Micromorphology of Resin/Dentin Interfaces Using 4th and 5th Generation Dual-curing Adhesive/Cement Systems: A Confocal Laser Scanning Microscope Analysis

Cesar A. G. Arrais[a]/Katsuia Miyake[b]/Frederick A. Rueggeberg[c]/David H. Pashley[d]/
Marcelo Giannini[e]

Purpose: This study evaluated the differential composition of resin/dentin interfaces of indirect restorations created by the application of 4th and 5th generation dual-curing luting systems (bonding agents/resin cements), when each material was either light cured or allowed to self-cure.

Materials and Methods: Occlusal flat dentin surfaces of 60 human third molars were assigned into 12 groups (n = 5) according to curing mode and dual-curing cementing system: 4th generation All Bond2 (AB2)/Duolink (Bisco) and 5th generation (B1) Bond1/Lute-it (Pentron). Fluorescein-labeled dextran (FDx) was mixed with the bonding agents, while rhodamine-labeled dextran (RhDx) was incorporated into resin cements and Pre-Bond resin from AB2. Resin cements were applied to 2-mm-thick, precured resin composite disks (Z250, 3M ESPE), which were fixed to dentin surfaces containing adhesive resin in either cured (light cured; LC) or uncured (self-cured; SC) states. The restored teeth were light activated (XL3000, 3M ESPE) according to the manufacturers' instructions (LRC) or allowed to self-cure (SRC), were stored for 24 h, and then vertically, serially sectioned into 1-mm-thick slabs, which were analyzed using confocal laser scanning microscopy. Fluorescent additives indicated where individual components of the bonding/cement systems were located. Additional specimens were prepared and analyzed using a conventional scanning electron microscope.

Results: AB2/LC and B1/LC exhibited nonuniform primer/adhesive layer thickness. AB2/SC showed adhesive resin penetration within the primed dentin, and resin cement penetration at the entrance of the dentin tubules. B1/SC/LRC demonstrated resin cement penetration within the hybrid layer and into the dentin tubules. More resin cement penetration was observed in B1/SC/SRC groups than in its LRC equivalent.

Conclusion: The morphological features and component interactions among materials at resin/dentin interfaces are related to the activation modes of the primer/adhesive layer and of the resin cement used.

Keywords: confocal laser scanning microscopy, dual-curing adhesive systems, dual-curing resin cements, adhesive interfacial morphology, indirect restorations.

J Adhes Dent 2009; 11: 15-26. *Submitted for publication: 08.10.07; accepted for publication: 20.12.07.*

The demand for tooth-colored indirect restorations has grown considerably in the past few years. The increase in this demand can be attributed to, among other factors, the reliable bond between adhesive cementing systems (resin cements/bonding agents) and mineralized dental tissues.[15,26] A stable and durable bond of indirect restorations to teeth is also related to good marginal adaptation, which prevents microleakage, recurrent caries, and pulpal irritation.[28] In an attempt to improve marginal adaptation and bond strength of indirect restorations using 4th or 5th generation adhesive systems,[10,29] different luting procedures have been proposed.

The luting procedure for indirect restorations most recommended by manufacturers is based on light activation of the adhesive resin before indirect restoration cementation. Because the bonding agent is accessible to direct light exposure in this procedure, maximal monomer conversion and bond strength can be achieved.[18] However, some studies have demonstrated that a thick, cured adhesive layer can affect full restoration seating, and thus marginal adaptation of indirect restorations.[11,13,19] Fur-

[a] *Assistant Professor, Department of Operative Dentistry, School of Dentistry, University of Guarulhos, Guarulhos SP, Brazil.*

[b] *Assistant Professor, Cell Imaging Core Facility, IMMAG, Medical College of Georgia, Augusta, GA, USA.*

[c] *Professor and Section Director, Dental Materials Section, Department of Oral Rehabilitation, School of Dentistry, Medical College of Georgia, Augusta, GA, USA.*

[d] *Professor, Department of Oral Biology and Maxillofacial Pathology, Medical College of Georgia, Augusta, GA, USA.*

[e] *Associate Professor, Department of Restorative Dentistry, Piracicaba School of Dentistry, State University of Campinas, Piracicaba, SP, Brazil.*

Correspondence: *Marcelo Giannini, Department of Restorative and Operative Dentistry, Piracicaba School of Dentistry, UNICAMP, Av. Limeira, #901, Piracicaba, SP, 13414-900– Brazil. Tel: +55-192-106-5338, Fax: +55-192-106-5218. e-mail: giannini@fop.unicamp.br*

Table 1 Manufacturers and compositions of dual-curing adhesive systems used

Product (code) (manufacturer)	Composition (batch number)	Manufacturer's instructions
All Bond 2 (AB2) (Bisco) 4th Generation	Primer A: acetone, ethanol, Na-N-tolylglycine, glycidyl-methacrylate (0500003574). Primer B: acetone, ethanol, biphenyl dimethacrylate (0500003579). Pre-Bond Resin: bis-GMA, TEG-DMA, benzoyl peroxide, BHT (0500004345).	Mix primers A and B. Apply 5 consecutive coats to dentin; dry all surfaces for 5 to 6 s with an air syringe, light cure 20 s. Apply thin layer of Pre-Bond Resin immediately prior to cementation. Air thin. Do not light cure.
Bond 1 (B1) (Pentron) 5th Generation	Activator: methacrylate monomers in ethanol and/ or acetone, benzoyl peroxide, acetone (128878). Resin: mixture of PMGDM, a condensation product of PMDA and glycerol, dimethacrylate HEMA and TMPTMA in ethanol and/or acetone with photoinitiator, amine accelerator and stabilizer, pyromellitic dianhydride (129121).	Mix one drop of Bond1 Dual Cure Activator with 2 drops of Bond1 Primer/Adhesive. Using a fully saturated brush tip each time, apply two coats of Bond1 Primer/Adhesive to tooth within 10 s. Apply a gentle stream of air for a minimum of 10 s. Hold air syringe 1 inch (2.54 cm) from site, positioned so as not to disturb resin surface. (Avoid excess of Bond1 Primer/Adhesive in internal line angles or point angles.)

TEG-DMA: triethylene glycol dimethacrylate; bis-GMA: bisphenol A diglycidyl ether methacrylate; PMDA: pyromellitic dianhydride; PMGDM: pyromellitic glycerol dimethacrylate; BHP: butylated hydroxytoluene; TMPTMA: trimethylolpropane trimethacrylate; HEMA: 2-hydroxyethyl methacrylate; GPDM: glycerophosphoric acid dimethacrylate.

thermore, polymerized adhesive layers created by 5th generation bonding agents usually contain residual water and have a high content of hydrophilic monomers. The presence of these components makes the polymerized bonding layer more susceptible to hydrolytic degradation than layers containing more hydrophobic monomers and less residual water, such as those created by 4th generation adhesive systems.[8,30]

To overcome these limitations, another clinical approach was developed in which the dentin bonding agent is left unpolymerized before application of the resin cement. Some studies demonstrate that the pressure of resin cement during delivery of a restoration may cause collapse of demineralized dentin.[9,17] However, this technique allows maximum restoration seating[11] and might create an adhesive layer composed of the mixture of bonding agent and resin cement. This new, combined layer would contain fewer hydrophilic monomers and more hydrophobic monomers that are attributed to the resin cement components, improving the long-term durability of the bonded interface as a consequence.[8,30] However, no information exists regarding the creation of such a layer when dentin bonding agents are left in the uncured state before resin cement application.

Confocal laser scanning microscopy (CLSM) is well suited to study the presence of bonding agent components and resin cement on the dentin surface or even within the hybrid layer. This technique permits accurate colocalization of resins by the incorporation of fluorescent markers prior to their application. CLSM is capable of individually exciting different fluorochromes by applying selective wavelengths.[6,7] Therefore, fluorochromes with well-separated emission spectra allow analysis of the mixture and interaction between adhesive resin and resin cement components.

