Marginal integrity between a prefabricated composite veneer and enamel, DEJ and dentine bonded by three adhesive resins

Short title: Evaluation of leakage-free restoration

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Abstract

Purpose: The stability of the interfacial layer and leakage patterns between enamel, DEJ, dentine and three adhesive systems as demonstrated by either a dye or a silver nitrate technique were examined to understand the marginal integrity of restored tooth substrates.
Material and Methods: Extracted human molars were cross-sectioned to prepare enamel, DEJ and dentine surfaces. Areas with the DEJ aligned in the middle were restored with prefabricated flat composite veneers using either Superbond C&B (SB), Panavia Fluorocement (PN) or Variolink II (VL) cements. Seven restored specimens for each cement were prepared to investigate the interfacial layer after chemical challenge using SEM. Sixteen restored and 16 control specimens for each cement were prepared for leakage tests by soaking either in 0.5% basic fuchsin or 50% silver nitrate solutions for 24 h. The penetration depth of tracer was measured using stereoscopy and a digital micrometer. An SEM backscattered electron image and energy dispersive x-ray spectrometry were used to detect the location of the silver.
Results: Prepared SB hybridised enamel, dentine and DEJ were stable against soaking in HCl followed by NaOCl solutions whereas prepared hybrids of DEJ and dentine were degraded, detached and thinner than that of enamel in VL and PN specimens. No leakage was found at the enamel-resin interface for all cements and at the DEJ- and dentine-resin interfaces for SB. Variolink specimens showed significantly greater leakage at the interface of dentine and DEJ than did PN specimens.
Significance: The stable hybridised tooth substrates in SB specimens were effective in inhibiting the demineralisation of hydroxyapatite and degradation of collagen suggesting leakage-free restoration margins. On the other hand, it was difficult to prepare impermeable barrier to dentine and DEJ in PN and VL specimens. A visualized leakage test with characterising tooth-resin interfaces is promising to expect the longevity of restored abutment against lactic acid demineralisation in oral cavity.

Keywords: impermeable barrier, hybridised dentine, leakage free, tracer as a model of lactic acid, adhesive resin cements.

Introduction

Previous studies have not shown that demineralisation of prepared dentine due to marginal percolation of lactic acid is a serious problem in dental care. Postoperative sensitivity, marginal discoloration, short-term detachment of restorations or prostheses, secondary caries and pulpal pathology sometimes occur after direct restorative and fixed prosthodontic dental treatment. One of the major contributors to these signs and symptoms is explained by leakage between restorative materials and dental hard tissues.[1-2] True adhesion between restorative materials and tooth structure to prevent microleakage has been the holy grail of dental restorative treatment for many decades.[3] The types of restorative materials and the accuracy of prostheses have been discussed as known contributors to the leakage.[3-6] Recently, it was reported that the microleakage, assessed by dye penetration, had taken place in demineralised dentine underneath set acid-base cements and in the remaining demineralised dentine because of incomplete infiltration of the resin.[7, 8]

Long-term success of restorations and fixed prostheses depending upon adequate retention had long been believed. Molecular entanglement by interpenetration of a polymer network into tooth substrate to form a hybrid layer provides better retention and marginal seal for restorative materials and is a technique which has been widely used over the last 20 years.[9,10] Dentine demineralised by H_3PO_4 collapses when air dried,[11] resulting in permeable demineralised dentine which allows dye penetration.[8] It is important to eliminate this permeable demineralised dentine, but it is difficult to identify its existence by tensile testing.[12] Controlling the water content of H_3PO_4-etched dentine in order to provide good bonding by monomer impregnation in wet bonding procedures was also difficult.[13,14] Water soluble dentinal polyelectrolytes (acidic non-collagenous proteins) were reported to play an important role in the collapse,[15] and the rate of demineralisation and dehydration of demineralised dentine which were quantitatively measured.[16] In intact dentine these polyelectrolytes are solidified with hydroxyapatite crystals, but they are dissolved when demineralised and are keen compounds in Caries Detector (Kuraray Co, Tokyo, Japan) and/or dyes for microleakage testing to visualise demineralised dentine. Conditioners which contain cations such as trivalent ferric ions can maintain the permeability of demineralized dentine to water and monomers by immobilization of the water soluble polyelectrolytes.[17,18] Thus impregnation of monomers into dentine is easier and results in a high quality hybridised dentine which contributes to the inhibition of leakage.[7,8]

