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Limited Decalcification/Diffusion of Self-adhesive Cements into Dentin

ABSTRACT

Resin cement diffusion into dentin may differ as a function of the pre-treatment regimen. Since self-adhesive cements do not require substrate pre-treatment for luting, penetration of and interaction with the underlying dentin are questioned. We hypothesized that differences in the resin cement diffusion into dentin may exist among current commercial adhesive cements. Composite cylinders were luted on mid-coronal dentinal surfaces by an etch-and-rinse cement (Calibra), a self-etching system (Panavia F 2.0), and 4 self-adhesive cements (Multilink Sprint, Rely X Unicem, G-Cem, Bis-Cem). Dentin/cement interfacial characteristics were analyzed by a staining technique (Masson's trichrome) and by scanning electron microscopy. Conventional acid etching resulted in partially infiltrated adhesive interfaces differing from those achieved with the application of self-etching primer. No hybrid layer and/or resin tag formation was detectable at the interfaces bonded with self-adhesive cements. Limited decalcification/infiltration was observed for self-adhesive cements into the underlying dentin. Self-adhesive cements were not able to demineralize/dissolve the smear layer completely.

KEY WORDS: self-adhesive cement, Masson's trichrome, smear layer, demineralization, exposed collagen.

INTRODUCTION

Resin-based dental luting agents, which are routinely used for luting gold, composite crowns, and all-ceramic restorations, have traditionally required a separate etching step to allow for subsequent adhesive infiltration (Diaz-Arnold *et al.*, 1999). Incomplete adhesive diffusion throughout the demineralized dentin has been reported for conventional dentin-bonding agents (Spencer and Swafford, 1999). The discrepancy between etching depth and adhesive penetration led to a large area of exposed collagen at the interface between the adhesive and prepared dentin surfaces. If this discrepancy occurs with luting agents that require a separate etching step, it is conceivable that there may be post-operative sensitivity as a result of the exposed collagen (Walshaw and McComb, 1996).

To overcome some of the limitations associated with dentin etching, investigators have proposed resin cements that include self-etching primers (Watanabe *et al.*, 1994). This approach has re-introduced the concept of using the smear layer as a bonding substrate, but with novel formulations that should etch beyond the smear layer into the underlying dentin (Reis *et al.*, 2005).

Interest has been increasingly focused on the use of self-adhesive cements. These systems were designed with the purpose of combining the favorable characteristics of different cements into a single product, in an attempt to satisfy demands for simplification of luting procedures and supposedly leaving little room for application mistakes induced by technique-sensitivity (De Munck *et al.*, 2004; Ibarra *et al.*, 2007).

Self-adhesive cements do not require any tooth surface pre-treatment, and their application is accomplished through a single clinical step, similar to the more conventional zinc-phosphate and polycarboxylate cements (Diaz-Arnold *et al.*, 1999).

Based on recent *in vitro* data, the behavior of the most-investigated self-adhesive cement to dentin (Rely X Unicem) and different restorative materials should not differ from that of multi-step resinous cements (Piwowarczyk *et al.*, 2004; Fabianelli *et al.*, 2005; Bitter *et al.*, 2006). However, concerns emerged regarding the bonding potential of these materials to enamel and dentin (Behr *et al.*, 2004; Gerth *et al.*, 2006). Although the basic adhesion mechanism appears similar for all self-adhesive cements, these materials are still relatively new, and detailed information on their composition and adhesive properties is limited.

The purpose of this study was a qualitative comparison of the dentin/cement interfacial characteristics of 6 current commercial adhesive cements that differ as a function of pre-treatment regimen. We used scanning electron microscopy (SEM) and a staining technique for optical microscopy that specifically identifies depth of decalcification/infiltration or exposed collagen at the dentin/cement interface. This study tested the hypothesis that resin cement diffusion into prepared dentin surfaces would differ as a function of the pre-treatment regimen.