The purpose of this study was to evaluate by CLSM and SEM the features of bonded interfaces of indirect resin composite restorations placed in extracted human teeth created by 4th and 5th generation dual-curing dentin bonding agents combined with their respective dual-curing resin cements when each was either allowed to self-cure or was exposed to light through a precured disk of resin composite. Visualization of specimen cross sections was made using both CLSM and conventional scanning electron microscopy (SEM). The null hypotheses were that: 1) when not light activated prior to resin cement application, the dentin bonding agent and resin cement components will be observed on the dentin surface and/or within the hybrid layer created by these materials used in their dual-curing form; and 2) light activation of the bonding agent prior to cementation will produce a uniform adhesive layer and only a superficial mixture between adhesive and resin cement with no presence of cement resin at or within the hybrid layer.

MATERIALS AND METHODS

Adhesive Resin Preparation for Confocal Laser Scanning Microscopy

Commercial 4th- and 5th-generation, dual-curing dentin adhesive systems and their corresponding dual-curing resin

Table 2 Manufacturers and compositions of the dual-curing resin cements used

Product (Manufacturer)	Composition	Batch number
Duolink (Bisco)	Base: bis-GMA, TEG-DMA, glass filler, urethane dimethacrylate. Catalyst: Bis-GMA, TEG-DMA, glass filler.	0500003751
Lute-It! (Pentron)	In both base and catalyst: UDMA, HDDMA, amine and inorganic pigments (in base only), benzoyl peroxide (in Catalyst only), UV Stabilizers (in both base and catalyst), barium glass, inorganic fluoride, borosilicate glass, silane silica zirconia.	Base: 130666 Catalyst: 126388

TEG-DMA: triethylene glycol dimethacrylate; bis-GMA: bisphenol A diglycidyl ether methacrylate; UDMA: urethane dimethacrylate; HDDMA: 1,6-hexanediol dimethacrylate.

cements were used (Table 1). Fluorescein-labeled dextran, neutral. 4000 average-molecular weight (FDx, 4KD; batch #123K0723, Fluorescein-Isothiocyanate-Dextran, FD4, Sigma; St Louis, MO, USA) was incorporated into Primers A and B of All Bond 2 (40 µg/ml in each bottle) and into Bond 1 primer/adhesive resin (Pentron; Wallingford, CT USA) (160 µg/ml in each bottle). Rhodamine B (RhDx; batch #121K3688, RITC/Rhodamine B, R6626, Sigma) was mixed with Pre-Bond resin of All Bond 2 (6.4 µg/ml) (Bisco; Schaumburg, IL, USA), as well as with the dual-curing resin cements Duolink (0.32 µg/mg) (Bisco) and Lute-it (0.32 µg/mg) (Pentron). When added to the resin cements (Table 2), the dye was incorporated into the base paste and was mixed with a spatula until the base paste changed shade uniformly. The dye-laden cement component was returned to the original syringe from which it was extruded. The dyes were added directly to the containers of the adhesive resins provided by the manufacturers and were continually agitated in a mixing device (Vortex Machine, Scientific Industries; New York, NY, USA) for at least 2 h to provide complete dye dissolution.

Indirect Restorative Bonding Procedures

Sixty freshly extracted, erupted human third molars, which were stored in saturated thymol solution at 5°C for no longer than three months, were used following a protocol approved by the Human Assurance Committee at The Medical College of Georgia (HAC #0403333). Teeth were transversally sectioned in the middle of the crown using a diamond blade (number 11-4244, Series 15HC Diamond, Buehler; Lake Bluff, IL, USA) on an automated sectioning device (Isomet 2000, Buehler) under water irrigation, exposing areas of middle-depth dentin. The exposed dentin surfaces were wet polished by machine (Supermet Grinder, item #48-1581, Buehler) with 600-grit SiC paper (pn 810-281-PRM, Silicon Carbide PSA Discs, Leco; St Joseph, MI, USA) to create a flat surface with standard smear layer formation before being bonded with the adhesive systems.[31]

Sixty light-activated composite resin disks (2 mm thick and 10 mm in diameter, A2 shade, Z250, lot #5LB, 3M ESPE; St Paul, MN, USA) were prepared to simulate overlying laboratory-processed indirect composite resin restorations. The surface of each precured resin disk that was to be bonded was sandblasted (air pressure: 80 psi; distance from the tip: 1.5 cm; Comco MB 1002, COMCO; Burbank, CA, USA) with 50-µm aluminum oxide particles (lot # 51116150, micron white, Danville Engineering; San Ramon, CA, USA) for 10 s.

Prepared teeth were randomly divided into twelve groups (n = 5). Flow charts of the fabrication of each specimen type are presented in Figs 1 and 2. All adhesive systems and resin cements were manipulated and applied to the dentin surfaces according to manufacturers' instructions (control): light activation (20 s, sn #202149, XL 3000, 3M ESPE), power density of 600 mW/cm^2 (as measured using a laboratory grade spectral radiometer DAS 2100, Labsphere; Sutton, NH, USA) of the Primer A and B mixture of All Bond 2. The mixture of Bond 1 and the activator component was left in the uncured state prior to placement of the respective resin cements (Table 1). For the experimental groups of the 4th generation product, the mixture of primers A and B were applied and left in the uncured state, relying completely on any self-curing mechanism, and Bond 1 was light activated for 20 s prior to the resin cement application. The penetration pattern of Pre-Bond resin into the primed dentin when All Bond 2 was used was evaluated using four additional experimental groups, in which rhodamine B was incorporated into the Pre-Bond resin instead of into the resin cement (Fig 1).

The mixed resin cement pastes were applied to the precured composite disk following manufacturers' instructions, and the disk was positioned and fixed to the adhesive-coated dentin surface under a load of 500 g for 5 min, during which the resin cement was allowed to self-cure. When the cementing materials were light activated through the precured composite disk, the curing unit tip was positioned against the composite disk surface and each specimen was exposed for 40 s (XL 3000, 3M ESPE).

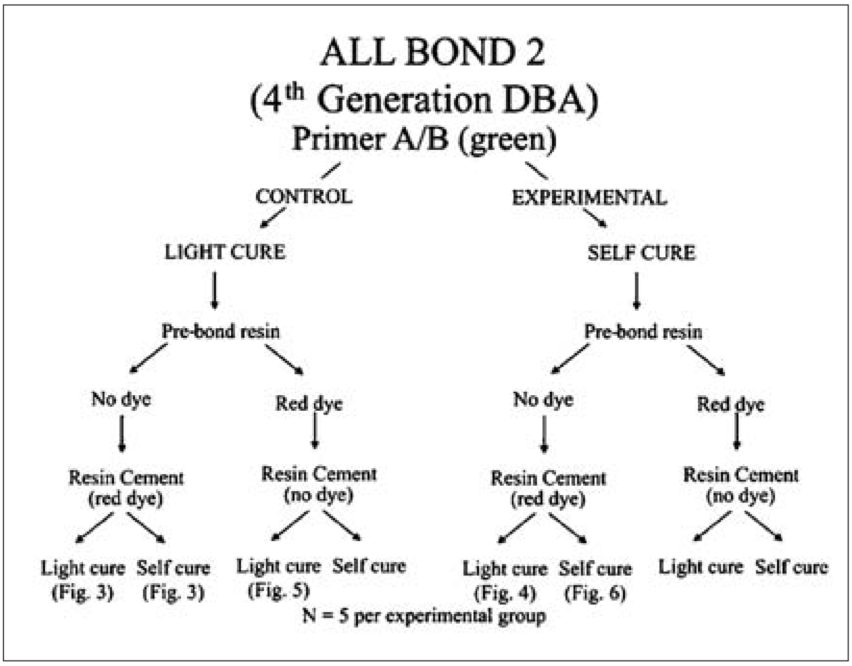

Fig 1 Schematic diagram of the experimental groups created according to the curing modes of the adhesive layer and resin cement for All Bond 2/Duolink.