Mostly bond strength data have been determined for adhesive resins; however such data provided only the mechanical property of bonded specimens, which are quite different and difficult to discuss the permeability and leakage. Bonding flaws, identified by silver deposition after soaking bonded specimens in silver nitrate solution, were reported to be degraded in the oral cavity over a period of 1-3 years,[19,20] but just what had degraded was not mentioned. Thus high tensile bond strength data using a testing method, which could not identify bonding defects may not predict the longevity of a restored tooth. In laboratory studies the prepared bonding interface should be characterised by soaking in acid followed by an NaOCl solution to show the stability of newly formed intermediate layer between the cured adhesive and tooth substrate. Otherwise, demineralized dentine which is not impregnated with adhesive resins may be mistaken for the hybrid layer.[19] Demineralised dentine, which is mainly collagen can be hydrolysed in the mouth thus limiting the longevity of the restoration. Hybridised dentine has been shown to be an impermeable layer *(or artificial enamel)*, which can reliably inhibit marginal leakage when exposed to acids such as lactic acid, which can demineralise hydroxyapatite and to resist against degradation with NaOCl. This may be an explanation for the development of secondary caries under restorations.[12] If the hybrid layer is permeable to stains and dyes,[21] careful examination may reveal the presence of secondary caries.

The characteristics of the hybrid layer can be examined using SEM or TEM without epoxy embedding, before and after sequential challenge with acid (HCl) and NaOCl, to demonstrate the resin content in conditioned enamel, DEJ and dentine.[8,12,22] Epoxy embedded specimens may give erroneous results as the epoxy resin may reinforce the bonded specimens and, of course, epoxy embedding cannot be applied in the clinical situation. How the amount and region of leakage at the enamel, DEJ, and dentine-resin interfacial layers relates to differences in the characteristics of their hybrid layers also has not been well studied. The hypothesis of this study was that identification of impermeable hybridised-dentine was difficult to detect by simple tensile testing but the bonding stability could be characterized by serial chemical modification of the restored dentine. If stable hybridised dentine is resistant to acid demineralisation and NaOCl degradation, this could provide leakage free restorations and may protect weakened prepared dentine in the mouth, which may make this type of dental treatment more reliable.

The objective of this study was to examine the characteristics of the enamel-, DEJ- and dentine-resin interfaces using three different resin systems before and after chemical modification, as related to the amount and region of leakage revealed by basic fuchsin dye and silver nitrate penetration methods.[23]

Materials and Methods

Preparation of resin composite veneers

Resin composite veneers of 2 x 4 x 1 mm were fabricated from a light-cured composite (3M Filtek, P60, 3M, USA) in a standardised mould. Each specimen was finished with silicon carbide paper #400 and #600 (Buehler-MET, USA) respectively. All the inner surfaces for bonding were grit blasted with 50μm alumina particles.

