layer and the particulate RMGIC. A representative example taken from Fuji II LC is illustrated in Fig. 3a. Similar absorption layers were also identified from the cement/dentine

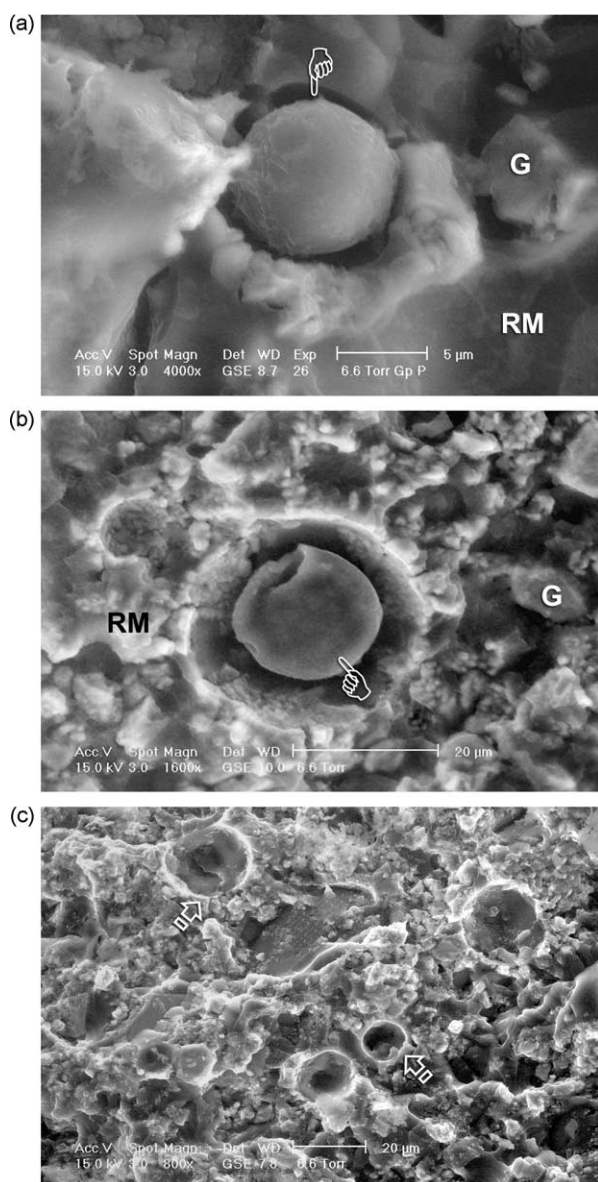


Figure 2 FE-ESEM micrographs of Photac-Fil Quick bonded to moist dentine. The non-dehydrated specimens were examined wet at 5 °C and 6.6 Torr (100% relative humidity). (a) Fracture that occurred naturally adjacent to the RMGIC/dentine interface. An intact spherical body (pointer) could be seen, and was distinctly different from the angular glass filler particles (G) that were found within the resin matrix (RM). (b) The solid nature of the spherical bodies was further confirmed with ESEM, as illustrated in this partially fractured spherical body (pointer) that was located adjacent to the RMGIC/dentine interface. G: glass filler; RM: resin matrix. (c) Photac-Fil Quick that was deliberately fractured 3 mm away from the bonded interface. Only empty air voids (open arrows) were observed.

interfaces in Photac-Fil Quick (not shown). In addition, spherical bodies were also observed within the particulate RMGICs when they were examined close to the bonded dentine. A sectioned spherical

body from Fuji II LC revealed a solid internal structure (Fig. 3b), consisting of a slightly electron-dense amorphous material that was intermingled with fragments of fractured glass particles and resin matrices. Similar to the SEM and FE-ESEM results, only empty air voids could be observed in resin matrices of Fuji II LC that were examined 3 mm distant from the bonded interfaces (Fig. 3c). A wide variation in the contents of the spherical bodies was observed as illustrated by examples taken from the examination of Photac-Fil Quick. The contents of the spherical bodies varied from predominantly amorphous electron-dense material (Fig. 3d), to a conglomerate that included fractured pieces of resin matrices and FASG fillers that were detached from the bulk of the RMGIC during ultramicrotomy and haphazardly attaching to the amorphous electron-dense material (not shown, similar to Fig. 3b). At a higher magnification, a more electron-dense layer could be identified along the periphery of the spherical body (Fig. 3e).

