Bonding of an auto-adhesive luting material to enamel and dentin

Jan De Muncka, Marcos Vargasa, Kirsten Van Landuyta, Kazuhiro Hikitaab,c, Paul Lambrechtsa, Bart Van Meerbeeka,*

aLeuven BIOMAT Research Cluster, Department of Conservative Dentistry, School of Dentistry, Oral Pathology and Maxillo-facial Surgery, Catholic University of Leuven, Kapucijnenvoer 7, Leuven 3000, Belgium
bDepartment of Operative Dentistry, University of Iowa, Iowa city, IA, USA
cInstitute of Medical Science, Health Sciences University of Hokkaido, Sapporo, Japan

Received 14 October 2003; received in revised form 2 February 2004; accepted 1 March 2004

KEYWORDS
Adhesion; Cement; Self-adhesive; Micro-tensile bond strength; Fe-SEM; TEM

Summary Objectives: The objectives of this study were (1) to assess the bonding performance of a new auto-adhesive cement (RelyX Unicem, 3M ESPE) to enamel and dentin, using a standard micro-tensile bond strength (μTBS) test set-up, and (2) to evaluate the interaction of this material with dentin by means of high-resolution electron microscopy.

Methods: The μTBS of RelyX Unicem with and without prior acid etching was determined to enamel and dentin after 24 h of water storage and compared to the bonding effectiveness of the control cement (Panavia-F, Kuraray). In addition, diamond-knife cut interfaces of RelyX Unicem and Panavia-F bonded to dentin were examined using field-emission scanning (Fe-SEM) and transmission electron microscopy (TEM).

Results: The μTBS of RelyX Unicem to enamel was significantly lower than that of the control cement, whereas no significant difference was found when both cements were bonded to dentin. Acid etching prior to the application of RelyX Unicem raised the enamel μTBS to the same level as that of the control, but was detrimental for the dentin bonding effectiveness. The latter must be attributed to inadequate infiltration of the collagen mesh as revealed by Fe-SEM and TEM. Morphological evaluation additionally revealed that RelyX Unicem only superficially interacted with enamel and dentin, and that application using some pressure is required to ensure close adaptation of the cement to the cavity wall.

Significance: (1) RelyX Unicem should always be applied with some pressure, to ensure that the relatively high viscous cement intimately adapts to the cavity wall; (2) The cement only interacted superficially with dentin and enamel; (3) The best bonding effectiveness with this new auto-adhesive cement was obtained by selectively acid-etching enamel prior to luting.

© 2004 Published by Elsevier Ltd on behalf of Academy of Dental Materials. All rights reserved.

*Corresponding author. Tel.: +32-16-33-75-87; fax: +32-16-33-27-52. E-mail address: bart.vanmeerbeek@med.kuleuven.ac.be


www.intl.elsevierhealth.com/journals/dema
Introduction

Resin-based adhesive luting materials are widely used for the fixation of inlays and onlays, crowns, posts and veneers. Currently, all resin cements are based upon the use of an etch-and-rinse or self-etch adhesive along with a low-viscosity resin composite. This multi-step application technique is complex and rather technique sensitive, and consequently may compromise bonding effectiveness. Recently, a new resin-based cement has been marketed that combines the use of adhesive and cement in one single application, eliminating the need for pre-treatment of both tooth and restoration. The adhesive properties are claimed to be based upon acidic monomers that demineralize and infiltrate the tooth substrate, resulting in micro-mechanical retention. Secondary reactions have been suggested to provide chemical adhesion to hydroxyapatite (3M ESPE RelyX Unicem product profile) a feature currently only proven for glass-ionomers.2,3

The purpose of this study was (1) to assess the bonding performance of a new auto-adhesive resin cement (RelyX Unicem, 3M ESPE) to enamel and dentin, using a standard micro-tensile bond strength (μTBS) test set-up, and (2) to evaluate the interaction of this material with dentin by means of high-resolution electron microscopy. The hypotheses tested were: (1) The use of a simplified application procedure does not decrease bonding effectiveness to both enamel and dentin. (2) The use of phosphoric acid prior to luting improves the bonding effectiveness. (3) The bonding mechanism of RelyX Unicem to dentin is similar to that of a self-etch adhesive.