Preparation of restored and control specimens (Fig. 1)

Extracted sound human molars showing no evidence of cracks were selected and root-embedded in acrylic blocks (Formatray, Kerr, USA). A 2 mm occlusal portion (Fig. 1-T1) was horizontally sectioned off using a sectioning machine (Isomet 1000 series 15, Buehler, USA) to expose surface, which was then abraded with #400 and #600 grit silicon carbide paper using a digital polishing system (Imptech, DPS3200, South Africa). Four prepared surfaces of 2 x 4 mm were outlined on each axial surface with the DEJ in the middle and 1 mm of enamel and dentine on either side. One of three resin cements: 1) Superbond C&B (Sun-Medical Co., Shiga, Japan) or C&B METABOND (Parkell Co., Edgewood, NY), 2) Panavia Fluorocement (Kuraray Co., Osaka, Japan), or 3) Variolink II (Ivoclar, Vivadent, North America), was randomly selected to fix the composite veneers on top of the outlined surfaces for each tooth (Fig. 1-T2). The manipulation of each resin system followed the manufacturer's recommendations (Table 1). The restored (Fig. 1-T2) and the control slabs (Fig. 1-T3) of 1 mm thickness for each tooth were horizontally sectioned off with a sectioning machine and vertically trimmed at the same location using a fissure diamond with a high-speed hand piece under air-water spray to make 2 x 4 x 2 mm (Fig. 1-a) and 2 x 4 x 1 mm (Fig. 1-b) specimens respectively. Sixteen restored samples and 16 corresponding control specimens for each group of resin cements were prepared for leakage (permeation) testing. Seven restored samples of 2 x 4 x 2 mm were prepared for investigation of the tooth-resin interfacial areas using SEM. All specimens were stored in distilled water at 37°C for 24 h before examination.

Examination of tooth-resin interfacial areas

Each restored specimen was cross-sectioned into two pieces each 1 mm thick. The surface to be examined was finished with #600 and #1200 grit abrasive papers and finally polished with 0.05 μm alumina paste and then ultrasonically cleaned for 20 min. One piece was soaked in 6 mol/L HCl for 30 s and then 1% NaOCl for 60 min. All specimens were desiccated and gold sputtered for SEM examination. The characteristics of the newly formed interfacial layer between tooth (enamel, DEJ, dentine) and cured resin were examined on the polished and chemically challenged specimens at x 500 and x 2000 magnifications.

Tracer penetration tests

All surfaces of control and restored specimens were finished with #400 grit grinding paper and coated twice with nail varnish with the exception of the surface, which contained the enamel, dentine and DEJ in control specimens and their resin interfaces in restored specimens, for tracer penetration (Fig. 2). The specimens in each cement group were divided into two sub-groups, each composed of eight restored and eight control specimens. Each subgroup was subjected to either testing by immersion for 24 h in 0.5% basic fuschin dye or 50% silver nitrate solutions.

Restored specimens were sequentially abraded from the opposite side of the tracer penetration (Fig. 2, W) until the leakage at the tooth-resin interface could be seen under a light microscope at x 40 and x 200 magnifications. The distance of tracer penetration when first shown on each layer of enamel-, dentine- and DEJ-resin interfaces was measured using a digital micrometer (Mitutoyo, Tokyo, Japan). The residual thickness, which could not be abraded off for each restored specimen was recorded as the final cut distance (Fig. 2, F). In the control groups, the distances of tracer penetration into tooth structures were measured under a light stereo-microscope at x 40, x 200 and x 400 magnification on a horizontally ground surface.

The restored specimens treated with silver nitrate penetration were carbon coated for 1 min and the location of the silver distribution related to the bonding interface was examined at x 500 and x 2000 using a backscattered electron image and SEM attached energy dispersive x-ray spectrometer (JSM-5008LV, JEOL, Japan) to confirm the deposition area of silver.

Results

SEM micrographs of the newly formed layer in enamel, dentine and the DEJ after chemical modification using HCl and NaOCl for SB specimens was consistent and continuous for 2-3 μm (Fig. 3), whereas those in the dentine and the DEJ were too thin to identify compared with that of enamel in PN specimens (Fig. 4). In VL specimens, the enamel-resin interface was clearly identified while DEJ and dentine demineralized by H_3PO_4 and not impregnated by the resin was detached and degraded (Fig. 5).