Representative EDX spectra taken from the FASG fillers, resin matrix, electron-dense periphery of the spherical body and the amorphous material within the spherical body are shown in Fig. 4. Cu, N and Mb (outside the range depicted by these spectra, data on file) were universally observed from all the spectra, with the first element corresponding to the composition of the copper grids, and the latter two elements corresponding to the composition of the formvar grid. Ca was only present in the resin matrix (Fig. 4b) and could not be found in the FASG filler (Fig. 4a) or the spherical bodies (Fig. 4d). The electron-dense periphery of the spherical body had a similar chemical composition as the surrounding resin matrix, but with a higher Al and Si content (Fig. 4c). The amorphous material within the spherical body was found to be organic in nature, consisting only of the elements C, and O (Fig. 4d).

Discussion

As the previously unreported spherical bodies were only observed adjacent to RMGIC/dentine interfaces and not within RMGICs that were remote from the moist dentine, we have to reject the null hypothesis that there is no difference in the ultrastructure of RMGICs fractured at different locations away from the bonded dentine. The association of these spherical bodies with the RMGIC/dentine interface, and their close resemblance of their composition with that of the absorption layer, suggested that both features

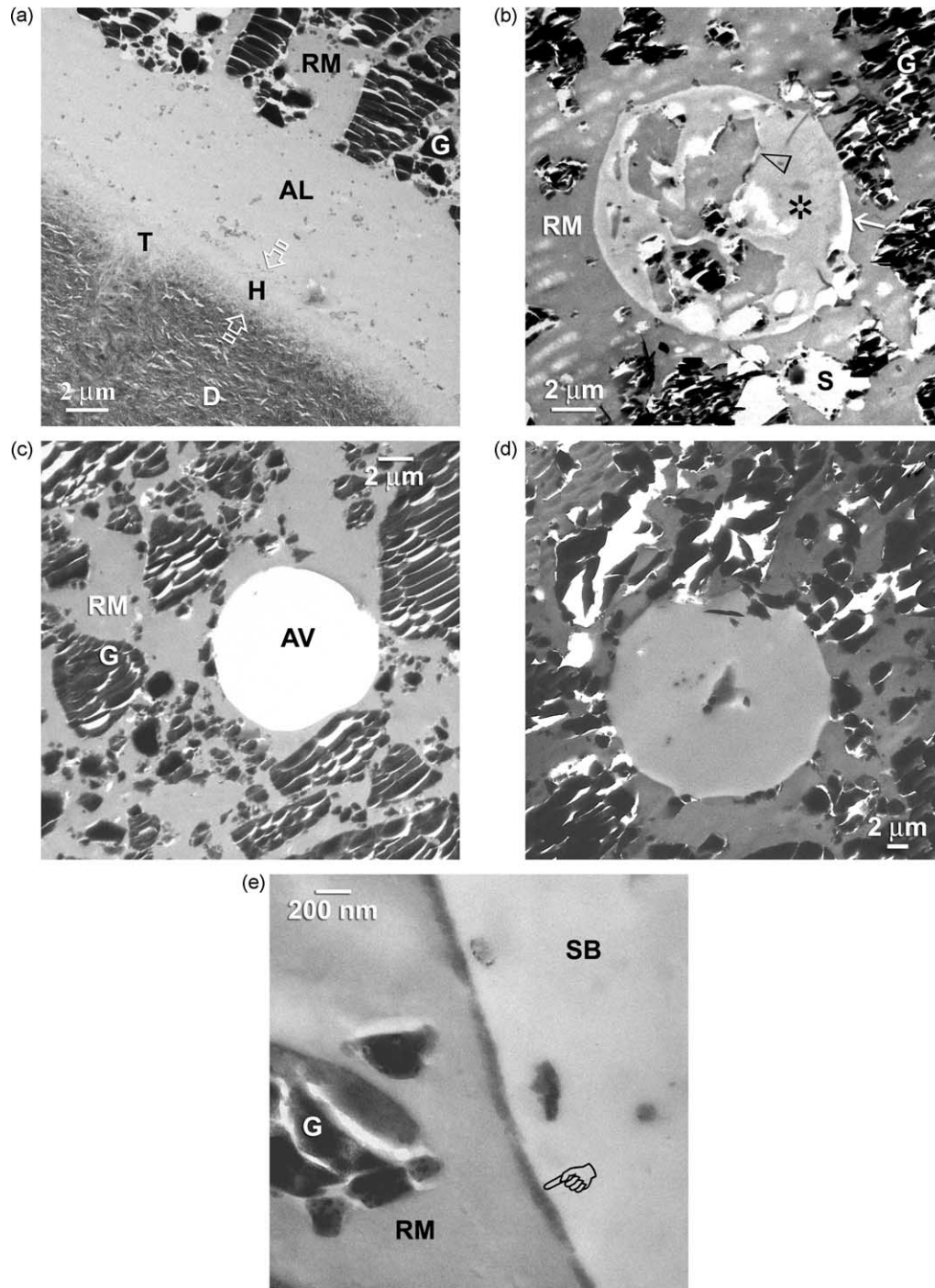


Figure 3 Unstained, undemineralised TEM micrographs of intact, unfractured RMGIC/dentine interfaces. The specimens were sectioned without infiltration with epoxy resin. Striations within the glass fillers (G) were artefacts caused by sectioning of the brittle material with a diamond knife; RM: resin matrix. (a) The RMGIC/dentine interface in Fuji II LC showing a distinctive resin-rich, non-particulate absorption layer (AL) between the hybrid layer (H; between open arrows) and the particulate RMGIC. T: dentinal tubule. D: intertubular dentine. (b) Fuji II LC that was examined adjacent to the bonded interface. A spherical body containing a slightly electron-dense material (asterisk) could be seen, in which part of this material has been detached from the resin matrix (arrow) as a result of dehydration. Additional fractured glass particles and resin matrix debris were also observed (open arrowhead) within the spherical body. (c) Fuji II LC that was examined 3 mm away from the bonded interface. Only empty air voids (AV) could be observed. (d) A spherical body observed in Photac-Fil Quick that contained a slight amount of fractured resin matrices and glass fillers within the spherical body. (e) A high magnification view of a spherical body, showing the existence of an electron-dense layer (pointer) around the periphery of the spherical body (SB).