Material and methods

Eighteen non-carious human third molars (gathered following informed consent, approved by the Commission for Medical Ethics of the Catholic University of Leuven) were stored in 0.5% chloramine in water at 4 °C and used within 1 month after extraction. The teeth were randomly divided into six experimental groups.

Enamel specimen preparation for μTBS

Lingual and/or buccal enamel was flattened using a high-speed diamond bur (842, Komet, Lemgo, Germany), mounted in the MicroSpecimen Former (University of Iowa, Iowa City, IA, USA). Prior to cementing procedures, pre-cured resin composite blocks (Paradigm MZ100, 3M ESPE, St Paul, MN, USA) were cut using a low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) to obtain a standard surface roughness. The bonding surface was prepared according to the manufacturers’ instructions of the respective cement (Table 1).

Next, the resin cement was applied on the enamel

<table>
<thead>
<tr>
<th>Group</th>
<th>Composition</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNICEM (3M ESPE, Seefeld, Germany)</td>
<td>Powder: glass powder, silica, calcium hydroxide, pigment, substituted pyrimidine, peroxo compound, initiator. Liquid: methacrylated phosphoric ester, dimethacrylate, acetate, stabilizer, initiator [001/0001]</td>
<td>Composite block: no pre-treatment. Tooth: no pre-treatment. Cement: mix capsule for 15° (Rotomix, 3M ESPE); apply on surface; lute resin block using light pressure; light cure for 20° from each side</td>
</tr>
<tr>
<td>H₃P0₄ + UNICEM</td>
<td>Etchant: 35% phosphoric acid, silica thickener (3M ESPE). RelyX Unicem see above</td>
<td>Composite block: no pre-treatment. Tooth: apply etchant for 15 s; rinse; air-dry without desiccation. Cement: as described above</td>
</tr>
<tr>
<td>Panavia-F (Kuraray, Osaka, Japan)</td>
<td>Primer A: HEMA, 10-MDP, 5-NMSA, water, accelerator [00108B], Primer B: 5-NMSA, accelerator, water, sodium benzene sulfinate [0015B]. Paste A: 10-MDP, hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, hydrophilic dimethacrylate, silanated silica, photoinitiator, dibenzoyl peroxide [00124A]. Paste B: hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, hydrophilic dimethacrylate, sodium aromatic sulfinate, accelerator, sodium fluoride, silanated barium glass [00046]</td>
<td>Composite block: no pre-treatment. Tooth: apply K-etchant for 5 s; rinse with water; air dry; apply mixture of Clearfil SE primer [00125C] with Porcelain bond activator [00107B] for 5 s; gently air blow; light cure for 10°. Tooth: mix ED primer (A&amp;B); apply undisturbed for 60°; gently air blow. Cement: mix cement, (A&amp;B); lute resin block using light pressure; light cure for 20° from each side; apply oxyguard [00334A] for at least 3°</td>
</tr>
</tbody>
</table>

HEMA: 2-hydroxyethyl methacrylate; 10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate; 5-NMSA: N-methacryloyloxy-5-aminosalicylic acid.
surface. The auto-adhesive cement was applied with and without prior phosphoric acid etching. The pre-cured composite block was pressed on the cement using light pressure, after which excess cement was removed. Light-curing was performed from four directions parallel along the cement interface using an Optilux 500 (Demetron/Kerr, Danbury, CT, USA) device with a light output not less than 550 mW/cm². At each moment during specimen processing, care was taken to prevent dehydration of the specimens.