Means and standard deviations for dye and silver nitrate penetration of control groups, leakage distances in restored specimens, and final cut distances are shown in Tables 2 and 3. Analysis of variance (ANOVA) and Scheffe's test found no significant difference between the silver nitrate penetration for the DEJ and dentine of control specimens ($p > 0.05$) (Table 3). No significant differences for penetrating distances in the enamel, dentine and DEJ of control specimens were found among cement groups either using dye or silver nitrate solutions. No significant differences for the final cut distances were found among the cement groups. No significant differences were found between the final cut distances and dye penetration in dentine of control specimens (Table 2).

No leakage using dye solution for the final cut was found at the enamel-resin interface for all cements, and at the dentine-resin and DEJ-resin interfaces for PN and SB restored specimens (Table 2, Fig. 6a). Statistically significantly greater dye leakage distance at the dentine-resin interface than for the DEJ-resin interfaces of VL restored specimens (Fig. 6b) was revealed ($p < 0.05$). No silver was found for the final cut at the enamel-resin interface for all cement groups, and in SB bonded specimen (Table 3, Fig. 7a). In PN specimens, the resin cement at the newly formed layer between the dentine-resin interface where the leakage occurred was stained by silver nitrate (Fig. 7b). The VL restored specimens showed significantly deeper leakage for silver nitrate than did PN restored specimens. Silver nitrate leakage distance was significantly deeper at the dentine than the DEJ-resin interfaces of VL groups (Fig. 7c). The restored specimens of SB showed no leakage either using dye or silver nitrate impregnation at any tooth-resin interfaces at the final cut distance (Figs. 6a, 7a).

SEM examination and energy dispersive x-ray spectrometer demonstrated no silver deposition at the tooth-resin interface of the final cut distance of SB-restored specimens (Fig. 8a). A distribution of silver in the tissue-resin interface of dentine and DEJ, and in the dentinal tubules was found in PN- and VL- restored specimens (Fig. 8b, 8c).

Discussion

The continuous and consistent thickness of the hybrid layer of enamel, DEJ and dentine after HCl and NaOCl immersion for SB restored specimens (Fig. 3a) suggested that this bonding system could provide impermeable and stable hybridised layers which can protect weak exposed dentine against acidic demineralisation and proteolytic degradation, and thus effectively inhibit secondary caries. The hybrid layer of the DEJ was clearly identified at a lower level than that of enamel and dentine. This suggested that the DEJ had a lower resistance to acidic demineralisation due to a lesser amount of hydroxyapatite than enamel and dentine. No dye or silver nitrate penetration at any tooth-resin interface was found at the final cut distance for SB restored specimens (Fig. 6a, 7a). Backscattered electron micrograph at the final cut distance of SB restored specimen demonstrated silver deposition from exposed dentinal tubules but no silver deposition at the interface of enamel, dentine, and DEJ was shown (Fig. 8a). A significantly less distance of final cuts for restored specimens in this group compared with natural penetration of silver nitrate for the control groups of DEJ and dentine was found (Table 3). These findings suggested that this impermeable hybridised dentine could efficiently inhibit leakage and block convection flow of dentinal fluid through dentinal tubules. This study used a green activator, which contained 10% citric acid and 3% ferric chloride in aqueous solution and 4-META/MMA-TBB resin in dry bonding. For the total conditioning of cut enamel, dentine and DEJ surfaces the solution was applied for 10 s (Table 1). The results suggested that 10 s conditioning with 10-3 was sufficient for impregnation of poly (4-META-co-MMA) to prevent leakage at the tooth-resin interface. No leakage occurred at the enamel- and dentine-resin interfaces which suggested there was no remaining demineralized dentine for the dye to penetrate as was previously reported for Superbond bonded bovine specimens.[8] This study could strongly suggest that restorations coupled with hybridised human dentine could inhibit demineralisation with acidic agents such as lactic acid thus preventing dentine and pulpal invasion from external stimuli.