Table. Chemical Composition and Application Mode of the Resin Cements Tested in the Study

Material	Composition	Application
Calibra (H_3PO_4) pH = 0.4	Caulk 34% Tooth Conditioner Gel (34% phosphoric acid). Prime&Bond NT: Acetone; Di- and Tri methacrylate resins; Urethane Dimethacrylate; PENTA; Nanofiller- amorphous silicone dioxide; Photoinitiators; Stabilizers; Cetylamine hydrofluoride. Calibra: Base: Barium boron fluoroalumino silicate glass; Bis-phenol A diglydidylmethacrylate;Polymerizable dimethacrylate resin; Hydrophobic amorphous fumed silica; Titanium dioxide; dl-camphoroquinone. Catalyst: Barium boron fluoroalumino silicate glass; Bis-phenol A diglydidylmethacrylate; Polymerizable dimetha-crylate resin; Hydrophobic amorphous fumed silica; Titanium dioxide; Benzoyl peroxide.	Apply etchant (30 sec). Water rinse (20 sec). Air-drying. Apply adhesive in a single coat. Gentle air-drying after 5 sec. Light cure for 20 sec. Mix Base and Catalyst (1:1). Apply and self-cure (5 min) Light-cure (40 sec).
Panavia F 2.0 pH = 2.4	ED Primer II: Liquid A: 10- methacryloloxydecyl dihydrogenphosphate; 2-hydroxyethyl methacrylate; N,N-diethanol-p-toluidine; N-methacryloyl 5-aminosalicylic acid; water. Liquid B: N,N-diethanol-p-toluidine; Sodium benzen sulphinate; N-methacryloyl 5-amino-salicylic acid; water. Panavia F: Paste A: Silanated barium glass; colloidal silica; Bisphenol A Polyethoxy Dimethacrylate; 10- methacryloloxydecyl dihydrogenphosphate; Hydrophilic dimethacrylate; Hydrophobic dimethacrylate; benzoil peroxide; dl-camphoroquinone. Paste B: Silanated barium glass; Silanated titanium oxide; Sodium fluoride colloidal silica; Bisphenol A Polyethoxy Dimethacrylate; Hydrophilic dimetha-crylate;Hydrophobic dimethacrylate; N,N-diethanol-p-toluidine; Sodium 2,4,6-Triiso-propyl benzene sulfinate.	Mix ED Primer A+B (1:1). Apply on the tooth. Gently air-blow after 30 sec. Mix Paste A+B (1:1) for 20 sec. Apply and self-cure (5 min) Light-cure (40 sec)
Rely X Unicem pH = 2.1	Powder: glass fillers, silica, calcium hydroxide, self-curing initiators, pigments, light-curing initiators. Liquid: methacrylated phosphoric esters, dimethacrylates, acetate, stabilizers, self-curing initiators, light-curing initiators	Mix cement. Apply, self-cure (5 min) and light-cure (40 sec)
Multilink Sprint pH = 4.2	Dimethacrylates; adhesive monomer; Fillers; initiators / stabilizers	Mix cement. Apply, self-cure (5 min) and light-cure (40 sec)
G-Cem pH = 2.7	UDMA; phosphoric acid ester monomer; 4-META; water; dimethacrylates; silica powder; initiators/stabilizers; fluoro-amino-silicate glass	Mix cement. Apply, self-cure (5 min) and light-cure (40 sec)
Bis-Cem pH = 2.1	Bis (Hydroxyethyl methacrylate) phosphate (Base); Tetraethylene glycol dimethacrylate; dental glass	Mix cement. Apply, self-cure (5 min) and light-cure (40 sec)

MATERIALS & METHODS

Crowns were removed (Isomet 4000, Buehler, Lake Bluff, IL, USA) from 30 extracted human third molars, stored at 4°C in 0.1% wt/vol Chloramine T solution, yielding mid-coronal dentin surfaces that were ground with 600-grit wet silicon carbide papers, creating a uniform thin smear layer. Human specimens were used following a protocol that was approved by the institutional review board (IRB) and with the informed consent of the donors.