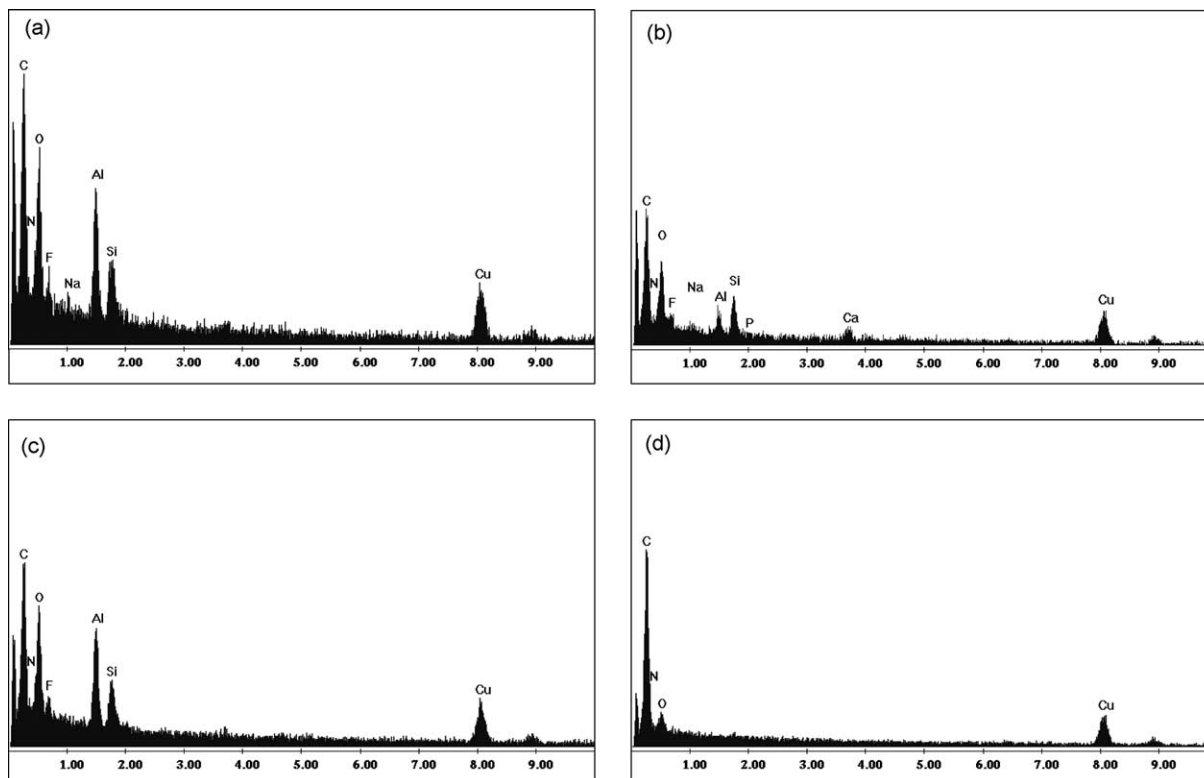


Figure 4 EDX spectrum of the composition of the various regions depicted in Fig. 3e. The element 'copper' originated from the copper grid. (a) Glass filler. (b) Resin matrix. (c) Electron dense periphery of the spherical body. (d) The amorphous material (i.e. without fractured resin matrices and glass fillers) within the spherical body.

were formed by a similar mechanism that is dependent upon the availability and diffusion of water from the underlying moist dentine. The term absorption layer was used by Sidhu and Watson¹¹ and Sidhu et al.¹³ to represent its association with water sorption within the maturing RMGICs when these materials were placed in deep, moist dentine. Diffusion of HEMA from the resin matrices of the RMGICs into the water-rich dentine surface probably resulted in the polymerization of the HEMA in the form of a soft poly(HEMA) hydrogel layer.²⁶ Based on this mechanism of formation of the absorption layer, we speculate that the internal core of the spherical bodies were also formed of a soft poly(HEMA) hydrogel. As water can diffuse from underlying moist dentin into HEMA in the resin matrix to form the absorption layer, it is not unreasonable to assume that diffusion of both water and HEMA may also occur into empty air voids that are close to the RMGIC/dentine interfaces. Presumably, these voids would become filled with water as the hydrophilic resins and polyalkenoic salt matrix create an osmotic gradient that draws water from the underlying dentine. Polymerisation of the HEMA in the presence of water may produce a soft hydrogel within the air

voids that results in the formation of the core of the spherical bodies. This probably provided an explanation for the putatively sticky nature of the spherical bodies that caused loose pieces of fractured resin matrices and FASG fillers to be transferred to the sectioned plane of the spherical bodies during ultramicrotomy. The absence of metallic ions within the core of the spherical bodies, as demonstrated by the EDX spectrum, further supported our interpretation that the cores of these spherical bodies were formed of poly(HEMA) rather than metallic salts of polyalkenoic acid.