After chemical modification the enamel-, DEJ- and dentine-resin interfaces were not consistent in PN specimens (Fig. 4a). SEM micrographs showed a very thin hybrid layer at the DEJ (Fig. 4c) and dentine (Fig. 4d) compared with that of enamel (Fig. 4b). No dye penetration was found at the tooth-resin interface; however the silver nitrate leakage distance at the dentine and DEJ-resin interfaces was not significantly different from the control groups. Silver was located in the newly prepared resin and connected dentinal tubules (Fig. 7b, 8b) suggesting that silver nitrate could penetrate into this area and was reduced, resulting in the deposition of silver in this area.[23] Silver staining of resin cement identified in PN restored specimens (Fig. 7b) suggested that its components could reduce silver nitrate to silver. PN is a self-etching system, and penetrates through a smear layer and is chemically cured in the absence of oxygen. The self-etching and priming resin was developed to avoid the need for acid etching to remove the smear layer and thus to reduce the procedural steps. Most of this system was bonded through smear layers and plugs. The hybridised smear layer might adversely affect the quality of the hybrid as it was the weakest structure when tested by a mini-dumbbell system.[24] Thus care is needed when using this type of self-etching systems on smear covered dentine. Outstandingly good clinical results after 10-years for restorations using a self-etching systems were recently published.[25] Thus more studies on the adverse effect of a weak smear layer on the restoration longevity is required.

The enamel, DEJ and dentine-resin interfaces of VL restored specimens after chemical modification was not consistent (Fig. 5a). The hybrid layer of enamel was thickest (Fig. 5b) compared with the other two groups while detachment and degradation at the DEJ- and dentine-resin interfaces took place as shown in Fig. 5c & 5d. This system uses H_3PO_4 etching which gives an adverse effect on the impregnation of resin and makes it harder to prepare impermeable hybridised dentine.[15, 18] This must be the reason that Variolink II specimens showed both dye and silver nitrate leakage at the dentine and DEJ-resin interfaces (Fig. 6b, 7c) which was significantly more severe than that of the control specimens and the other two cement groups. Silver was mostly found distributed at the lower part of the interfacial layer and connected tubules (Fig. 8c). This resin cement is a wet bonding system using 37% H_3PO_4 for total surface etching (Table 1). Water permeability of this demineralised dentine is too low to be dehydrated by acetone primer as had been supposed previously.[14] Water movement in this kind of resin,[26] could be explained by the adverse effect of H_3PO_4 on the dentin.

Preparing impermeable hybridised-dentine is very important in preventing leakage to achieve true adhesion. Post-operative hypersensitivity of exposed dentine resulting from convection flow of dentinal fluid through tubules under pulpal pressure can be experienced. Also many microorganisms have the potential to invade the body. Therefore they can invade through this incomplete barrier and lead to pulpal pathology. Furthermore, demineralisation of dentine due to marginal leakage of lactic acid produced by microorganisms in the mouth can occur. Severe microleakage results suggesting that the DEJ or dentine restored with VL cement had a higher risk of hypersensitivity, secondary caries, pulpal pathology and restoration detachment in the short term. This was similar to the results reported for acid-base cemented restorations.[3] Silver nitrate permeation occurring in the PN group might suggest reduced longevity of the restorations, as lactic acid could diffuse through the same pathway as the silver nitrate permeated in this study. Demineralised dentine should be eliminated from restored dentine, as it is hydrolyzed even in water and is permeable to dye and acids resulting in restoration loosening and/or detachment.[12] While dentine is stable in water, dentine demineralised even by acid-base cements allows diffusion of lactic acid in the mouth. The demineralised dentine remaining due to the use of resin adhesives also behaves in the same way as when acid-base dental cements have been used.[3]