We made composite cylinders by layering 2-mm-thick increments of a micro-filled hybrid composite (Gradia Direct Anterior, GC Corp., Tokyo, Japan, shade A3) in a split-aluminum mold (8 mm diameter/4 mm height). Each increment was light-cured for 40 sec (VIP, Bisco Inc., Schaumburg, IL, USA; output, 600 mW/cm^2). The specimen was removed from the mold, and additionally light-cured from 5 aspects for 40 sec each on the portions previously in contact with the metallic surface of the mold.

The prepared dentin surfaces (n = 5 each group) were luted with: (1) Calibra dual-cured etch-and-rinse cement (Dentsply DeTrey GmbH, Konstanz, Germany); (2) Panavia F 2.0 dual-cured self-etch cement (Kuraray Co. Ltd, Osaka, Japan); (3)

Multilink Sprint (Ivoclar-Vivadent, Schaan, Liechtenstein); (4) Rely X Unicem (3M ESPE, St. Paul, MN, USA); (5) G-Cem (GC Corporation, Tokyo, Japan); and (6) Bis-Cem (Bisco, Schaumburg IL, USA) dual-cured self-adhesive cements.

pH measurements were performed for all tested luting agents. After being mixed, the agents were dispensed on narrow-range pH acid indicator strips (0.0-1.8; 1.8-3.8; 3.8-5.5; Panreac Química, Barcelona, Spain). The compositions, pH, and application modes of the tested resin cement systems are reported in the Table.

The luting procedure for composite cylinders on dental substrates was performed by a constant pressure of 40 g/mm^2 exerted during the initial five-minute self-curing period (Goracci *et al.*, 2006).