It is interesting that the periphery of the spherical bodies was formed of a very thin shell that is rich in Al and Si. This suggested that there is probably a continuing GI reaction during the maturation phase of the RMGIC. As water is not available from the external environment, the water required for the additional GI reaction may be derived from the surrounding resin matrix, or from bound water within the poly(HEMA) hydrogel. We have previously observed the existence of spherical bodies in six conventional GICs.²⁷ However, unlike the spherical bodies found in RMGICs, those that were observed in conventional GICs were consistently hollow in

appearance, consisting only of a thicker layer of the metallic ion-rich peripheral layer. We believe that the spherical bodies observed in conventional GICs and RMGICs were formed by a similar mechanism, in response to water permeation from the underlying moist dentine. However, as HEMA is absent in conventional GICs, the spherical bodies so formed must necessarily be hollow, comprising only of the peripheral layer of the silicon-rich phase that was formed during the aging of GICs.^{28,29} Conversely, photopolymerisation of the HEMA greatly reduces the acid-base reaction during the early setting reaction of RM-GIC.³⁰ As a result, the acid-base reaction takes place at a slower rate in the air-void with deposition of only a thin film of silicon- and aluminum-rich phase around the poly(HEMA) hydrogel.

As the specimens in the present study were immediately sealed with resins, the only source of water available was that derived from the underlying dentine. This explains why the spherical bodies and the absorption layer were formed adjacent to the moist dentine, and not along the 'free' outer surfaces of the restorations. Water sorption in RMGICs is influenced by the HEMA content.³¹ HEMA is added to the RMGIC liquid as a co-solvent for the organic and aqueous components. The concentration of HEMA in RMGIC liquids was found to vary between 18 and 32%³² (35-40 wt% for Fuji II LC and 25-50 wt% for Photac-Fil Quick, based on the MSDS information supplied by the respective manufacturer). HEMA improves the physical properties of RMGICs^{33,34} as well as their bond strength to dentine.³⁵ The manifestation of spherical bodies in RMGICs may be analogous to the phenomenon of 'osmotic blistering' that was recently reported with the use of single-step self-etch adhesives on moist dentine.³⁶ In osmotic blistering, water diffuses from the moist dentine and form water droplets along the adhesive-composite interface, with the adhesive layer acting as a permeable membrane. In the context of RMGICs, it is possible that the hydrophilic resin matrix containing polyalkenoate salts create an osmotic gradient that attracts water into the RMGIC. As HEMA is a monofunctional monomer, water movement is probably enhanced by a more loosely-arranged, non-cross-linked resin matrix.

It is worth mentioning that no spherical bodies were observed along fractured RMGIC/dentine interfaces when Vitremer (3M ESPE) was bonded to moist dentine (Yiu, unpublished results). This is likely to be due to the compositional differences among various RMGICs. Vitremer uses a self-etching primer that contains HEMA and a copolymer of

polyacrylic acid with a photoinitiator. The primer is applied on the smear-layer covered dentine, which is then air-dried and light-cured. As the smear layer and plugs are partially present, the permeability of the moist dentine is probably significantly reduced.³⁷ The set cements of Fuji II LC and Photac-Fil consist mainly of crosslinked polyalkenoate networks, entangled with non-crosslinked poly(HEMA) chains. In Vitremer, however, the polyalkenoate network and the polymer chains are further connected by means of pendant methacrylate groups on the polyalkenoate molecules to form ionic and covalent crosslinks.³³ The presence of a more highly crosslinked polymer network and the decreased availability of free HEMA may restrict water permeation across the Vitremer-moist dentine interface.