Enamel can resist dye and silver nitrate penetrations significantly better than the DEJ and dentine (Table 2, 3). Removal of enamel must be minimized during dental treatment as the natural enamel on dentine is an excellent barrier to protect weak porous dentine against acidic demineralization, which may induce caries. The newly prepared hybrid of restored enamel in all cement groups resisted chemical challenge and no leakage at the interface was found. This supported that restorations on enamel abutments could survive longer than that on dentine abutments. No significant difference in distance between the silver nitrate penetration of control enamel and the final cut which had no leakage at the interface (Table 3), suggesting that hybridised-DEJ and -dentine using SB cement could resist tracer penetration as well as did enamel. Bonding technology had been hypothesised to inhibit detachment of restorations. However, good strong adhesives showing higher bond strength data did not provide impermeable hybridised dentine for long-term durability.[19, 20] A permeable hybrid layer resulting from poor infiltration of resin leads to leakage and consequently secondary caries. Leakage levels are therefore of importance when characterising tooth-resin interfaces and it can be assumed that leakage free data could be more informative than bond strength data in assessing the long-term survival of the restored tooth. It may be necessary to review or reject the previous hypothesis as it would be better to study how to provide a barrier to protect exposed dentine from external stimuli as well as does the enamel barrier in order to preserve tooth for life-long function.

Conclusion

Complete hybridisation of resin into conditioned dentine and the DEJ is very important in order to eliminate the remaining smear layer, demineralised dentine and water which cause bonding defects that lead to leakage pathways. The quality of the bonding interface depends on the resin content in the hybrid layer, which can be identified by immersion in acid and NaOCl solutions. A chemically resistant hybridised-dentine leading to a leakage-free interface can be produced using Superbond C&B. Restored teeth with leakage-free hybridised-dentine can achieve long-term survival because weak prepared dentine is as well protected against acidic demineralisation in the oral cavity as is enamel.

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Table 1 Tooth-conditioning and resin cement manipulation

Procedures	Superbond C&B	Panavia F	Variolink II
Conditioner	10-3	ED primer (self-etching)	37% H_3PO acid
Tooth-conditioning	Applied 10 s, rinsed off 10 s, air-dried 10 s	Applied 30 s, air-dried 2-3 s (repeated)	Applied 10 s, rinsed off 15 s, air-dried 2-3 s, Applied Excyte 10 s, Gently air-dried 2-3 s, light-cured for 20 s
Resin cement	4-META/MMA:TBB:PMMA 4 drops : 1 drop : 1 scoop	Base : Catalyst 1 : 1	Base : Catalyst 1:1
- Manipulation	Brush-dip and hand mixed, cemented	Hand mixed, cemented	Hand mixed, cemented
- Polymerisation	self-cured	light-cured 20 s (Oxygard coated)	light-cured 40 s

Table 2 Distances of dye penetration (Mean ± SD, mm) in control and restored specimens, & the final cut distances (FCD) of restored specimens (n = 8)

Cements	Control specimens			Restored specimens			
	Enamel	DEJ[a]	Dentine[b]	FCD[b]	Enamel	DEJ	Dentine
SB	0	0.014±0.024	0.197±0.029	0.178±0.022	0	0	0
PN	0	0.019±0.032	0.193±0.057	0.191±0.017	0	0	0
VL	0	0.026±0.036	0.190±0.055	0.200±0.011	0	0.287±0.090[b]	0.423±0.144[c]

0 = no dye penetration for control and no leakage at interface for the restored final cuts
Values connected with a straight line were not significantly different ($p > 0.05$).
Differences in distances between groups for control or restored specimens that were significant are indicated by the different letters ($p < 0.05$).

Table 3 Distances of silver nitrate penetration (Mean ± SD, mm) in control and restored specimens, and the final cut distances (FCD) of restored specimens (n = 8)

Cements	Control specimens				Restored specimens		
	Enamel[a]	DEJ[b]	Dentine[b]	FCD[a]	Enamel	DEJ	Dentine
SB	0.230±0.043	0.389±0.064	0.421±0.054	0.201±0.010	0	0	0
PN	0.247±0.040	0.395±0.070	0.429±0.045	0.195±0.011	0	0.416±0.110[b]	0.517±0.085[b]
VL	0.226±0.029	0.376±0.055	0.407±0.036	0.193±0.011	0	0.971±0.137[c]	1.146±0.116[d]

0 = no leakage found at the interface for the restored final cuts
Values connected with a straight line were not significantly different ($p > 0.05$).
Differences in distances between groups for control or restored specimens that were significant are indicated by the different letters ($p < 0.05$).