Trichrome Stain and Microscopic Observation

After 24-hour storage (37°C at 100% humidity), 3 samples from each group were sectioned perpendicular to the bonded surface into 1-mm-thick slabs by means of a water-cooled, low-speed diamond saw (Isomet 4000). In total, 12 sections were analyzed for each dentin treatment. Slabs were glued onto methacrylate supports with photo-curing adhesive (Technovit 7200 VLC, Kulzer, Norderstedt, Germany) and ground with an Exakt polishing machine (EXAKT
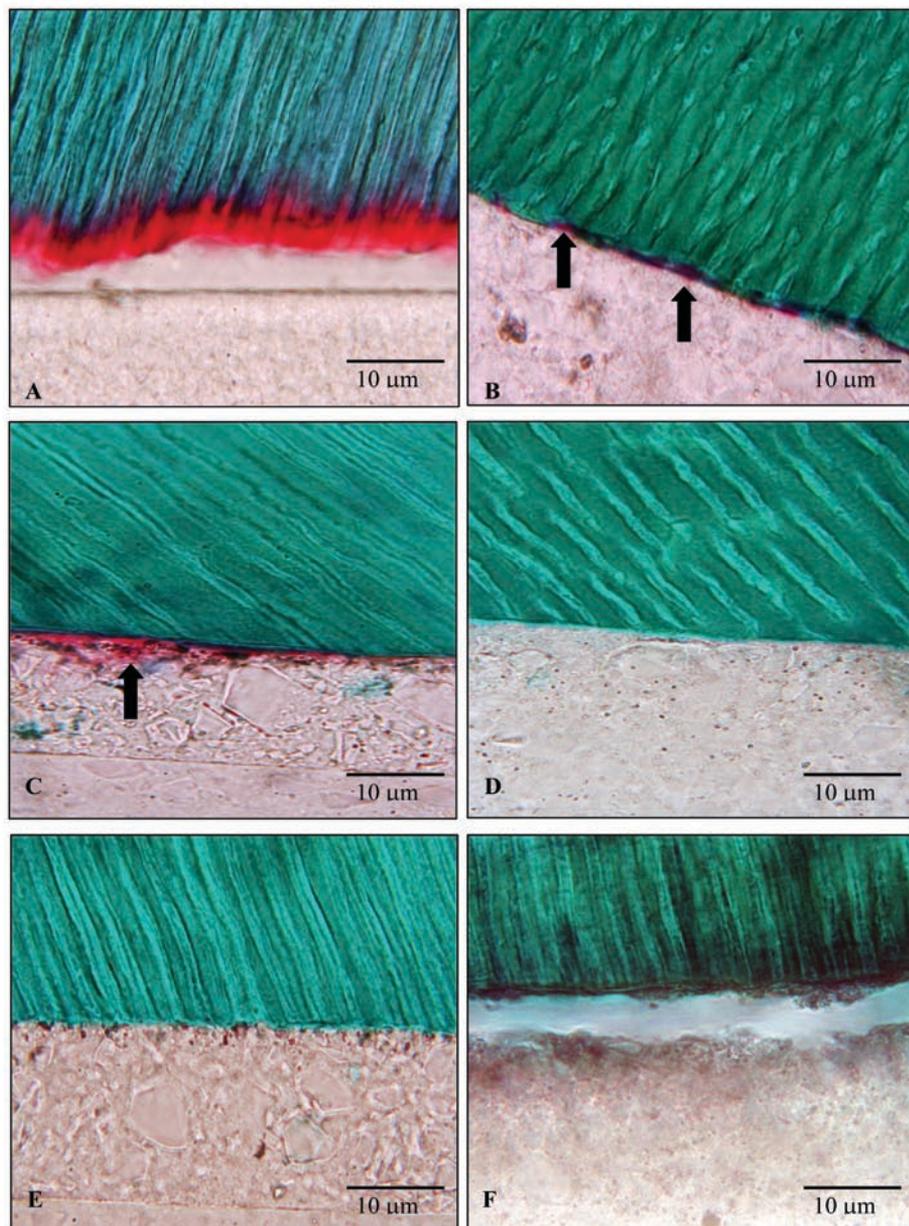
Figure 1. Representative light micrographs of cement/dentin interfaces stained with Masson's trichrome: mineralized dentin (green), resin cement (clear with filler particles), exposed protein (red). **(A)** A distinct red zone of exposed protein was identified in the sections recovered from specimens etched with phosphoric acid (Calibra). **(B,C)** A slight purple line representing collagen partially reacted with resin cement is detectable at the interface between dentin and the self-etching primer (B; Panavia F 2.0) or Multilink Sprint (C) (arrows). **(D-F)** No signs of demineralization and/or exposed protein (red stain) are detectable at the cement/dentin interface of Rely X Unicem (D), G-Cem (E), and Bis-Cem (F). Original magnification, 100x; bar = 10 μm.

SEM evaluation. Samples were sectioned perpendicular to the bonded surface (Isomet 4000). Each section was polished with wet abrasive SiC papers, gently decalcified (37% phosphoric acid/10 sec), de-proteinized (2% NaOCl solution/1 min), ultrasonicated in 96% ethanol for 2 min, and air-dried. Samples were mounted on stubs, sputter-coated with gold (Polaron Range SC7620; Quorum Technology, Newhaven, UK), and observed by scanning electron microscopy (SEM; JSM-6060 LV, JEOL, Tokyo, Japan) at different magnifications to evaluate for resin tags and hybrid layer formation. Impressions of the restored teeth and positive impression replicas were fabricated (Chersoni *et al.*, 2004) and observed by SEM to control for artefact formation.

RESULTS

According to the Masson's trichrome-staining technique, the mineralized dentin stains green, whereas the resin cement is clear with filler particles. The staining reaction of proteins is non-specific, and some non-collagenous proteins may have been stained. Type I collagen represents 90% of the dentin organic matrix. Thus, it is likely that the protein staining (red) resulted from dentin collagen unprotected by mineral and/or resin.

With the conventional etch & rinse luting agent (Calibra), a distinct red zone of denuded collagen at the base of the bonded interface was observed. Tubules were opened (Fig. 1A), and a hybrid layer with resin tag formation was identified by SEM (Fig. 2A). At the dentin/cement interface of teeth luted with the self-etching cement (Panavia), a narrow purple line representing mild collagen demineralization was detectable at the intact dentin surface (Fig. 1B). After Multilink Sprint application, the dentin surface appeared red (decalcified), but was not resin-infiltrated (Fig. 1C).