Confocal Laser Scanning Microscopy Analysis

The restored teeth were stored in vegetable oil (Crisco Pure Vegetable Oil, J. M. Smucker Company; Orrville, OH, USA; expiration date Oct 2007) for 24 h to prevent loss of water and dye and were vertically, serially sectioned into several 1-mm-thick slabs using a diamond blade (Buehler; Lake Bluff, IL, USA) on a sectioning device (Isomet Low Speed Saw, Buehler; Evanston, IL, USA) under oil lubrication. The slabs were stored in vegetable oil for 24 h and were analyzed under CLSM (LSM 510 Meta Confocal Microscope, Zeiss; Göttingen, Germany) performed at the Cell Imaging Core Facility of the Medical College of Georgia. Images were recorded onto DVD disks for further analysis by Zeiss Image Examiner Physiology image analysis software. An argon laser at 488 nm and He-Ne laser at 543 nm provided excitation energies. The intensity of the excitation source and photomultiplier amplification were kept constant during the investigation period. CLSM images were recorded in the fluorescent mode. The visualized layer was selected approximately 10 µm below the sample surface and images were recorded with an oil immersion objective (40X, numerical aperture 1.3). The sizes of the recorded images were 230.3 x 230.3 μm^2 and 76.8 x 76.8 μm^2, at a resolution of 1024 x 1024 pixels. Images were recorded at magnifications of 770X and 3000X from three different regions along the bonded interface of each specimen. The different dyes provided specific emission wavelengths for each resinous component at the resin/dentin interface. Therefore, the mixture of primers A and B from All Bond 2 and Bond 1 emitted a green shade and Pre-Bond resin and the resin cements emitted a red shade when excited by the appropriate lasers. Because this was an observational evaluation only, no statistical analysis was performed and only visual differences among experimental groups were considered as findings. The general appearance of the 5 replications from each experimental group was used to characterize trends observed for that test condition.

Scanning Electron Microscopy Analysis

An additional 24 extracted, caries-free, erupted human third molars were prepared, restored with the same dual-curing cementing systems and pre-cured composite disks as described before, and were stored in water at 37°C for 24 h. The restored teeth were vertically sectioned in the middle using a diamond blade (Buehler; Lake Bluff, IL, USA) on a sectioning device (Isomet Low Speed Saw, Buehler) to expose the resin/dentin interface. The bonded interfaces were wet polished with 1200- and 2000-grit SiC paper and with 6- and 3-µm diamond pastes. A 5N HCl solution was applied to the specimen interfaces for 10 s to remove the inorganic components from the dentin surface. The specimens were washed with water, immersed in 2.5% NaOCl for 10 min, and then ultrasonicated for 1 min to remove all exposed collagen after treatment with acid, so only the hybrid layer and resin tags remained at the adhesive interface. Afterwards, the specimens were allowed to dry overnight at 37°C. They were then sputter coated with gold (MED 010, Balzers; Balzers, Liechtenstein) and examined using a scanning electron microscope (VP 435, Leo; Cambridge, UK) at 770X and 3000X.

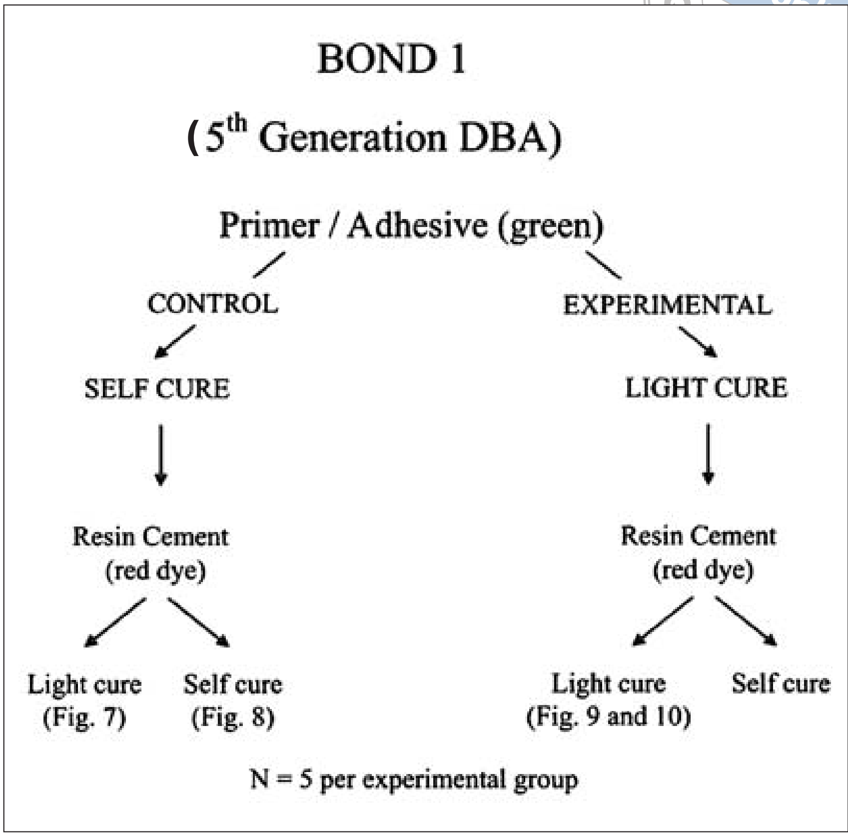

Fig 2 Schematic diagram of the experimental groups created according to the curing modes of the adhesive layer and resin cement for Bond 1/Lute-it.

RESULTS

Figures 3A, 3B, and 3C show CLSM images, and Fig 3D presents the SEM image of bonded interfaces created when All Bond 2/Duolink was applied according to manufacturer's instructions (control), where the mixture of primers A and B and the resin cement were each light activated separately. The primer light activation formed two different interfacial morphologies, one exhibiting a nonuniformly thick primer layer (arrows, green shade, Fig 3A) and the other one showing the bonded interface without primer layer (Fig 3C). A thin yellow line was observed between the adhesive and resin cement layers (Fig 3B). When the primer layer was not evident on the SEM image (Fig 3D), it was noticed that the resin cement penetrated into the entrance of the dentinal tubules (asterisks, red shade, Fig 3C) and a red line was noted between the dentin surface and the resin cement layer (arrows). Similar morphological features were observed (not presented here) when All Bond 2 was applied according to the manufacturer's instructions and the resin cement was allowed to self-cure.

Figures 4A and 4B present the bonded interface created when All Bond 2 was applied to dentin, the mixture of primers A and B was left in the uncured state, and the resin cement was light activated through the pre-cured composite disk. Figure 4C shows an SEM image from specimens restored following the same experimental protocol. In this experimental group, fluorescein (green) was added to the primer and rhodamine (red) was added to the resin cement. A superficial penetration of the resin cement was observed at the top of the hybrid layer (arrows, Fig 4B), and dark spots, related to the presence of Pre-Bond resin, were noted within the resin cement layer at the top of the dentin surface (asterisks, Figs 4A and 4B). SEM images demonstrated a thinner hybrid layer than that observed in CLSM images.

Figures 5A and 5B show the bonded interface using CLSM. Figure 5C presents the SEM image created when All Bond 2 was applied to the dentin surface, the primer was left in the uncured state, and the resin cement was light activated, but rhodamine was added to the adhesive resin (Pre-Bond) instead of to the resin cement. Pre-Bond resin (red) penetrated into all dentin tubules to apparently similar depths, approximately 12 µm (Fig 5A), and changes in the red shade (from light to dark red) were also observed at the adhesive/resin cement layer (asterisks, Fig 5B). A light orange-shaded area was noted at the top of the hybrid layer (arrows, Fig 5B), indicating the mixture between primer and Pre-Bond resin at the top of the hybrid layer. When compared to the hybrid layer in the CLSM image, the thinner hybrid layer in the SEM image (Fig 5C) after acid-base challenge seemed to correspond to the hybrid layer region containing both primer and Pre-Bond resin.