Legends

Fig. 1 Schematic preparations of tooth surfaces (T1), restored specimens (T2, a) and control specimens (T3, b); E = enamel, D = dentine, V = veneer

Fig. 2 A schematic of restored specimen coated with nail varnish except for the tracer penetration surface (coloured), abraded direction (W) and final cut distance (F); E = enamel, D = dentine, V = veneer

Fig. 3 SEM micrograph of Superbond restored specimens after HCl and NaOCl immersion demonstrating: **a)** the consistent and continuous thickness of hybrid layer (≈ 2-3 μm) in enamel, DEJ and dentine (original x 500), **b)** hybridised enamel, **c)** the hybrid layer at the DEJ **d)** hybridised dentine (original x 2000), ME = modified enamel, MDEJ = modified DEJ, MD = modified dentine, H = hybrid layer, S = cured Superbond

Fig.4 SEM micrograph of Panavia restored specimens after HCl and NaOCl immersion demonstrating: **a)** the inconsistent thickness of the interfacial layer in enamel, DEJ and dentine (original x 500), **b)** the thickness of interfacial layer in enamel (≈ 2-3 μm), **c)** the thinner interfacial layer in DEJ and dentine, **d)** the hardly identified interfacial layer in dentine (original x 2000), ME = modified enamel, MDEJ = modified DEJ, MD = modified dentine, H = tooth-resin interfacial layer, P = Panavia resin cement

Fig. 5 SEM micrograph of Variolink restored specimens after HCl and NaOCl immersion demonstrating: **a)** the inconsistent thickness of interfacial layer in enamel, DEJ and dentine (original x 500), **b)** the clearly identified interfacial layer in enamel, **c)** a thinner interface of interfacial layer in DEJ and dentine than that of enamel, **d)** the degraded and detached interfacial layer of dentine (original x 2000), ME = modified enamel, MDEJ = modified DEJ, MD = modified dentine, H = tooth-resin interfacial layer, VL = Variolink cement

Fig. 6 Light stereoscope images of restored specimens using dye penetration demonstrating: **a)** no leakage at the enamel, DEJ and dentine-resin interfaces (arrowed) at the final cut distance (0.183 mm) of Superbond (original x 40), **b)** leakage at the DEJ & dentine-resin interfaces (arrowed) at 0.317 mm distance of Variolink (original x 200), E = enamel, D = dentine, V = composite veneer, R = resin cement

Fig. 7 Light stereoscope images of restored specimens using silver nitrate penetration demonstrating: **a)** no leakage at the enamel, DEJ- and dentine-resin interfaces (arrowed) at the final cut distance (0.193 mm) of Superbond (original x 40), **b)** leakage at the DEJ- and dentine-resin interfaces (arrowed) at 0.348 mm distance of Panavia & **c)** at 0.927 mm distance of Variolink (original x 400), E = enamel, D = dentine, V = composite veneer, R = resin cement

Fig. 8 Back scattered electron micrograph demonstrating: **a)** no silver deposition at tooth-resin interface of Superbond-restored final cut specimens, silver deposition from exposed dentinal tubule (arrowed) was identified, **b)** silver distribution (arrowed) in the tooth-resin interface at the DEJ, dentine and connected tubules for Panavia restored specimens, **c)** silver distribution (arrowed) at the tooth-resin interface of DEJ, dentine & dentinal tubules for Variolink restored specimens (original x 2000), E = enamel, D = dentine, H = tooth-resin interfacial layer