It is usual to observe artefactual cracks within fractured RMGICs when specimens were examined under SEM. Similar artefacts were seen when water-based GICs were examined in a high vacuum, desiccated environment.^{38,39} However, it was rather surprising to see similar cracks when the RMGICs were examined under FE-ESEM. Conversely, no artefactual cracks could be observed in the polyalkenoate matrices of conventional GICs when similarly fractured specimens were examined under FE-ESEM.²⁷ Thus, we speculate that the cracks identified by FE-ESEM were not simply artefacts caused by dehydration shrinkage. The unusual findings of cracks in RMGICs may be attributed to the 'intrinsic setting shrinkage' inherent in RMGICs following the free-radical setting reaction^{40,41} and 'self-desiccation' of the developing matrix that may occur as a result of the depletion of water that is present in the original cement formulation for acid-base reaction.⁴² It is known that acid-base reaction proceeds more slowly in RMGICs when compared to conventional GICs, as some of the water has been replaced by HEMA. Two separate phases of GI reaction have been proposed for RMGICs.⁴² The initial phase involves the use of water from the original cement formulation, and terminates as the intrinsic water is consumed. The subsequent phase of GI reaction depends on water absorption from the environment. As the RMGIC specimens in the study were sealed immediately after light-activation to prevent environmental water gain or loss, self-desiccation of the RMGICs may occur, in the absence of sufficient water from an external source to relax the stress developed in final setting matrix. Conversely, the conventional GIC sets more slowly and the initial rubbery hydrogel stage that the cement passes through during the setting process allows for stress relief.

Although the clinical implications of the presence of spherical bodies along the RMGIC/dentine interface is not fully understood, it is hypothesized that these bodies, similar to the absorption layer, may serve to deflect or blunt any cracks that attempt to propagate through the matrix, thereby toughening the material. The spherical bodies may play an adjunctive role by obliterating porosities in the resin matrix adjacent to the dentine and delay the growth of inherent cracks to catastrophic sizes in this region under loading.⁴³ It has been shown that the mode of failure of RM-GIC restoration is usually cohesive within the RMGIC/absorptive layer,¹² with the absorption layer remaining firmly attached to dentine. On the basis of these findings, it is dubious whether RMGICs should really be protected from initial water uptake,⁴⁴ in contrast to conventional GICs in which this is essential. Clinical studies have shown that Fuji II LC exhibited 100% retention rate in class V cavities after 2 years when bonding was applied to moist dentine with low RMGIC powder/liquid ratio.⁴⁵ Reduced post-operative sensitivity has been observed in class I cavities lined with a RMGIC as compared with resin bonding at 24 h and 7 days post-operatively.⁴⁶ The formation of the absorption layer and the spherical bodies adjacent to the RMGIC/dentine interfaces may be viewed upon as a 'autonomic self-healing' mechanism⁴⁷ to compensate for the setting stresses of RM-GICs and to prevent spontaneous fracture at the bonded interfaces. Such a capacity is definitively lacking in bonding techniques that rely on the use of contemporary dentine adhesives.⁴⁸ Further studies are required to investigate the long-term effects of spherical bodies and water absorption on physical properties of the bond between RMGICs and dentine.

Conclusions

Within the limitations of the present in vitro study, it may be concluded that:

1. The spherical bodies in air voids in RMGICs represented a continuation of GI reaction and poly(HEMA) hydrogel formation.
2. The spherical bodies were formed at the RM-GIC/dentine interface as a result of water diffusion from the underlying moist dentine.
3. Together with the previously reported absorption layer, the existence of the spherical bodies provides morphological evidence for absorption of water along the RMGIC/dentine interfaces.