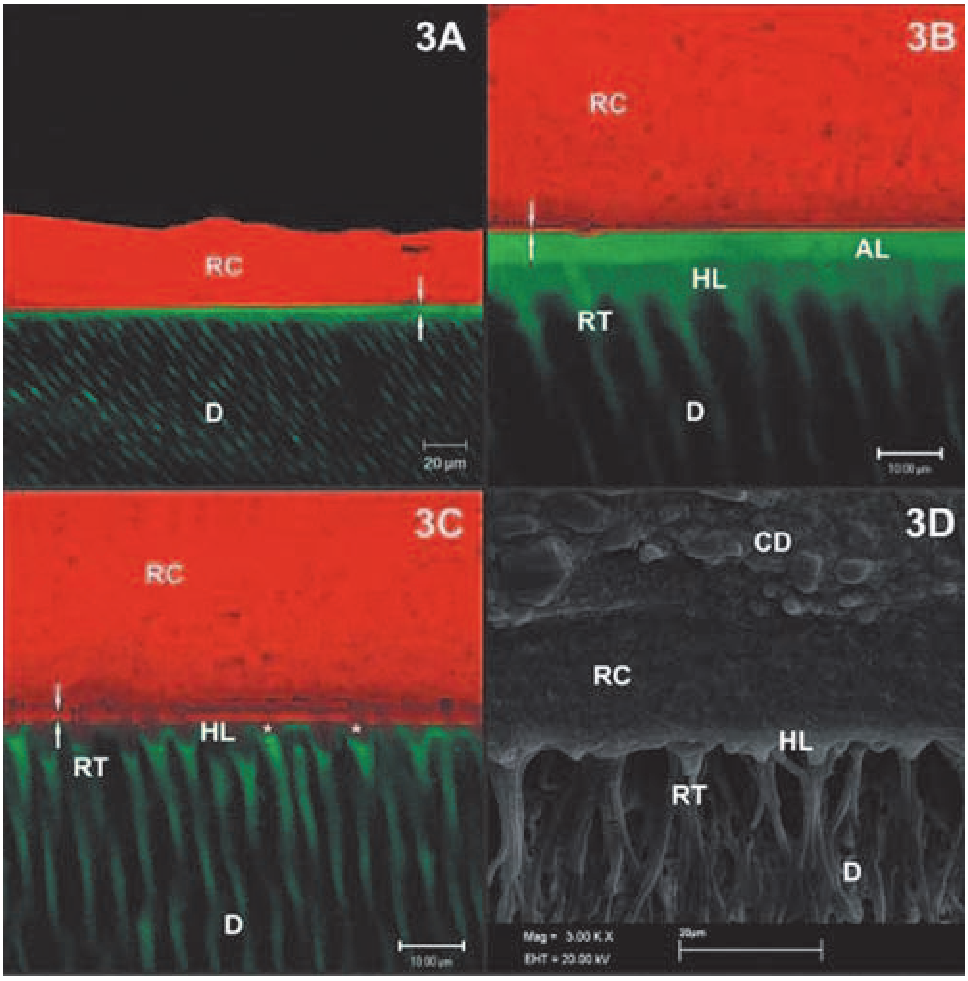

Fig 3 Bonded interface created when All Bond 2/Duolink was applied to the dentin surface, and the primer (green) and the resin cement were light activated for the 40 s as recommended by the manufacturers (control). RC – resin cement; HL – hybrid layer; RT – resin tag; D – dentin; AL – adhesive layer; CD – composite disk. (A) Representative CLSM image exhibiting a nonuniform primer layer (arrows). (B) The presence of a primer layer (AL) and the mixture between oxygen-inhibited uncured primer and resin cement components were noted (between arrows). (C) No primer layer was observed in some regions, which showed the resin cement penetration at the entrance of dentin tubules (asterisks) and a red line corresponding to the deposition of organic matrix from the resin cement (between arrows). (D) Representative SEM image of the bonded interface.

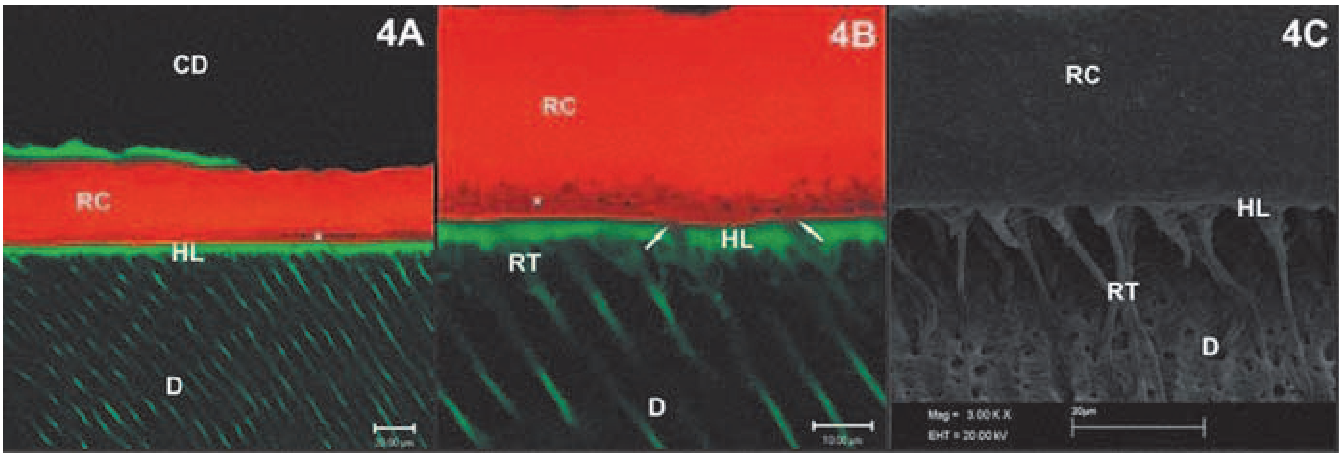

Fig 4 Bonded interface created when All Bond 2/Duolink was applied to the dentin surface; the primer (green) was left in the uncured state (experimental group) and the resin cement (red) was light activated. (A) A uniform hybrid layer and a dark red layer (asterisk) were noted along the whole extension of the dentin surface. The green shade above the resin cement corresponds to the primer AB, which was applied to the resin disk's surface according to the manufacturer's instructions. (B) Superficial resin cement penetration (arrows) was noted at the entrance of the dentin tubules, and a dark red layer (asterisk) corresponding to the presence of Pre Bond resin was observed within the resin cement layer (RC). (C) Representative SEM image of the bonded interface. HL – hybrid layer; RT – resin tag; D – dentin; CD – composite disk.