**Dentin specimen preparation for μTBS**

The occlusal third of the molars was removed using the Isomet slow-speed diamond saw. Dentin surfaces were controlled for absence of enamel and/or pulp tissue using a stereomicroscope (Wild M5A, Heerbrugg, Switzerland). The teeth were mounted in a chuck and a standard smear layer was produced by removing a thin layer of the surface using a high-speed medium-grit (100 μm) diamond bur (842, Komet) mounted in the MicroSpecimen Former. The cements were subsequently applied following the methodology described above.

**μTBS testing**

After 24 h of water storage, the teeth were sectioned perpendicular to the adhesive-tooth interface using the Isomet saw to obtain rectangular sticks of about 1.8 × 1.8 mm wide and 8–9 mm long. Specimens were trimmed at the biomaterial-tooth surface to a cylindrical hourglass shape with a diameter of about 1.2 mm using the MicroSpecimen Former and fine cylindrical diamond burs (Ø = 1.2 mm, 835KREF, Komet, Lemgo, Germany) under continuous air/water spray. Next, the cross-sectional diameter was precisely (accuracy = 0.001 mm) measured using a precision measuring instrument transformed from a x-y multi-purpose modular microscope (Leitz, Wetzlar, Germany). Specimens were then fixed to Ciucchi’s jig (fixation height 4–6 mm) with cyanoacrylate glue (Model Repair II Blue, Dentsply-Sankin, Ohtawara, Japan) and stressed at a crosshead speed of 1 mm/min until failure in a LRX material testing device (LRX, Lloyd, Hampshire, UK). The μTBS was expressed in MPa, as derived from dividing the imposed force (N) at the time of fracture by the bond area (mm²). When specimens failed before actual testing, the μTBS was determined from the specimens that survived specimen processing with an explicit note of the number of pre-testing failures. The means of the different groups were compared using one-way ANOVA analysis and a Scheffe’s multiple comparisons test at a significance level of 0.05. All statistics were performed using the Statistica software package (StatSoft, Tulsa, OK, USA). The mode of failure was determined at a magnification of 50× using a stereomicroscope (Wild M5A, Heerbrugg, Switzerland).

**Electron microscopy (Fe-SEM and TEM)**

Bonding effectiveness to dentin was also ultra-morphologically assessed by electron microscopy. Two dentin surfaces were prepared for each group in the same way as for μTBS testing. RelyX Unicem was applied using proper pressure during application by pressing the cement with a glass plate. The same cement was also applied to two dentin surfaces that were fractured with a forceps and consequently were free of smear layer debris. Next, a thin layer of a low-viscosity resin composite (Clearfil Protect Liner F, Kuraray) was applied. Then, the resin-bonded dentin specimens were cross-sectioned perpendicular to the resin-dentin interface to 1 mm wide sticks using the Isomet diamond saw. Half of the specimens were demineralized and fixed simultaneously in a 10% formaldehyde-formic acid solution (Gooding and Stewart Fluid, Prosan, Gent, Belgium) for at least 36 h.
Table 2  $\mu$TBS results.

<table>
<thead>
<tr>
<th></th>
<th>UNICEM</th>
<th>H$_2$PO$_4$ + UNICEM</th>
<th>PANAVIA-F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enamel</td>
<td>Dentin</td>
<td>Enamel</td>
</tr>
<tr>
<td>Mean</td>
<td>19.6a</td>
<td>15.9a</td>
<td>35.6a</td>
</tr>
<tr>
<td>SD</td>
<td>6.0</td>
<td>3.9</td>
<td>13.2</td>
</tr>
<tr>
<td>n</td>
<td>15</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>ptf</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD, standard deviation; n, total number of specimens prepared; ptf, pre-testing failures; means with the same superscript are not significantly different ($p < 0.05$, Scheffe multiple comparisons).

Table 3  Failure analysis from light microscopy.