No demineralization/infiltration of dentin was evident for the self-adhesive cements Rely X Unicem (Fig. 1D), G-Cem (Fig. 1E), and Bis-Cem (Fig. 1F), which produced similar interfacial patterns. All light microscopy sections from the Bis-Cem group debonded at the cement/dentin interfacial level during laboratory preparation procedures (Fig. 1F).

A scanning electron micrograph of Bis-Cem bonded to dentin revealed an intimate adaptation with the substrate. However, no signs of hybrid layer formation with the underlying dentin could be noticed; no resin tags were observed

Technologies, Inc., Oklahoma City, OK, USA) with SiC abrasive wet papers (800-, 1200-, 2500-, and 4000-grit) until a thickness of 5-6 μm was achieved. Differential staining was accomplished with Masson's trichrome, a classic bone stain (Erhardt *et al.*, 2008). After samples were cover-slipped with mounting media, they were examined by light microscopy (BH2, Olympus, Tokyo, Japan) at 100x magnification.

Scanning Electron Microscopy

Two additional specimens for each group were prepared for

(Fig. 2B). All the tested auto-adhesive luting cements recorded an acidic pH ranging from 2.1 and 4.2 after mixing (Table).

DISCUSSION

Within the limitations of this study, the combined application of the trichrome technique and SEM examination provided information about demineralized dentin depth, extent of adhesive diffusion, and hybrid layer formation (Spencer and Swafford, 1999). Differences in resin cement diffusion into the prepared dentin surfaces as a function of the pre-treatment regimen were evidenced. The interfacial patterns of the tested simplified self-adhesive cements were not comparable with those of conventional resin-based systems. Thus, the null hypothesis had to be rejected.

The substantial zone of demineralization produced by the etch-and-rinse pre-treatment facilitated resin penetration, but infiltration of Prime&Bond NT was not complete, since it was encountered when compared with other etch & rinse systems (Spencer *et al.*, 2004). It seems that the solvent (acetone) was unable to generate interfibrillar spaces wide enough to accommodate the infiltrating adhesive (Pashley *et al.*, 2002). This adhesive does not contain monomers capable of enhancing diffusion and lowering the initial viscosity of the mixture (HEMA or TEGDMA) (Toledano *et al.*, 2006).

Differing from the application of the etch & rinse system, the mild etching-priming blend (Panavia; pH = 2.4) produced minimal dentin demineralization, but resin penetration was identified. It contains ambiphilic monomers (HEMA, 10-MDP, 5-NMSA), with low molecular weight, that may have selectively diffused into dentin (Al-Assaf *et al.*, 2007), forming the hybridized complex (Walker *et al.*, 2002; Reis *et al.*, 2005).