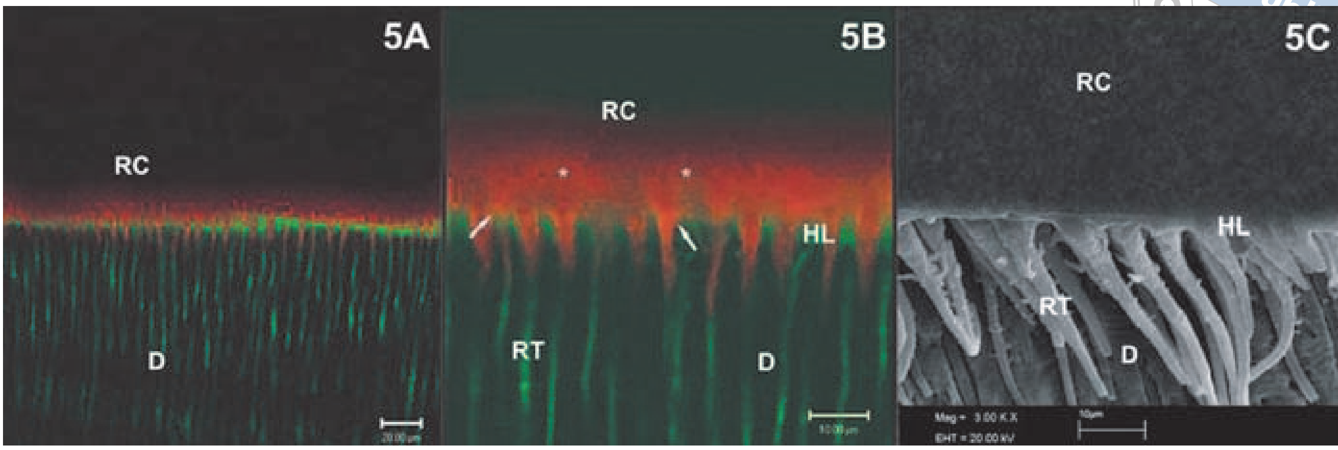

Fig 5 Bonded interface created when All Bond 2/Duolink was applied to the dentin surface, the primer (green) was left in the uncured state (experimental) and the resin cement was light cured for 40 s, but rhodamine was incorporated to Pre-Bond resin (red) instead of in the resin cement (RC). (A) Pre-Bond resin penetration (red) into the dentin tubules and at the top of the hybrid layer was noted throughout the length of the dentin surface. (B) Orange areas indicating the mixture between Pre-Bond resin (red) and the primer (green) within the hybrid layer (arrows) and the mixture between Pre-Bond resin and resin cement (darker red areas, asterisks) were noted more clearly at higher magnification. (C) Representative SEM image showing corresponding areas of the bonded interface. RC – resin cement, HL – hybrid layer; RT – resin tag; D – dentin.

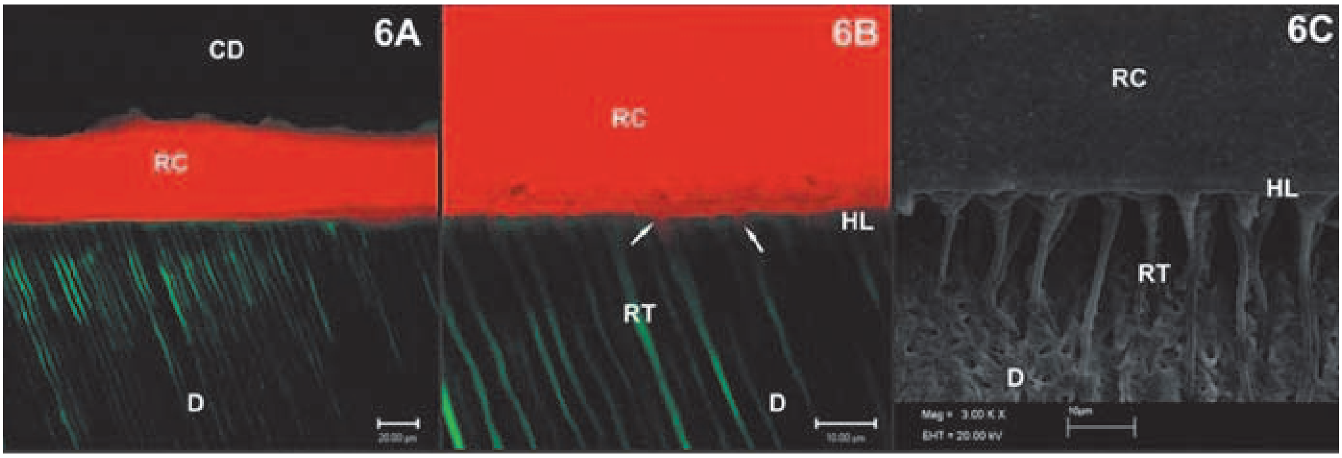

Fig 6 Bonded interface created when All Bond 2/Duolink was applied to the dentin surface. The mixture of primers A and B (green) was left in the uncured state (experimental) and the resin cement (red) was allowed to self-cure. (A) The penetration of the resin cement components (red) was noted at the entrance of dentin tubules along the whole extension of the bonded interface. (B) Deeper resin cement penetration (red, arrows) at the entrance of the dentin tubules was observed when compared to the resin cement penetration observed when the resin cement was light activated (Fig 4B). (C) Representative SEM image showing the bonded interface. RC – resin cement layer; HL – hybrid layer; RT – resin tag; D – dentin; CD – composite disk.

Figures 6A and 6B show the bonded interfaces created by All Bond 2/Duolink on the dentin surface when the mixture of primers A and B was left in the uncured state and the resin cement was allowed to self-cure. Figure 6C corresponds to the SEM images obtained from dentin restored following a similar experimental protocol. The penetration of resin cement was observed at the entrance of most dentin tubules (Figs 6A and 6B, arrows). However, no morphological evidence of the presence of resin cement in the dentin tubules, such as the presence of filler particles at the entrance of the dentin tubules, was found in SEM images (Fig 6C).

Figures 7A and 7B present CLSM images of the bonded interfaces. Figure 7C shows the SEM interface created when Bond 1 was left in the uncured state and the resin cement was light activated through cured composite resin. The change in shade from green to light yellow, orange, or even to red represents mixing between the bonding agent and resin cement within the hybrid layer (Figs 7A and 7B). The SEM image indicates a hybrid layer thickness similar

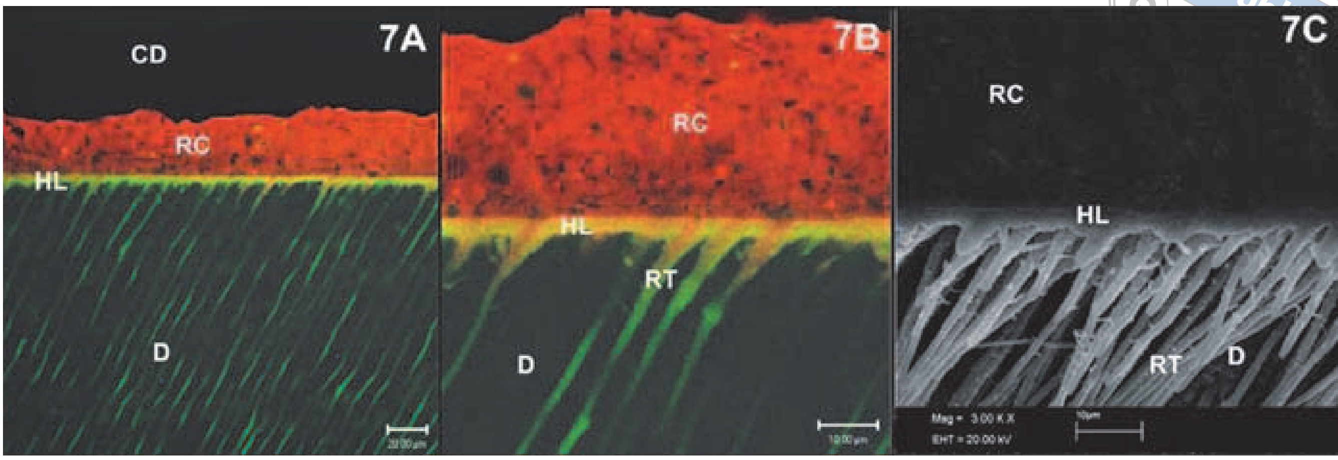

Fig 7 Bonded interface created when Bond 1/Lute-it was applied to the dentin surface, the bonding agent (green) was left in the uncured state and the resin cement was light activated (manufacturer's recommended directions, control). (A) Resin cement (red) penetration was observed into the entrance of all dentin tubules. A change in shade from green to yellow within the hybrid layer is seen denoting mixture between resin cement components (red) and bonding agent (green) along the whole bonded interface. (B) Higher magnification showing the resin cement (red) penetration into the dentin tubules and within the hybrid layer. (C) Representative SEM image of the bonded interface. RC – resin cement; HL – hybrid layer; RT – resin tag; D – dentin; CD – composite disk.