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References

1. Wilson AD. Developments in glass-ionomer cements. *International Journal of Prosthodontics* 1989;2:438–46.
2. Mitra SB. Adhesion to dentin and physical properties of a light-cured glass-ionomer liner/base. *Journal of Dental Research* 1991;70:72–4.
3. Sidhu SK, Watson TF. Resin-modified glass ionomer materials. A status report for the American Journal of Dentistry. *American Journal of Dentistry* 1995;8:59–67.
4. Nicholson JW, Anstice HM, McLean JW. A preliminary report on the effect of storage in water on the properties of commercial light-cured glass-ionomer cements. *British Dental Journal* 1992;173:98–101.
5. Wilson AD. Resin-modified glass-ionomer cements. *International Journal of Prosthodontics* 1990;3:425–9.
6. Lin A, McIntyre NS, Davidson RD. Studies on the adhesion of glass-ionomer cements to dentin. *Journal of Dental Research* 1992;71:1836–41.
7. Carvalho RM, Yoshiyama M, Horner JA, Pashley DH. Bonding mechanism of VariGlass to dentin. *American Journal of Dentistry* 1995;8:253–8.
8. Pereira PN, Yamada T, Tei R, Tagami J. Bond strength and interface micromorphology of an improved resin-modified glass ionomer cement. *American Journal of Dentistry* 1997;10:128–32.
9. Glasspoole EA, Erickson RL, Davidson CL. Effect of surface treatments on the bond strength of glass ionomers to enamel. *Dental Materials* 2002;18:454–62.
10. Tanumiharja M, Burrow MF, Cimmino A, Tyas MJ. The evaluation of four conditioners for glass ionomer cements using field-emission scanning electron microscopy. *Journal of Dentistry* 2001;29:131–8.
11. Sidhu SK, Watson TF. Interfacial characteristics of resin-modified glass-ionomer materials: a study on fluid permeability using confocal fluorescence microscopy. *Journal of Dental Research* 1998;77:1749–59.
12. Sidhu SK, Sherriff M, Watson TF. Failure of resin-modified glass-ionomers subjected to shear loading. *Journal of Dentistry* 1999;27:373–81.