Similar to self-etching primer formulations, self-adhesive cements contain multifunctional phosphoric acid methacrylates that are claimed to react with the hydroxyapatite of the hard tooth tissue (Fu *et al.*, 2005; Moszner *et al.*, 2005; Hikita *et al.*, 2007). However, no evidence of decalcification/infiltration into dentin was found in any of the tested self-adhesive cements. To achieve a correct infiltration pattern, these cements should be able to etch the substrate in a relatively short time, requiring optimal wetting properties to ensure a fast interaction with dental hard tissues (Moszner *et al.*, 2005). Ideally, the thin smear layer evaluated in this study should allow acidic
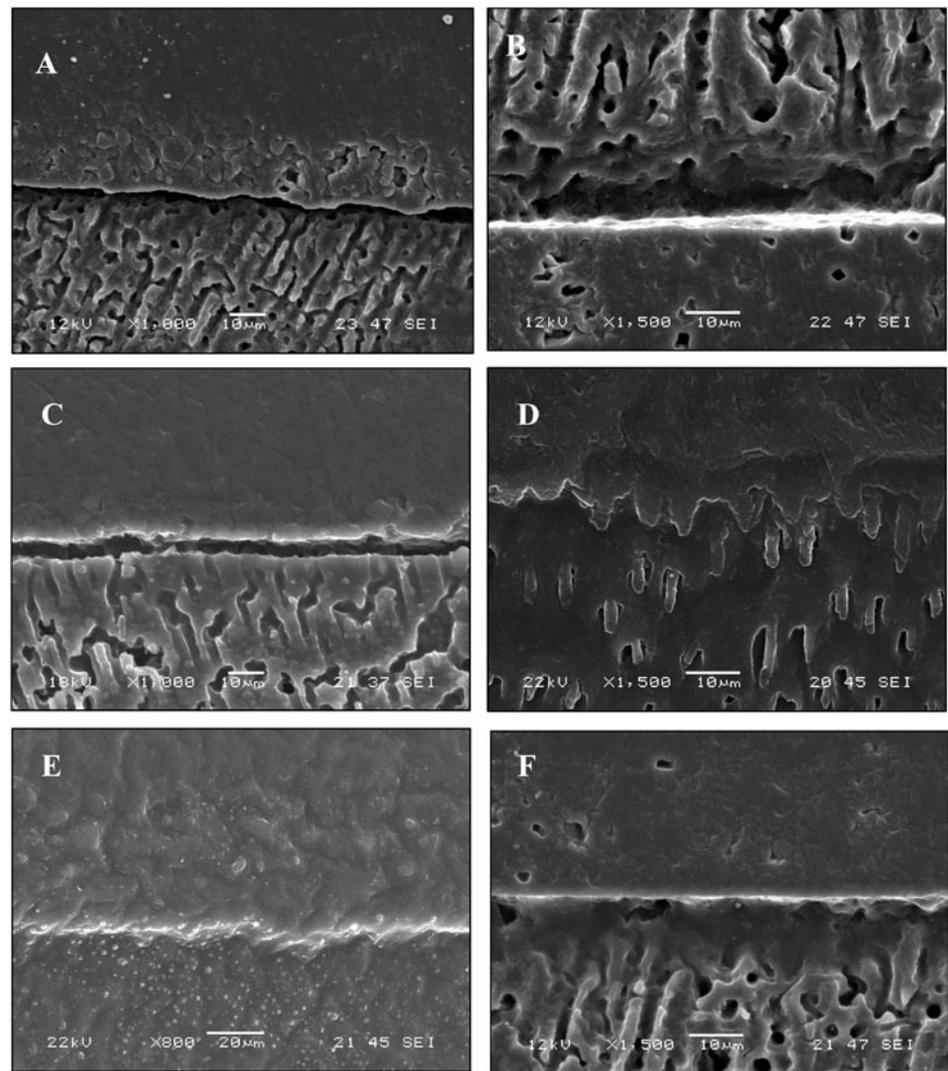
Figure 2. Scanning electron micrographs of G-Cem (**A**), Rely X Unicem (**B**), Multilink Sprint (**C**), Calibra (**D**), Panavia F 2.0 (**E**), and Bis-Cem (**F**). When the multi-step resin cement (Calibra) was used, dentin was demineralized and consecutively infiltrated by resin. Resin tags and a resin cement/hybrid zone are identified. When luted with self-adhesive cements, tubules were not infiltrated by resin, but intimate adaptation was seen, and no distinct morphological manifestation of interaction with dentin was observed.

monomers free access to the mineralized tissue underneath (Reis *et al.*, 2005), but this did not occur. Despite the initial acidic pH, Rely X Unicem and Bis-Cem did not produce any evidence of dentin demineralization and/or hybridization at the micrometer level (Yang *et al.*, 2006; Al-Assaf *et al.*, 2007). The adhesive interface appeared essentially similar to that of some conventional luting agents (silicate cements or zinc-phosphate) (Behr *et al.*, 2004).