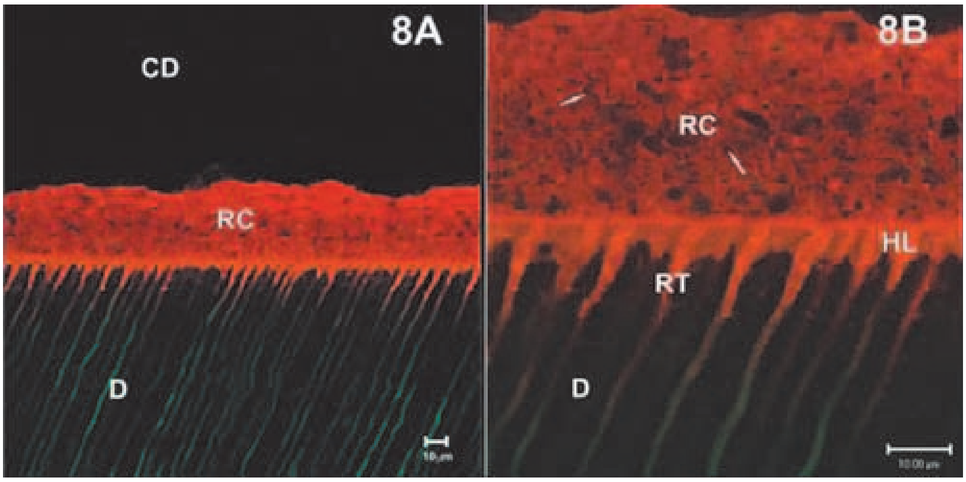

Fig 8 Bonded interface created when Bond 1/Lute-it was applied to the dentin surface, the bonding agent was left in the uncured state (control) and the resin cement was allowed to self-cure. (A) Intense resin cement penetration (red) was observed into all dentin tubules and within the hybrid layer along the entire bonded interface. (B) Higher magnification showing the change in shade from green to red within the hybrid layer denoting the higher concentration of resin cement components within the hybrid layer. The distribution of filler particles (dark areas, arrows) was also noted within the resin cement layer, but none were present in the hybrid layer. RC – resin cement; HL – hybrid layer; RT – resin tag; D – dentin; CD – composite disk.

to that observed in the CLSM images; however, no evidence of mixed components was seen.

When Bond 1 was not light activated and the resin cement was allowed to self-cure (Figs 8A and 8B), a darker red shade was observed at the bottom of the hybrid layer in comparison to the shade observed within the hybrid layer when the resin cement was light activated (Figs 7A and 7B). Deeper penetration of resin cement components (red) into dentin tubules along the entire bonded interface was also observed (Figs 8A and 8B). Filler particles within the resin cement layer were visible (arrows), but no such particles were noted within the dentin tubules.

Figures 9A and 9B show the bonded interfaces using CLSM, and 9C presents the SEM images created by Bond 1/Lute-it when the adhesive and resin cement layers were each light activated separately. A nonuniformly thick adhesive layer was observed (Fig 9A). Some regions exhibited thick adhesive layers (Fig 9B, approximately 22 µm) and the mixture between resin monomers from the oxygen-inhibited uncured adhesive and resin cement layers was

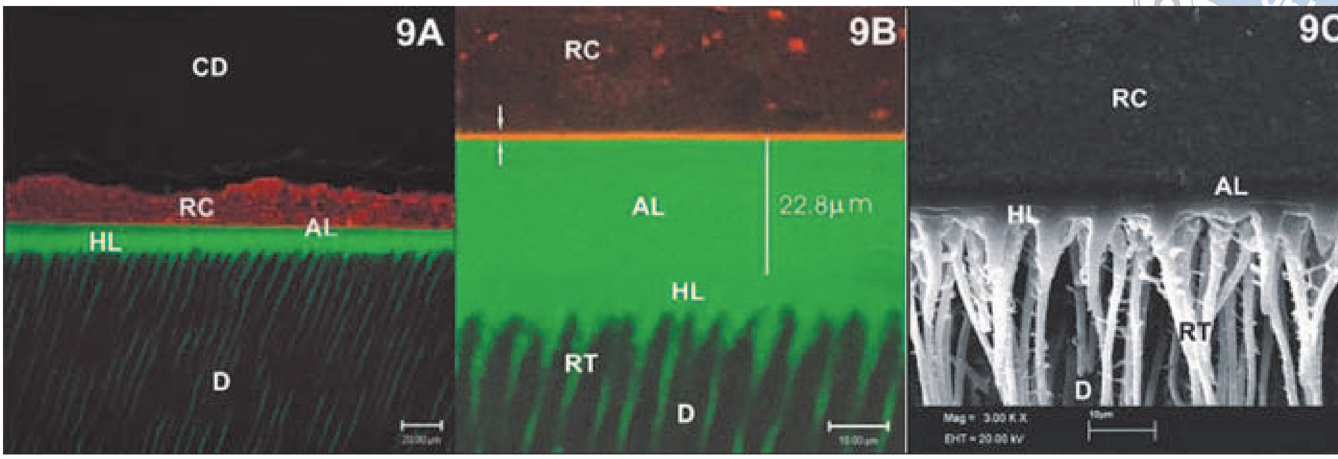

Fig 9 Bonded interface created in the 5th generation product when Bond 1/Lute-it was applied to the dentin surface and bonding agent and resin cement were light activated separately (experimental). (A) A nonuniform adhesive layer (green) and a 9-µm thick hybrid layer (green) were observed along the entire bonded interface. (B) A thick hybrid layer (green) and a yellow/orange line denoting the mixture between uncured, air-inhibited dentin bonding layer (AL) and resin cement (between arrows) were noted. (C) Representative SEM image of the bonded interface. HL – hybrid layer; RT – resin tag; RC – resin cement; D – dentin; AL – air-inhibited layer; CD – composite disk.

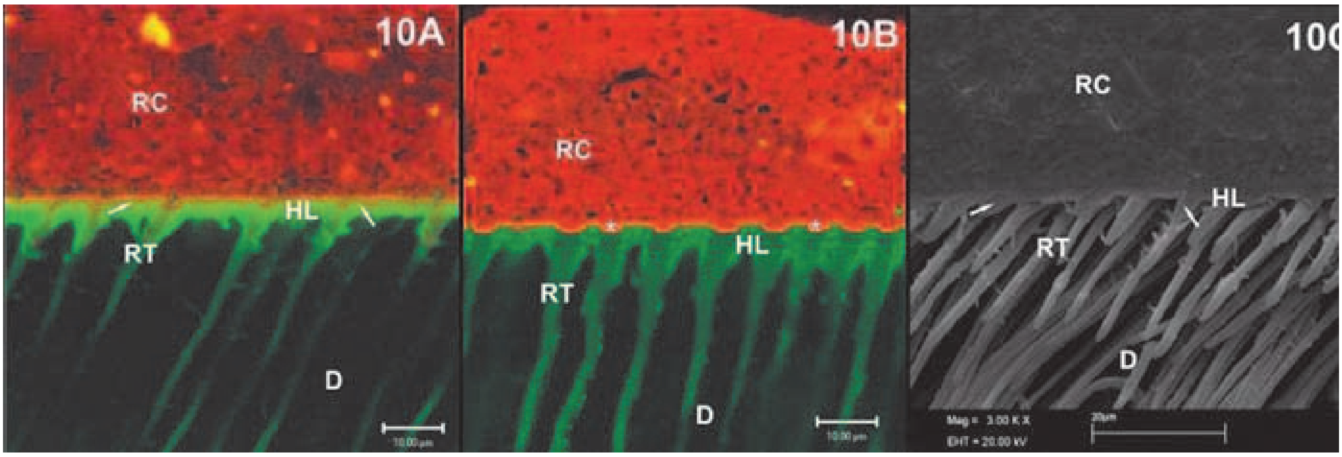

Fig 10 Bonded interface created when Bond 1/Lute-it was applied to the dentin surface and bonding agent (green) and resin cement (red) were light activated separately. (C) Representative SEM image of the bonded interface. (C) No adhesive layers were observed at some regions of the bonded interface when the bonding agent was light activated and resin cement penetration (red) into the dentin tubules (arrows) in those regions. (B) Resin cement penetration was also observed into the demineralized dentin (yellow-orange area, asterisks). RC – resin cement; HL – hybrid layer; RT – resin tag; D – dentin.

noted along the entire interface length (Fig 9B, orange line, arrows). Separate adhesive and hybrid layers were also evident in the SEM images. The same application technique of Bond 1/Lute-it also created bonded interfaces without a separate adhesive layer above the hybrid layer (Figs 10A to 10C). Those interfaces were characterized by resin cement infiltration at the top of the hybrid layer (Fig 10B, arrows) and at the entrance of the dentinal tubules (Fig 10C, asterisks).