13. Sidhu SK, Pilecki P, Cheng PC, Watson TF. The morphology and stability of resin-modified glass-ionomer adhesive at the dentin/resin-based composite interface. *American Journal of Dentistry* 2002;15:129–36.
14. Koibuchi H, Yasuda N, Nakabayashi N. Bonding to dentin with a self-etching primer: the effect of smear layers. *Dental Materials* 2001;17:122–6.
15. Stokes DJ, Rea SM, Porter AE, Best SM, Bonfield W. Characterization of biomedical materials, cells and interfaces using environmental SEM (ESEM). *Materials Research Society Symposium Proceedings* 2002;711:113–8.
16. Watson TF, Billington RW, Williams JA. The interfacial region of the tooth/glass ionomer restoration: a confocal optical microscope study. *American Journal of Dentistry* 1991;4:303–10.
17. Watson TF, Pagliari D, Sidhu SK, Naasan MA. Confocal microscopic observation of structural changes in glass-ionomer cements and tooth interfaces. *Biomaterials* 1998;19:581–8.
18. Watson TF, Azzopardi A, Etman M, Cheng PC, Sidhu SK. Confocal and multi-photon microscopy of dental hard tissues and biomaterials. *American Journal of Dentistry* 2000;13:19D–24D.
19. Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Perdigão J, Lambrechts P, Vanherle G. Microscopy investigations. Techniques, results, limitations. *American Journal of Dentistry* 2000;13:3D–18D.
20. Royall C, Stokes D, Hopkinson I, Donald A. Confocal microscopy and environmental SEM for polymers. *Polymer News* 2001;26:226–33.
21. Danilatos GD. Introduction to the ESEM instrument. *Microscopy Research and Technique* 1993;25:354–61.
22. Donald AM. Environmental scanning electron microscopy for the study of 'wet' systems. *Current Opinion in Colloid and Interface Science* 1998;3:143–7.
23. Cowan AJ, Wilson NHF, Wilson MA, Watts DC. The application of ESEM in dental materials research. *Journal of Dentistry* 1996;24:375–7.
24. Tay FR, Pashley EL, Huang C, Hashimoto M, Sano H, Smales RJ, Pashley DH. The glass-ionomer phase in resin-based restorative materials. *Journal of Dental Research* 2001;80:1808–12.
25. Hatton PV, Brook IM. Characterisation of the ultrastructure of glass-ionomer (poly-alkenoate) cement. *British Dental Journal* 1992;173:275–7.
26. Chirila TV, Chen YC, Griffin BJ, Constable IJ. Hydrophilic sponges based on 2-hydroxyethyl methacrylate I: effect of monomer mixture composition on the pore size. *Polymer International* 1993;32:221–32.
27. Yiu CKY, Tay FR, King NM, Pashley DH, Sidhu SK, Neo JCL, Toledano M, Wong SL. Interaction of glass-ionomer cements with moist dentin. *Journal of Dental Research* 2004;83:289–95.
28. Wasson EA, Nicholson JW. New aspects of the setting of glass-ionomer cements. *Journal of Dental Research* 1993;72:481–3.
29. Matsuya S, Maeda T, Ohta M. IR and NMR analyses of hardening and maturation of glass-ionomer cement. *Journal of Dental Research* 1996;75:1920–7.
30. Kakaboura A, Eliades G, Palaghias G. An FTIR study on the setting mechanism of resin-modified glass ionomer restoratives. *Dental Materials* 1996;12:173–8.