<table>
<thead>
<tr>
<th></th>
<th>Cohesive in tooth</th>
<th>Tooth/adhesive</th>
<th>Adhesive</th>
<th>Adhesive/resin</th>
<th>Cohesive in resin</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNICEM Enamel</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>3</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Dentin</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>H$_2$PO$_4$ + UNICEM Enamel</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Dentin</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>PANAVIA-F Enamel</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>8</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Dentin</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>10</td>
</tr>
</tbody>
</table>

Figure 2  Fe-SEM photomicrographs of RelyX Unicem bonded to bur-cut dentin. (a) RelyX Unicem (Un) applied to a flat surface. At dentin (D), tubules were not obturated by resin tags (hand indicator). Within the RelyX Unicem cement and especially at the interface with dentin, many porosities can be detected (arrow). A = Air bubble resulting from mixing. (b) Higher magnification of (a), clearly showing porosities at the interface (arrow). Within the cement, glass particles (Gp) as well as voids can be noted. (c) Fe-SEM overview of a resin composite inlay (Ci)/RelyX Unicem (Un)/dentin (D) interface (inlay cemented under pressure). The cement is approximately 80 $\mu$m thick, packed with glass particles ranging in size from 1 to 5 $\mu$m and larger. No voids can be observed at the interface with dentin. (d) Higher magnification of (c), though RelyX Unicem adapted pore-free with dentin, no distinct morphological manifestation of interaction with unaffected dentin can be observed.
Further specimen preparation of both the demineralized and non-demineralized sections was performed in accordance with common procedures used for ultra-structural Transmission Electron Microscopy (TEM) examination of biological tissues. Then, 70–90 nm thick sections were cut through the fractured plane using a diamond-knife (Diatome, Bienne, Switzerland) in an ultramicrotome (Ultracut UCT, Leica, Vienna, Austria). For evaluation of collagen, TEM sections were positively stained with 5% uranyl acetate (UA) for 20 min and saturated lead citrate (LC) for 3 min prior to TEM examination (Philips CM10, Eindhoven, The Netherlands). Unstained sections were evaluated as well. The remaining diamond-knife cut blocks, from which the TEM sections were prepared, were gold-sputtered and evaluated by field-emission scanning electron microscopy (Fe-SEM, Philips XL30, Eindhoven, The Netherlands). To evaluate the importance of applying the cement under pressure, also two Class I cavities were prepared with the gingival floor ending into mid-coronal dentin, after which resin composite inlays were cemented using RelyX Unicem. Consequently, in this group the cement was applied using a clinically relevant pressure, opposed to the first groups that were applied to a flat surface using a glass plate, which results in very light pressure.

Results

μTBS testing

The mean μTBS values are presented per experimental group in Fig. 1 and Table 2. The values of the control cement (Panavia-F) were always among the highest values obtained (Table 2), this when bonded to enamel as well as to dentin. The μTBS of RelyX Unicem to enamel was significantly lower than that of the control cement, whereas no significant difference was found when both cements were bonded to dentin (Table 2). The application of RelyX Unicem to acid-etched enamel significantly increased the μTBS to a level that was not significantly different from that of Panavia-F bonded to (non-etched) enamel. However, the μTBS of RelyX Unicem to acid-etched dentin was significantly lower than when dentin was not acid etched, and significantly lower than when

![Figure 3 TEM photomicrographs of RelyX Unicem bonded to bur-cut dentin. (a) Unstained non-demineralized section. No intermediary zone of interaction between RelyX Unicem and dentin (D) can be detected. Smear plugs (Sp) still occlude the dentinal tubules. Pd: Peri-tubular dentin, Gp: glass particle. (b) Stained non-demineralized section. An irregular interaction layer (I), ranging from nearly 0 to 1.5 μm is present in between cement and unaffected dentin. (c) High magnification of an unstained non-demineralized section at a site of limited interaction. The interaction zone (I) is only a few hundred nanometers thick. Hand pointer = nanofiller. (d) Stained demineralized section. Under the thick irregular interaction zone (I), a deeper zone (Z) showed some heavy-metal stained strings.](image-url)
Panavia-F was bonded to dentin. Failure analysis of the μTBS fracture surfaces in general corroborated these results (Table 3), as nearly all non-etched enamel specimens failed adhesively, in contrast to mostly 'mixed' and 'cohesive' failures in the acid-etched enamel group. Noteworthy for the dentin specimens is that after phosphoric acid etching, all specimens failed solely 'adhesively' between RelyX Unicem and dentin, whereas the application of RelyX Unicem without prior acid etching on the other hand resulted in mostly 'mixed' failures.