DISCUSSION

The results of this study demonstrated that resin cement and bonding agent components created a combined, mixed layer on the dentin surface when both the 4^{th} and 5^{th} generation adhesive systems were applied to dentin and left in the uncured state. Therefore, the first null hypothesis was validated for the cementing systems. However, only the bonded interfaces created by the 5th generation adhesive and its respective resin cement exhibited a new combined layer within the hybrid layer.

The bonded interfaces created by the 5^{th} generation bonding agent differed from those created by the 4^{th} generation system (All Bond 2/Duolink), which demonstrated penetration of resin cement components only at the entrance of the dentin tubules (Figs 4 and 6). A singular feature of 4th generation adhesives is the use of separate bottles containing primer and bonding resin. According to the manufacturer's instructions, Pre-Bond bonding resin must be applied to the dentin surface after application and light activation of the mixture of primers A and B. The adhesive resin functions to link resin composite to primed dentin, and is composed of a high concentration of hydrophobic monomers, such as bis-GMA (Table 1). This monomer may be responsible for the high viscosity observed in Pre-Bond resin. As can be observed in Fig 3, Pre-Bond resin combines with the resin cement during seating of the indirect restoration. Therefore, it is possible to speculate that presence of the viscous Pre-Bond resin impairs resin cement diffusion through it and within the hybrid layer when the primer was left in the uncured state. This speculation may be confirmed by the observation that an apparently deeper penetration of the resin cement at the entrance of the dentin tubules was observed when the resin cement was allowed to self-cure only (Fig 6).

Surprisingly, the 5th generation dual-curing cementing system (Bond 1/Lute-it) showed resin cement components not only into the dentin tubules, but also within the hybrid layer (Figs 7 and 8). The darker orange and red shades correspond to higher concentrations of resin cement components within the hybrid layer, while the yellow color represents lower concentration. Therefore, higher concentration of resin cement components was observed at the top of the hybrid layer and into the dentin tubules as well, and lower concentration of those components was observed at the bottom of the hybrid layer. The infiltration of such resin cement components within the hybrid layer can be attributed not only to the low viscosity of the 5th generation bonding agent Bond 1, but also to the low viscosity of Lute-it when compared to that of Duolink (Table 2). The resultant effects of this mixture between dentin bonding agent and resin cement components on the mechanical properties at the hybrid layer will depend on the chemical compatibility among components, monomer conversion, and curing mode as well.[1,2,24] However, assuming confluence, it is reasonable to assume that presence of the more hydrophobic resins from the cement at and into the hybrid layer would substantially improve the physical properties of the polymer formed in those areas.

Self-curing components, or co-initiators, were added to these bonding systems to overcome the chemical incompatibility between dual-curing resin cements and acidic monomer from the bonding agents.[14] Otherwise, the tertiary amines from the peroxide-amine component can react with acidic monomers to form a charge transfer complex (CT complex)[4] and lose their ability as reducing agents in the redox reaction.[23,24,34] As a consequence, a poor polymerization reaction would be expected from the combined adhesive/resin cement layer or from the mixture between the primer/adhesive oxygen-inhibited layer[21,23] and resin cement components at the top of the light-activated bonding agent layer. This mixture between the primer/adhesive oxygen-inhibited layer was represented by the light orange line between the primer/adhesive and resin cement layers (arrows) (Figs 3B and 9B). Therefore, this interaction between resin cement and bonding agent components is only acceptable when dual-curing adhesive systems are used, even when the primer and adhesive layer are light activated prior to resin cement application.

When Bond 1 was applied to dentin and left in the uncured state (the manufacturer-recommended condition), a significant change in shade within the hybrid layer was observed when self-cured resin cement groups were compared with the light-activated ones. A light yellow hybrid layer was observed when the resin cement was light activated, while a dark red hybrid layer was noted when Lute-it was allowed to self-cure. Such differences in shade are probably related to the longer resin cement setting time promoted by its self-polymerization, which can take several minutes.[20,22] Therefore, the longer setting time allowed deeper resin cement penetration within the hybrid layer and co-mixture of these compounds with those of the primer/adhesive material. This change in monomer composition within the hybrid layer may result in higher content of hydrophobic monomers, which can contribute to longer durability at not only the adhesive layer but also within the hybrid layer.[8,30]

A nonuniformly thick adhesive layer, which was totally absent at some bonded interfaces, was observed when the primer/adhesive layers of both 4th and 5th generation bonding agents were light activated before the indirect restoration was seated (Figs 3A, 3C, 10A, 10B). Therefore, the second hypothesis of the current study was rejected. Although the 3-D features of the CLSM images do not provide a clear indication about differences in dentin tubule density, it is possible that a thinner or absent primer/adhesive layer is related to the higher density of dentin tubules when the bonding agents were applied to these deep dentin surfaces.[12] More primer/adhesive resin would be necessary in deep dentin to compensate its higher permeability due to the higher dentin tubule density, and consequently create a uniform layer on the dentin. On the other hand, the same amount of primer/adhesive resin may create a thick layer when applied to other dentin surfaces where tubule size and density may be less (Figs 3B and 9B). The layer thickness observed in some regions (approximately 25 µm, Fig 9B) can compromise the internal adaptation of indirect restorations and, as a consequence, may promote a thicker luting space at some internal and marginal areas of the restoration.[11,13,19] Several clinical and in vitro investigations revealed the luting space as being the weakest part of a ceramic inlay restoration.[16,25,27] For these reasons, in order to avoid a nonuniform or thick polymerized adhesive layer (Figs 3B, 9B, 9C), care must be taken when a gentle air stream is applied before light activation, in order to create a uniform layer not only at the preparation surfaces, but also at its internal line angles. Moreover, careful visual analysis of the bonded restoration may allow the clinician to distinguish regions with a thick adhesive layer before light activation.

The SEM analysis (Figs 3D, 4C, 5C, 6C, 7C, 9C, 10A) was a useful tool to distinguish and confirm the micromorphological structures observed at the adhesive interface of the CLSM images. The acid treatment (5N HCl) combined with 2.5% NaOCl was used to provide clear observation of the hybrid layer morphology and resin tags: demineralization of dentin and removal of unprotected collagen fibrils. However, other studies demonstrated that specimen polishing and acid treatment can damage the micromorphology and change hybrid layer composition.[5,32,33] On the other hand, as specimen preparation for CLSM analysis does not involve chemical superficial treatments, and imaging is obtained below the surface, the micromorphology of the adhesive interface is preserved.[3] Furthermore, the CLSM images provided detailed information about component distribution from the adhesive resin and resin cements at the resin cement layer and within the hybrid layer as well, while such distinctions were not possible using SEM analysis.

In summary, bearing in mind the limitations of this study, the following statements can be made:

- A combined layer composed of primer/bonding agent and resin cement was observed on the dentin surface and at the entrance of the dentinal tubules when a 4th generation dual-curing adhesive system was used; the 5th generation dual-curing bonding agent used created the combined layer not only on the dentin surface, but also within the hybrid layer and in the dentin tubules.
- Light activation of the primer for All Bond 2 and bonding agent for Bond 1 before cementing an indirect restoration provided a nonuniform adhesive layer, which was also totally absent in some regions, and did not allow monomer infiltration from the resin cement or Pre-Bond resin into the hydrophilic bonding agent and dentin.
- The option of not light curing the bonding agent prior to the application of resin cement can be a reliable technique considering the changes in composition and morphology of the adhesive interface. However, only evaluation of the mechanical properties of adhesive interfaces created when this technique is used will confirm its effectiveness.