31. Yap AU. Resin-modified glass ionomer cements: a comparison of water sorption characteristics. *Biomaterials* 1996;17:1897–900.
32. Ikeda K, Fujishima A, Suzuki M, Inoue M, Sasa R, Miyazaki T. Resin content in cement liquids of resin-modified glass ionomers. *Dental Material Journal* 1999;18:248–58.
33. Eliades G, Palaghias G. In vitro characterization of visible light-cured glass ionomer liners. *Dental Materials* 1993;9:198–203.
34. de Gee AJ, Leloup G, Werner A, Vreven J, Davidson CL. Structural integrity of resin-modified glass ionomers as affected by the delay or omission of light activation. *Journal of Dental Research* 1998;77:1658–63.
35. Ruzs JE, Antonucci JM, Eichmiller F, Anderson MH. Adhesive properties of modified glass-ionomer cements. *Dental Materials* 1992;8:31–6.
36. Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. Single-step adhesives are permeable membranes. *Journal of Dentistry* 2002;30:71–82.
37. Pashley DH, Livingston MJ, Greenhill JD. Regional resistance to fluid flow in human dentine, in vitro. *Archives of Oral Biology* 1978;23:807–10.
38. Xie D, Brantley WA, Culbertson BM, Wang G. Mechanical properties and microstructure of glass-ionomer cements. *Dental Materials* 2000;16:129–38.
39. Yip HK, Tay FR, Ngo HC, Smales RJ, Pashley DH. Bonding of contemporary glass ionomer cements to dentin. *Dental Materials* 2001;17:456–70.
40. Feilzer AJ, Kakaboura AI, de Gee AJ, Davidson CL. The influence of water sorption on the development of setting shrinkage stress in traditional and resin-modified glass ionomer cements. *Dental Materials* 1995;11:186–90.
41. Watts DC, Kisumbi BK, Toworfe GK. Dimensional changes of resin/ionomer restoratives in aqueous and neutral media. *Dental Materials* 2000;16:89–96.
42. Young AM. FTIR investigation of polymerisation and polyacid neutralisation kinetics in resin-modified glass-ionomer dental cements. *Biomaterials* 2002;23:3289–95.
43. Inglis CE. Stresses in a plate due to the presence of cracks and sharp corners. *Proceedings of the Institute of Naval Architects* 1913;55:219–41.
44. Watson TF, Sidhu SK, Griffiths BM. Ionomers vs. composites at the tooth interface. In: Hunt P, editor. *Proceedings of the Second International Symposium on Glass Ionomer Cements. International Symposia in Dentistry, Philadelphia*; 1994. p. 123–30.
45. Wilder AD, Boghosian AA, Bayne SC, Heymann HO, Sturdevant JR, Roberson TM. Effect of powder/liquid ratio on the clinical and laboratory performance of resin-modified glass-ionomers. *Journal of Dentistry* 1998;26:369–77.
46. Akpata ES, Sadiq W. Post-operative sensitivity in glass-ionomer versus adhesive resin-lined posterior composites. *American Journal of Dentistry* 2001;14:34–8.
47. Davidson CL. Glass ionomer cement, an intelligent material. *Bulletin du Groupement International pour la Recherche Scientifique en Stomatologie et Odontologie (Bruxelles)* 1998;40:38–42.
48. Tay FR, Pashley DH. Dental adhesives of the future. *Journal of Adhesive Dentistry* 2002;4:91–103.