**Morphology**

Fe-SEM analysis revealed that the adaptation of RelyX Unicem to the dentin surface substantially improved when it was used to lute a composite inlay in a Class-I cavity. This indicates that some pressure during luting is necessary to prevent voids at the interface (Fig. 2), which otherwise must be detrimental to the bonding longevity. Though with some pressure, an intimate adaptation to the cavity walls could be achieved, RelyX Unicem only interacted superficially with the underlying dentin (Fig. 2). No hybrid layer nor resin tags were observed using SEM. The cement itself is heavily packed with glass particles, ranging in diameter from less than 1 up to 5 μm and more.

TEM analysis revealed that no real hybrid layer was formed. Nevertheless, an irregular interaction zone was disclosed ranging from nearly 0 up to 2 μm (Fig. 3). Below this interaction zone, cement components appeared to have infiltrated deeper, as disclosed by a deeper heavy-metal stained zone (Fig. 3d). To exclude any smear layer effects and to elucidate the actual interaction with unaffected dentin, the cement was applied to fractured dentin. Hardly any interaction was detected (Fig. 4), apart from a very thin, more densely stained layer at the dentin-cement interface. As fractured dentin tubules are not occluded by smear plugs, the cement also infiltrated into the tubules. Within the tubules, RelyX Unicem interacted with the tubule wall in a similar way as at the intertubular dentin surface. The body of the resin tag seemed, however, to have picked up more staining.

When RelyX Unicem was applied to phosphoric acid-etched dentin, the weak link in the bonding

![Figure 4](image-url)
complex was clearly located at the level of the exposed collagen matrix, as shown by failure analysis (Table 3) and Fe-SEM evaluation of the \( \mu \)TBS fracture surfaces (Fig. 5a,b). This was substantiated by TEM evaluation, which revealed the presence of a non-infiltrated demineralized collagen mesh in between cement and unaffected dentin (Fig. 5b,c).

Opposite to RelyX Unicem, the application of Panavia-F resulted in the formation of a ‘true’ hybrid layer (Fig. 6). Demineralization of the surface was however incomplete, so that much hydroxyapatite remained within the hybrid layer (Fig. 6b). On top of this thin (0.5-1 \( \mu \)m) hybrid layer, irregular, amorphous, hybridized remnants of the smear layer can be observed (Fig. 6c).

**Discussion**

In this study, the bonding effectiveness of a new cement with a simplified application procedure was compared to that of a conventional composite cement. The first hypothesis that the use of a simplified application procedure does not decrease bond strengths must be rejected, as the \( \mu \)TBS of the experimental cement to enamel was significantly lower than that of the control cement Panavia-F. Nevertheless, the \( \mu \)TBS of RelyX Unicem to dentin was in the same range of that of Panavia-F to dentin. The \( \mu \)TBS values obtained in this study for the control cement were comparable to those obtained by Mak et al.\(^1\) In that study, however, Panavia-F underscored the other cements investigated. The authors ascribe this to the inhibition of polymerization of the cement by acidic monomers. This effect must have been negligible in this study as the cement was light cured directly upon luting.