CONCLUSION

Based on the findings of this study, it is recommended that care be taken when dual-curing 4th or 5th generation adhesive systems are applied and indirect restorations are cemented on bonded dentin. Much more knowledge is needed to reliably provide adequate marginal adaptation and proper formation of a combined layer at the bonded interface. Further in vivo studies are needed to evaluate the effects of dentinal fluids under pulpal pressure on the penetration of dual-curing resin cements into dentin tubules and within the hybrid layer, as well as to validate the clinical performance of the mixture created by dual-curing bonding agents and resin cements such as those observed in this study.

ACKNOWLEDGMENTS

The authors are indebted to Bisco Inc. and the Pentron Corporation for providing all restorative materials, and to Dr. E. W. Kitajima (NAP-MEPA/ESALQ-USP) for support of the microscopy procedures used. This study was supported by grants from FAPESP (#03/03645-0) and CAPES (#BEX 0184/05-5) and the Medical College of Georgia School of Dentistry, Augusta, GA, USA.

REFERENCES

1. Arrais CA, Giannini M, Rueggeberg FA, Pashley DH. Effect of curing mode on microtensile bond strength to dentin of two dual-cured adhesive systems in combination with resin luting cements for indirect restorations. Oper Dent 2007;32:37-44.
2. Arrais CA, Giannini M, Rueggeberg FA, Pashley DH. Microtensile bond strength of dual-polymerizing cementing systems to dentin using different polymerizing modes. J Prosthet Dent 2007;97:99-106.
3. Bitter K, Paris S, Martus P, Schartner R, Kielbassa AM. A Confocal Laser Scanning Microscope investigation of different dental adhesives bonded to root canal dentine. Int Endod J 2004;37:840-848.
4. Bowen RL, Cobb EN, Rapson JE. Adhesive bonding of various materials to hard tooth tissues: improvement in bond strength to dentin. J Dent Res 1982;61:1070-1076.
5. Carvalho RM, Yoshiyama M, Horner JA, Pashley DH. Bonding mechanism of VariGlass to dentin. Am J Dent 1995;8:253-258.
6. D'Alpino PH, Pereira JC, Svizero NR, Rueggeberg FA, Pashley DH. Use of fluorescent compounds in assessing bonded resin-based restorations: a literature review. J Dent 2006;34:623-634.
7. D'Alpino PH, Pereira JC, Svizero NR, Rueggeberg FA, Carvalho RM, Pashley DH. A new technique for assessing hybrid layer interfacial micromorphology and integrity: two-photon laser microscopy. J Adhes Dent 2006;8:279-284.
8. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, Lambrechts P, Vanherle G. Four-year water degradation of total-etch adhesives bonded to dentin. J Dent Res 2003;82:136-140.
9. Dietschi D, Spreafico R. Current clinical concepts for adhesive cementation of tooth-colored posterior restorations. Pract Periodontics Aesthet Dent 1998;10:47-54; quiz 56.
10. Dong CC, McComb D, Anderson JD, Tam LE. Effect of mode of polymerization of bonding agent on shear bond strength of autocured resin composite luting cements. J Can Dent Assoc 2003;69:229-234.
11. Frankenberger R, Sindel J, Krämer N, Petschelt A. Dentin bond strength and marginal adaptation: direct composite resins vs ceramic inlays. Oper Dent 1999;24:147-155.
12. Giannini M, Carvalho RM, Martins LR, Dias CT, Pashley DH. The influence of tubule density and area of solid dentin on bond strength of two adhesive systems to dentin. J Adhes Dent 2001;3:315-324.
13. Hahn P, Schaller H, Hafner P, Hellwig E. Effect of different luting procedures on the seating of ceramic inlays. J Oral Rehabil 2000;27:1-8.
14. Ikemura K, Endo K. Effect of adhesion of new polymerization initiator systems comprising 5-monosubstituted barbituric acids, sulfinate amides, and ter-butyl peroxymaleic acid in dental adhesive resin. J Appl Polymer Sci 1999;72:1655-1668.
15. Inokoshi S, Willems G, Van Meerbeek B, Lambrechts P, Braem M, Vanherle G. Dual-cure luting composites: Part I: Filler particle distribution. J Oral Rehabil 1993;20:133-146.
16. Krejci I, Lutz F, Reimer M. Wear of CAD/CAM ceramic inlays: restorations, opposing cusps, and luting cements. Quintessence Int 1994;25:199-207.
17. Magne P, Douglas WH. Porcelain veneers: dentin bonding optimization and biomimetic recovery of the crown. Int J Prosthodont 1999;12:111-121.
18. McCabe JF, Rusby S. Dentine bonding – the effect of pre-curing the bonding resin. Br Dent J 1994;176:333-336.
19. Pashley DH. In vitro simulations of in vivo bonding conditions. Am J Dent 1991;4:237-240.
20. Peters AD, Meiers JC. Effect of polymerization mode of a dual-cured resin cement on time-dependent shear bond strength to porcelain. Am J Dent 1996;9:264-268.
21. Rueggeberg FA, Margeson DH. The effect of oxygen inhibition on an unfilled/filled composite system. J Dent Res 1990;69:1652-1658.

22. Rueggeberg FA, Caughman WF. The influence of light exposure on polymerization of dual-cure resin cements. Oper Dent 1993;18:48-55.
23. Ruyter IE. Unpolymerized surface layers on sealants. Acta Odontol Scand 1981;39:27-32.
24. Sanares AM, Itthagarun A, King NM, Tay FR, Pashley DH. Adverse surface interactions between one-bottle light-cured adhesives and chemical-cured composites. Dent Mater 2001;17:542-556.
25. Shinkai K, Suzuki S, Leinfelder KF, Katoh Y. Effect of gap dimension on wear resistance of luting agents. Am J Dent 1995;8:149-151.
26. Sjögren G, Molin M, van Dijken J, Bergman M. Ceramic inlays (Cerec) cemented with either a dual-cured or a chemically cured composite resin luting agent. A 2-year clinical study. Acta Odontol Scand 1995;53:325-330.
27. Suzuki S, Leinfelder KF, Shinkai K. Wear resistance of resin cements. Am J Dent 1995;8:83-87.
28. Swift EJ, Jr., Perdigao J, Heymann HO. Bonding to enamel and dentin: a brief history and state of the art, 1995. Quintessence Int 1995;26:95-110.
29. Swift EJ Jr, May KN Jr, Wilder AD Jr. Effect of polymerization mode on bond strengths of resin adhesive/cement systems. J Prosthodont 1998;7:256-260.
30. Tanaka J, Ishikawa K, Yatani H, Yamashita A, Suzuki K. Correlation of dentin bond durability with water absorption of bonding layer. Dent Mater J 1999;18:11-18.
31. Tay FR, Carvalho R, Sano H, Pashley DH. Effect of smear layers on the bonding of a self-etching primer to dentin. J Adhes Dent 2000;2:99-116.
32. Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Perdigao J, Lambrechts P, Vanherle G. Microscopy investigations. Techniques, results, limitations. Am J Dent 2000;13:3D-18D.
33. Wang Y, Spencer P. Exploring the nature of acid-resistant hybrid layer with wet bonding. Oper Dent 2004;29:650-655.
34. Yamauchi J. Study of dental adhesive containning phosphoric acid methacrylate monomer. Japanese J Dent Mater 1986;5:144-154.

Clinical relevance: A new combined resin cement/bonding agent layer is created during the cementation of indirect resin composite restorations when the bonding agent is left in the uncured state prior to the application of the resin cement. This combined layer would be more hydrophobic than the layer composed of solely bonding agent due to the addition of hydrophobic monomers from the resin cement.