Some reasons may be proposed for the limited capacity of these cements to diffuse and decalcify the underlying dentin effectively: (1) High viscosity (De Munck *et al.*, 2004), which may rapidly increase as an acid-base reaction (ionic bond formation and setting), reminiscent of conventional cements (i.e., silicate or glass ionomers), is supposed to occur (Fukuda *et al.*, 2003); and (2) a neutralization effect may occur during setting, since these chemical reactions involve water release and alkaline filler that may raise

the pH level (Behr *et al.*, 2004; Al-Assaf *et al.*, 2007); this neutralization effect may also be exerted by dentin-buffering components contained in the smear layer (Oliveira *et al.*, 2003; Reis *et al.*, 2005).

In an attempt to improve diffusivity, one could propose a reduction in the initial pH of the cement formulations, but this would impair the hydrolytic stability of acidic methacrylate phosphates (Moszner *et al.*, 2005), may reduce polymerization efficacy (Nunes *et al.*, 2006), and could leave an unprotected interface prone to degradation.

The presence of the smear layer has been recognized as the "weak" link in bonding of glass ionomers to dentin, and this may also be the case with self-adhesive cements (Al-Assaf *et al.*, 2007). Phosphoric acid etching, prior to the application of the self-adhesive cement, has been shown to be detrimental to effective dentin bonding (De Munck *et al.*, 2004). Most likely, the choice of milder acidic agents to remove the superficial loosely bound fraction of the smear layer could somewhat enhance adhesion.

In the case of G-Cem, a self-adhering capacity to dentin may be due to the incorporation of 4-META, which bonds by a chelating reaction to calcium ions in apatite (Abo *et al.*, 2004). Water in the cement composition is expected to aid the conditioning reaction, reducing the time needed for interacting with the substrate. However, the relatively weak bonding potential and the high molecular weight of the functional monomer are expected to contribute poorly to the supposed chemical reaction, within a clinically reasonable time (Yoshida *et al.*, 2004).

The mild discrepancy between demineralization and the infiltration depths recorded for Multilink Sprint may be the result of a deeper diffusion of non-cured non-neutralized acidic monomers below the smear layer. Similar to self-etching primers, these residuals may retain their etching potential, forming an unprotected dentin zone and jeopardizing adhesion (Carvalho *et al.*, 2005; Wang and Spencer, 2005a,b).

A bond strength study performed under the same laboratory conditions (Mazzitelli *et al.*, 2008) attained results that correlate with the present findings. Calibra attained the highest bond strength and Bis-Cem the lowest (68% of Bis-Cem specimens produced pre-testing failures); Rely-X and G-Cem recorded bond strengths somewhat higher than those of Multilink Sprint and Bis-Cem, but due to the high standard deviations, differences were not identified. Long-term bond strength results remain to be ascertained.

Intimate adaptation of self-adhesive cements to dentin was observed by SEM, but no hybrid layer or resin tag formation was in evidence. Other mechanisms (as chemical interactions) had been advocated to occur at these complex interfaces (De Munck *et al.*, 2004; Hikita *et al.*, 2007). It should also be noted that decalcification of underlying dentin was not produced by any of these cements, so ionic bonding may also be impaired. Following the adhesion/decalcification concept, demineralization is a surface-controlled phenomenon involving interaction with hydroxyapatite and depends upon adsorption of the acid anions onto hydroxyapatite. Acidity of the cements/adhesives may not be as determining as previously thought (Yoshida *et al.*, 2001).

It is worth mentioning that a standardized cementation pressure was applied in this experiment in consideration of previous investigations (Goracci *et al.*, 2006). The cement viscosity most likely decreased while undergoing shear, producing better adaptation and reducing cement film thickness. However, such a thixotropic behavior does not necessarily allow for a deeper interaction of auto-adhesive cements with the substrate (De Munck *et al.*, 2004).

In conclusion, self-adhesive cements were unable to demineralize/dissolve the smear layer completely, and no decalcification/infiltration of dentin was observed. The presence of a partially demineralized/infiltrated smear layer and/or micromechanical retention with dentin may be responsible for the previously reported adhesion values, always weaker than those of conventional resin-based cements (De Munck *et al.*, 2004; Mazzitelli *et al.*, 2008).

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