Although the pH of the mixed material is very low (<2 during the first minute, data supplied by 3M ESPE), nearly no demineralization of the dentin surface was noticed (Figs. 2-4). This may be due to the relatively high viscosity of the material and the limited penetration/interaction time (the material was light cured directly after application). These factors along with the thixotropic properties of this cement can also explain the improved adaptation of
the cement when applied under pressure, as evidenced by a reduced amount of porosities at the interface (Fig. 2). This is clinically very relevant, as most indirect restorations are luted with pressure, as for example crowns, inlays and onlays.

TEM analysis revealed a very irregular interaction zone in between the cement and dentin. This zone probably corresponds to the rough and irregular smear layer, as produced by the regular-grit diamond bur. This smear layer was partially demineralized and subsequently infiltrated by the resin cement. Below this infiltrated smear layer, a vague deeper infiltration was detected when the specimens were stained (Fig. 3). This probably corresponds to some phosphoric-ester monomer infiltration, as this monomer was previously shown to be highly stainable. The low demineralization effect of RelyX Unicem, despite its low initial pH, was confirmed by applying it to fractured dentin. Even though the interaction was not hampered due to the lack of an intermediary smear layer, dentin did not appear to be demineralized at all in the unstained sections (Fig. 4a). Staining with heavy metals on the other hand disclosed some limited interaction within a range of 100–200 nm (Fig. 4c, d). This probably corresponds to the deeper infiltration of phosphoric-acid monomers below the smear layer, as seen in Fig. 3d. Consequently, the third hypothesis that the bonding mechanism of RelyX Unicem to dentin is similar to that obtained with a self-etch adhesive, must be rejected, as no distinct demineralization and hybridization was observed, as commonly seen with self-etch materials (Fig. 6).

Acid etching prior to the application of RelyX Unicem raised the enamel μTBS to the same level as that of the control (Table 2), thus corroborating the second hypothesis that use of phosphoric acid prior to luting improves the bonding effectiveness to enamel. This hypothesis must, however, be rejected for the dentin group, as the μTBS was
significantly lower than when applied without phosphoric acid. The phosphoric acid thus decreased the dentin bonding effectiveness, whilst an increased bonding effectiveness was expected, because the ‘weak’ smear layer was removed by phosphoric acid.\textsuperscript{10} Apparently, the thick and compact collagen mesh prevented the viscous cement from reaching the deeper unaffected dentin (Fig. 5c). Consequently, a weak layer of hydroxyapatite-depleted collagen remained in between RelyX Unicem bonded to acid-etched dentin specimens (Table 3), (2) significant lower bond strengths were recorded, despite the formation of particle-reinforced resin tags and complete smear layer removal (Table 2), and because (3) remnants of the poorly infiltrated collagen mesh remained attached to the resin cement after \textit{μ}TBS testing (Fig. 5b). Such a demineralized, non resin-reinforced, collagen layer is known to be prone to degradation processes\textsuperscript{11,12} and will also enhance other degradation processes as resin elution and nanoleakage.\textsuperscript{13–15} Hence, a phosphoric acid pre-treatment should be limited to enamel only. It might however be worthwhile to investigate if other smear layer removing procedures that do not demineralize dentin, as for example air abrasion, may improve the \textit{μ}TBS of this cement to dentin.

\section*{Conclusion}

(1) RelyX Unicem should always be applied with some pressure to ensure that the cement intimately adapts to the cavity wall. (2) The cement only interacted superficially with dentin and enamel. (3) The best bonding effectiveness with this new auto-adhesive cement was obtained by selectively acid-etching enamel prior to luting.

\section*{Acknowledgements}

This study was supported in part by a Research Grant of the Fund for Scientific Research Flanders (F.W.O.-grant ‘Krediet aan Navorsers’ 1.5.142.02), in part by 3M ESPE (Seefeld, Germany) and in part by a fund of the Toshio Nakao Chair for Adhesive Dentistry inaugurated at the Catholic University of Leuven with B. Van Meerbeek and P. Lambrechts awarded as chairholders. We thank the respective manufacturers for the generous donation of materials.

\section*{References}